Immunization Safety Review: Multiple Immunizations and Immune Dysfunction

Kathleen Stratton, Christopher B. Wilson and Marie C. McCormick, Editors, Immunization Safety Review Committee, Board on Health Promotion and Disease Prevention

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Board on Health Promotion and Disease Prevention

INSTITUTE OF MEDICINE

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Willing is not enough; we must do.

—Goethe

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by **Robert Lawrence**, Johns Hopkins Bloomberg School of Public Health, and **Floyd Bloom**, The Scripps Research Institute. Appointed by the National Research Council and Institute of Medicine, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Foreword

Vaccines are among the greatest public health accomplishments of the past century. In recent years, however, a number of concerns have been raised about the safety of, and need for, certain immunizations. Indeed, immunization safety is a contentious area of public health policy, with discourse around it having become increasingly polarized and exceedingly difficult. The numerous controversies and allegations surrounding immunization safety signify an erosion of public trust in those responsible for vaccine research, development, licensure, schedules, and policy-making. Because vaccines are so widely used—and because state laws require that children be vaccinated to enter daycare and school, in part to protect others—immunization safety concerns should be vigorously pursued in order to restore this trust.

It is in this context that the Institute of Medicine (IOM) was approached more than a year ago by the Centers for Disease Control and Prevention and the National Institutes of Health to convene an independent committee that could provide timely and objective assistance to the Department of Health and Human Services in reviewing emerging immunization-safety concerns.

The IOM was chartered by the National Academy of Sciences in 1970 to serve as an adviser to the federal government on issues affecting the public's health, as well as to act independently in identifying important issues of medical care, research, and education. The IOM thus brings to this mission three decades of experience in conducting independent analyses of significant public health policy issues. In particular, as described in more detail in this report, the IOM has a long history of involvement in vaccine safety. The IOM published its first

major vaccine-safety report in 1977, followed by a subsequent report in 1988; both focused on the safety of polio vaccines. Two subsequent major reports, published in 1991 and 1994, examined the adverse events of childhood vaccines. Since then, the IOM has conducted several smaller studies and workshops focused on various vaccine-safety topics. These studies were all well received by both the public and policy makers, and previous IOM committees on vaccine safety issues have been viewed as objective and credible.

Given the sensitive nature of the present immunization safety review study, the IOM felt it was especially critical to establish strict criteria for committee membership. These criteria prevented participation by anyone with financial ties to vaccine manufacturers or their parent companies, previous service on major vaccine-advisory committees, or prior expert testimony or publications on issues of vaccine safety.

The rationale for imposing these stringent criteria was twofold. First, given growing public concern about vaccine safety and the public scrutiny surrounding this committee's work, it was important to establish standards that would preclude any real or perceived conflict of interest or bias on the part of the committee members. While the committee members all share a belief in the benefits of vaccines to the public health, none of them has any vested interest in any of the vaccine safety issues that will come before them. Second, the IOM wanted to ensure consistency in the committee membership and avoid having members recuse themselves from the deliberations because they had participated in the development or evaluation of a vaccine under study.

Thus, the IOM has convened a distinguished panel of 15 members who possess significant breadth and depth of expertise in a number of fields, including pediatrics, neurology, immunology, internal medicine, infectious diseases, genetics, epidemiology, biostatistics, risk perception and communication, decision analysis, public health, nursing, and ethics. The committee members were chosen because they are leading authorities in their respective fields, are well respected by their colleagues, and have no conflicts of interest. This committee brought a fresh perspective to these critically important issues and approached its charge with impartiality and scientific rigor.

The IOM does not propose the use of the criteria it has laid out above in selecting members for federal vaccine advisory committees. The IOM committee was convened for a very different purpose from the usual federal vaccine advisory committees and, as such, required different standards.

As with all reports from the IOM, the committee's work was reviewed by an independent panel of experts. The purpose of the review process is to enhance the clarity, cogency, and accuracy of the final report and to ensure that the authors and the IOM are creditably represented by the report published in their names. The report review process is overseen by the National Research Council's (NRC) Report Review Committee (RRC), comprised of approximately 30 members of the National Academy of Sciences, National Academy of Engineer-

ing, and IOM. The IOM, in conjunction with the RRC, appoints a panel of reviewers with a diverse set of perspectives on key issues considered in the report. Unlike the selection criteria for committee membership (discussed above), many reviewers will have strong opinions and biases about the report topic. The composition of the review panel is not disclosed to the committee until after the report is approved for release. While the committee must consider and evaluate all comments from reviewers, it is not obligated to change its report in response to the reviewers' comments. The committee must, however, justify its responses to the reviewers' comments to the satisfaction of the RRC's review monitor and the IOM's review coordinator. A report may not be released to the sponsors or the public, nor may its findings be disclosed, until after the review process has been satisfactorily completed and all authors have approved the revised draft.

This report represents the unanimous conclusions and recommendations of that dedicated committee whose members deliberated a critical health issue. The report's conclusions and recommendations should be of value to all concerned about these important matters.

Kenneth I. Shine
President, Institute of Medicine

Acknowledgments

The committee would like to acknowledge the many speakers and attendees at its open meeting held on November 12, 2001, in Seattle. The discussions were informative and helpful. The committee would also like to thank those people who submitted information to the committee through the mail or e-mail. Finally, the committee would like to thank the IOM staff for their dedication to this project. Without their commitment, attention to detail, creativity, sensitivity, and hard work, this project would be unworkable.

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Executive Summary

ABSTRACT

By two years of age, healthy infants in the United States can receive up to 20 vaccinations to protect against 11 diseases. Although most people know that vaccines effectively protect against serious infectious diseases, approximately one-quarter of parents in a recent survey believe that infants get more vaccines than are good for them, and that too many immunizations could overwhelm an infant's immune system. The Immunization Safety Review Committee reviewed the evidence regarding the hypothesis that multiple immunizations increase the risk for immune dysfunction. Specifically, the committee looked at evidence of potential biological mechanisms and at epidemiological evidence for or against causality related to risk for infections, the autoimmune disease type 1 diabetes, and allergic disorders.

There are reasonable theories for how vaccines could cause these effects. However, for allergic disease and type 1 diabetes, the evidence from animal and clinical studies is weak that relevant biological mechanisms operate in humans after receipt of vaccines. The biological mechanisms evidence regarding increased risk for infections is strong. However, the committee found that the epidemiological evidence (i.e., from studies of vaccine-exposed populations and their control groups) favors rejection of a causal relationship between multiple immunizations and increased risk for infections and for type 1 diabetes. The epidemiological evidence regarding risk for allergic disease, particularly asthma, was inadequate to accept or reject a causal relationship.

These immune disorders carry heavy individual and societal burdens, and serious vaccine-preventable disease could increase if parents unnecessarily avoid immunizing their children due to continuing concerns about this issue.

Because vaccines are given to healthy children to protect others in addition to themselves, it is important to understand fully the possible risks of serious adverse consequences of vaccines. Therefore, the committee recommends continued attention in the form of policy analysis, research, and communication strategy development. However, the committee does not recommend a review by national and federal vaccine-related advisory bodies of the licensure or schedule of administration of the vaccines administered to infants in the United States on the basis of concerns about immune dysfunction. See Box ES-1 for a summary of all conclusions and recommendations.

Immunization to protect infants and children from vaccine-preventable diseases is one of the greatest achievements of public health. Immunization is not without risks, however. It is well established, for example, that the oral polio vaccine can on rare occasion cause paralytic polio.

The Immunization Safety Review Committee was established by the Institute of Medicine (IOM) to evaluate the available evidence on a series of immunization safety concerns. While all of the committee members share the view that immunization is generally beneficial, none of them has a vested interest in the specific immunization safety issues that come before the group.

For each hypothesis to be examined, the committee assesses both the scientific evidence and the significance of the issue for society.

> The *scientific* assessment has two components: an examination of the epidemiological and clinical evidence regarding a possible causal relationship between the immunization and the adverse event, and an examination of experimental evidence for any biological mechanism(s) relevant to the hypothesis.
>
> The *significance* assessment addresses such considerations as the burden of the health risks associated with the vaccine-preventable disease and with the adverse event in question, as well as the level of public concern about the safety issue.

In this report, the committee examines the hypothesis that receipt of multiple immunizations adversely affects the developing immune system.

The examination of experimental evidence for biological mechanisms has been referred to in previous reports of this committee (IOM, 2001a, 2001b) and others (IOM, 1991, 1994) as an assessment of "biological plausibility." The committee has noted, however, that the term is a source of confusion on at least two fronts. First, it is associated with a particular set of guidelines (sometimes referred to as the Bradford Hill criteria) for causal inference from epidemiological evidence; and second, readers sometimes regard the term with a degree of certainty or precision the committee never intended. For example, a relationship

between immunization and a particular adverse event may be found to be biologically plausible at the same time that the epidemiological evidence is found to be inadequate to accept or reject a causal relationship.

Given the resulting lack of clarity, the committee believes that the adoption of new terminology and a new approach to its discussions of experimental biological data are warranted. The committee will thus review evidence regarding "biological *mechanisms*" that might be consistent with the proposed relationship between immunization and a given adverse event. This biological assessment section of the report is written distinct from any argument regarding the causality of such relationships.

Beginning with this report, the committee will summarize the biological mechanisms as theoretical only, or as having derived from either experimental evidence in animals or *in vitro* systems or from mechanism-related, biological evidence in humans of response to vaccine or infectious disease antigen. If there is either experimental evidence (e.g., from animals) or evidence in humans for a mechanism, the committee will designate it as weak, moderate, or strong. Though the committee tends to judge biological evidence in humans to be "stronger" than experimental evidence, the strength of the evidence also depends on other factors, such as experimental design and sample size. The conclusions drawn from this review will depend both on evidence and scientific judgment.

UNDER REVIEW

Over the past two decades, the pediatric immunization schedule has grown more complicated. In 1980, infants received immunizations against four diseases (diphtheria, tetanus, pertussis, and polio). Today, a healthy infant immunized in complete accord with the recommended childhood immunization schedule receives up to 15 doses of five vaccines to protect against seven diseases by 6 months of age and up to 20 doses of seven vaccines to protect against 11 diseases by 2 years of age. According to a recent survey, a substantial minority of parents (23–25%) believes that getting too many immunizations weakens a child's immune system and that children get more immunizations than are good for them (Gellin et al., 2000).

The Immunization Safety Review Committee was asked to address the hypothesis that multiple immunizations can adversely affect the developing immune system. One particular concern, for example, is related to increases in the incidence of diseases such as asthma and type 1 diabetes—conditions associated with immune system dysfunctions. Although genetic factors are known to affect the risk of these diseases, increases in their incidence seem more likely to reflect changes in environmental exposures than in the genetic makeup of a population. Immunization has been proposed as one possible adverse environmental modifier of immune function.

To conduct its review, the committee had to establish a clear statement of the question before it, as well as a manageable scope of inquiry. The committee focused on exposure to multiple immunizations during infancy (less than two years of age), a period of active immune system development. The committee included studies of "one vaccine" if it contained antigens against more than one disease or more than one strain of infectious agent. For example, the diphtheria and tetanus toxoids and pertussis vaccine would be considered to represent "multiple immunization." The committee restricted its considerations regarding causality to those vaccines used in the United States.

Because immune system dysfunction is a broad term—adverse outcomes can result from stimulation of harmful immune responses or suppression of beneficial immune responses—the committee had to define it for the purposes of this study. The scope of the committee's inquiry can be summarized in the following three questions:

1. Do multiple immunizations have adverse short-term effects on the developing infant immune system that are reflected in increased susceptibility to heterologous infection (infections other than those targeted by the immunization)?
2. Does exposure to multiple antigens, as administered in vaccines, directly and permanently redirect or skew the immune system toward autoimmunity, as reflected in type 1 diabetes?
3. Does exposure to multiple antigens, as administered in vaccines, directly and permanently redirect or skew the immune system toward allergy, as reflected in asthma?

The committee was unable to address the concern that repeated exposure of a susceptible child to multiple immunizations over the developmental period may also produce atypical or non-specific immune or nervous system injury that could lead to severe disability or death (Fisher, 2001). There are no epidemiological studies that address this. Thus, the committee recognizes with some discomfort that this report addresses only part of the overall set of concerns of some of those most wary about the safety of childhood immunization.

The committee collected information from several sources. At an open scientific meeting in November 2001 (see Appendix C), academic researchers gave presentations on specific scientific issues germane to the topic. All information presented to the committee at that meeting can be viewed on the project website (www.iom.edu/imsafety). In addition, an extensive review was performed of the published, peer-reviewed scientific and medical literature. (see Appendix D).

Autoimmune Diseases

Collectively, diseases of autoimmunity affect 3 to 5 percent of the population in the United States (Jacobson et al., 1997). Autoimmune diseases are mediated by T cell and/or T cell-dependent B cell responses directed against self-

antigens, and the T cell responses in most autoimmune diseases are dominated by interferon-_ producing CD4 T cells, commonly referred to as Th1 T cells (Marrack et al., 2001). An autoimmune process can target individual organs, such as the central nervous system in multiple sclerosis, or can operate throughout the body, as in systemic lupus erythematosus. For this report, the committee focused on type 1a diabetes, which is associated with an autoimmune-mediated loss of insulin-secreting pancreatic cells. (Type 1b refers to diabetes associated with a loss of insulin secretion, for reasons unknown. Many epidemiological studies do not distinguish between these two types.) Type 2 diabetes is not associated with destruction of insulin-secreting cells. Type 1 diabetes has been referred to as "childhood" or insulin-dependent diabetes, while type 2 diabetes has been referred to as "adult-onset" diabetes. However, the onset of either form of the disease can occur at any age.

Worldwide, estimates of the incidence of type 1 diabetes in children under 14 years of age range from 0.1 per 100,000 in parts of China and Venezuela to 36.8 per 100,000 in Sardinia and 36.5 per 100,000 in Finland (Karvonen et al., 2000). As reported by Karvonen and colleagues (2000), estimated incidence for the early 1990s in the United States locations range from 11.7 per 100,000 in Chicago to 17.8 per 100,000 in Allegheny County, Pennsylvania.

Allergic Diseases

Allergy is responsible for a variety of acute and chronic health problems, including anaphylaxis, rhinitis, asthma, and allergic eczema. These conditions reflect an overreaction of the immune system to allergens—normally harmless environmental agents such as pollens, dust mites, insect venom, and certain foods. Under certain circumstances, exposure to an allergen primes the immune system for hypersensitivity reactions involving allergen-specific IgE antibodies and Th2 cells.

The committee focused on allergic asthma. Characteristic symptoms of asthma are episodes of shortness of breath, coughing, wheezing, and chest tightness. These symptoms reflect an acute bronchial hyperresponsiveness to specific allergens and other environmental factors, and a chronic inflammation of the airways (IOM, 2000; Parham, 2000).

The prevalence of asthma has increased in the United States and other countries over the past 30 years (Grant et al., 1999). An international study of asthma in children found that prevalence was higher in more developed countries (Asher and Weiland, 1998). In the United States, the prevalence rates of self-reported asthma rose from 3.1 percent in 1980 to 5.4 percent in 1994, an increase of 74 percent (Mannino et al., 1998). For children aged 0 to 4 years, rates increased by 159 percent during this period (from 2.2 percent to 5.7 percent). Increases in asthma prevalence were seen in all race, sex, age, and regional groups in the United States.

Antigen Load

Central to the concerns about multiple childhood immunizations is whether the recommended schedule overloads an infant's immune system. That is, are there quantitative or qualitative aspects of the antigens to which an infant is exposed through immunization that lead to an inability of the developing immune system to respond appropriately?

Calculations reviewed by the committee (Kollman, 2001; Offit et al., 2002) suggest that the number of antigens contained in the complete set of vaccines that comprise the recommended childhood immunization schedule has actually decreased over the past 20 to 30 years, despite the increased number of vaccines and vaccine doses administered. The removal from the schedule of two vaccines, smallpox and the whole cell pertussis vaccine, accounts for this decrease. Routine use of the smallpox vaccine, which contained approximately 200 distinct and potentially antigenic elements, was discontinued in the United States in 1971. The whole-cell pertussis vaccine was replaced by an acellular vaccine, the first of which was approved by the FDA in 1991. The whole-cell vaccine contained approximately 3,000 distinct and potentially antigenic components, whereas the acellular vaccine contains only 2–5 antigens.

Vaccines added to the immunization schedule over the past 20 years have relatively few antigens. For example, the hepatitis B vaccine contains only one antigen. Therefore, the decrease in vaccine antigens from the removal of smallpox and whole cell pertussis vaccines far exceeds the increase of antigens from the addition of newer vaccines added to the schedule.

Another question is whether infants are capable of responding adequately to the antigens presented by immunization. Although the numbers of different T cell receptors present in human neonates has not been determined directly, their diversity has been shown by several groups to be similar to that of the adults. This is the basis for the notion that human infants have the capacity to respond to the substantial number of foreign molecules (e.g., bacterial antigens) to which they are exposed shortly after birth. This is consistent with the theoretical estimates presented to the committee, which suggest that the capacity of the infant's immune system is at least 1000 times greater than that maximally required to respond to vaccines (Kollman, 2001; Offit et al., 2002).

Over the course of several decades, the antigen load presented to the developing immune system has undergone significant qualitative changes, particularly in the context of the total antigen exposures during infancy and childhood. Approximately a decade ago, researchers interested in the changing epidemiology of several diseases began formulating the "hygiene hypothesis." This hypothesis suggests that the increasingly aseptic environment in which children in developed countries live has led to changes in the development of the immune system, causing an increase in allergic disease (Rook, 2000; Strachan, 2000; Wills-Karp et al., 2001). In keeping with the hygiene hypothesis, factors that

decrease the risk for allergy include the presence of pets, infections through the fecal-oral route, and rural living.

The proposed explanation for an immune system role in these epidemiological observations is that early exposure to infectious diseases and environmental microbes "shapes" the developing immune system toward a Th1[1] cell responsiveness, which is generally considered a protective immune response (i.e., to host defense against intracellular pathogens and allergy). Eliminating these early exposures through hygienic practices and altered behaviors is thought to predispose the immune system toward a Th2[2] cell responsiveness, which is associated with allergy.

The most recent refinement of the hygiene hypothesis includes regulatory cell imbalance (Rook, 2001; Wills-Karp et al., 2001). This Th2-skewing or regulatory cell imbalance, some theorize, is exacerbated by exposure to vaccines, many of which evoke a Th2 response instead of the Th1 response that would be generated by wild-type infections with the diseases that the immunizations prevent.

Not yet clear is the role vaccines may have in directly altering the development of the immune system, or the relative contribution of immunization-related changes in the context of the hygiene hypothesis. Vaccine-induced immune responses may differ from those resulting from wild-type infection because of differences in context, including differences in their timing, either in terms of age at exposure or of the sequence of antigen exposure. Most certainly, the route of exposures—that is, an injection rather than a respiratory or gastrointestinal exposure—is different from what it had been. Under debate is whether that difference in exposure is associated with adverse health outcomes.

In any case, the number of infections prevented by immunization is actually quite small compared with the total number of infections prevented by other hygienic interventions such as clean water, food, and living conditions.

SCIENTIFIC ASSESSMENT

Causality

Heterologous Infection

The committee reviewed several case-control or cohort studies (Black et al., 1991; Burstein and Fleisher, 1994; Davidson, 1991; Griffin et al., 1992; Kristensen et al., 2000) and a randomized controlled trial (Otto et al., 2000). Vaccine

[1] Th1 (h stands for helper) cells travel to the site of infection and secrete cytokines that mainly activate macrophages. Upon activation, macrophages will phagocytose extracellular pathogens and then kill them.

[2] The primary function of Th2 cells is to stimulate B cells to make antibodies which bind to extracellular bacteria and virus particles. Th2 cells work within secondary lymphoid tissue.

exposure varied among the studies but fit the committee's definition of exposure to "multiple immunizations." The studies examined the effects of adding one vaccine to an existing immunization schedule, of one vaccine dose consisting of antigens from more than one infectious agent or strain of virus (e.g., DTP, OPV, or MMR), or of several vaccines received at the same time. Outcome measures in the studies also varied, with the "disease" group including subjects who had a positive culture to invasive bacterial disease, who had symptoms related to infectious diseases, or who had died. Limitations of the studies included a potential health care utilization bias and high dropout rates. Despite these variations and limitations, the overall findings from the studies consistently demonstrated either no effect or a beneficial effect of multiple immunizations on heterologous disease. **Therefore, the committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of heterologous infections.**

Type 1 Diabetes

The committee found five controlled studies (Blom et al., 1991; DeStefano et al., 2001; EURODIAB, 2000; Heijbel et al., 1997; Karvonen et al., 1999) and three ecological studies (Classen, 1996; Hiltunen et al., 1999; Hyoty et al., 1993) that examined this relationship. The studies looked at the effects of adding one vaccine to an existing immunization schedule, of one vaccine dose consisting of antigens from more than one infectious agent or strain of virus (e.g., DTP, OPV, or MMR), or of several vaccines received at the same time. Despite these variations, the overall findings from the studies consistently demonstrated no effect of multiple immunizations on the incidence of type 1a diabetes. **Therefore, the committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of type 1 diabetes.**

Allergic Disease

The committee reviewed five studies that utilized controls (Farooqi and Hopkin, 1998; Hurwitz and Morgenstern, 2000; Kemp et al., 1997; Wickens et al., 2001), including a randomized controlled trial (Nilsson et al., 1998) and one ecological study (Anderson et al., 2001). Outcomes assessed included allergic symptoms (wheezing) and allergic disorders (hay fever and asthma). All the studies examined exposure to DTaP or DTwP, and other vaccines given concurrently, such as MMR and polio vaccines, but no two studies examined exactly the same exposure.

While many of these studies reported elevated odds ratios linking immunizations to some allergic outcome, some of which were statistically significant, methodological weaknesses within individual studies, as well as the pattern of

results across studies diminish the confidence that the observed associations reflect causal relationships. In the two studies that reported a significant positive effect of DTP or tetanus immunization or the pertussis component of DTwP (Farooqi and Hopkin, 1998; Hurwitz and Morgenstern, 2000), potential sampling bias, caused by substantial losses to follow-up or restriction to subjects with regular medical care, could have distorted the relationship between immunization and allergies.

A problem in most of the studies was that the number of unvaccinated children was small, limiting the ability to control for potentially confounding factors, which are numerous and strong for the outcomes of asthma and atopy, and particularly complex when considering risk over an entire childhood. Adequate control of confounding is a serious issue for observational designs, particularly in this domain, as nonimmunized children typically differ on baseline characteristics from immunized children in ways that are not always measurable.

Finally, the findings of the studies, taken as a whole, did not show a consistency of findings that would outweigh the concerns about individual studies. While some studies pointed to the pertussis vaccine as a risk factor for allergic syndromes with no effect of MMR, another found that MMR vaccine was the strongest risk factor. The ecological study indicated a protective DPT effect, and the only randomized study indicated minimal or no effect of pertussis vaccines, with a non-significant reduction in risk from the whole-cell vaccine.

Given the design weaknesses in the observational studies, and a randomized trial study that does not support the risk factor most frequently implicated in the observational studies, **the committee concludes that the epidemiological and clinical evidence is inadequate to accept or reject a causal relationship between multiple immunizations and an increased risk of allergic disease, particularly asthma.**

Biological Mechanisms

Although biological data do not provide an independent basis for evaluating causality, they can help validate epidemiologically based conclusions for or against causal associations; such data can also guide further investigation when epidemiological evidence is inconclusive. The mechanisms considered by the committee represent two possible pathways to adverse outcomes: stimulation of harmful immune responses, or suppression of beneficial immune responses. The stimulation of harmful immune responses involves the mechanisms of molecular mimicry,[3] bystander activation, [4] and nonspecific or polyclonal T-cell and/or B-

[3] Molecular mimicry is the antigenic similarity between a pathogen antigen and a cellular antigen which results in the induction of antibodies or T cells that act against the pathogen but also cross-react with the self antigen (Parham, 2000).

cell activation. The suppression of beneficial immune responses is addressed in terms of the hygiene hypothesis and the prevention of potentially protective infections through immunization.

In theory, molecular mimicry, bystander activation, and impaired immunoregulatory mechanisms might act in an additive or synergistic manner to affect the risk of autoimmunity. There is, however, no experimental evidence for molecular mimicry by any of the vaccines in the current routine childhood immunization schedule to create an antigenic epitope[5] capable of cross-reaction with self epitopes. **Therefore, in the absence of experimental or human evidence regarding molecular mimicry or mercury-induced modification of any vaccine component to create an antigenic epitope capable of cross-reaction with self epitopes as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that these mechanisms are only theoretical.**

There is some evidence of a bystander effect associated with immunization, but this effect is 1) relatively modest compared to those resulting from wild-type infection, 2) most evident to co-administered vaccine antigens rather than other environmental antigens or infections and 3) inconsistently shown. Current vaccines have, on balance, weak or no Th1-inducing activities. BCG appears to demonstrate the principle for co-administered antigens. However, BCG is not used in the U.S., so the relevance for this mechanism in the effects of the U.S. recommended schedule is not demonstrated. Viral vaccines carry some potential for bystander activation, but likely would have a small effect, if it occurs at all. The data on DTaP vaccine indicates that Th1 dominance is not prominent. There is also no evidence in humans that vaccine antigens lead to the pathophysiological disease state. The limited evidence from humans that does exist regards surrogates of the disease process, that is, just some components of the events that would need to take place for the appearance of clinically relevant pathophysiology. **Thus, the committee concludes that there is weak evidence for bystander activation, alone or in concert with molecular mimicry, as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.**

In the absence of experimental or human evidence regarding loss of protection against a homologous infection as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that

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[4] Bystander activation results when an infection creates environmental conditions that allow the activation of self-reactive T and B cells that are normally held in check. It does not require that antigens of the infectious agent be structurally similar to self-antigens.

[5] An epitope is a molecule's specific antigenic site that is bound by an antibody.

this mechanism is only theoretical. On balance, the current recommended childhood immunization schedule in the United States appears less likely to act as an initiator or facilitator of autoimmunity than the schedule of the past.

On a numerical basis, vaccine-preventable infections represent a minute fraction of the overall infectious and microbial exposure in childhood. For immunization to have an impact on autoimmunity under the hygiene hypothesis, it would be necessary for one or more vaccine-preventable diseases to be particularly important for conditioning immunoregulatory immune responses. The gastrointestinal tract is thought to play a particularly critical role in this process, so it would follow that immunizations that affect infection or colonization of the gut would be good candidates, but none of the childhood vaccines currently in use do so. Data from animal models suggest that no one infection is likely to be key, but rather a global reduction in microbial contact could be a factor.

The theory by which the hygiene hypothesis, originally proposed on the basis of epidemiological data, could explain an increase in incidence of autoimmune (or allergic) disease is substantial, and the biological evidence in support of the hygiene hypothesis is moderate. However, the potential contribution of vaccine-preventable diseases as part of this model is minimal. **Therefore, in the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.**

Considering molecular mimicry, bystander activation, and impaired immunoregulation collectively rather than individually, the committee concludes that there is weak evidence for these mechanisms as means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.

The biological mechanisms by which immunizations that contain microbial stimuli favor Th1 responses and immunizations containing alum favor Th2 responses are well established. Although the impact of immunization on heterologous allergic responses is unknown, on balance the current routine childhood immunization schedule in the United States is less likely to favor Th1 responses to heterologous antigens and more likely to favor Th2 responses. **The committee concludes that there is weak evidence for bystander activation as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.**

The theory by which the hygiene hypothesis could explain an increase in incidence of allergic diseases is substantial. However, the potential contribution of vaccine-preventable diseases as part of this hypothesis is minimal. **In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an indi-**

vidual's risk of allergy, the committee concludes that this mechanism is only theoretical. The committee also concludes that there is weak evidence for the existence of any biological mechanisms, collectively or individually, by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

The committee concludes that there is strong evidence for the existence of biological mechanisms by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk for heterologous infections.

SIGNIFICANCE ASSESSMENT

The committee's assessment of the significance of concerns about possible immune system dysfunctions took several factors into consideration: the burden of the possible adverse outcomes of autoimmune diseases such as type 1 diabetes and allergic diseases such as asthma; indications of the extent of the concern about multiple immunizations; and views regarding the framework for immunization policy-making.

Although parents appear to value immunization, a substantial minority (23-25%) believes that multiple immunizations could be harmful (Gellin et al., 2000). Autoimmune and allergic diseases are common in the United States, after all, and the incidence of these conditions appears to be increasing. As represented by type 1 diabetes and asthma, these conditions are life-threatening if not adequately treated and are associated with substantial health care costs.

A better understanding of parents' perceptions of risk and decisionmaking may be necessary in order to prevent decreases in immunization rates and increases in vaccine-preventable disease. Current approaches to immunization policy-making emphasize epidemiological and economic considerations, but a recent paper suggests that these policies may benefit from greater attention to ethical issues, including personal liberty and equity in allocation of the benefits and burdens of immunization (Feudtner and Marcuse, 2001). **Thus, the committee concludes that concern about multiple immunizations has been, and could continue to be, of societal significance in terms of parental worries, potential health burdens, and future challenges for immunization policy-making.**

RECOMMENDATIONS REGARDING PUBLIC HEALTH RESPONSE

With government and professional recommendations calling for young children to receive increasing numbers of immunizations, it is important to respond to concerns about possible increases in risk of allergic or autoimmune diseases. Although the committee's review points to no causal relationship between multiple immunizations and type 1 diabetes or risk of infection, and the

review is inconclusive for asthma, the biological evidence does provide weak support for increased risk of allergy and autoimmunity and strong support for increased risk of infection (see Table ES-1 for summary). Further study of such associations poses difficult scientific challenges, and relevant epidemiological evidence remains limited. Several important scientific and policy issues, therefore, deserve further public health attention.

Policy Review

The nature of the childhood immunization schedule is likely to change in response to such factors as the development of new vaccines and utilization of novel delivery systems. Changing perceptions of disease risks—derived from antibiotic resistance, threats of bioterrorism, or (re)emerging infectious diseases—could also lead to wider use of existing vaccines not currently included in the immunization schedule. As the array of available vaccines and disease targets expands the current emphasis on universal recommendations and state mandates for vaccine use should be reassessed (Feudtner and Marcuse, 2001). **The committee recommends that state and federal vaccine policymakers consider a broader and more explicit strategy for developing recommendations for the use of vaccines.**

Feudtner and Marcuse (2001) have provided a beginning for such discussion by urging consideration of a range of perspectives (e.g., those of individuals, families, organizations, society) regarding the benefits, risks, and ethical implications of vaccine use and immunization policies. Priorities can be expected to differ among the diverse perspectives, and policymakers must consider how to achieve an equitable balance. These issues require long-term planning and evaluation; a reactive response to the next schedule addition will be much less effective than a proactive assessment and strategy development across-the-board.

As part of this overall effort, the committee encourages an exploration of the merits of accommodating requests for alternative vaccine-dosing schedules and the development of appropriate clinical guidance for any such alternatives. A more flexible schedule might allow for a reduction in the number of vaccines administered at one time. Such a change would respond to some concerns about multiple immunizations; but it would also have disadvantages, such as requiring more health care visits, that might contribute to lower rates of immunization coverage in the population and consequent increases in morbidity and mortality. In addition, such a change would require extensive communication with health-care providers and health plans in order that appropriate immunizations occur and are reimbursed equivalently to those on the “traditional” schedule.

By issuing the recommendation above, the committee does not intend to signal concern about health consequences of the multiple immunizations in the recommended childhood immunization schedule. In fact, **the committee does not recommend a policy review—by the CDC’s Advisory Committee on**

Immunization Practices (ACIP), the American Academy of Pediatrics' Committee on Infectious Diseases, and the American Academy of Family Physicians—of the current recommended childhood immunization schedule on the basis of concerns about immune system dysfunction.

The committee does not recommend a policy review by the Food and Drug Administration's Vaccines and Related Biologic Products Advisory Committee of any currently licensed vaccines on the basis of concerns about immune system dysfunction.

Research

The committee concluded that the findings available from epidemiological sources and consideration of possible biological mechanisms do not at this time warrant specialized studies of possible associations between multiple immunizations and immune system dysfunction. Instead, the committee encourages epidemiological studies on immunization safety conducted within the framework of ongoing research and surveillance programs on allergy, autoimmune disease, and vaccine safety; it also encourages additional basic research on the immune system and on allergy and autoimmune diseases.

The committee emphasizes the need for continuing surveillance of vaccine recipients and possible adverse events. Changes in the immunization schedule may present opportunities to study whether or not the incidence of adverse health outcomes also changes. Several vaccine-related data resources already exist, including the Vaccine Adverse Event Reporting System (VAERS), the Vaccine Safety Datalink (VSD), and state and local immunization registries. **The committee recommends exploring the feasibility of using existing vaccine surveillance systems, alone or in combination, to study safety questions related to asthma and other important allergic disorders, as well as to study type 1 diabetes and other important autoimmune diseases.** An important component of such research will be the use of uniform standards of observation and evaluation.

In addition, surveillance of autoimmune diseases and allergic disorders should be strengthened. Disease registries and long-term research programs that identify individuals with these diseases, or with known genetic risk factors, could be an efficient means of finding subjects for either retrospective or prospective studies of possible vaccine-related risks. **The committee recommends exploring the use of such cohorts for research on possible vaccine-related disease risks. Furthermore, the committee recommends that disease registries and research programs for autoimmune and allergic disorders routinely collect immunization histories as part of their study protocols.**

Research on the developing human immune system, especially in relation to vaccines, is limited. Studies of animal models are essential to advancing knowledge of the immune system, but those studies have limits because of important

differences between humans and animals. Thus, **the committee recommends continued research on the development of the human infant immune system.**

Genetic factors are known to be an important source of variability in the responses of the human immune system and in the risk of allergic or autoimmune disease. But understanding of the complex interactions among genetic variables, as well as of the interactions between those variables and environmental exposures (including vaccines and wild-type viral and bacterial agents), remains incomplete. **The committee endorses current research efforts aimed at identifying genetic variability in human immune system development and immune system responsiveness as a way to gain a better understanding of genetic susceptibility to vaccine-based adverse events.**

For some autoimmune and allergic disorders, surrogate biological markers of disease or disease risk have been identified. In particular, in individuals at risk for type I diabetes, the development of multiple autoantibodies to GAD65 (glutamic acid decarboxylase), IA-2 (protein tyrosine phosphatase-like molecule), and insulin correlate strongly with later development of overt type I diabetes (Notkins and Lernmark, 2001). However, there are to date no other surrogate markers that have sufficient predictive power to be useful in monitoring risk for other autoimmune diseases in children receiving routine immunizations (Leslie et al., 2001). For allergic disorders, the clinical history of allergic diseases should be collected in follow-up evaluations, and the feasibility of specific tests for atopy considered. In theory, collecting data on known markers in the course of vaccine research and testing would present an opportunity to study the prevalence of such markers before and after vaccination. Similarly, it might also be possible to study whether the prior presence of a marker was associated with differences in the response to a vaccine. **The committee recommends exploring the feasibility of collecting data on surrogate markers for type I diabetes and clinical history of allergic diseases in the vaccine testing and licensing process.** Such might also be useful in vaccine-related studies in high-risk cohorts. **The committee recommends exploring surrogates for type I diabetes and clinical history of allergic diseases in existing cohort studies of variations in the immunization schedule.**

Communication

Along with the increasingly complicated immunization schedule has come a dramatic increase in the complexity of immunization safety issues, and it appears that some people have redefined their conceptions of the related risks and benefits. The focus seems to have shifted from whether children will get a disease if they are not vaccinated to whether children will experience temporary or potentially longer-term adverse events if they *are* vaccinated (McPhilips and Marcuse, 2001).

The committee is not convinced, however, that available reports on such attitudes provide an adequate scientific basis for understanding either these

changes in perception or the groups that are experiencing them. More information is needed in order to develop effective risk-benefit communication strategies on immunization and immunization safety.

A deeper understanding of why and how people make decisions as they do is needed, but relying on impressions, assumptions, or any single research method (e.g., survey, focus group, mental modeling, decision analysis) will be too limited. Therefore, **the committee recommends that an appropriate panel of multidisciplinary experts be convened by the Department of Health and Human Services. It would develop a comprehensive research strategy for knowledge leading to the optimal design and evaluation of vaccine risk-benefit communication approaches.**

SUMMARY

A review of the possible biological mechanisms for any adverse effects of multiple immunization on immune function does not support the hypothesis that the infant immune system is inherently incapable of handling the numbers of antigens presented during routine immunization.

A review of the clinical and epidemiological literature suggests that multiple immunizations do not lead to risk of infection or type 1 diabetes, and that the possible role in the risk of allergy is indeterminate. Meanwhile, the biological evidence that immunization might lead to infection, autoimmune disease, or allergy is more than only theoretical. This literature base is somewhat limited, however, and the concern is great among a significant minority of parents.

Therefore the committee recommends limited but continued public health attention to this issue in terms of exploiting current research efforts. No recommendations for policy change are made, but the committee does recommend considering new frameworks for immunization policy, particularly as the number of licensed vaccines increases.

TABLE ES-1 Biological Mechanisms for the Possible Role of Immunizations in Increasing the Risk of Immune Dysfunction

Adverse Health Outcome	Mechanism	Committee Conclusion About the Weight of the Biological Evidence
Autoimmune disease	Molecular mimicry	Theoretical only
	Bystander effect	Weak
	Loss of protection induced by homologous infection	Theoretical only
	Via the hygiene hypothesis	Theoretical only
	Collective mechanistic possibilities	Weak
Allergic disease	Bystander effect	Weak
	Via the hygiene hypothesis	Theoretical only
	Collective mechanistic possibilities	Weak
Heterologous Infections	Carrier-induced epitope suppression	Strong
	Competition for antigen presentation	

BOX ES-1 Committee Conclusions and Recommendations

SCIENTIFIC ASSESSMENT

Causality Conclusions

The committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of heterologous infections.

The committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of type 1 diabetes.

The committee concludes that the epidemiological and clinical evidence is inadequate to accept or reject a causal relationship between multiple immunizations and an increased risk of allergic disease, particularly asthma.

Biological Mechanisms Conclusions

Autoimmune Disease

In the absence of experimental or human evidence regarding molecular mimicry or mercury-induced modification of any vaccine component to create an antigenic epitope capable of cross-reaction with self epitopes as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that these mechanisms are only theoretical.

The committee concludes that there is weak evidence for bystander activation, alone or in concert with molecular mimicry, as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.

In the absence of experimental or human evidence regarding loss of protection against a homologous infection as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.

In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.

Considering molecular mimicry, bystander activation, and impaired immunoregulation collectively rather than individually, the committee concludes that there is weak evidence for these mechanisms as means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.

Allergic Disease

The committee concludes that there is weak evidence for bystander activation as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy, the committee concludes that this mechanism is only theoretical.

The committee concludes that there is weak evidence for the existence of any biological mechanisms, collectively or individually, by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

Heterologous Infection

The committee concludes that there is strong evidence for the existence of biological mechanisms by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk for heterologous infections.

SIGNIFICANCE ASSESSMENT

Conclusions

The committee concludes that concern about multiple immunizations has been, and could continue to be, of societal significance in terms of parental worries, potential health burdens, and future challenges for immunization policy-making.

PUBLIC HEALTH RESPONSE RECOMMENDATIONS

Policy Review

The committee recommends that state and federal vaccine policymakers consider a broader and more explicit strategy for developing recommendations for the use of vaccines.

The committee does not recommend a policy review—by the CDC's Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics' Committee on Infectious Diseases, and the American Academy of Family Physicians—of the current recommended childhood immunization schedule on the basis of concerns about immune system dysfunction.

The committee does not recommend a policy review by the Food and Drug Administration's Vaccines and Related Biologic Products Advisory Committee of any currently licensed vaccines on the basis of concerns about immune system dysfunction.

Research

Epidemiological Research

The committee recommends exploring the feasibility of using existing vaccine surveillance systems, alone or in combination, to study safety questions related to asthma and other important allergic disorders, as well as to study type 1 diabetes and other important autoimmune diseases.

The committee recommends exploring the use of cohorts for research on possible vaccine-related disease risks. Furthermore, the committee recommends that disease registries and research programs for autoimmune and allergic disorders routinely collect immunization histories as part of their study protocol.

Basic Science and Clinical Research

The committee recommends continued research on the development of the human infant immune system.

The committee endorses current research efforts aimed at identifying genetic variability in human immune system development and immune system responsiveness as a way to gain a better understanding of genetic susceptibility to vaccine-based adverse events.

The committee recommends exploring the feasibility of collecting data on surrogate markers for type I diabetes and clinical history of allergic diseases in the vaccine testing and licensing process.

The committee recommends exploring surrogates for type I diabetes and clinical history of allergic diseases in existing cohort studies of variations in the immunization schedule.

Communication

The committee recommends that an appropriate panel of multidisciplinary experts be convened by the Department of Health and Human Services. It would develop a comprehensive research strategy for knowledge leading to the optimal design and evaluation of vaccine risk-benefit communication approaches.

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Immunization Safety Review: Multiple Immunizations and Immune Dysfunction

Immunization to protect infants and children from vaccine-preventable diseases is one of the greatest achievements of public health. Immunization is not without risks, however. It is well established, for example, that the oral polio vaccine can on rare occasion cause paralytic polio, that some influenza vaccines have been associated with a risk of Guillain-Barré syndrome, and that vaccines sometimes produce anaphylactic shock. Thus public concern about the safety of immunizations has increased. A recent survey suggests that a substantial minority of parents (23–25%) believes that getting too many immunizations weakens a child's immune system and that children get more immunizations than are good for them (Gellin et al., 2000). Given the widespread use of vaccines, state mandates requiring vaccination of children for entry into school or day care, and the importance of ensuring that trust in immunization programs is justified, it is essential that safety concerns receive assiduous attention.

The Immunization Safety Review Committee was established by the Institute of Medicine (IOM) to evaluate the evidence on possible causal associations between immunizations and certain adverse outcomes, and to then present conclusions and recommendations. The committee's mandate also includes assessing the broader significance for society of these immunization safety issues. In this report, the committee examines the hypothesis that receipt of multiple immunizations, as recommended by public health authorities, adversely affects the developing immune system.

THE CHARGE TO THE COMMITTEE

Since the mid-1990s, challenges to the safety of immunizations seem to have gained prominence in public and scientific debate. Given these persistent and growing concerns about immunization safety, the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) recognized the need for an independent, expert group to address immunization safety in a timely and objective manner. The IOM has been involved in such issues since the 1970s. (A brief chronology can be found in Appendix A.) In 1999, as a result of IOM's previous work and its access to independent scientific experts, CDC and NIH began a year of discussions with IOM to develop the Immunization Safety Review project to address vaccine safety issues both existing and emerging.

The Immunization Safety Review Committee is responsible for examining a broad variety of immunization safety concerns. Committee members have expertise in pediatrics, neurology, immunology, internal medicine, infectious diseases, genetics, epidemiology, biostatistics, risk perception and communication, decision analysis, public health, nursing, and ethics. While all the committee members share the view that immunization is generally beneficial, none of them has a vested interest in the specific immunization safety issues that come before the group. Additional discussion of the committee composition can be found in the Foreword written by Dr. Kenneth Shine, President of the IOM.

The committee is charged with examining three immunization safety hypotheses each year during the three-year study period (2001–2003). These hypotheses are selected by the Interagency Vaccine Group (IAG)—made up of officials from the National Vaccine Program Office at the Department of Health and Human Services (DHHS), the National Immunization Program and the National Center for Infectious Diseases at the CDC, the National Institute for Allergy and Infectious Diseases at the NIH, the Department of Defense, the Food and Drug Administration (FDA), the National Vaccine Injury Compensation Program at the Health Resources and Services Administration (HRSA), the Centers for Medicare and Medicaid Services (CMS, formerly the Health Care Financing Administration), and the Agency for International Development. For each topic, the committee reviews relevant literature and submissions by interested parties, and holds an open scientific meeting, followed directly by a one- to two-day closed meeting, to formulate its conclusions and recommendations. The committee's findings are released to the public in a brief consensus report 60–90 days after its meeting.

For each hypothesis to be examined, the committee assesses both the scientific evidence and the significance of the issue for society.

The *scientific* assessment has two components: an examination of the epidemiological and clinical evidence regarding a possible causal relationship

between the vaccine and the adverse event, and an examination of experimental evidence for any biological mechanism(s) relevant to the hypothesis.

The *significance* assessment addresses such considerations as the burden of the health risks associated with the vaccine-preventable disease and with the adverse event in question. Other considerations may include the perceived intensity of public or professional concern, or the feasibility of additional research to help resolve scientific uncertainty regarding causal associations.

The findings of the scientific and significance assessments provide the basis for the committee's recommendations on public health response, which includes immunization policy review, current and future research, and effective communication strategies. There are limits to the committee's charge, however. For example, recommending a change in the licensure, scheduling, or administration of a vaccine would exceed the committee's authority. If it concluded that the scientific evidence or other important factors justified such action, it could recommend convening the appropriate advisory group(s) to examine the question. See Figure 1 for a schematic of the committee's charge.

THE STUDY PROCESS

The committee held an initial organizational meeting in January 2001. CDC and NIH presented the committee's charge at the meeting, and the committee conducted a general review of immunization safety concerns and determined its methodology for assessing causality. This approach would be used for the hypotheses to be considered at subsequent meetings. A website (www.iom.edu/imsafety) and a listserv were created to facilitate communication with the committee and provide public access to information about its work. The committee's conclusions and recommendations in the first two reports, *Immunization Safety Review: Measles-Mumps-Rubella Vaccine and Autism* (IOM, 2001a) and *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders* (IOM, 2001b), are summarized in Appendix B.

To evaluate the hypothesis on multiple immunizations and immune system dysfunction, the committee collected information from several sources. At an open scientific meeting in November 2001 (see Appendix C), academic researchers gave presentations on specific scientific issues germane to the topic. All information presented to the committee at that meeting can be viewed on the project Website (www.iom.edu/imsafety). In addition, an extensive review was performed of the published, peer-reviewed scientific and medical literature (see Appendix D). A reference list of material reviewed by the committee, even if not cited in this report, can be found on its website as well.

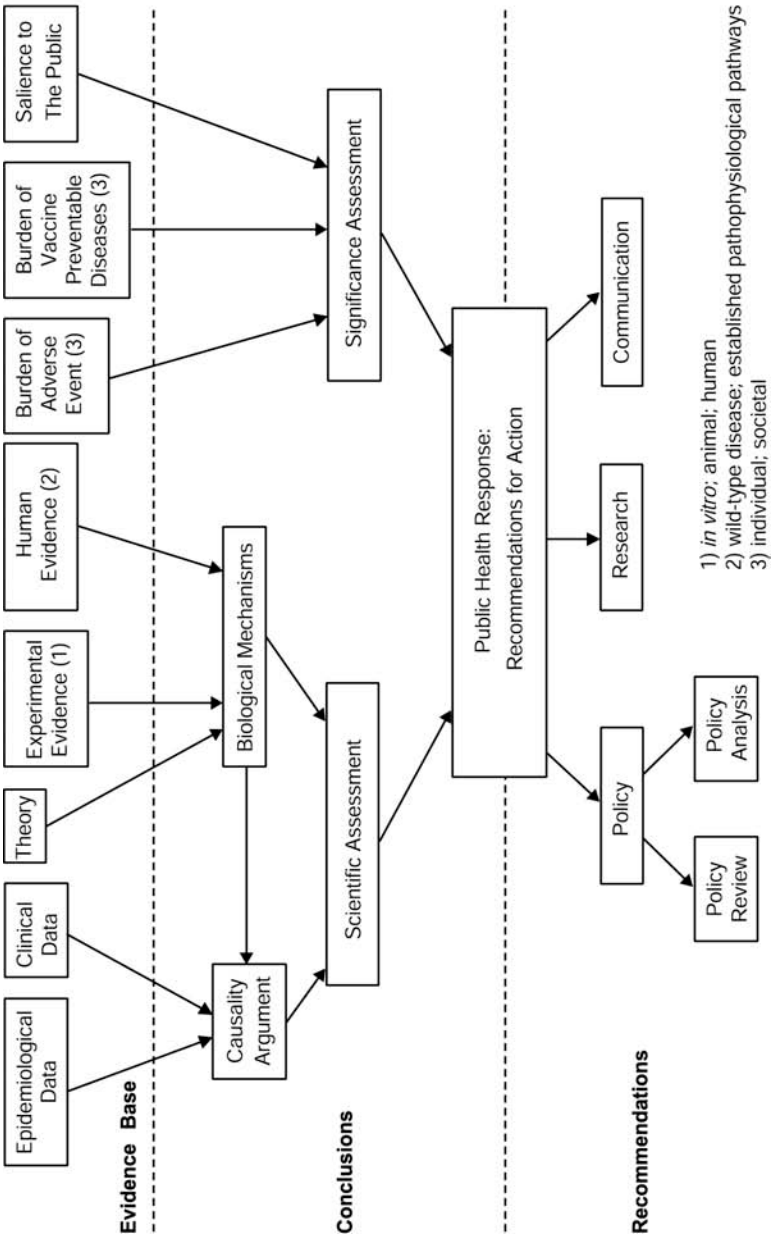


FIGURE 1 Committee Charge

THE FRAMEWORK FOR SCIENTIFIC ASSESSMENT

Causality

The Immunization Safety Review Committee has adopted the framework for assessing causality developed by its predecessors (convened by the IOM in 1991 and 1994) to address questions of immunization safety. The categories of causal conclusions used by the committee are as follows:

1. No evidence
2. Evidence is inadequate to accept or reject a causal relationship
3. Evidence favors rejection of a causal relationship
4. Evidence favors acceptance of a causal relationship
5. Evidence establishes a causal relationship.

Assessments begin from a position of neutrality regarding the specific vaccine safety hypothesis under review. That is, there is no presumption that a specific vaccine (or vaccine component) does or does not cause the adverse event in question. The weight of the available clinical and epidemiological evidence determines whether it is possible to shift from that neutral position to a finding for causality ("the evidence favors acceptance of a causal relationship") or away from causality ("the evidence favors rejection of a causal relationship"). The committee does not conclude that the evidence favors rejecting causality merely if the evidence is inadequate to support causality. Instead, it maintains a neutral position, concluding that the "evidence is inadequate to accept or reject a causal relationship". For some relationships that fall into this category, data are plentiful but the results are conflicting or not strongly convincing. For other relationships that fall into this category, the data specifically addressing the causal relationship are scarce. Some authors of similar assessments use phrases such as "the evidence does not presently support a causal association." The committee believes however that such language does not make the important distinction between evidence that a relationship does not exist (category 3) and evidence that is indeterminate with regard to causality (category 2).

Although there are no firm rules for an amount of or quality of evidence required to support a specific category of causality conclusion, standard epidemiological criteria are used to guide the decision. The strongest category is "establishes causality," which is reserved for those relationships where the causal link is unequivocal, such as with OPV and vaccine-associated paralytic polio, or certain anaphylactic reactions to vaccine administration. The next category is "favors acceptance" of a causal relationship. This is evidence that is strong and generally convincing, although it is not firm enough to be described as unequivocal or established. "Favors rejection" is the strongest category in the negative direction. There is no "establishes no causal relationship" category since it is virtually impossible to prove the absence of a relationship with the same certainty that one can establish the presence of one. Finally, if the evidence

is not reasonably convincing in either the causal or non-causal direction, it is placed in the category "inadequate to accept or reject a causal relationship." Evidence that is sparse, conflicting, of weak quality, or just suggestive falls into this category.

The sources of evidence considered by the committee in its scientific assessment of causality include epidemiological and clinical studies directly addressing the question at hand. That is, the data relate to the effects of the vaccine(s) under review and the specific adverse health outcome(s) under review—in the case of this report, the effects of multiple immunizations on developing immune system function. Epidemiological studies carry the most weight in a causality assessment; these studies measure health-related exposures or outcomes in a defined sample of subjects and make inferences about the nature and strength of associations between exposures and outcomes in the overall population from which the study sample was drawn. Epidemiological studies can be categorized as observational or experimental (clinical trial), and as uncontrolled (descriptive) or controlled (analytic). Among these various study designs, experimental studies generally have the advantage of random assignment to exposures and therefore carry the most weight in assessing causality. Uncontrolled observational studies are important but are generally considered less definitive than controlled studies. In uncontrolled observational studies where observations are made over time, confounding (e.g., changing case definitions and improving case detection) may influence the incidence and prevalence of the adverse outcomes studied.

Case reports and case series are generally inadequate by themselves to establish causality. Despite the limitations of case reports, the causality argument for at least one vaccine-related adverse event (the relationship between vaccines containing tetanus toxoid and Guillain-Barré syndrome) was strengthened most by a single, well-documented case report on recurrence of the adverse event following re-administration of the vaccine, a situation referred to as a "rechallenge" (IOM, 1994).

Biological Mechanisms

Evidence considered in the scientific assessment of biological mechanisms includes human, animal, and *in vitro* studies related to biological or pathophysiological processes by which immunizations could cause immune system dysfunction. This kind of review has been referred to in previous reports of this committee (IOM, 2001a, 2001b) and others (IOM, 1991, 1994) as an assessment of the "biological plausibility" of a causal relationship. The committee has previously described biological plausibility as existing on a spectrum, ranging from not plausible to established. An agreed upon hierarchy of evidence required for assessments of biological plausibility does not exist, nor does an associated terminology (Weed and Hursting, 1998).

The committee has noted, moreover, that the term biological plausibility is a source of confusion on at least two fronts. First, it is associated with guidelines (sometimes referred to as the Bradford Hill criteria) for causal inference from epidemiological evidence (Hill, 1965). In that context, an assessment of the biological plausibility of an association demonstrated by epidemiological analysis is meant to ensure that such an association is consistent with current biological knowledge. Evidence regarding biological plausibility can never prove causality. Therefore, it is also meant to guard against attributions of causality to biologically implausible statistical associations that might result from studies that have not adequately accounted for important variables.

For example, although a strong statistical relationship might exist between a woman's risk of breast cancer and the number of bathrooms in her home, there is no mechanism based on knowledge of cancer biology that could indicate the relationship is causal. Rather, the number of bathrooms is associated with socioeconomic status, which is associated with such factors as diet that can be linked mechanistically to cancer biology. The biological implausibility of an association between the number of bathrooms in a house and the risk of breast cancer weakens the argument for a causal relationship. In other cases, a review of the biological plausibility of an association might add reassurance that the epidemiological findings point toward or reflect causality. Occasionally an epidemiological observation has been explained by a reasonable biological mechanism that, on further investigation, appeared not to be relevant for the pathophysiology.

This committee, however, is often faced with a set of circumstances in which the epidemiological evidence is judged inadequate to accept or reject a causal association between a vaccine exposure and an adverse event of concern. It is then left with the task of examining proposed or conceivable biological mechanisms that might be operating if an epidemiologically sound association *could* be shown between vaccine exposure and an adverse event. Identification of sound mechanisms could influence the development of an appropriate research agenda and give support for policymakers, as decisions frequently must be made in situations of incomplete information regarding causality. Finally, there is often value in understanding and pursuing possible biological mechanisms even if the epidemiological evidence suggests a lack of a causal association. New epidemiological studies could question that existing causality assessment and the biological data would gain prominence in the new assessments. Also, a review of biological data could give support to the negative causality assessment or could cause one to reconsider or pursue the epidemiological findings further.

Second, the committee understands that some readers of its reports are confused by what are perceived as contradictory findings. Although the committee has previously stated that biological plausibility can range across a spectrum, readers sometimes regard the term with a degree of certainty or precision the committee never intended. When other evidence of causality is available, bio-

logical plausibility adds an additional piece of supportive evidence. However, in the absence of other evidence pointing to a causal relationship, use of the term biological plausibility, as ingrained in the language of causal inference, seems to add confusion.

Thus the committee finds that for the purpose of its reports, the lack of clarity in the phrase "biological plausibility" warrants the adoption of new terminology and a new approach to its discussion of biological data. The committee will review evidence regarding "biological mechanisms" that might be consistent with the proposed relationship between a vaccine exposure and given adverse events. This biological assessment section of the report is written distinct from any argument regarding the causality of such relationships. This is not meant to imply that current understanding of biological processes does not shape or guide assessments of causality. In fact, the current thinking of a possible biological explanation for a relationship between immunization and an adverse event will influence some of the important controls used in a good epidemiological analysis. The important consideration of "confounders" in epidemiological studies comes from understanding biological phenomena that could underlie or explain the observed statistical relationship. Only when important confounders are considered can the statistical observation be considered for evidence of causality. However, absent evidence of a statistical association, or convincing clinical evidence, biological mechanisms cannot be invoked to prove causality.

There are three general categories of evidence on biological mechanisms:

Theoretical only: A reasonable mechanism can be hypothesized that is commensurate with scientific knowledge and that does not contradict known physical and biological principles, but it has not been demonstrated in humans or animal models.

Experimental evidence: The evidence can be derived under highly contrived conditions. For example, the results require extensive manipulation of the genetics of an animal system or extreme vaccine antigen exposures *in vivo* or *in vitro* in terms of dose, route, or duration. Other experimental evidence is derived under less contrived conditions. For example, a compelling animal or *in vitro* model exists whereby administration of a vaccine antigen under conditions similar to human use results in a pathological process analogous to a human disease pathology. Experimental evidence often describes effects on just one or a few of the steps in the pathological process required for expression of disease. As more components of the theoretical pathways are shown to operate in reasonable experimental models, the more confident one is that the mechanisms could possibly result in disease in humans.

Evidence that the mechanism results in known disease in humans: For example, a wild-type infection causes the adverse health outcome, or another vaccine has been demonstrated to cause the same adverse outcome by the same or similar mechanism. Data from population-based studies of the effects of the vaccine

administration on the occurrence of the adverse outcomes under review contribute not to the biological mechanisms argument but to the causality argument.

Beginning with this report, the committee will summarize the biological mechanisms as theoretical only, or as having derived from either experimental evidence or mechanism-related evidence in humans. If there is evidence in experimental models or humans for a mechanism, we will designate it as weak, moderate, or strong. Though the committee tends to judge evidence in humans to be "stronger" than experimental evidence from animals or *in vitro* systems, the strength of the evidence also depends on other factors, such as the experimental design and sample size. Obviously, the conclusions drawn from this review will depend both on evidence and scientific judgment. To ensure that its own summary judgment is defensible, the committee intends to be as explicit as possible regarding the strengths and limitations of the biological data.

Published and Unpublished Data

Published reports that have been subjected to a rigorous peer review process carry the most weight in the committee's assessment. Unpublished data and other reports that have not undergone peer review do have value, and they are often considered by the committee; they could be used, for example, in support of a body of published literature with similar findings. If the committee concluded that the unpublished data were well described, had been obtained using sound methodology, and presented very clear results, the committee could report, with sufficient caveats in the discussion, how those data fit with the entire body of published literature. But only in extraordinary circumstances could an unpublished study refute a body of published literature. In general, the committee cannot rely heavily on unpublished data in making its scientific assessments (regarding either causality or biological mechanisms) because they have not been subjected to a rigorous peer review process, and therefore must be interpreted with caution.

The committee acknowledges that its approach differs from the state of the art for evidence-based reviews of clinical practices in medicine, which does not include consideration of unpublished or non-peer-reviewed information or of studies with flawed experimental designs (U.S. Preventive Services Task Force, 1996). However, the Immunization Safety Review Committee was convened specifically to assess topics that are often of immediate and intense concern. In some cases, the committee's review will take place as data are only beginning to emerge. Thus, given the unique nature of this project, the committee thought it was important to review and consider as much information as possible, including unpublished information. The committee did not perform primary or secondary analyses of unpublished data, however. In reviewing unpublished material, the committee applied generally accepted standards for assessing the quality of scientific evidence, as described above. (All unpublished data reviewed by the

committee and cited in this report are available—in the form reviewed by the committee—through the public access files of the National Academies at 202-334-3543 or www.national-academies.org/publicaccess.)

UNDER REVIEW: MULTIPLE IMMUNIZATIONS AND IMMUNE DYSFUNCTION

Over the past two decades, the pediatric immunization schedule has grown more complicated. In 1980, the youngest infants received vaccines against four diseases (diphtheria, tetanus, pertussis, and polio). Today, a healthy child immunized in complete accord with the recommended childhood immunization schedule receives up to 15 doses of five vaccines to protect against seven diseases by 6 months of age and up to 20 doses of seven vaccines to protect against 11 diseases by 2 years of age (see Figure 2). Furthermore, the immunization schedule seems likely to expand in the next decade, with more vaccines for infants and children being developed or considered.

The increase in the number of vaccines and vaccine doses given to children has led to concerns among some about possible adverse effects of individual vaccines or of the aggregate vaccine exposure. One such concern has been prompted by increased incidence of conditions associated with immune system dysfunctions—for example, asthma and type 1 diabetes, often referred to as insulin-dependent diabetes mellitus. Although genetic factors are known to affect the risk of these diseases, increases in their incidence seem more likely to reflect changes in environmental exposures than in the genetic makeup of a population. Increased exposure to vaccines has been proposed as one possible environmental modifier of immune function.

For others, however, the concern is that having to administer many injections in a short period of time could adversely affect the acceptance of the immunization schedule by parents and health care providers, leading to reduced vaccination rates and greater risk of vaccine-preventable disease. Combination vaccine products can mean fewer injections per visit, but, they give little comfort to those who worry about the safety of multiple vaccine exposures. In addition, if adverse effects occur after the receipt of a combination vaccine product it may be difficult to determine which individual vaccine is responsible. Another concern is that development of novel vaccine delivery systems (i.e., nasal sprays and patches) may further complicate the issue of the effects of vaccines on the developing infant immune system.

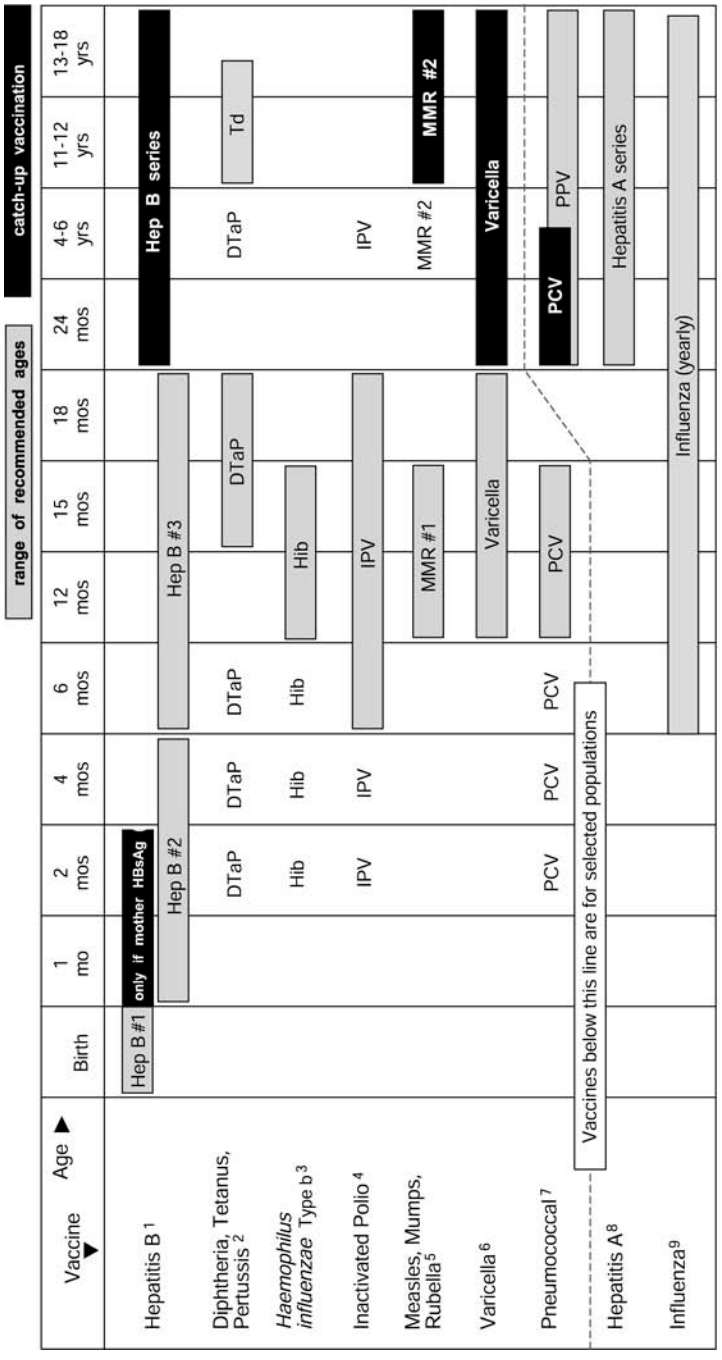
Framing the Question

The Interagency Vaccine Group asked the Immunization Safety Review Committee to address the concern that multiple immunizations can adversely affect

the developing immune system. To conduct its review, the committee had to establish a clear statement of the question before it, as well as a manageable scope of inquiry. Both "multiple immunization" and "immune system dysfunction" must be defined for the purposes of this report. First, multiple immunization has several possible meanings. A single dose of vaccine may present multiple antigens for a single disease (e.g., polio or pneumococcal vaccines) or multiple antigens for multiple diseases (e.g., measles-mumps-rubella [MMR] vaccine). Also, individual doses of several separate vaccines may be administered at a single health care visit. And further "repeat" doses of a single vaccine are administered, alone or with other vaccines, at specified intervals (e.g., 2, 4, and 6 months of age). The committee intended its primary focus to be on exposure to multiple vaccine antigens during infancy and childhood. However, as described in the section below on causality, the literature base is not large, and relevant studies often addressed the effects of incremental exposure differences, such as four vaccines compared to three.

The committee restricted its considerations to those vaccines used in the United States. Thus, data regarding BCG vaccine, which is used against tuberculosis in other countries, did not contribute directly to the committee's causality arguments. (Studies of BCG did, however, help inform the committee's understanding of the biological arguments for and against the hypotheses.) Nor did the committee address possible effects of smallpox vaccine, which has not been used in the United States for 30 years. The committee included studies of "one vaccine" if it contained antigens against more than one disease or more than one strain of infectious agent. For example, the diphtheria and tetanus toxoids and pertussis (DTP) vaccine—whether whole-cell (DTwP) or acellular (DTaP) preparations—would be considered to represent a "multiple immunization," as would the polio vaccines, which contain live or killed viruses against three distinct strains of poliovirus.

Second, immune system dysfunction is a broad term. A brief review of the literature about immunization safety indicates that three types of immune system injury are of concern to vaccine safety advocates: risk of infection, risk of allergic diseases, and risk of autoimmune diseases. These concerns have gained prominence due to a generic consideration of biological mechanisms and due to studies, mostly ecological analyses, that are occasionally salient in the lay and scientific literature. The committee considered two possible pathways to adverse outcomes: stimulation of harmful immune responses or suppression of beneficial immune responses. The committee addressed infections only as distinct from those the vaccines are intended to protect against—referred to as heterologous infection—and in lieu of trying to sweep broad categories of allergic and autoimmune diseases, the committee narrowed its focus to specific conditions. It appeared to the committee that much of the concern, and a large component of the evidentiary base, centered around the allergic disease of asthma and the autoimmune form of diabetes—that is, type 1a diabetes, one of two types of



This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2001, for children through age 18 years. Any dose not given at the recommended age should be given at any subsequent visit when indicated and feasible. ■ Indicates age groups that warrant special effort to administer those vaccines not previously given. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' package inserts for detailed recommendations

FIGURE 2 Recommended Childhood Immunization Schedule, United States, 2002

NOTES

1. Hepatitis B vaccine (Hep B). All infants should receive the first dose of hepatitis B vaccine soon after birth and before hospital discharge; the first dose may also be given by age 2 months if the infant's mother is HBsAg-negative. Only monovalent hepatitis B vaccine can be used for the birth dose. Monovalent or combination vaccine containing Hep B may be used to complete the series; four doses of vaccine may be administered if combination vaccine is used. The second dose should be given at least 4 weeks after the first dose, except for Hib-containing vaccine which cannot be administered before age 6 weeks. The third dose should be given at least 16 weeks after the first dose and at least 8 weeks after the second dose. The last dose in the vaccination series (third or fourth dose) should not be administered before age 6 months.

Infants born to HBsAg-positive mothers should receive hepatitis B vaccine and 0.5 mL hepatitis B immune globulin (HBIG) within 12 hours of birth at separate sites. The second dose is recommended at age 1-2 months and the vaccination series should be completed (third or fourth dose) at age 6 months.

Infants born to mothers whose HBsAg status is unknown should receive the first dose of the hepatitis B vaccine series within 12 hours of birth. Maternal blood should be drawn at the time of delivery to determine the mother's HBsAg status; if the HBsAg test is positive, the infant should receive HBIG as soon as possible (no later than age 1 week).

2. Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP). The fourth dose of DTaP may be administered as early as age 12 months, provided 6 months have elapsed since the third dose and the child is unlikely to return at age 15-18 months. **Tetanus and diphtheria toxoids (Td)** is recommended at age 11-12 years if at least 5 years have elapsed since the last dose of tetanus and diphtheria toxoid-containing vaccine. Subsequent routine Td boosters are recommended every 10 years.

3. *Haemophilus influenzae* type b (Hib) conjugate vaccine. Three Hib conjugate vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB® or ComVax® [Merck]) is administered at ages 2 and 4 months, a dose at age 6 months is not required. DTaP/Hib combination products should not be used for primary immunization in infants at age 2, 4 or 6 months, but can be used as boosters following any Hib vaccine.

4. Inactivated poliovirus vaccine (IPV). An all-IPV schedule is recommended for routine childhood poliovirus vaccination in the United States. All children should receive four doses of IPV at age 2 months, 4 months, 6-18 months, and 4-6 years.

5. Measles, mumps, and rubella vaccine (MMR). The second dose of MMR is recommended routinely at age 4-6 years but may be administered during any visit, provided at least 4 weeks have elapsed since the first dose and that both doses are administered beginning at or after age 12 months. Those who have not previously received the second dose should complete the schedule by the visit at age 11-12 years.

6. Varicella vaccine. Varicella vaccine is recommended at any visit at or after age 12 months for susceptible children (i.e. those who lack a reliable history of chicken-pox). Susceptible persons aged $\geq$13 years should receive two doses, given at least 4 weeks apart.

7. Pneumococcal vaccine. The heptavalent **pneumococcal conjugate vaccine (PCV)** is recommended for all children aged 2-23 months and for certain children aged 24-59 months. **Pneumococcal polysaccharide vaccine (PPV)** is recommended in addition to PCV for certain high-risk groups. (See CDC, 2000b).

8. Hepatitis A vaccine. Hepatitis A vaccine is recommended for use in selected states and regions, and for certain high-risk groups; consult your local public health authority. (See CDC, 1999c).

9. Influenza vaccine. Influenza vaccine is recommended annually for children age $\geq$6 months with certain risk factors (including but not limited to asthma, cardiac disease, sickle cell disease, HIV and diabetes; (see CDC, 2001d) and can be administered to all others wishing to obtain immunity. Children aged $\leq$12 years should receive vaccine in a dosage appropriate for their age (0.25 mL if age 6-35 months or 0.5 mL if aged $\geq$3 years). Children aged $\leq$8 years who are receiving influenza vaccine for the first time should receive two doses separated by at least 4 weeks.

Additional information about vaccines, vaccine supply, and contraindications for immunization, is available at www.cdc.gov/nip or at the National Immunization Hot-line, 800-232-2522 (English) or 800-232-0233 (Spanish).

what are often referred to as insulin-dependent diabetes mellitus (IDDM). The committee also considered neurological disorders for which the injury is known to be caused by the immune response, including MS and Guillain-Barré syndrome, but did not include these in its causality considerations due to the paucity of epidemiological/clinical information that addresses the possible role of multiple immunizations rather than individual vaccines. In addition, the committee will likely address at least some of these adverse outcomes in subsequent reports.

The scope of the committee's inquiry can be summarized in the following three questions:

1. Do multiple immunizations have adverse short-term effects on the infant immune system that are reflected in increased susceptibility to heterologous infection?
2. Does exposure to multiple antigens, as administered in vaccines, directly and permanently redirect or skew the immune system toward autoimmunity, as reflected in type 1 diabetes?
3. Does exposure to multiple antigens, as administered in vaccines, directly and permanently redirect or skew the immune system toward allergy, as reflected in asthma?

The committee was unable to address the concern of some that repeated exposure of a susceptible or fragile child to multiple vaccines over the developmental period may also produce atypical or nonspecific immune or nervous system injury that could lead to severe disability or death (Fisher, 2001b). Such adverse health outcomes may not be "classical" diseases but variants of diseases. Variants would not necessarily be picked up in epidemiological or clinical investigations that use strict diagnostic criteria. There are no epidemiological studies that address this, either in terms of exposure or outcome. That is, there is no study that compares an unvaccinated control group with children exposed to the complete immunization schedule, nor are there any studies that looked at health outcomes other than those classically defined, such as infections, allergy, or diabetes. Thus the committee recognizes with some discomfort that this report addresses only part of the overall set of concerns of some who are most wary about the safety of childhood vaccines.

Key Features of Immune Response

The immune system of humans and other vertebrates has the capacity both for generalized and specialized responses to organisms, such as bacteria, viruses, and parasites. Generalized responses are produced by mechanisms of innate immunity, while the mechanisms of adaptive immunity generate highly specialized responses to a diverse array of antigens; these are presented by microbes or by products such as vaccines, which may incorporate only specially selected

antigens. The capacity for highly specialized immune responses carries with it the possibility that those responses will be directed against antigens of the body's own cells, the process known as autoimmunity, or against normally harmless environmental materials, such as foods and pollens, a process known as allergy.

Antigen-specific immunity is mediated by T and B lymphocytes (also referred to as T and B cells)[1] and their products. These cells carry antigen-specific receptors on their surface. B lymphocyte receptors, the immunoglobulins (e.g., IgA, IgE, IgG), can potentially react with a wide variety of molecular structures. T lymphocyte receptors recognize short pieces of proteins (peptides) bound to self-major histocompatibility (MHC) molecules, which in humans are referred to as HLA (human leukocyte antigen) molecules. As an individual's T and B cells develop, an enormous diversity of receptors is formed, allowing those cells to recognize and respond to the variety of antigens that might be encountered over a lifetime. This diversity is achieved by a nearly random process of genetic recombination of the genes that encode T- and B-cell receptors. When T and B cells are activated by antigens encountered through infection or vaccines, they multiply and differentiate into effector cells tailored to respond to those antigens. The effector T cells include two types of "helper" cells, designated Th1 and Th2.

During their development and at all stages of their subsequent existence, T and B lymphocytes are "educated" by their environment. Initially, their antigen receptors have no intrinsic bias—that is, they are as likely to recognize antigens from the individual (self antigens) as from a foreign source (e.g., a microbe). As part of the education of T lymphocyte precursors in the thymus, those cells must show that their receptors are capable of reacting with self-peptides bound to self-MHC molecules. Cells that do not react or react very strongly to these self antigens usually die. Cells that react weakly are allowed to mature, leave the thymus, and go to the secondary lymphoid tissues as naïve T cells (na_ve refers to the immune system at birth). This process ensures that T cells have the potential to be useful in that they can react with self-MHC. Similarly, strongly self-reactive B lymphocytes are removed during development.

The censoring of more strongly self-reactive T and B lymphocytes is imperfect, however. As a result, some strongly self-reactive lymphocytes with the potential to produce autoimmunity can be found in the blood and secondary lymphoid tissues of most apparently normal individuals. Usually, these self-reactive T and B lymphocytes do not induce autoimmunity. For the most part, they simply do not encounter self antigens in a context that can trigger lymphocyte activation and expansion. Others are unable to respond even on encounter with self-antigen because they are anergic and/or short-lived cells that will soon die. Even when naïve self-reactive T cells do encounter self antigens, these

[1] The designations of these cells reflect the sites where they mature. T lymphocytes migrate from the bone marrow to mature in the thymus, whereas B lymphocytes mature in the bone marrow.

T cells are held in check in most individuals by counter-regulatory mechanisms, including suppressive cytokines and regulatory (suppressor) T lymphocytes that prevent the self-reactive T (and B) lymphocytes from responding (Ermann and Fathman, 2001; Letterio and Roberts, 1998; Maloy and Powrie, 2001; Moore et al., 2001; Roncarolo and Levings, 2000; Rook et al., 2000; Shevach, 2000; Wills-Karp et al., 2001; Zhang et al., 2001). Self-reactive B lymphocytes are also held in check by the lack of T cell help, without which they are unable to replicate and produce higher affinity antibodies in greater quantities.

In some individuals, however, these regulatory processes fail, allowing self-reactive T and B cells to replicate, differentiate into effector cells, and cause autoimmunity—which is likely to be a multistep process. A common initial event may be the proliferation and differentiation of naïve self-reactive T (and in some cases B) cells into effector/memory cells. Thereafter, other mechanisms may further amplify the T cell response sufficiently to produce, sustain, or trigger a relapse of clinical autoimmune disease. There may also be cases where mixed Th1 and Th2 T cell responses can mediate autoimmunity (Benoist and Mathis, 2001; Marrack et al., 2001).

Genetic factors have been shown, through a substantial body of data from both human studies and animal models, to play a critical role in determining risk for autoimmunity. In some rare disorders, single-gene defects are uniformly associated with the development of autoimmunity.[2] However, the vast majority of human autoimmune diseases appear to be complex traits in which multiple genetic factors determine disease susceptibility and environmental factors determine whether disease develops (Ermann and Fathmann, 2001; Robles and Eisenbarth, 2001; Wanstrat and Wakeland, 2001). Familiar examples of such diseases include type 1a diabetes mellitus, systemic lupus erythematosus (SLE), and multiple sclerosis (MS) (Noseworthy et al., 2000; Robles and Eisenbarth, 2001; Steinman, 2001; Wakeland et al., 2001; Wanstrat and Wakeland, 2001; Wucherpfennig and Eisenbarth, 2001).

Considerable progress has been made in identifying genetic factors that determine risk for autoimmune disorders. One major risk factor—common to type 1a diabetes, SLE, MS, and many other autoimmune diseases—is related to the MHC/HLA locus, which encodes the molecules that bind and present antigenic peptides to T lymphocytes. Genetic differences among individuals—in the ability of their MHC molecules to bind specific antigenic peptides in such a way that a portion of the peptide (the epitope[3]) is recognized by their T lymph o-cytes—influences the differences among individuals in the generation of T

[2] Examples include two rare forms of polyendocrine autoimmunity (Aaltonen and Bjorses, 1999; Bennett et al., 2001; Wildin et al., 2001) and the autoimmune lymphoproliferative syndrome (Jackson and Puck, 1999). Similarly, more than 90 percent of individuals with a genetic absence of the complement protein C1q will develop systemic lupus erythematosus (Wanstrat and Wakeland, 2001).

[3] Epitope is a "portion of an antigenic molecule that is bound by an antibody or gives rise to the MHC-binding peptide that is recognized by a T-cell receptor" (Parham, 2000).

lymphocytes able to respond to self or foreign antigenic peptides. The other genes that confer risk for autoimmune disease are less completely characterized, but the best candidates are genes that regulate the amplitude and quality of the immune response or that affect the generation of specific antigenic epitopes.

Atopy or allergy refers to diseases resulting from IgE-associated immune responses to innocuous environmental substances, such as certain foods or pollens. Allergic individuals have a hereditary predisposition to mount IgE responses when they encounter such substances, which are referred to as allergens, and they develop "atopic" (allergic) diseases such as asthma. This predisposition results in part from a bias favoring the generation of Th2 T cell responses to allergens, which produce the cytokines IL-4, IL-5, and IL-13 that favor the production of IgG4 and IgE antibodies by B cells and are implicated in allergic types of inflammation. By contrast, non-allergic individuals either do not mount an immune response to environmental allergens or mount a Th1 T cell response, which cells produce interferon-γ (IFN-_) and favors the production of IgG1 antibodies. Multiple genetic factors appear to involved in the predisposition to atopy, but these are at present incompletely understood.

In humans, the immune system begins development in early gestation. Although the human fetus has the potential to respond to foreign antigens by midgestation, exposure is very limited and the immune system is often referred to as naïve at birth. Active immunity in the neonate includes B and T cell responses, although the responses are not identical to those of older children. Infants' B cell responses to T cell-independent antigens, such as polysaccharide antigens, is less vigorous than in adults. Thus, pure polysaccharides (including unconjugated *H. influenzae* and *S. pneumoniae* polysaccharides) do not induce an effective antibody response in children under approximately 2 years of age. However, if these polysaccharides are conjugated (linked) to protein antigens, they become T cell-dependent and induce protective antibody responses even in young infants. The effectiveness of such vaccines reflects the substantial maturity of T cell and T cell-dependent B cell responses, and the diverse repertoire of antigens that can be recognized by T cell and B cell receptors in the human infant. There are certain functional differences compared to adult T cells, including the apparent tendency in favor of Th2 responses, which differences appear to reflect in large part the naïve status of the neonatal immune system and lack of exposure to bacteria and other microbes, which encourage Th1 responses, prior to birth (reviewed in English et al., 2001; Lewis and Wilson, 2001; Prescott et al., 1998; Siegrist, 2001).

Antigen Exposure Through Vaccines

Central to the safety concerns about multiple childhood immunizations is the question of whether the increasingly complex recommended schedule of immunizations overloads a child's immune system. That is, have there been quantitative or qualitative changes in the antigens to which a child is exposed through

vaccines that lead to an inability of the immune system to respond appropriately? A related question involves the "hygiene hypothesis." These two issues are reviewed here, prior to the review of evidence regarding possible adverse health effects—specifically infection, autoimmune disease, or allergy—of multiple immunizations on the developing immune system.

A vaccine directed against a single disease can contain one antigen or can contain multiple antigens, each of which can have multiple epitopes. For example, the polio vaccine has always been directed against three strains of poliovirus. The pneumococcal polysaccharide vaccine (recommended for children older than 24 months of age and for adults) contains antigens for 23 distinct strains of pneumococcal bacteria, and the pneumococcal polysaccharide-protein conjugate vaccine (for children between 2 and 23 months of age) contains antigens for seven distinct strains of pneumococcal bacteria. With other vaccine products, such as the DTaP vaccine or the MMR vaccine, a single inoculation (or "shot") is directed against several different diseases. Some vaccines are much simpler. For example, the vaccine directed against the hepatitis B virus contains only one protein antigen.

Quantitative Considerations

Calculations presented to the committee (Kollman, 2001; Offit et al., 2002) suggest that the number of antigens contained in the complete set of vaccines that comprise the recommended childhood immunization schedule has decreased over the past 20 to 30 years, despite the increased number of vaccines and vaccine doses administered. The removal of two vaccines from the schedule account for this decrease. First, routine use of the smallpox vaccine was discontinued in the United States in 1971; the World Health Assembly certified the elimination of wild-type smallpox in May 1980 (CDC, 2001c). The smallpox vaccine contained approximately 200 distinct and potentially antigenic elements. Second, the DTwP vaccine—the whole-cell pertussis (wP) vaccine generally given in combination with diphtheria (D) and tetanus (T)—was replaced by an acellular vaccine DTaP, the first of which was approved by the FDA in 1991. The whole-cell vaccine contained approximately 3,000 distinct and potentially antigenic components, whereas the acellular vaccine contains only 2–5 antigens. As of 1997, the acellular pertussis vaccine is the vaccine of choice in the United States, although the whole-cell preparation is still used elsewhere.

The vaccines added to the immunization schedule over the past 20 years have relatively few antigens. For example, the hepatitis B vaccine, a genetically engineered product, contains only one antigen, and the *Haemophilus influenzae* type b (Hib) vaccine contains only two. The varicella vaccine, a live viral vaccine, contains approximately 70 antigens (see Table 1). Thus, with the elimination of smallpox vaccine and the changeover to the acellular pertussis vaccine, the total number of immunogenic proteins or polysaccharides in childhood

vaccines has decreased to a level well below that of the vaccines given widely even as recently as 1980 (Kollman, 2001; Offit et al., 2002).

Certain caveats must be made regarding these calculations. First, they rely on counting numbers of unique molecules (e.g., proteins) in smallpox and whole cell *B. pertussis* vaccines—some of which may not be antigenic and others of which contain multiple epitopes to which the immune system responds. The calculations also do not address the effects of changes in the presence or absence of contaminating proteins. For example, the use of antibiotics, growth media, animal proteins, or carrier proteins could alter these preliminary calculations. In addition, there is no attempt to consider inter- or intra-manufacturer differences in vaccine preparations.

The other side of the quantitative question regarding antigen load is whether infants are capable of responding adequately to the antigens presented by vaccines. Adult humans have a T cell receptor repertoire (the numbers of unique T cell receptors and thus the number of different epitopes to which the T cells of an individual could respond) of ~2.5 x 10^7 (Arstila et al., 1999). Although the numbers of different T cell receptors present in human neonates has not been determined directly, their diversity has been shown by several groups to be similar to that of adults. Thus, the range of different epitopes that human neonates can recognize is almost certainly >10^7. The diversity of antigens to which B cells can make specific antibodies is thought to be even greater, and although there are some qualitative differences from adults, it appears that the overall diversity of antigens to which B cells can respond is similar to adults by 6–8 weeks of age in humans (English et al., 2001; reviewed in Lewis and Wilson, 2000; Marolleau, 1998). This is the basis

TABLE 1 Number of Immunogenic Proteins and Polysaccharides Contained in Vaccines Over the Past 40 Years

1960		1980		2000	
Vaccine	Protein	Vaccine	Protein	Vaccine	Protein
Smallpox	~200	Diphtheria	1	Diphtheria	1
Diphtheria	1	Tetanus	1	Tetanus	1
Tetanus	1	WC-Pertussis	~3000	AC-Pertussis	2–5
WC-Pertussis	~3000	Polio	15	Polio	15
		Measles	10	Measles	10
Polio	15	Mumps	9	Mumps	9
		Rubella	5	Rubella	5
Total	**~3217**			Hib	2
		Total	**~3041**	Varicella	69
				Pneumococcus	8
				Hepatitis B	1
				Total	**123–126**

SOURCE: Adapted from Offit et al., 2002
NOTE: WC-Pertussis = whole cell pertussis, AC = acellular pertussis

for the notion that human infants have the capacity to respond to the substantial number of foreign molecules (e.g., bacterial antigens) to which they are exposed shortly after birth. This is consistent with the theoretical estimates presented to the committee, which suggest that the capacity of the infant's immune system is at least 1000 times greater than that maximally required to respond to vaccines (Kollman, 2001; Offit et al., 2002).

It is the judgment of several scientific groups, including the Immunization Safety Review Committee, that the antigen load of the recommended childhood immunization schedule has decreased, not increased, in the last 20 years or so and that the infant immune system has an adequate capacity to respond to that number of antigens.

Qualitative Considerations

In considering whether the additional exposure to vaccine antigens might "overwhelm" the infant immune system, reference has been made to the fact that the fetus moves from a sterile environment in the womb into the birth canal and outside world that is coinhabited by an almost infinite array of microorganisms (IOM, 1994). Within hours, the newborn's skin and upper respiratory and intestinal tracts are colonized by a variety of bacteria and fungi, and exposure to viruses begins. Thus, the baseline exposure to microbial antigens by an infant is very large.

The normal infant develops a "commensal" relationship with these bacteria and fungi, almost always without preceding expression of overt disease—a sort of truce between the host and microbe that allows the microbe to colonize but not invade. During this process, the immune system is stimulated by these exposures, as illustrated by the presence of detectable antibody and lymphocyte responses to organism-specific antigens. Antibodies against the common pathogens *H. influenzae* and pneumococci has also been demonstrated in infants not recognized to have had disease caused by these bacteria (Anderson et al., 1972; Gray et al., 1981; Sell et al., 1973). Moreover, genetic immunodeficiency diseases represent "experiments of nature" that show that abnormality of any single component of the host defense system, including antibody and lymphocyte function, can result in serious, often lethal disease caused by pathogens or by one or another of the commensal organisms. Thus a vigorous immune response is required to protect the human infant against infection by a broad variety of organisms that have the potential to cause disease, and the infant must be able to mount this response consistently and repeatedly. Within this context, it seems unlikely that immunizations constitute a significant departure from the magnitude of the antigenic challenges endured under natural circumstances by any normal infant.

Over the course of several decades, the antigen load presented to the developing immune system has undergone significant qualitative changes,

particularly in the context of the total antigen exposures during infancy and childhood. Approximately a decade ago, researchers interested in the changing epidemiology of several diseases began formulating the "hygiene hypothesis," which has generated an extensive descriptive and research literature (e.g., Rook, 2000; Wills-Karp et al., 2001). Fundamentally, the hygiene hypothesis suggests that the increasingly aseptic environment in which children in developed countries are reared has led to changes in the development, or maturation, of the infant immune system. Many ecological analyses correlate the rise of allergic and autoimmune diseases in many parts of the world with increased economic development (Rook and Stanford, 1998). Epidemiological literature in support of the hygiene hypothesis includes findings of a negative correlation between risk for allergic diseases and a host of factors that would increase a child's exposure to bacteria and other infectious agents. These risk factors include, for example, the number of older siblings, the presence of pets, infections through the fecal-oral route, and rural living. Changes other than in hygienic behavior, such as increases in environmental pollutants, have also occurred in the developed world and may contribute to the changing epidemiology of some diseases.

The proposed explanation for an immune system role in these epidemiological observations is that early exposure to infectious diseases and environmental microbes "shapes" the developing immune system toward a Th1-cell responsiveness, which is generally considered a protective immune response (i.e., to host defense against intracellular pathogens and allergy). Eliminating these early exposures through hygienic practices and altered behaviors is thought to predispose the immune system toward a Th2 cell responsiveness, which is associated with allergy. This "skewing" or "biasing" of the immune system as a result of the elimination of many kinds of antigen exposures, some theorize (Rook, 2001), is exacerbated by exposure to vaccines, many of which evoke a Th2 response instead of the Th1 response that would be generated by wild-type infections with the diseases that the vaccines prevent. The most recent refinement of the biological mechanisms proposed to explain the hygiene hypothesis looks beyond the idea of a simple Th1-Th2 imbalance into the realm of regulatory cell imbalance (Rook, 2001; Wills-Karp et al., 2001). Under this scenario, a major contributor to altered immune responses is a decrease in T regulator cells, along with the alteration in T effector cells (Th1 or Th2).

Not yet clear is the role immunizations may have in directly altering development of the immune system, or the relative contribution of vaccine-related changes in the context of the hygiene hypothesis. Vaccine-induced immune responses may, however, differ from those resulting from wild-type infection because of differences in context, including differences in their timing, either in terms of age at exposure or of the sequence of antigen exposure. For example, through immunization, many American children currently mount a simultaneous immune response to diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b, and three strains of poliovirus three times during the first 6

months of life. It is unlikely that the timing of this vaccine-induced immune response would have been mimicked under conditions of nonvaccination.

In any case, the number of infections prevented by vaccines is actually quite small compared with the total number of infections prevented by other hygienic interventions, such as clean water, food, and living conditions. And, although it is true that the developing immune system is frequently bombarded with many antigens at one time, most of the antigens do not pose a threat inherent to the infant. Most certainly, the route of antigen exposures through vaccines—that is, an injection rather than a respiratory or gastrointestinal exposure—is different than what occurs in wild-type infection.

Actually, the history of vaccine development shows that the immune response to a vaccine is sometimes devastatingly different from the response to wild-type infection. Early experience with killed-virus vaccines directed against measles and respiratory syncytial virus (RSV) saw the appearance of atypical and virulent disease in vaccinated individuals that were subsequently infected with wild-type virus.

In the 1960s, some children developed an atypical form of measles after receiving the killed measles virus vaccine (Fulginiti and Helfer, 1980). Atypical measles is described as a delayed, severe hypersensitivity reaction (Krause et al., 1978; Redd et al., 1999). Symptoms of high fever, headache, abdominal pain, myalgia, and cough (Redd et al., 1999) are followed in 48–72 hours by the appearance of a maculopapular, pruritic rash on the extremities that spreads inward toward the trunk and may become vesicular, purpuric, or petechial (Brodsky, 1972). Patients become severely ill during the first few days of illness, but atypical measles is self-limited and resolves in 7–14 days (Brodsky, 1972).There was only one report of a possible fatality among cases seen in the 1960s following use of the inactivated measles vaccine (Redd et al., 1999).

Children with atypical measles were found to lack the antibody to the measles virus F protein, which is responsible for the virus's hemolytic and cell fusion properties (Annunziato et al., 1982; Redd et al., 1999). In contrast, the H, or hemagglutin protein, the other measles virus surface protein, was found in the sera of the ill patients. This indicated that children given the killed-virus vaccine formed antibody against the H protein even though they did not do so against the F protein (Annunziato et al., 1982). There was also a suggestion of an exaggerated cellular immune response to measles antigens in patients (Redd et al., 1999), although more recent studies in rhesus monkeys suggest that the induction of humoral and CD4 T cell-mediated immunity but not cytotoxic T cells directed against viral antigens may be an important factor in the adverse response to subsequent infection with wild-type virus which resulted in the production of extremely high levels of circulating antibody (Polack et al., 1999; Redd et al., 1999). Years after being vaccinated with killed virus, patients who contracted measles were still developing a clinical illness that, aside from the

initial symptoms, was quite different from regular measles (Annunziato et al., 1982).

A similar effect was seen when a vaccine against RSV infection—the leading cause of lower respiratory tract illness among infants and children—was tested. Susceptible children were vaccinated with a concentrated, formalin-inactivated, adjuvant-enhanced vaccine. Children who received this vaccine initially developed high levels of neutralizing and complement-fixing antibody. However, upon exposure to wild-type RSV, they developed more severe infections than unvaccinated children did (CDC, 2000a; IOM, 1985). Thus, the experimental RSV vaccine was never used clinically.

Atypical measles syndrome and the severe RSV infections are two disturbing consequences of immunization. These two examples resulted from an incomplete understanding of the immune system's response to the live and killed form of these viruses. Thus, it is clear that the response to vaccines cannot be predicted, even with caution and reserve and therefore should be assessed carefully.

Autoimmune and Allergic Diseases

Autoimmune Disease

Collectively, diseases of autoimmunity affect 3 to 5 percent of the population in the United States. (Jacobson et al., 1997).Autoimmune diseases are mediated by T cell and/or T cell-dependent B cell responses directed against self-antigens, and the T cell responses in most autoimmune diseases are dominated by interferon-_ producing CD4 T cells, commonly referred to as Th1 T cells (Marrack et al., 2001). An autoimmune process can target individual organs, such as the brain and spinal cord in multiple sclerosis, or can operate throughout the body, as in systemic lupus erythematosus. For this report, the committee focused on the autoimmune form of diabetes, referred to as type 1a diabetes. Type 1b refers to diabetes associated with an idiopathic loss of insulin secretion. Type 2 diabetes is not associated with destruction of insulin-secreting pancreatic islet cells. Type 1 diabetes has frequently been referred to as "childhood" or insulin-dependent diabetes, while type 2 diabetes has been referred to as "adult-onset" diabetes. It is now recognized that onset of either form of diabetes can occur at any age.

Type 1a diabetes results from the immunological destruction of pancreatic islet ß cells (Atkinson and Eisenbarth, 2001). (The destruction of the islet cells in type 1b diabetes is idiopathic). The beta cells produce insulin, which the body requires to process glucose. Symptoms of type 1 (a and b) diabetes include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. Once diagnosed, the condition can be treated with regular injection of supplemental insulin, but various long-term complications (e.g., diabetic retinopathy, kidney failure, vascular disease) are common. The

development of clinical disease is preceded by an asymptomatic period of progressive islet destruction that may last for many years. As understanding of type 1a diabetes increases, the preclinical period may provide a window for interventions that can delay or prevent clinical onset (Knip, 1997).

The etiology of type 1a diabetes and other autoimmune diseases is multifactorial, involving genetics and environmental exposures. Genetic susceptibility, arising from combinations of multiple genetic factors, appears to be a necessary but not sufficient risk factor for the disease. Monozygotic twins of persons with type 1a diabetes are at increased risk compared with other family members, but as few as 23 percent of these twins developed the disease in one report (Abiru and Eisenbarth, 2000). Although the disease can cluster in families, more than 80 percent of type 1 diabetes cases are reported to occur in persons with no family history of the disease (Dorman et al., 1995). Certain genetic factors may also provide protection from type 1 diabetes.

Environmental factors are thought to serve as triggers or promoters of the autoimmune process in genetically susceptible individuals. In particular, dietary and viral exposures have been suspected in type 1 diabetes. Some studies found that early exposure to cow's milk was associated with increased risk (Gerstein, 1994), as was breastfeeding for less than 3 months (Gerstein, 1994). Newer prospective studies, however, have found no association with these factors (Graves et al., 1999; Hummel et al., 2000; Norris et al., 1996). Congenital rubella syndrome (CRS) shows a clear association with type 1 diabetes, with about 20 percent of CRS patients in the United States also having diabetes (Menser et al., 1978; Rubinstein et al., 1982). Studies of Coxsackie B virus infections have produced conflicting evidence regarding their possible contribution to type 1 diabetes (Robles and Eisenbarth, 2001). Atkinson and Eisenbarth (2001) described a model of progression to clinical disease that depends not on exposure to a single triggering environmental agent but rather on the cumulative effect of various exposures over time.

Worldwide, estimates of the incidence of type 1 diabetes[4] in children under 14 years of age range from 0.1 per 100,000 in parts of China and Venezuela to 36.8 per 100,000 in Sardinia and 36.5 per 100,000 in Finland (Karvonen et al., 2000). As reported by Karvonen and colleagues (2000), estimated incidence for the early 1990s for United States locations range from 11.7 per 100,000 in Chicago to 17.8 per 100,000 in Allegheny County, Pennsylvania. In most populations, incidence is highest in the oldest age group (10–14 years). The disease is also diagnosed in adults, but incidence data are limited. Data from Rochester, Minnesota, for 1945–1969 suggest an incidence rate of 9.2 per 100,000 among persons age 20 and older (Melton et al., 1983).

Rates have generally been lower in more tropical countries and higher in populations of European origin. These patterns may be related to differences

[4] Most population-based studies do not distinguish between types 1a and 1b diabetes.

among racial and ethnic groups in the distribution of genetic risk factors or to the differences in exposure to environmental factors (Atkinson and Eisenbarth, 2001). In any case, the worldwide incidence of type 1 diabetes appears to be increasing 3% a year, and the rate of increase is greatest where incidence has been low (Onkamo et al., 1999). Data from Europe also indicate that incidence is increasing more rapidly among the youngest children (ages 0–4 years) (EURODIAB, 2000).

Allergy

Allergy is responsible for a variety of acute and chronic health problems, including anaphylaxis and allergic rhinitis, asthma, and eczema. These conditions reflect an overreaction of the immune system to allergens—normally harmless environmental agents such as pollens, dust mites, insect venom, and certain foods—that can be encountered through inhalation, ingestion, injection, or skin contact. Under certain circumstances, exposure to an allergen primes the immune system for hypersensitivity reactions involving allergen-specific IgE antibodies and Th2 cells.

The committee focused its attention on allergic asthma for this report. Characteristic symptoms of asthma are episodes of shortness of breath, coughing, wheezing, and chest tightness. These symptoms reflect an acute bronchial hyperresponsiveness to specific allergens and other environmental factors, and a chronic inflammation of the airways (Busse and Lemanske, 2001; IOM, 2000; Kay, 2001; Parham, 2000). The acute response involves activation of mast cells in the lower airways and their release of histamine, cytokines, and other molecules. These mast-cell mediators induce accumulation of fluid, secretion of mucus, and contraction of the smooth muscle around the airways. A “late phase” response includes persistent or recurrent bronchial constriction and infiltration of airway tissue by inflammatory cells. The inflammation can produce temporary or permanent tissue damage.

Exposures to allergens and other environmental factors are known to induce new episodes of asthma when the disease is established, but the underlying factors that account for the development of this type of hyperresponsiveness are not fully understood. Several genetic factors may combine in various ways to establish susceptibility, but they remain poorly defined (Barnes, 2000; Kay, 2001). Environmental exposures may also influence the development of asthma, but current evidence is mixed for exposure such as cockroach allergens or cat dander (e.g., IOM, 2000; Lau et al., 2000; Litonjua et al., 2001; Peat et al., 1993). The presence of older siblings or attendance at day-care (factors that may be markers for the nature or timing of certain exposures) has shown protective effects (Ball et al., 2000). Early exposure to certain viral infections also has shown protective effects (Illi et al., 2001; Openshaw and Hewitt, 2000), but some

respiratory infections have been associated with increased risks (Nafstad et al., 2000), as has early exposure to antibiotics (Droste et al., 2000).

The prevalence of asthma has increased in the United States and other countries over the past 30 years (Grant et al., 1999). An international study of asthma in children found that prevalence was higher in more developed countries (Asher and Weiland, 1998). In the United States, the prevalence rates of self-reported asthma rose from 3.1 percent in 1980 to 5.4 percent in 1994, an increase of 74 percent (Mannino et al., 1998). For children age 0–4 years, rates increased by 159 percent during this period (from 2.2% to 5.7%). Increases in asthma prevalence were seen in all race, sex, age, and regional groups in the United States. No national estimates of the incidence of new asthma cases in the United States are available.

SCIENTIFIC ASSESSMENT

Causality

As has been specified, the committee's review of the safety of multiple immunizations focused on three possible adverse outcomes: heterologous infections; autoimmune disease in the form of type 1a diabetes; and allergy, especially asthma. For each of these outcomes, the epidemiological evidence is summarized (in the text and in accompanying Tables 2, 3, and 4) and the committee's conclusion regarding causality is presented. The search strategies used to identify relevant published reports are described in Appendix D.

Heterologous Infections

Controlled Epidemiological Studies

Guinea-Bissau. Kristensen and colleagues (2000) studied the relationship between vaccination and childhood survival in a population of 8,752 children born to mothers participating in a longitudinal mortality study in Guinea-Bissau. Recommended childhood vaccines in Guinea-Bissau include BCG, OPV, DTP, and measles. Vaccination status was determined by inspection of immunization cards kept by the children's parents. Children were excluded if the card could not be examined, which was the case for more than a third of children.

Vaccine exposure for BCG, OPV, and DTP was assessed during a first visit when children were 0–6 months of age. Mortality was assessed at a subsequent visit approximately 6 months later. For surviving children, vaccination status, including measles vaccine exposure as well as that of BCG, OPV, and DTP, was updated. Mortality was assessed again at a third visit, approximately 6 months after the second visit.

A Cox proportional hazards model was used to calculate the mortality ratio for vaccinated and unvaccinated children. The overall mortality for any vaccine was nonsignificant (RR = 0.74, 95% CI 0.53–1.03). Receipt of BCG vaccine was associated with lower mortality (adjusted RR = 0.55, 95% CI 0.36–0.85), as was measles vaccine (adjusted RR = 0.48, 95% CI 0.27–0.87). The mortality ratio for one dose of DTP vaccine versus none was 1.84 (95% CI 1.10–3.10), but the ratio for two to three doses was not significantly elevated (RR = 1.38, 95% CI 0.73–2.61). The pattern was similar for OPV, with an elevated mortality ratio for one dose (RR = 1.81, 95% CI 1.07–3.05), and nonsignificant ratio for two to three doses (RR = 1.39, 95% CI 0.73–2.64).

The authors conclude that receipt of BCG and measles vaccines may have a protective effect against mortality, while receipt of a single dose of DTP and polio vaccines may carry a higher mortality risk compared with receiving no vaccinations. The results also suggest that DTP vaccine may negate the positive effects associated with BCG vaccine.

However, the interpretation of these findings warrants caution. The vaccination status of some children was unclear and more than a third of the children did not have records available. Many children may have been underimmunized, contributing to the increased mortality rates and reflecting limited access to health care. Vaccinated children were also more likely to receive health care than unvaccinated children, which may mean that getting vaccinated is associated with access to or use of other interventions that improve survival. Mothers of children vaccinated with DTP were younger than mothers of children vaccinated with BCG or measles vaccine, which means maternal age may have contributed to infant mortality risk. The adjustment procedure for potential confounders was also unclear. For the United States, the findings regarding BCG vaccine are not relevant since the vaccine is not routinely used in this country.

United States-Boston. In a case-control study, Burstein and Fleischer (1994) examined the relationship between vaccination and the risk of occult bacteremia. Cases and controls were patients treated in the emergency department at Children's Hospital in Boston between November 1987 and December 1990. Cases were 54 children age 3 to 36 months who participated in a multicenter antibiotic study. Pathogens isolated from these children included *S. pneumoniae, E. coli, S. aureus, H. influenzae,* or *Salmonella spp*. The 108 controls were matched to cases according to age. Each case had two controls. One control group included febrile nonbacteremic children. The other group included nonfebrile children who were treated for symptoms not related to infectious diseases. Vaccination history, including DTwP, was obtained from medical records.

The authors found no significant difference between cases and controls for time since last vaccination of any type, or for time since last DTwP vaccination. Limitations of this study included weak statistical power. It was also unclear which vaccines, other than DTwP, the children received.

United States-Tennessee. Griffin and others (1992) examined the ass o-ciation between DTwP immunization and the risk of invasive bacterial infection. The incidence of invasive bacterial diseases (*H. influenzae*, *N meningitidis*, *Streptococcus pneumoniae*, group B *Streptococcus*, or *Listeria monocytogenes*) was measured in a cohort of 64,591 children who received at least one dose of DTwP vaccine through any of the four largest Tennessee county health clinics from 1986 to 1987. Based on surveillance data, 158 children diagnosed with invasive bacterial infection after receiving DTwP vaccine were identified in this cohort. Using a Poisson regression model and controlling for age, the relative risk for infections during the early post-immunization periods (0–7, 8–14, 15–28 days) compared with the later period (29 or more days) was nonsignificant. The authors concluded that there was no increase in the risk for invasive bacterial infection following receipt of DTwP vaccine, especially during the early post-immunization period. Interpretation of the study is limited by the lack of an unvaccinated comparison group. In addition, the analysis was limited to cases of serious culture-confirmed infections.

United States-Kaiser Permanente Northern California. In a case-control study, Black and colleagues (1991) examined the relationship between vaccination and the risk of heterologous invasive bacterial disease. Cases and controls were identified from member records in the Kaiser Permanente Medical Care Program of Northern California. As cases, 223 children between 1 month and 2 years of age who were diagnosed with invasive bacterial disease (*Pneumococcus*, *H. influenzae*, *E. coli*, and *Meningococcus*) between 1986 and 1988. Invasive bacterial disease status was identified from a computerized microbiology laboratory database. The 446 controls were matched according to age, sex, zip code, and length of plan membership. For cases, all vaccines received within three months prior to disease onset were identified through medical chart review. For matched controls, the date of diagnosis for the corresponding case was the reference date used to obtain vaccination histories. Children had received one or more of the following vaccines: DTP, OPV, and MMR.

A conditional logistic regression model was used to estimate the effect of recent immunization on disease; odds ratios were calculated from the regression results for each vaccine. A separate analysis controlled for the effect of well care visits and day care attendance (information available for 72 percent of the subjects). Odds ratios were calculated for separate time intervals from date of vaccine receipt to date of disease diagnosis: 0–7 days, 8–30 days, 31–60 days, and 61–90 days. Receipt of individual vaccines was associated with a lower risk of disease in all time intervals, with significant effects for DTP at any interval after 7 days and for OPV at 8–30 days and 31–60 days. After adjustment for day-care attendance and well-care visits, however, no individual vaccine had a significant effect on risk of disease. But there was a significant protective effect in the adjusted analysis from the receipt of any vaccine within 30 days (OR = 0.26, 95% CI 0.09–0.76) or 90 days (OR = 0.31, 95% CI 0.13–0.73).

The authors conclude that vaccines do not increase the risk for invasive bacterial disease and that they may provide a protective effect against disease, especially within 3 months after vaccination. However, children who received well care visits were also less likely to develop invasive bacterial disease than those who did not receive them. A health care effect may account for the observed protection of vaccines against heterologous invasive bacterial disease.

United States-Alaska. In a two-part study, Davidson and colleagues (1991) examined risk of disease following receipt of DTwP vaccine among Alaskan Native children. They first conducted a case-control study to examine the risk of invasive bacterial disease. The 186 cases were children 2 to 24 months old who received at least one DTwP vaccine and were identified through surveillance reports for *H. influenzae* type b and *S. pneumoniae* diseases. There were 186 controls matched according to age, sex, residence, and number of DTwP vaccines received. The time interval between last DTwP vaccination and date of disease diagnosis (the reference date for controls) was obtained. There were no significant differences in DTwP vaccine intervals for Hib disease cases. For *S. pneumoniae* disease, significantly more cases had been immunized 31–60 days earlier (OR = 3.3, 95% CI 1.1–10.0), but differences were not significant for shorter or longer intervals. The authors conclude that there was no clear association between the timing of DTwP vaccination and risk of invasive bacterial disease. The authors note that a possible explanation for the lack of difference observed in the study is overmatching. Overmatching is where cases and controls closely resemble each other on factors related to the exposure of interest. In this study, matching based on the number of DTP vaccines received may have resulted in the lack of difference in the timing of DTwP immunization between cases and controls. However, the authors observed that matching according to the number of DTP vaccines was necessary and reduced potential confounders such as those related to health status.

Subjects in the second part of the study included 104 cases and controls from the earlier part who had complete medical records available. Cases and controls were combined to compare the occurrence of any illnesses within 30 days before and after receipt of DTwP vaccine. Comparisons were made for the occurrence of any illness, any infectious disease, otitis media, other respiratory infections, temperature greater than 38°C, or hospitalization. There was a higher incidence of any illness during the 30 days prior to DTP vaccination than in the 30 days following vaccination (53% versus 43%, $p = .004$). There were no significant differences between the pre- and post-vaccination periods for the other indicators of illness. The authors again conclude that DTwP vaccination does not increase the risk of other illnesses. The authors note that the higher frequency of disease in the pre-DTP group compared to the post-DTP group may have resulted from a "well-child effect," whereby immunization of children with illnesses was postponed until they were well.

Randomized Controlled Trial

Germany. Otto and colleagues (2000) examined differences between vaccinated and unvaccinated children in the risk of morbidity associated with infectious diseases. A total of 662 children, born between January 1995 and December 1996, were randomized to receive their first vaccine (against diphtheria, pertussis, tetanus, *Haemophilus influenzae type B,* and poliomyelitis) at either 60 days or 90 days after birth.[5] Children were observed during the third month of life, beginning at the sixth day after vaccine receipt. Mothers kept a daily journal and recorded any occurrence of symptoms. Morbidity was assessed in terms of the incidence of coughing, signs of rhinitis, restlessness, vomiting, rash, pain, poor food or fluid intake, fever, and respiratory embarrassment.

A total of 166 children were excluded from the analysis, mostly because of missed vaccination. The authors observed a significant ($p < 0.01$) increase in vomiting, cough, rhinitis, restlessness, rash, and pain in the unvaccinated group compared with the vaccinated group. Hospitalization was more frequent among unvaccinated children ($n = 4$) than vaccinated children ($n = 1$). The authors concluded that children who received vaccinations during the third month of life did not demonstrate an increased risk of infectious-disease symptoms and may experience some protective effect from vaccination. Study limitations included observer bias in that the mothers, who were responsible for monitoring morbidity, were not blinded as to vaccination status. The high exclusion and dropout rate (39% versus 11% in the unvaccinated group), especially in the vaccinated group, may also effect interpretation of the study results.

Other Studies

The committee reviewed additional articles that reported adverse events after receipt of multiple immunizations. These studies helped inform the committee's assessment of risk for heterologous infection but did not contribute to the causality argument. Most of these articles reported on randomized controlled trials that primarily investigated the safety, efficacy, and/or immunogenicity of various vaccines. They did not specifically examine the incidence of heterologous infections in children post-immunization, compare the incidence of such infections between different exposure groups, or report statistical analyses from which to make inferences or extrapolations related to heterologous infections. The articles reviewed are briefly summarized below.

Shinefield and colleagues (1999) examined the safety and immunogenicity in infants of a heptavalent pneumococcal CRM_{197} conjugate vaccine administered concurrently with DTwP, Hib, and OPV vaccines. Hepatitis B vaccine was

[5] In Greiswald, Germany, immunization recommendations call for children to receive their first vaccine during the third month of life. Children participating in the study received their vaccines on either the first day (60 days after birth) or the 30th day of the third month (90 days after birth), complying with the recommended immunization schedule.

administered concurrently or at least two weeks earlier or later. Children received DTaP vaccine at a later stage of the study. The authors reported on adverse events observed following immunization. In the study, 302 infants age 2 months were randomized to receive the pneumococcal vaccine or meningococcal Group C conjugate vaccine. During the study, 12 emergency room visits occurred within 30 days of any vaccine dose. These visits were for croup, otitis, febrile illness, and urinary tract infection, but none were considered vaccine-related. Following the primary doses of vaccines, eight children were hospitalized, two within 30 days after vaccination. Following the booster doses, there were four emergency department cases (of viral illness, otitis media [two cases], and burn) and four hospitalizations (pneumonia, otitis media, elective surgery, asthma, and cough). The authors did not believe that these events were vaccine-related.

Olin and others (1997) compared the efficacy of three types of DTaP vaccine to the DTwP vaccine used in the United Kingdom. A sample of close to 83,000 infants age 2–3 months were randomized to different DTP vaccine exposures and also given Hib and IPV vaccines. The authors reported on adverse events following vaccine receipt. Those that may have involved heterologous infections included two deaths from pneumonia within 4–7 days of a trial vaccine dose, 20 cases of invasive bacterial infections, and one case of suspected encephalitis

Afari and colleagues (1996) examined the immunogenicity and reactogenicity of two types of DTaP vaccine (a freeze-dried, heat-stable product and a liquid product) and DTwP vaccine. Of the 403 infants who were studied, 136 were randomized to receive the freeze-dried DTaP, 130 to receive liquid DTaP product, and 137 to receive DTwP. The authors reported that three children who received the freeze-dried vaccine died of measles or malaria, two children who received liquid DTaP died of malaria or diarrheal disease, and two children who received DTwP died of multiple boils or malaria. Differences in mortality rates between either DTaP group and the DTwP group were not statistically significant.

Simondon and colleagues (1996) compared the safety and immunogenicity of DTaP and DTwP vaccines in a randomized clinical trial involving 286 Senegalese infants. Children also received BCG and IPV during the study. Six deaths occurred within 2 months after vaccination. On the basis of verbal autopsies, the four deaths in the DTwP group were attributed to diarrhea, malaria, and pneumonia. The two deaths in the DTaP group were attributed to meningitis and diarrhea. The authors suggested caution in drawing conclusions regarding the number and causes of deaths in the study. The infant mortality rate in the study region is high, and verbal autopsies are an imprecise means of determining cause of death.

Riordan and colleagues (1995) reported two cases of bacterial meningitis after receipt of MMR vaccine. Fever and rash occurred in two children, age 12 months and 13 months, within 4 days after MMR vaccine, and were initially attributed to the vaccine. After diagnostic tests, both children were found to have

a high level of C-reactive protein and were diagnosed with a bacterial infection. Meningococcal infection was confirmed in one case and suspected in the other. The authors noted that the median age of children admitted to their hospital with meningococcal disease is 14 months.

Avendano and colleagues (1993) evaluated the safety and immunogenicity of a Hib vaccine made with purified polyribosylribitol phosphate conjugated to tetanus toxoid (PRP-T). The 287 infants in the study were randomized to receive either the Hib vaccine combined in a single injection with DTP, separate injections of Hib vaccine and DTP, or separate injections of DTP and a placebo. The one death during the study, resulting from pneumonia and sepsis, occurred 38 days after the second dose of combined DTP and PRP-T. Cultures obtained from the infant were positive for *Streptococcus pneumoniae*.

Chazono and colleagues (1991) described the side effects following use of DTaP vaccine in Japan and compared that experience with the side effects reported in a Phase III trial of the acellular pertussis vaccine component in Sweden in 1986. Pediatricians in four health centers in Japan collected information on the number of children diagnosed with any infectious diseases after receiving DTaP vaccine, as well as information on cases of pertussis or abnormal reactions such as febrile seizures. Information was obtained from medical charts or from parents or guardians contacted by telephone or mail. Of the 940 infants for whom information was available, three children had an infectious disease (one case each of measles, mumps, and varicella). The authors contrasted their findings with those from the Phase III acellular pertussis trial in Sweden, where three deaths from severe invasive bacterial infection (i.e., Hib, pneumococcal, and meningococcal infections) occurred among 1,385 children.

Ruuskanen and colleagues (1980) examined antibody responses and adverse reactions following receipt of inactivated polio vaccine. Children in the study received 2 doses of IPV as well as 4 doses of DTP between the ages of 3 months and 24 months. Information on reactions following receipt of IPV was obtained from questionnaires returned by parents of 225 of 380 children in the study. Fever (greater than or equal to 37.5°C) was reported for about 17 percent of children and was clinically significant (greater than or equal to 38.5°C) in 5 percent of the children. Fevers usually began the same day as vaccination and lasted up to 2 days. In a few children (exact number unspecified), fevers started 6 or more days following vaccination. The authors believed that these fevers were caused by infection, and not vaccination, although the basis for their belief was not stated.

Causality Argument

The committee reviewed several case-control or cohort studies (Black et al., 1991; Burstein and Fleisher, 1994; Davidson et al., 1991; Griffin et al., 1992; Kristensen et al., 2000) and a randomized controlled trial (Otto et al., 2000) (see

Table 2). Vaccine exposure varied among the studies but fit the committee's definition of exposure to "multiple immunizations." The studies examined the effects of the addition of one vaccine to an existing immunization schedule, of one vaccine consisting of antigens from more than one infectious agent or strain of virus (e.g., DTP, OPV, or MMR), or of several vaccines received at the same time. Outcome measures in the studies also varied, with the "disease" group including subjects who had a positive culture to invasive bacterial disease, who had symptoms related to infectious diseases, or who had died. Limitations of the studies included a potential health care utilization bias and high dropout rates. Despite these variations and limitations, the overall findings from the studies consistently demonstrated either no effect or a beneficial effect of multiple immunizations on heterologous disease. **Therefore, the committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of heterologous infections.**

Autoimmune Disease: Type 1 Diabetes

Uncontrolled Observational Studies

An ecological analysis by Hyoty and colleagues (1993) and an update by Hiltunen et al. (1999) examined the incidence of type 1 diabetes before and after introduction of MMR vaccine in Finland in 1982. Periodic mumps epidemics had been suggested as a contributing factor in the incidence of Type 1 diabetes, and the introduction of MMR vaccine resulted in almost complete disappearance of mumps. Data on the incidence of type 1 diabetes among children ages 0–14 years was obtained from a national registry for the years 1966–1996. The authors found a continuing increase in the incidence of diabetes over the period, especially among children ages 0–4 years and 5–9 years, but no cohort effect associated with the introduction of MMR vaccine was observed. The authors concluded that neither wild-type mumps nor MMR vaccine were related to the continuing increase in diabetes.

In a letter reporting on an ecological analysis, Classen (1996) examined IDDM incidence in children born before and after the introduction of a hepatitis B immunization program in New Zealand in 1988. At the time, children in New Zealand were also routinely immunized with DTP, MMR, and OPV vaccines. Exposure was based on birth year, and diabetes cases were identified through the diabetes registry in Christchurch. The author reported an increase in average annual incidence of diabetes after introduction of hepatitis B vaccine, although no control was made for the general secular trend of increasing diabetes incidence rates. The incidence rate for 1982–1987 was 11.2 cases per 100,000 per year, and the rate increased to 18.2 cases per 100,000 per year for 1989–1991. The authors proposed a possible link between the hepatitis B vaccine, and the timing of its

administration, and the rising incidence of type 1 diabetes, but the ecological nature of the study limits the ability to make inferences about causation.

Controlled Epidemiological Studies

Vaccine Safety Datalink. In a case-control study, DeStefano and colleagues (2001) examined the association between childhood vaccines and the risk of developing type 1 diabetes. Data for both cases and controls were obtained from the four health maintenance organizations (HMOs) that participate in CDC's Vaccine Safety Datalink project. The cases were 252 children diagnosed with type 1 diabetes and the 768 controls were matched to individual cases on HMO, sex, date of birth (within 7 days), and length of health plan enrollment. For each case, vaccination history prior to the date of diabetes diagnosis was gathered from a medical chart review. The same reference date was used to obtain vaccination histories for the matched controls.

The vaccines evaluated included DTaP, DTwP, MMR, varicella, Hib, and hepatitis B. Oral polio vaccine was not included in the analysis because so few children had not received it (one case and three controls). Also tested was the effect of the timing of the first hepatitis B vaccine (never vaccinated; birth to 14 days, 15 to 55 days, or 56 or more days). In addition, the effect of differences in the schedule of Hib immunization (one dose at 21 to 27 months of age versus 3 doses in the first 8 months plus a fourth dose at 12 to 18 months) was examined.

On the basis of a conditional logistic regression model stratified by the matching variables, the odds ratio for each of the vaccines was nonsignificant. However, after adjusting for race/ethnicity and family history of type 1 diabetes, the odds ratio for whole-cell pertussis was 0.23 (95% CI 0.06–0.93). The highest adjusted odds ratio was for MMR (1.43, 95% CI 0.71–2.86) but was not statistically significant. Variations in the timing of hepatitis B vaccine produced no significant differences in the risk of type 1 diabetes. Similarly, there were no significant differences among various Hib immunization schedules. However, the ability to compare the different Hib schedules was limited, since only a few children received either no Hib vaccine or received one dose at 21 to 27 months. The authors concluded that neither the receipt of routine childhood vaccines nor the timing of certain vaccines was associated with an increased risk of type 1 diabetes.

EURODIAB. In a case-control study, a group examined infections and vaccinations as risk factors for type 1 diabetes (EURODIAB, 2000). Cases and controls were identified through seven European centers, each of which operates a population-based diabetes registry. As cases, there were 900 children with diabetes onset before age 15. The 2,302 controls were matched to the cases by age distribution. Vaccination history was obtained from parents through interviews or questionnaires and validated by official sources or entries in child health records held by the parent. Vaccines received included BCG, polio, tetanus, diphtheria, pertussis, rubella, measles, mumps, and Hib.

The odds ratios for all nine vaccines were nonsignificant using either a Mantel Haenszel analysis stratified by center, or a logistic regression analysis with adjustment for center, age group, breast feeding, birth weight, maternal age, jaundice at birth, asthma, and vitamin D supplementation. There was no evidence, the authors concluded, that vaccinations increase the risk of type 1 diabetes. The study may have been compromised by ascertainment bias. About 75% of responders had validated vaccination records available. Validation was based on either a review of official records or on parental recall of exact vaccination dates, even if the investigator did not see a record. The latter may have contributed to imprecision in assigning vaccine status.

Finland. Karvonen and colleagues (1999a) studied the relationship between multiple vaccines and type 1 diabetes by examining the effect of adding Hib vaccine to the routine childhood immunization schedule. Incidence of type 1 diabetes was compared in cohorts of Finnish children born before or after a Hib vaccine efficacy trial and followed for 10 years. One cohort of 128,936 children was born between October 1983 and September 1985, prior to the Hib vaccine trial and thus was not exposed to the vaccine. Children born between October 1985 and August 1987 participated in the Hib vaccine efficacy trial. These children were divided into two cohorts: 59,238 children who were born on odd days were vaccinated with Hib at 3, 4, 6, and 14 to 18 months; 57,114 children born on even days were vaccinated at 24 months only. All children were assumed to have received BCG, diphtheria-tetanus-pertussis, polio, and measles-mumps-rubella vaccines. Newly diagnosed cases of diabetes among all three cohorts were ascertained from a national hospital discharge registry (1983–1986) or a nationwide prospective childhood diabetes registry (1987–1997).

There was no significant difference in the risk of diabetes by age 10 between the children who did not receive the Hib vaccine and children who were vaccinated at 24 months of age. Similarly, no difference in risk was found between the children first vaccinated at 3 months of age and those vaccinated at 24 months. For each of the comparisons, the relative risk was near 1.0. The authors concluded that neither the addition of Hib vaccine to the immunization schedule nor the timing of Hib vaccine increased the risk of type 1 diabetes in children. Estimates of both vaccine exposure and diabetes cases were based on aggregate data from three cohorts and from the population as a whole. Thus, interpreting the results at the level of the individual is difficult.

Sweden. Heijbel and colleagues (1997) examined the effect of pertussis vaccination in infancy on the risk of developing type 1 diabetes. Cumulative incidence of type 1 diabetes at ages 0 to 12 years was compared in cohorts of children born before or after the pertussis vaccine was removed from the routine immunization schedule in Sweden. Specifically, cohorts of children born in 1977 (96,057 children) and in 1978 (93,248 children) received pertussis vaccine

TABLE 2 Evidence Table: Controlled Epidemiological Studies—Vaccines and Heterologous Infections

Citation	Design	Population	Exposure Measure	Outcome Measure	Results	Comment	Contributions to Causality
Kristensen et al. (2000)	Cohort; two visits required	8,752 children born to women participating in a longitudinal mortality study (Guinea-Bissau)	Vaccine status by inspection of immunization card. Vaccines included BCG, polio, diphtheria-tetanus-pertussis,* and measles vaccines	Mortality by parental report	**RR (95% CI) mortality** Any vaccine = 0.74 (0.53–1.03) BCG = 0.55 (0.36–0.85) Measles = 0.48 (0.27–0.87) DTP = 1.84 (1.10–3.10) Polio = 1.81 (1.07–3.05)	vaccination status unclear; records not available for more than one-third; vaccinated children more likely to receive health care; maternal age differences in cohorts; adjustment for potential confounds not clear; DTP may negate the positive effect of BCG	weak evidence relevant to causality; favors beneficial effect of measles and BCG and negative effect of DTP in country with a high infant mortality of uncertain relevance to U.S.; BCG data not relevant to U.S.
Otto et al. (2000)	Randomized controlled trial comparing vaccinated and unvaccinated children	662 children born between Jan 1995 to Dec 1996 at a single hospital; 166 children excluded from final analysis (most missed vaccination) (Germany)	Vaccinated: 1st vaccination (diphtheria, pertussis,* tetanus, (ital.)*Haemophilus influenzae type B*, and poliomyelitis) 60 days after birth Unvaccinated: 1st vaccination at 90 days after birth	Any "Unspecific morbidity" from parental diary on days 66 to 90 after birth. Unspecific morbidity = coughing, signs of rhinitis, restlessness, vomiting, rash, pain, poor	Vomiting, cough, rhinitis, restlessness, rash, pain more common in unvaccinated group (all $p < 0.01$); 4 hospitalizations in unvaccinated group vs. 1 in vaccinated group	Not blinded; drop out/exclusion rate 25%, disproportionately in early-vaccination group	Weak evidence relevant to causality; favors no effect or beneficial effect of vaccines

				food/fluid intake, fever and respiratory embarrassment			
Burstein & Fleischer (1994)	Case-control	54 case children, age 3–36 months, participating in multicenter antibiotic study and 108 age-matched controls (United States)	Vaccination history, including DTwP, obtained from medical records. Hib not yet available for these children	Children with occult bacterimia: *S. pneumoniae*, *E. coli*, *S. aureus*, *H. influenzae*, or *Salmonella spp*	Difference between cases and controls NS ($p > 0.05$) Interval (days) since last Vaccine Cases = 128.8 +/- 111.5 Febrile = 154.9 +/- 99.6 Trauma = 163.0 +/- 157.8 Interval (days) since last DTwP vaccine Cases = 161.5 +/- 134 Febrile = 188.8 +/- 128.8 Trauma = 184.1 +/- 172.1	Specific vaccines, other than DTwP, administered to children are unknown.; weak statistical power	Weak evidence relevant to causality; favors no effect of vaccines or of DTwP vaccine
Griffin et al. (1992)	Cohort	64,591 children immunized through Tennessee county health clinics	At least one DTP vaccination in 1986 or 1987; exposure based on information in linked TN immunization-birth certificate database	Invasive bacterial infections (*H. influenzae*, *N .meningitidis*, *Streptococcus pneumoniae*, *group B Streptococcus*, or *Listeria monocytogenes*. in children 72 mos. or younger, from surveillance of hospital infection control	**Age-adjusted RR (95% CI)** Of all invasive bacterial infections by days since DTP immunization Comparing early post-immunization periods (0–7, 8–14, 15–28 days) to later period (29+ days): 0–7 days: 1.0 (0.5–2.0) 8–14 days: 0.8 (0.4–1.7) 15–28 days: 1.2 (0.7–1.9)	No unvaccinated comparison group; analysis limited to cases of serious culture-confirmed cases	Weak evidence relevant to causality; favors no effect of DTP vaccine

Continued

Continued

Black et al. (1991)	Case-control	223 cases of invasive bacterial disease in children between 1 month and 2 years from 1986 to 1988; 446 controls matched on age, sex, zip code, duration of plan membership (U.S.Kaiser Permanente)	Immunization by record review; diphtheria-tetanus-pertussis,* measles-mumps-rubella, and oral polio immunization within 3 months of disease onset during first 2 years of life	Invasive bacterial disease status identified from a computerized microbiology laboratory database *Pneumococcus, H .influenzae, E. coli, Meningococcus* among diseases identified	**Unadjusted OR (95% CI)** Within 8–30 days DTP = 0.37 (0.10–1.41) OPV = 0.13 (0.02–1.11) MMR = 0.16 (0.01–2.33) Any = 0.29 (0.09–0.95) Within 61–90 days DTP = 0.31 (0.04–2.50) Any = 0.21 (0.03–1.63) **Adjusted OR (95% CI),** any vaccine (DTP, OPV, MMR), adjusted for day-care attendance and well-care child visits Within 30 days = 0.26 (0.09–0.76) Within 90 days = 0.31 (0.13–0.73)	No demonstration of an increased risk for invasive bacterial disease following immunization with DTP, MMR, and OPV	Weak evidence relevant to causality; favors no effect or beneficial effect
Davidson et al. (1991)	Case-control	study #1 186 cases of invasive bacterial disease; 186 controls matched on age, sex, residence, number DTwP immunizations (US-Alaska natives)	Exposure to DTwP† vaccination determined from public health immunization records and computerized Indian Health Service medical records.	Cases with invasive bacterial disease, specifically, *H. influenzae* type b or *S. pneumoniae*, detected through active and passive surveillance reports	**ORs (95% CI)** ≤ 3 day interval Hib disease = 1.0 (0.05–20.9) 31–60 day interval Hib disease = 1.0 (0.5–2.0) Sp = 3.3 (1.1–10.0) Total = 1.4 (0.8–2.5) > 120 interval days Hib disease 1.0 (0.5–2.2) Sp 0.3 (0.08–1.0) Total = 0.7 (0.3–1.3)	potential for over matching; well child effect likely	Weak evidence relevant to causality; favors no effect of DTwP vaccine

Davidson et al. (1991)	Cohort	study #2 (subset of study #1) 104 cases for whom all medical records were available (U.S-.Alaska natives)	Exposure to DTwP[†] vaccination determined from public health immunization records and computerized Indian Health Service medical records	Occurrence of all illness during the 30-day period before and after DTwP administration. Categories include: any illness; any infectious disease; otitis media; other respiratory infections; temperature > 38°C; and hospitalization	For all illness, 53% of all children had illnesses during 30 days before DTwP immunization and 43% had illnesses during 30 days after DTwP immunization (p = .004); Other illness categories were non-significant; any infectious disease (p = .084); otitis media (p = .501); other respiratory infections (p = 0.285); temp > 38°C (p = .724); hospitalization (p = .424)	potential for over matching	Weak evidence relevant to causality; favors no effect of DTwP vaccine

BCG = Bacille Calmette-Guerin vaccine
MMR = measles-mumps-rubella vaccine
DTaP = diphtheria-tetanus-acellular pertussis
DTwP = diphtheria-tetanus-whole-cell pertussis
Hep B = hepatitis B vaccine
Hib = *Haemophilus influenzae b* vaccine
OPV = oral polio virus
HMO: health maintenance organization
NS = not significant
CI = confidence interval
OR = odds ratio
RR = relative risk

*These studies do not specify whether acellular or whole-cell pertussis vaccine was administered. It is assumed that whole-cell pertussis vaccine was administered to children in these studies.

[†] Diphtheria-tetanus-whole cell pertussis vaccine

through DTP vaccinations. Children born in 1980 (97,064 children) and in 1981 (94,065 children) received DT vaccine.

Rates of exposure to DT or DTP vaccine were assessed from yearly vaccination reports of child health care centers. Estimates of pertussis vaccine exposure among children born in 1980 or 1981 were based on reports of vaccine doses released. Data from the national diabetes registry were used to determine the cumulative incidence of type 1 diabetes in each birth cohort. No difference was found in the cumulative type 1 diabetes incidence for any of the birth cohorts. The population-level immunization data limits ability to make extrapolations at the individual level.

In another study in Sweden, Blom and colleagues (1991) used a case-control approach to examine infections and vaccinations as possible risk factors for type 1 diabetes. A total of 339 children age 0 to 14 years and newly diagnosed with diabetes from September 1985 through August 1986 were identified from the Swedish childhood diabetes register. The study included 528 controls matched on age, sex, and county, who were identified from the Swedish population register. Vaccination histories in the two groups were collected from local child health care centers and school health units. The vaccines received by these children included BCG, smallpox, DTP, DT, tetanus, polio, MMR, measles, mumps, and rubella. Similar proportions of children in both the case and control groups had received each of these vaccines.

The odds ratios for risk of type 1 diabetes for individual vaccines (or combination products) were nonsignificant. The odds ratio for measles vaccine alone was 0.74 (95% CI 0.55–1.00), but when the measles-mumps-rubella and/or measles vaccine was considered, the odds ratio suggested a significant reduction in risk (OR = 0.69, 95% CI 0.48–0.98). The authors concluded that the evidence did not support an increased risk of type 1 diabetes after vaccination and that the measles vaccine may have a protective effect against type 1 diabetes. The study was well conducted, but the number of cases of diabetes was small, so that the study may have been under-powered to detect significant differences in the 13 categories of vaccination examined. Power calculations for Type II errors were not provided. The isolated finding of a possible protective effect (just at the 0.05 level of statistical significance) of the measles vaccine is difficult to interpret given the multiple comparisons made in the analysis.

Other Studies

The committee reviewed additional studies examining the relationship between diabetes and BCG or smallpox vaccination (Classen and Classen, 1996, 1997, 1999; Dahlquist and Gothefors, 1995; Parent et al., 1997). But because these vaccines are not routinely administered to children in the United States, the studies were not considered directly relevant to the committee's task.

Causality Argument

The committee found five controlled studies (Blom et al., 1991; DeStefano et al., 2001; EURODIAB, 2000; Heijbel et al., 1997; Karvonen et al., 1999a) (Table 3) and three ecological studies (Classen, 1996; Hiltunen et al., 1999; Hyoty et al., 1993) that studied this relationship. The studies looked at the effects of the addition of one vaccine to an existing immunization schedule, of one vaccine consisting of antigens from more than one infectious agent or strain of virus (e.g., DTP, OPV, or MMR), or of several vaccines received at the same time. Despite these variations, the overall findings from the studies consistently demonstrated no effect of multiple immunizations on the incidence of type 1 diabetes. **Therefore, the committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of type 1 diabetes.**

Allergic Disease

Uncontrolled Observational (Ecological) Study

Anderson and colleagues (2001) examined the relationship between immunization and allergic disease by comparing trends in immunization rates with the prevalence of allergic disease symptoms. Allergy data, specifically for asthma, allergic rhinoconjunctivitis, and atopic eczema, were obtained for children ages 6 to 7 years and 13 to 14 years from centers participating in the International Study of Asthma and Allergies in Childhood (ISAAC). Those data were compared with national and local immunization rates for BCG, DTP, and measles.

For children age 6 to 7 years, allergy data were obtained from 91 centers, with a median of 2,996 children per center (range 1,104–6,533). Local immunization rates were available for 57 centers. Allergy data for children age 13 to 14 years were available from 154 centers with a median of 2,064 children (range = 1,046–11,400); 92 centers had local immunization data.

In the 13- to 14-year-old age group, the authors observed a significant negative association (rank correlations, $p < .05$, adjusted for socioeconomic factors) between local DTP rates and wheezing (-0.53, 95% CI, -1.49, 0.43), rhinoconjunctivitis (-0.60, -1.02, -0.19), and atopic eczema (-0.27, -0.76, 0.21). For measles vaccine, significant negative correlations were found for rhinoconjunctivitis (-0.47, -.98, 0.04) and atopic eczema (-0.42, -0.98, 0.13). No associations were observed for children age 6 to 7 years. The authors concluded that DTP vaccines are not risk factors for allergic disease at the population level. However, the authors noted that because immunization data were available only at the population level, associations at the individual level could not be excluded.

TABLE 3 Evidence Table: Controlled Epidemiological Studies—Vaccines and Type 1 Diabetes

Citation	Design	Population	Exposure Measure	Outcome Measure	Results	Comment	Contribution to Causality Argument
DeStefano et al. (2001)	Case-control	252 children from 4 HMOs that participate in Vaccine Safety Datalink project of the CDC along with their 768 matched controls (United States)	From chart review: vaccine exposure prior to diabetes incidence date: DTaP, DTwP, measles-mumps-rubella, varicella; Hib, HepB; Timing of Hep B (never vaccinated; birth to 4 days, 15 to 55 days, and GTE 56 days); Hib schedule (3 doses in first 8 mos + 4th dose at 12–18 mos vs. 1 dose at 21–27 months)	Cases in HMO registries with ICD-9 diagnosis of type 1 diabetes	**Adjusted OR (95% CI):** Vaccines and Type I diabetes Hep B = 0.73 (0.45–1.19) Hib = 1.23 (0.53–2.89) whole-cell pertussis = 0.23 (0.06–0.93) acellular pertussis = 1.12 (0.63–1.99) MMR = 1.43 (0.71–2.86) Varicella = 1.02 (0.61–1.71) Hep B timing and risk of type 1 diabetes 0–14 d = 0.66 (0.27–1.59) 15-55 d = 0.65 (0.21–2.0) ≥ 56 d = 0.74 (0.45–1.21) Hib schedule and risk of type 1 diabetes: 1 dose only at 21–27 mo: 0.45 (0.15–1.30) Other schedules = 0.71 (0.41–1.24) Not vaccinated = 0.64 (0.22–1.81)	Limited ability in comparing Hib schedules since only a few children received no Hib or only 1 dose at 21–27 months	Weak evidence relevant to causality. Favors no effect of specific vaccine or the timing of Hepatitis B and Hib.
EURODIAB Study Group (2000)	Case-control	900 cases with diabetes and 2302 controls matched on age	Vaccination history by interview or questionnaire; Vaccines included BCG, polio,	Diabetes cases from a population-based register	**Adjusted OR (95% CI)** BCG = 0.83 (0.57–1.20) Polio = 1.20 (0.57–2.52) Tetanus = 1.56 (0.73–3.33)	Ascertainment bias	Weak evidence relevant to causality; Favors no effect

		from 7 European centers with population-based diabetes registers	tetanus, diphtheria, pertussis,* rubella, measles, mumps, and Hib	of childhood onset diabetes			of any vaccine
Karvonen et al. (1999a)	Cohort	10-year follow-up of three cohorts of Finnish children: Cohort 1 = 128,936 (born 1983–1985); Cohort 2 = 59,238 Cohort 3 = 57,114 (born 1985–1987), assignment to cohort 2 or 3 based on odd (2) or even (3) day of birth	3 Vaccine exposure cohorts: 1) born before Hib availability; 2) participating in Hib efficacy trial, vaccination at 3, 4, 6, and 14–18 months; and 3) Hib at 24 months only Children assumed to also have received BCG, diphtheria-tetanus-pertussis,* polio, and measles-mumps-rubella vaccines	New IDDM cases in each cohort, reported in nationwide hospital disease registry (1983-1986) or nationwide prospective childhood diabetes registry (post-1987)	Diphtheria = 1.27 (0.63–2.56) Pertussis = 0.83 (0.63–1.09) Rubella = 1.27 (0.93–1.72) Measles = 1.10 (0.84–1.42) Mumps = 1.03 (0.82–1.30) Hib = 0.75 (0.30–0.92) **RR of diabetes by age 10 (P-value) :** Cohort 3 vs. Cohort 1 = 1.01 (p = 0.228) Cohort 2 vs. Cohort 3 = 1.06 (p = 0.545)	Vaccine exposure and diabetes cases based on aggregate data from three cohorts and from population. Interpretation at level of individual is difficult	Weak evidence relevant to causality. Favors no effect of adding Hib to the vaccine schedule
Heijbel et al. (1997)	Cohort	Birth cohorts of Swedish children with and without routine pertussis vaccination for the following years: 1977 (n = 96,057), 1978 (n =	Exposure to diphtheria-tetanus or diphtheria-tetanus-pertussis* determined from yearly vaccination reports from child health centers. Pertussis vaccine exposure based on reported released doses of vaccines	Cumulative incidence of IDDM to age 12 in each birth cohort, determined from national registry	No difference in cumulative IDDM incidence in birth cohorts	Immunization data population-only. Not known if children received other vaccines besides DT or DTP. No statistical analysis	Weak evidence relevant to causality. Favors no effect of pertussis vaccine

		93,248), 1980 (n = 97,064), and 1981 (94,065)					
Blom et al. (1991)	Case-control	Children ages 0–14: 339 cases (newly diagnosed diabetes); 528 controls (matched on age, sex, county) (Sweden)	Vaccination history from local child health care centers, school health units. Vaccines included BCG, smallpox, diphtheria-tetanus-pertussis,* diphtheria-tetanus, tetanus, polio, measles-mumps-rubella, measles, mumps, and rubella.	Diabetes cases identified from national registry	**Odds ratios (95% CI)** BCG = 1.04 (0.77–1.40) Smallpox = 1.07 (0.77–1.49) DTP = 0.94 (0.70–1.28) DT = 0.96 (0.71–1.30) Tetanus = 0.96 (0.70–1.31) Polio = 1.04 (0.17–6.25) MMR = 0.95 (0.71–1.28) Mumps = 1.75 (0.54–5.70) Rubella = 1.24 (0.41–3.73) Measles 0.74 (0.5–1.00) MMR and/or measles -0.69 (0.48–0.98). MMR and/or mumps -0.95 (0.70–1.27). MMR and/or rubella- 0.95 (0.71–1.28)	Small number of cases with diabetes; power calculations for type II were not provided; protective effect of measles difficult to interpret due to multiple comparisons made in analysis	Weak evidence relevant to causality. Favors no effect for most vaccines, beneficial effect for MMR and/or measles vaccine

BCG = Bacille Calmette-Guerin vaccine
CI = confidence interval
DTaP = diphtheria-tetanus-acellular pertussis
DTwP = diphtheria-tetanus-whole-cell pertussis
Hep B = hepatitis B vaccine
Hib = *Haemophilus influenzae b vaccine*
MMR = measles-mumps-rubella vaccine
HMO: health maintenance organization
NS = not significant
OR = odds ratio
RR = relative risk

*These studies do not specify acellular or whole-cell pertussis vaccine. It is assumed that whole-cell pertussis vaccine is being administered to children in these studies.

Controlled Observational Studies

Wellington, New Zealand. Wickens and colleagues (2001) studied p o-tential risk factors for asthma in a case-control study of children age7 to 9 years. Cases were 233 children selected from the Wellington, New Zealand, arm of ISAAC who currently had asthma. Controls were 241 children who never had asthma or asthma-like symptoms. Information on demographics and risk factors for asthma, such as parental smoking history, was collected through interviews with parents. Vaccination histories were gathered from the medical records of study participants. Vaccines to which case and control children were exposed included DTP, DT, hepatitis B, polio, MMR, measles, and BCG.

Odds ratios were individually calculated for the following vaccine categories: DPT, DT or DPT, hepatitis B, polio, measles or MMR, MMR, and BCG. Only a few children were unvaccinated (no DTP = 38; no polio = 25; no MMR = 333), and children in the unvaccinated group may have been misclassified, as some of their vaccination records were missing. In an univariate analysis, the asthma odds ratios for most vaccine exposures were non-significant. For MMR, however, the odds ratio was 1.62 (95% CI 1.06–2.47), though for measles or MMR combined the risk for asthma was not significantly increased (OR = 1.52, 95% CI 0.89–2.58). In a multivariate analysis, the association between asthma and MMR vaccine was nonsignificant (OR = 1.43, 95% CI 0.85–2.41). The authors suggested that the significant univariate association observed between MMR vaccination and asthma was confounded by measles infection and polio vaccination and concluded that there was no association between vaccination and asthma, commenting that the (non-significant) elevated odds ratios could be due to selection bias, exposure misclassification or multiple comparisons.

United States. Hurwitz and Morgenstern (2000) examined the association between vaccination and allergies (as well as allergy-related respiratory symptoms) among children and adolescents, using data collected between 1988 and 1994 for the third National Health and Nutrition Examination Survey. For 13,944 infants, children, and adolescents ages 2 months through 16 years, information was gathered regarding DTP or tetanus vaccination, lifetime history of physician-diagnosed allergy (asthma or hay fever), and allergy symptoms in the past 12 months. Children's parents or guardians answered the survey.

Of the 13,944 subjects, 284 children reported no receipt of DTP or tetanus immunization and were classified as unvaccinated. Subjects with missing data (n = 332) were excluded from the analysis. Adjusted odds ratios were calculated for nine different definitions of allergy related symptoms in the vaccinated and unvaccinated groups. Three closely related outcomes were statistically significant: allergy-related symptoms in the past 12 months (adjusted OR = 1.63, 95% CI 1.05–2.4), any lifetime allergy history or 12-month allergy symptoms (OR = 1.7, 95% CI 1.1–2.6), and nose and eye symptoms (OR = 2.2, 95% CI 1.3–3.7).

Odds ratios for asthma, severe allergic reactions, sinus problems, and wheezing ranged from 1.2 to 2.0 and were not statistically significant. The prevalence of atopy as determined by skin testing with 10 environmental allergens was similar in the two groups (49% and 51%). The authors concluded that DTP and tetanus vaccination increases the risk of clinical allergy but not atopy in children and adolescents. The authors noted several limitations. Subjects excluded from the analysis were more likely to have risk factors related to being unvaccinated than subjects included in the analysis. Excluded subjects were also more likely to have allergies than unvaccinated subjects. These limitations may have biased the estimated effects towards a positive effect. The authors' reanalysis of the data, assuming that the excluded subjects were all unvaccinated, lowered the estimated effects. The limitations reported by the authors included the cross-sectional design, self-reported vaccination status and allergy symptoms, use of proxy respondents, missing data on 2.4% of subjects, small number of unvaccinated subjects (2% of the population), lack of clinical information about the clinical nature of symptoms, recall bias, selection bias for care-seeking behavior, and unmeasured confounding. To this could be added the very limited ability to control for confounders due to the small numbers of subjects in the unvaccinated groups, particularly when analyses were restricted to children over 5 years of age, when the number of unvaccinated children for different outcomes ranged between 1 and 8, and the inclusion of children under two years of age, in whom allergic symptoms are difficult to separate from infectious ones.

United Kingdom. Farooqi and Hopkin (1998) examined the relationship between childhood infections and subsequent allergic disorders, along with other potential risk factors, such as immunizations. Of the 3,062 children born between 1975 and 1984 and seen at a general medical practice in the United Kingdom, 1,934 children (63% of original cohort) who remained registered at the practice and who had complete immunization records were included in the study. Information was obtained from medical records and a regional child health database on vaccines received (i.e., diphtheria-tetanus-whole-cell pertussis/diphtheria-tetanus, polio, and measles) and diagnoses of allergic diseases such as hay fever and asthma.

A higher proportion of children with allergic disease received pertussis vaccine, which showed a statistically significant association with allergy, asthma, and hay fever. The unadjusted odds ratio for DTP immunization (either complete or incomplete course) and allergy was 1.57 (95% CI 1.28–1.96); for asthma, the odds ratio was 1.44 (95% CI 1.17–1.85); and for hay fever, the odds ratio was 1.56 (95% CI 1.21–2.02). A multiple logistic regression analysis showed pertussis immunization statistically associated with allergic disease (OR = 1.76, 95% CI 1.39–2.23), along with maternal atopy (OR = 2.0, 95% CI 1.5–2.7) and antibiotic treatment in the first two years of life (OR = 2.1, 95% CI 1.6–2.6). The authors concluded that whole-cell pertussis vaccination significantly increased the odds of developing asthma, and they noted as well that the

association persisted when children with a history of allergic disease prior to immunization were excluded from the analysis. The authors also noted the possibility of health care-seeking bias in their results, but they found no difference between vaccinated and unvaccinated children in the number of health care visits. However, the removal of 36 percent of potential subjects who were no longer registered at the practice, and their exclusion from the study suggests a potential health care seeking bias in the sampled population. Because of this bias, interpretation of the study's findings warrants caution.

Christchurch, New Zealand. Kemp and others (1997) examined the r elationship between immunization and allergic disease in infants. Data on vaccines received, asthma, and allergic diseases were collected for 1,265 children born in 1977 and participating in a New Zealand health study. Children in this health study were expected to have received DTP and polio vaccines at ages 3 and 5 months and measles vaccine at 12 to 15 months. Information on vaccines and the occurrence of asthma and/or allergic diseases (including rhinitis) at ages up to 5, 10, and 16 years was obtained from parents' medical diaries and physicians' records, plus additional inquiries directed to parents. Of the children in the study, 23 did not receive either DTP or polio vaccine at the scheduled age and were classified as "nonvaccinated."

For children age 0 to 10 years, a greater proportion in the immunized group reported asthma episodes, asthma consults, and allergy consults, compared with children in the nonvaccinated group, for whom there were no reports of asthma or consultations for asthma or allergy. Because no events occurred in the nonvaccinated group, the relative risk was infinite (95% CI 1.03–infinity). For children age 11 to 16 years, among whom 5 allergic and asthma consultations/episodes occurred in the nonvaccinated group, the relative risks for vaccinated children (2.7 to 5.6) were not significant. No association was seen between measles vaccine and risk of asthma episodes in children age 0 to 10 years (OR 1.0, 95% CI 0.9–1.1). Minimal effect of potential confounders was seen when each were considered singly in multivariate models, and the unvaccinated children differed from vaccinated children in ways that should have put them at higher risk for asthma-related symptoms (e.g., lower SES, more parental smoking, more family history of allergy, more pet ownership). The authors concluded that infant immunization for DTP and polio may increase the risk of developing asthma in childhood. But limitations of the study, including the small number of unvaccinated children, the marginal significance of the results (with high sensitivity to a change of even one subject in the unvaccinated group), the potential for a health care utilization bias, and difficulty in adjusting for confounders (due to the small number of unvaccinated children), warrant caution in interpreting these findings.

Controlled Clinical Trials

Sweden. Nilsson and others (1998) studied the development of allergic disease during a randomized controlled trial of pertussis vaccines in Sweden (Gustafsson et al., 1996). A total of 669 children were randomized to one of four exposure groups: 2-component DTaP, 5-component DTaP, DTwP, or DT (control). As part of the recommended immunization schedule in Sweden, children were also given inactivated polio and Hib vaccines two or more weeks later or at the same time. Parent questionnaires, clinical findings, and medical record information provided data on diagnoses of bronchial asthma, atopic dermatitis, allergic rhinoconjunctivitis, urticaria, and food allergy. Children were followed from ages 2 months to 2.5 years.

Cumulative incidence of allergic disease at age 2.5 years in the three pertussis vaccine groups was similar to that in the DT group after adjusting for family history of allergy. Among children whose parents had no history of allergic disease, those who received DTwP were estimated to have an 8% relative reduction in risk (one-sided 95% CI = 28% increase) of allergic disease compared with the control group, which had a predicted risk of 22.5%. The combined DTaP group had an estimated 10% relative increase in risk (one-sided 95% CI = 41% increase). When both parents had a history of allergy, DTwP was associated with a 6% relative reduction in risk (one-sided 95% CI = 18% increase) and DTaP with a 8% relative increase in risk (one-sided 95% CI = 27% increase). With the authors' one-tailed test for increased risk, they calculated that with the size of the sample, they had approximately 80% power to detect a 50% increase in risk, roughly the magnitude reported in some other studies. Differences between the DTwP and DTaP groups were not significant. Interestingly, subsequent asthma was elevated over two-fold ($p = 0.03$) by the development of clinical pertussis, which occurred in 14.5% of unimmunized and 4.4% of immunized children, although the authors raised the possibility that this could partly be due to transient bronchial hyperreactivity after whooping cough.

This study had a nonstandard technique of calculating confidence limits, so the upper limits on the relative risk increase may not be directly compared to those of other studies. Although they used a logistic regression model for analysis, which would ordinarily generate odds ratios (and attendant Is) for the risk of asthma due to various immunizations, they appeared (not described) to use the regression model to estimate absolute risks, and calculate relative risk estimates based on these fitted absolute risks. It is also not clear how upper confidence limits on these relative risks were calculated. In addition, their use of one-sided upper limits for the relative risk would be lower than the more conventional two-sided limits by roughly 4–8 percentage points (e.g., 34% [two-sided] vs. 28% [one sided]). The combination of these two factors makes the upper confidence limits reported in this study somewhat difficult to interpret and to compare to other studies, which used odds ratios. For example, the one-sided upper

limit of a 28% relative risk increase reported by these authors for whole cell vaccine (over a baseline risk of 22.5%) corresponds approximately to a two-sided upper limit of a 50% increase in the odds of asthma.

The authors concluded that there was no evidence supporting an increase in allergic disease after pertussis immunization of the magnitude reported in some other studies, and if there was any increased risk, they thought it was most likely to be associated with the acellular pertussis vaccines. However, limitations included wide confidence intervals restriction to symptom development before 2.5 years of age, the unclear and nonstandard statistical approach, and the focus only on the pertussis component of the immunization schedule. Strengths included the randomized, prospective design and small (<5%) loss to follow-up.

Causality Argument

The committee reviewed five studies that utilized controls (Farooqi and Hopkin, 1998; Hurwitz and Morgenstern, 2000; Kemp et al., 1997; Wickens et al., 2001), including a randomized controlled trial (Nilsson et al., 1998) (see Table 4) and one ecological study (Anderson et al., 2001). Outcomes assessed included allergic symptoms (wheezing) and allergic disorders (hay fever and asthma). All the studies examined exposure to DTaP or DTwP, and other vaccines given concurrently, such as MMR and polio vaccines, but no two studies examined exactly the same exposure.

While many of these studies reported elevated odds ratios linking immunizations to some allergic outcome, some of which were statistically significant, methodological weaknesses within individual studies, as well as the pattern of results across studies diminish the confidence that the observed associations reflect causal relationships. In the two studies that reported a significant positive effect of DTP or tetanus immunization or the pertussis component of DTwP (Farooqi and Hopkin, 1998; Hurwitz and Morgenstern, 2000), potential sampling bias, caused by substantial losses to follow-up or restriction to subjects with regular medical care, could have distorted the relationship between immunization and allergies.

A problem in most of the studies was that the number of unvaccinated children was small, limiting the ability to control for potentially confounding factors, which are numerous and strong for the outcomes of asthma and atopy, and particularly complex when considering risk over an entire childhood. Adequate control of confounding is a serious issue for observational designs, particularly in this domain, as nonimmunized children typically differ on baseline characteristics from immunized children in ways that are not always measurable. Control can be compromised by imperfect and possibly biased confounder and outcome measurement introduced by retrospective, unblinded review of records or parental report of outcomes or exposures that occurred in the past.

TABLE 4 Evidence Table: Controlled Epidemiological Studies—Vaccines and Allergic Disorders

Citation	Design	Population	Vaccines	Outcome Measures	Results	Comment	Contribution to Causality Argument
Wickens et al. (2001)	Case-control	233 cases with wheezing or asthma; 241 controls; ages 7–9 years (New Zealand)	DTP,* DT, polio, MMR, measles, BCG, HepB from medical records	Wheezing or asthma in last 12 months by proxy or self-report	**Unadjusted ORs (95% CI):** DTP = 1.51 (0.77–2.97) DT/DTP = 1.43 (0.69–2.96) HepB = 0.72 (0.50–1.06) Polio = 2.11 (0.90–4.90) Measles/MMR = 1.52 (0.89–2.58) MMR = 1.62 (1.06–2.47) BCG = 1.23 (0.41–3.72) **Adjusted ORs (95% CI)** HepB = 0.66 (0.42–1.05) Polio = 2.48 (0.83–7.41) MMR = 1.43 (0.85–2.41)	Potential selection bias, exposure misclassification, or multiple comparisons	weak evidence relevant to causality; favors no effect of any vaccine
Hurwitz & Morgenstern (2000)	cross-sectional survey (NHANES III)	13,944 infants and children ages 2 months through 16 years (United States)	DTP* or tetanus, by proxy (information obtained from children's parent or guardian)	history of physician-diagnosed asthma, hay fever; self-report of allergic reactions by proxy; atopy by skin testing with 10 allergens	**Estimated Crude ORs (95% CI)/Adjusted ORs (95% CI) of DTP or tetanus vaccination on following** Asthma = 2.20 (0.70–6.84)/ 2.00 (0.59–6.74) Hay fever = 1.21 (0.21–6.83)/ 0.82 (0.16–4.35) Severe allergic reaction = 2.11 (9.42–10.45)/ 1.50 (0.33–6.89) Any allergy/allergic reaction = 2.11 (0.81–5.49)/ 1.66 (0.67–4.14) Sinusitis/sinus problems = 2.16 (0.77–6.06)/ 1.81 (0.69–4.71) Wheezing/whistling = 1.03 (0.68–1.57)/ 1.23 (0.78–1.95) Nose & eye symptoms = 2.44	Study limitations included the following: cross-sectional design, recall bias; missing data on 2.4% of unvaccinated subjects; small number of unvaccinated children; lack of clinical information; selection bias for care-seeking behavior; unmeasured confounding; limited ability to control for con-	weak evidence relevant to causality; favors effect of DTP or tetanus vaccine on clinical history of allergic disorder but no effect on atopy defined by skin test reactivity

Citation	Design	Population	Vaccines	Outcome Measures	Results	Comment	Contribution to Causality Argument
					(1.57–3.78)/ 2.22 (1.30–3.77) Any allergy-related respiratory symptom (past 12 mos) = 1.68 (1.09–2.59)/1.63 (1.05–2.54) Any lifetime allergy history/12 mo. Symptoms = 1.79 (1.16–2.76)/1.69 (1.10–2.59)	founders	
Farooqi & Hopkin (1998)	Cohort	1934 patients born in 1975–1984 (United Kingdom)	DTwP/DT, polio, measles immunization from regional Child Health database	Clinical diagnosis of allergic disorder (eczema, hay fever, or asthma).	**Unadjusted OR for DTwP immunization (either complete or incomplete course) (95%CI)** Allergy = 1.57 (1.28–1.95); Asthma = 1.44 (1.17–1.85), Hay fever = 1.56 (1.21–2.02). **Multiple logistic regression: pertussis immunization** Allergic disorders = 1.76 (1.39–2.23)	Potential health care-seeking bias; exclusion of 36% of potential subjects.	Weak evidence relevant to causality. Favors effect of pertussis component of DTwP
Nilsson et al. (1998)	Randomized controlled trial	669 children participating in pertussis vaccine trial (Sweden)	Randomization to DTaP (2- or 5-component), DTwP, and DT given at 2 months. All children were given Hib and inactivated polio virus. Children followed for 2.5 years	Diagnosis based on questionaires, clinical findings, and information on medical records for bronchial asthma, atopic dermatitis, allergic rhinitis, urticaria, and food	**Estimated increase in risk:** No parental history of allergic disease (one-sided 95% CI): DTwP = 8% reduction (28% increase) DTaP = 10% increase (41% increase) Both parents had history of allergic disease DTwP = 6% reduction (18% increase) DTaP = 8% increase (27% increase)	Differences non-significant; one-tailed analysis; wide confidence intervals; restriction to development before 2.5 years of age, and focus only on pertussis component of immunization schedule.	Weak evidence relevant to causality. Favors no effect of DTaP

Citation	Design	Population	Vaccines	Outcome Measures	Results	Comment	Contribution to Causality Argument
				allergy			
Kemp et al. (1997)	cohort	1,265 children born in 1977; followed through age 16 years (New Zealand)	DTP,* polio, measles by proxy	asthma, other allergic diseases by diary or questionnaire by proxy, and by medical records	**Risk ratios** for asthma episodes, asthma consults, and allergy consults for immunized children ages 0–10 years were infinite (95% CI 1.03–infinity) because no events occurred in non-immunized group; **Risk ratios** (95% CI), age 0.16 yrs; Asthma episodes: 2.9 (0.8–23.6) Asthma consults: 2.7 (0.7–22.3) Allergy consults: 5.6 (1.0–222.6) **Risk ratios** for asthma episodes age 0–10 and measles vaccination: 1.0 (0.9–1.1)	weaknesses in study design include small number of unvaccinated children, marginal significance of results, potential for health care utilization bias; difficulty in adjusting for confounders (due to small number of unvaccinated)	weak evidence relevant to causality; favors no effect of any vaccine.

BCG = Bacille Calmette-Guerin vaccine
DTaP = diphtheria-tetanus-acellular pertussis
DTwP = diphtheria-tetanus-whole-cell pertussis
Hep B = hepatitis B vaccine
Hib = *Haemophilus influenzae b vaccine*
MMR = measles-mumps-rubella vaccine
HMO: health maintenance organization
NS = not significant
OR = odds ratio
RR = relative risk
CI = confidence interval

*These studies do not specify acellular or whole-cell pertussis vaccine. It is assumed that whole-cell pertussis vaccine is being administered to children in these studies.

Finally, the findings of the studies, taken as a whole, did not show a consistency of findings that would outweigh the concerns about individual studies. While some studies pointed to the pertussis vaccine as a risk factor for allergic syndromes with no effect of MMR, another found that MMR vaccine was the strongest risk factor. The ecological study indicated a protective DPT effect, and the only randomized study indicated minimal or no effect of pertussis vaccines, with a non-significant reduction in risk from the whole-cell vaccine.

Given the design weaknesses in the observational studies, with effect sizes and modest degrees of statistical significance that are not robust to probable biases, and a randomized trial study that does not support the risk factor most frequently implicated in the observational studies, **the committee concludes that the epidemiological and clinical evidence is inadequate to accept or reject a causal relationship between multiple immunization and an increased risk of allergic disease, particularly asthma.**

Biological Mechanisms

Although biological data do not provide an independent basis for evaluating causality they can help validate epidemiologically based conclusions for or against causal associations; such data can also guide further investigation when epidemiological evidence is inconclusive.

This section discusses evidence regarding immune system responses and mechanisms by which multiple immunizations might be related to autoimmunity, allergy, or risk for infection. The mechanisms considered represent two possible pathways to adverse outcomes: stimulation of harmful immune responses, or suppression of beneficial immune responses. The stimulation of harmful immune responses is discussed in terms of the mechanisms of molecular mimicry, bystander activation, and nonspecific or polyclonal T-cell activation. The possible suppression of beneficial immune responses is addressed in terms of the hygiene hypothesis and the prevention of potentially protective infections through immunization. The discussion includes consideration not only of exposure to vaccine antigens but also of exposure to vaccine adjuvants and of injection as the principal route.

Issues related to autoimmunity are considered first, in an extensive discussion beginning with autoimmune injury related to wild-type infection. This relationship is important to understand because vaccines are intended to act as surrogates for wild-type infection and so might be expected to pose similar risks for harmful autoimmune responses. Inherent in the hypothesis that multiple vaccines predispose to autoimmunity or allergy is an effect, in genetically predisposed individuals, of the vaccines on the developing immune system that somehow increases the risk of such immune disorders at some later time, from childhood to adulthood. Thus, the effect would not be detectably associated in time with the receipt of the immunizations. Allergy is considered next, with the primary focus

being the mechanisms by which vaccine exposure might affect susceptibility (see Figure 3). The discussions in both cases considers the possibility of either stimulation of harmful responses or suppression of beneficial ones.

Multiple Immunizations and Autoimmunity

Proposed Mechanisms for Induction of Autoimmunity Through Infection

Infection can induce immune-mediated tissue injury. In most cases, this injury is short-lived and resolves as the immune system eliminates active infection. The injury is a consequence of the immune response to the foreign invader, and when the invader is eliminated, the damaging immune process ceases. In some diseases, however, infection appears to induce an injurious immune response directed, at least in part, against self antigens. Nevertheless, true autoimmune injury must be distinguished from immune-mediated injury resulting from persistent but undetected infection. If the infectious agent was not detected, ongoing immune-mediated responses to that agent and the resulting injury of host tissues could be interpreted as autoimmunity, when in fact the immune response was directed against the foreign microbe and not against self.

Two major mechanisms—molecular mimicry and bystander activation—are proposed to account for the activation of self-reactive T and B cells and the induction of autoimmunity by infection (Albert and Inman, 1999; Bach and Chatenoud, 2001; Benoist and Mathis, 2001; Davidson and Diamond, 2001; Marrack et al., 2001; Regner and Lambert, 2001; Rose, 2001; Singh, 2000; Wucherpfennig, 2001; Zinkernagel, 2001).

Also described below is the possible link between infection and autoimmunity through the mechanism of nonspecific or polyclonal T cell activation. The evidence regarding the possibility that these mechanisms actually contribute to autoimmune diseases is discussed in a later section.

Molecular mimicry. An antigenic epitope from a microbe that is structurally similar to (mimics) an epitope of a self-molecule has the potential to trigger the activation of self-reactive, naïve T or B lymphocytes. Once activated, self-reactive T cells could expand in number and mature into effector (memory) T cells that have a lower threshold for activation by self antigens. These cells would also gain the ability to migrate to specific tissues, produce additional mediators/cytokines, and to mediate injury on contact with cross-reacting self antigens. In addition, they would gain the potential to help B cells that are responding either to the same antigen as the T cells or to other self-antigens that are physically linked to it.

Bystander activation. Bystander activation results when an infection creates environmental conditions that allow the activation of self-reactive T and B

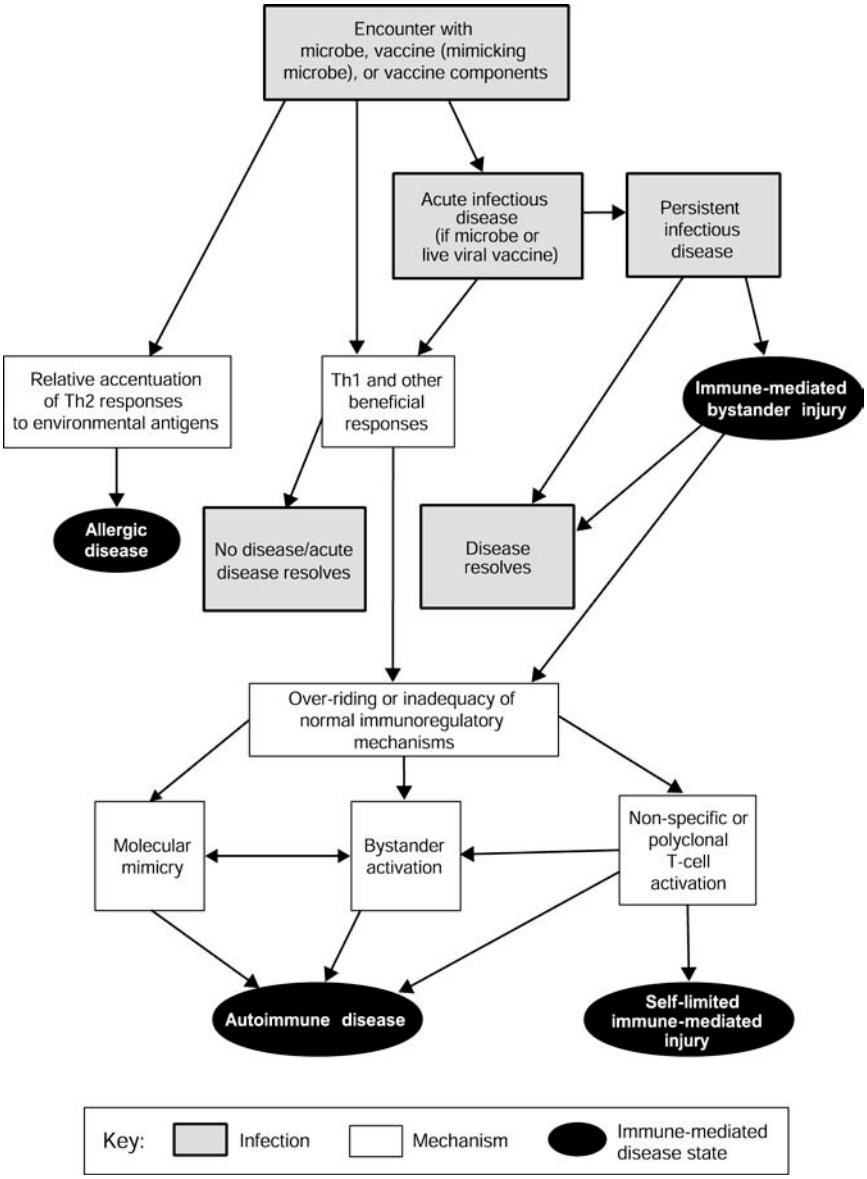


FIGURE 3 Theoretical Basis for Immunization-induced Immune Dysfunction

cells that are normally held in check. It does not require that antigens of the infectious agent be structurally similar to self-antigens. Bystander activation may be mediated in part by infection-induced death of host cells, which results in the release of greater amounts of self peptides or in the generation of novel self-peptides (i.e., novel or cryptic epitopes not normally found in the absence of the infection). As part of this process, molecules derived from the microbes (and perhaps also from the necrotic host cells—e.g., heat-shock proteins) would stimulate other components of the immune system.

In particular, antigen presenting cells (i.e., dendritic cells) gain an increased abundance of self peptide/MHC complexes and costimulatory molecules (e.g., B7 molecules) and produce cytokines, including interleukin 12 (IL-12) and tumor necrosis factor (TNF). Collectively, these changes can allow the activation of naïve self-reactive T cells that are induced to differentiate into Th1 effector T cells, which play a central role in most forms of human autoimmunity (reviewed in Bach and Chatenoud, 2001; Marrack et al., 2001; Martin et al., 2001; Parham, 2000; Robles and Eisenbarth, 2001; Singh, 2000; Wills-Karp et al., 2001). In turn, these self-reactive Th1 T cells would be available to provide help to self-reactive B cells, responding either to the same antigen as the T cells or to other antigens that are physically linked to them.

Nonspecific or polyclonal T cell activation. It is also possible that infections could activate a variety of T or B lymphocytes that would otherwise respond only to certain antigens. This non-antigen-specific response is referred to as a polyclonal or oligoclonal response. For example, toxins produced by *Streptococcus pyogenes* and *Staphylococcus aureus* can act as superantigens, binding both to T cell receptors and to MHC molecules and activating a substantial fraction (greater than 5 percent) of the total T cells of an individual. This overactivation of the immune system leads to the acute "toxic-shock" syndromes associated with the toxin-producing strains of these bacteria. Another example is polyclonal B cell activation following Epstein-Barr virus (EBV) infection and capable of enhancing autoimmune reactions. These nonspecific immune responses are usually self-limited, however, and resolve as the infection is cleared.

If self-reactive T cells are activated by a nonspecific immune response, they can induce autoimmunity. Superantigen-induced activation normally ends in the programmed death (apoptosis) of the activated cells, terminating the response. However, other microbial products, like endotoxin, that trigger the innate immune response can enhance the survival of superantigen-activated T cells (Vella et al., 1995, 1997), and could—in the context of genetic differences in mechanisms controlling the death of activated T cells—prolong the survival of self-reactive T cells, in theory allowing them to mediate self-injury.

Examples of Molecular Mimicry and Bystander Activation in Autoimmunity

The processes of molecular mimicry and bystander activation are not mutually exclusive. These mechanisms may act in synergy or at different stages in the initiation and progression of autoimmunity. Mimicry can be thought of as an initiator, because it triggers the initial activation of cross-reactive T cells, which then replicate and differentiate into effector T cells with a lower threshold for activation by self-antigens. The bystander activation of antigen presenting cells facilitates further replication of these T cells and their differentiation into Th1 T cells and also facilitates their recruitment to the target tissue.

Bystander effects are almost certainly essential for molecular mimicry to succeed in overcoming normal regulatory mechanisms (see Figure 3). Through a process referred to as epitope spreading, the initial activation by an infection of cross-reactive T cells that react to a single self-antigen may play a critical role in facilitating, in a bystander-like manner, the activation of T cells that recognize other self antigens. Thus, even after the infection has resolved and cross-reactive T cells are no longer stimulated by foreign antigens, the autoimmune process would be perpetuated and amplified by self-reactive T cells. These mechanisms have been delineated in animal models, which provide illustrative examples:

Herpes simplex-induced keratitis. In some strains of mice, acute herpes simplex virus infection of the cornea is followed by the development of autoimmune corneal injury (keratitis) (Benoist and Mathis, 2001; Panoutsakaopoulou et al., 2001; Zhao et al., 1998). Genetic factors, including MHC type, account for the differences in susceptibility between strains of mice. In susceptible mice, the development of keratitis depends on the presence of a specific viral antigen, which triggers the generation of T cells that are cross-reactive with a self-protein. These activated, cross-reactive T cells migrate via the blood back to the cornea, where they induce the keratitis.

Infection at sites other than the cornea does not induce the disease, even though cross-reactive T cells are generated. This most likely reflects the need for multiple non-antigen-specific bystander effects of the viral infection. For example, injury to the cornea releases cross-reactive self-peptides that help amplify the number of self-reactive T cells that mature into injurious Th1 effector T cells. The injury to the cornea also triggers the production of inflammatory cytokines that recruit antigen presenting cells and T cells to the infected eye, where the T cells are activated and produce keratitis. (In humans, a similar bystander process likely accounts for trauma-induced sympathetic ophthalmia, in which injury of one eye leads to an autoimmune attack against both the injured and uninjured eye (Chan and Mochizuki, 1999).

Consistent with an important role for non-antigen-specific or bystander effects in herpes simplex-induced keratitis is the ability to induce the disease with mutant strains of herpes simplex virus that lack the cross-reactive epitope. But keratitis can be induced by these mutant strains only if the numbers of

potentially self-reactive T cells have been artificially increased. Simply increasing the number of T cells is not sufficient, however; local infection or injury to the eye is necessary to initiate the disease process.

Experimental allergic encephalomyelitis. Experimental allergic e ncephalomyelitis (EAE) is a demyelinating disorder induced in rodents by immunization with myelin extracts, proteins, or peptides found in myelin (Goverman et al., 1997; Zamvil and Steinman, 1990).[6] Although EAE is one of two commonly used models of MS, it may more accurately model post-infectious acute disseminated encephalomyelitis (ADEM). As with most autoimmune disorders, only some strains of mice are susceptible to EAE, and susceptibility is determined in part by the MHC type. Molecular mimicry is not involved in EAE, since the auto-antigen is the inducing agent.

To induce this disorder, mice are immunized with myelin protein or peptides in a potent adjuvant. The adjuvant must not only increase the persistence of the antigen, but must stimulate antigen-presenting cells to more effectively present these peptides and to produce cytokines, including IL-12, that induce the formation of Th1 T cells that in turn can migrate to the central nervous system and produce injury.

The most commonly used adjuvant in animal studies is complete Freund's adjuvant, which contains dead mycobacteria in a water-in-oil emulsion. Heat-killed whole *Bordetella pertussis* or pertussis toxin can also be use as an adjuvant in this model (Blankenhorn et al., 2000; Goverman et al., 1997; Jee and Matsumoto, 2001; Zamvil and Steinman, 1990). It is the presence of dead mycobacteria that makes Freund's adjuvant effective. Multiple components of the mycobacteria (including those of their membranes and DNA) trigger the innate immune response via Toll-like receptors (Akira et al., 2001; Medzhitov and Janeway, 2000), causing the maturation of antigen presenting cells and the production of IL-12 and other potent cytokines. The inclusion in the initial immunization not only of complete Freund's adjuvant but of biologically active pertussis toxin is common and increases the frequency of clinical disease. The pertussis toxin probably acts as adjuvant and enhances the ability of the T cells to migrate into the central nervous system (Goverman et al., 1997).

Once the demyelinating process has been initiated, exacerbations can be induced by bystander mechanisms alone with the administration of IL-12 or microbial agonists (endotoxin, dsRNA [double-stranded RNA], and bacterial DNA) that stimulate the production of IL-12 and activate other aspects of the innate immune response (Constantinescu et al., 1998; Goverman et al., 1997; Segal et al., 1997).

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[6] For historical reasons this process is called allergic rather than autoimmune encephalomyelitis. But the injury in this disease is mediated by Th1 T cells, not Th2 T cells and IgE antibodies, which are the mediators of allergic diseases.

Human Inflammatory and Autoimmune Conditions Induced by Infection

Various specific infections are associated with the development of self-injury. For many of these infections, there is considerable evidence that the injury is caused, at least in part, by autoimmunity and is not simply a consequence of the immune response to the infectious agent. For example, group A streptococcal infection in humans is clearly linked to the induction of rheumatic fever. Inflammation also occurs in the skin and joints, but the major disability results from injury to the heart or, in the case of Syndenham's chorea and perhaps pediatric autoimmune neuropsychiatric disorders (PANDAS), to the brain (Marrack et al., 2001; Perlmutter et al., 1998; Wucherpfennig, 2001). Human (and mouse) antibodies to certain streptococcal M proteins cross-react with cardiac myosin and can injure cardiac muscle cells in culture, suggesting that molecular mimicry is a factor. However, it is not certain that these antibodies are the cause of the clinical damage observed following infection, nor is there clear evidence that infection leads to the generation of antibodies or T cells that are truly self-reactive rather than cross-reactive. That is, there is no clear evidence of epitope spreading and autoimmunity that persists after infection is eradicated.

Other examples in humans of infection-induced acute inflammatory injury that appears to be autoimmune in nature include Guillain-Barré syndrome, ADEM, reactive arthritis, and herpes simplex keratitis (IOM, 1991, 1994; Marrack et al., 2001; Stuve and Zamvil, 1999; Wucherpfennig, 2001). Guillain-Barré syndrome is most strongly associated with *Campylobacter jejuni* infection, which may account for up to 30 percent of cases (McCarthy and Giesecke, 2001). It has also been associated with a variety of other, mostly viral, infections. ADEM has been associated with measles and mumps virus infections (IOM, 1994). In these conditions, the injurious immune and inflammatory response resolves over time once the infection is eradicated. The damage produced during the period of active infection may, however, result in death or persistent disability, and injury may be re-induced by recurrent infection with the inciting agent. Nonetheless, the resolution of active immune-mediated injury suggests that the loss of self-tolerance is not permanent, and the precise contribution of the various mechanisms cited remains uncertain.

Arguably the strongest evidence for true persistent autoimmunity induced by a specific infectious agent in humans is chronic Lyme disease arthritis. In this disease, there is clear evidence of genetic predisposition (linked to HLA type). A putative molecular mimic has been identified, with bacterial OspA (outer surface protein A) as a mimic of human LFA-1 (lymphocyte function-associated antigen). The disease persists in the absence of any evidence of persistent infection, and cross-reactive T cells are present in increased numbers in the joints of these patients (reviewed in Benoist and Mathis, 2001; Wucherpfennig, 2001).

As noted above, there is also strong evidence for a causal association between congenital rubella infection and type 1a diabetes: approximately 20

percent of individuals with congenital rubella develop diabetes by adulthood (Robles and Eisenbarth, 2001). The pathogenesis of diabetes in these cases shares many features with typical type 1a diabetes. The similar preponderance of HLA class II alleles and the presence of T cells reactive to GAD65 (glutamic acid decarboxylase) peptides (Ou et al., 1999; Robles and Eisenbarth, 2001) suggests that the disease results, at least in part, from congenital rubella-induced autoimmunity. By contrast, there is no evidence for a similar link between acquired rubella infection after birth and type 1 diabetes.

With the exception of chronic Lyme arthritis and congenital rubella-induced diabetes, the role of infection and of specific infectious agents in the induction or exacerbation of the major chronic autoimmune diseases in humans— including type 1a diabetes, MS, systemic lupus erythematosus, and rheumatoid arthritis—is uncertain. A number of associations have been made (see Table 5), but findings across studies have not been consistent and experimental evidence to support a causal link is currently lacking (Benoist and Mathis, 2001; Marrack et al., 2001; Wucherpfennig, 2001). It is worth noting, however, that of the possible mechanisms by which infection might contribute to autoimmunity, only molecular mimicry requires a specific association between an infectious agent and autoimmunity. The evidence that more than one infectious agent can trigger the onset of reactive arthritis and demyelinating neurological diseases is consistent with the notion that bystander effects may be a common feature of infection-induced autoimmune injury. Also, the evidence that a given T cell may be able to recognize multiple peptide-MHC complexes (Marrack et al. 2001; Wucherpfennig, 2001) means it is clearly possible that the proliferation of T cells that are cross-reactive with self-antigens might be induced by more than one infectious agent.

Vaccine-Preventable Diseases and the Possibility of Vaccine-Induced Autoimmunity

The discussion above provided examples and a mechanistic framework from which to consider infection-induced immunological injury and autoimmunity. If the vaccine acts as a surrogate for the infectious disease it is designed to prevent, it might trigger the same type of immune-mediated injury as the infection itself. In previous reviews by the IOM and others (Chen, RT et al., 2001; IOM, 1991, 1994), the plausibility of this notion, and the evidence for or against causation in response to individual vaccines, were reviewed. A causal relationship between a vaccine and an autoimmune disorder was found for MMR and thrombocytopenia, OPV and Guillain-Barré Syndrome (GBS), and tetanus-containing vaccines and GBS. In addition, some types of influenza vaccine are associated with GBS and rubella with arthritis. Vaccinia is associated with acute disseminated encephalomyelitis. None of these findings, however, assessed the risk of autoimmune disease secondary to a “skewing” of immune response

TABLE 5 Putative Examples of Molecular Mimicry in Human Autoimmune Disorders with Proposed Cross-Reactive Autoantigens and Infection-Derived Antigens

Disease	**Organ/ Autoantigen**	**Infection/ Antigen**	**Cross reaction on:**
Lyme arthritis	Joints/LFA-1	*Borrelia burgdorferi*/ Osp A	T cells
Rheumatoid arthritis	Joints/Hsp60	Mycobacteria/ Hsp65	T cells
Multiple sclerosis	Brain/Myelin basic protein	Papillomavirus/ L2	T cells
Type I diabetes	Pancreatic ß-cells/GAD	Coxsackie B/ P2-C	T cells
Stiff man syndrome	GABA-ergic neurons/GAD	hCMV/DNA binding protein	T cells
Primary biliary cirrhosis	Bile duct/pyrdeH complex	*E.coli*/PDC-E2	T cells
Rheumatoid arthritis	Joints/DRB1*04 01	*E.coli*/DNAJ/ EBV/gp110	T cells/Antibody
Multiple sclerosis	Brain/Myelin basic protein	EBV/capsid	T cells/Antibody
Myocarditis	Heart/myosin	Chlamydia/60kD protein	T cells/Antibody
Rheumatic fever	Heart/cardiac myosin/heart valves/kidney/ CNS	Streptococci/ M protein b	Antibody
Chagas disease	Heart/ß-1 adren-ergic receptor	*Trypanosoma cruzi*/ribosomal protein	Antibody
Myasthenia gravis	Muscle/ acetylcholine receptor	Herpes simplex/gpD	Antibody
Guillain-Barré syndrome	Peripheral nerve gangliosides	*Campylobacter jejuni* LPS	Antibody

Source: Adapted from Marrack et al., 2001; Wucherpfennig, 2001

caused by multiple immunizations early in life. These studies do provide epidemiological evidence for a causal link between certain vaccines and immune-mediated injury and disease. However, in several cases in which the infection itself is known to trigger immune-mediated diseases (examples are measles, mumps, rubella, *Borrelia burgdorferi* infections), a causal link between immunization against the infection and the disease has not been established.

Assessment of Biological Mechanisms by Which Multiple Immunizations Might Contribute to Autoimmunity

As discussed previously, the total number of different vaccines and vaccine doses administered to children in the first years of life has increased substantially over the past 20 years in the United States. However, the number of individual proteins or polysaccharide antigens administered has declined from a peak of more than 3,000 in the early 1990s to about 130 with the current immunization schedule. By comparison, a single bacterial infection (e.g., *H. influenzae* or group A streptococcus) results in exposure to many more antigens, of which 50 or more induce B or T cell responses (Cunningham, 2000; Halsey, 2001). Thus, the number of antigens in current vaccines resembles the number to which the immune system responds in the physiological context of an infection. Nonetheless, the overall content and context of vaccine administration has changed because of changes in the number of vaccines administered, their timing, the nature and formulation of the vaccines, and the main vaccination route.

The current vaccine formulations and immunization schedule have been shown to induce protection or concentrations of antibody responses sufficient to mediate protection (Blackwelder, 1995; Halsey, 2001). In some cases, achieving protective efficacy has required modification of the amounts of individual components in multivalent or combination vaccines, such as the trivalent polio virus vaccines and the MMR vaccine. The need for such modifications is consistent with the notion that prior or simultaneous infection, or immunization with multiple vaccines or antigens, can influence the magnitude and quality of the immune response to individual antigens (Chen, HD et al., 2001; Gomez et al., 1997; Insel, 1995; IOM, 2001a; Selin et al., 1998, 1999; Vekemans et al., 2001).

That factors related to infection and immunization can affect immune responses raises the theoretical possibility that changes to vaccine formulations or the vaccine schedule might also alter the potential for vaccines to induce, facilitate, or amplify immune-mediated injury or autoimmunity. Theoretically, concomitant or sequential administration of multiple vaccines could have several possible effects, alone or in combination. Molecular mimicry might be enhanced through an additive or synergistic mechanism in which more than one vaccine induces cross-reactive T cells. Another possibility might be the generation of cryptic or novel epitopes that are not found when vaccines are given separately. In addition, the nature or magnitude of bystander effects might be altered.

Molecular mimicry. Molecular mimicry appears to be a mechanism by which Lyme disease causes rheumatoid arthritis. This constitutes evidence for a biological mechanism by which the OspA Lyme vaccine (which is not recommended for routine use, seen in Figure 2) could possibly induce immune-mediated injury. It remains theoretically possible that molecular mimicry occurs in response to other vaccines but that the extent of the immune response induced has been insufficient to induce clinically evident disease. If this were the case, the addition of new vaccines that also induced cross-reactive immune responses might initiate injury and epitope spreading sufficient to result in clinically manifest autoimmunity.

The following example illustrates this hypothetical possibility. In post-infectious encephalitis, a rare but known complication of measles virus infection, the injury appears to be mediated by infection-induced expansion and entry into the nervous system of T cells directed against myelin basic protein (also a target antigen in MS) (Johnson, 1987; Liebert, 1997). Whether this reflects a cross-reactive immune response induced by measles virus or a bystander effect, or both, is uncertain. If measles vaccine (or another vaccine) weakly stimulated T cells cross-reactive with myelin basic protein and were co-inoculated with a second vaccine that also weakly stimulated such cross-reactive T cells, clinically apparent autoimmunity might result from their combined effects, even though it might not result from use of either vaccine alone.

A related theoretical mechanism is altered molecular mimicry. Ingestion of high amounts of inorganic mercury or methylmercury can induce an autoimmune disease in genetically susceptible strains of mice and rats. This effect appears to reflect the modification by mercury of self-proteins—laminin in the rat and fibrallarin in the mouse (Hultman and Nielsen, 2001; Pollard et al., 2000). These modified self-molecules are perceived as foreign by the host, inducing a T and B cell response that may spread to include a response to the native (unmodified) self-protein. At very high concentrations of mercury a polyclonal B cell response is induced, but the basis for this is not known. Mercury-induced immune injury (hypersensitivity) has also been described in some humans, but it is infrequent and the mechanisms are less clearly defined than in the rodent models (Enestrom and Hultman, 1995; Griem and Gleichman, 1995; Pollard and Hultman, 1997).

It is theoretically possible that administration of ethyl mercury-containing vaccines along with vaccines not previously given at the same time (and in the same extremity/site) could result in a mercury-induced modification of a constituent of the coadministered vaccine, creating an antigenic epitope capable of cross-reaction with self epitopes that might activate T cells cross-reactive to self proteins. No experimental evidence is available to support this effect, however. Moreover, the concentrations of mercury required to induce autoimmunity in rodents (blood levels of mercury commonly >100 μg/ml) (Hultman and Nielsen, 2001) are markedly greater than those theoretically achievable through infant

immunization, even before the mercury-containing preservative thimerosal was removed from routine childhood vaccines (Ball, 2001).

In the absence of experimental or human evidence regarding molecular mimicry or mercury-induced modification of any vaccine component to create an antigenic epitope capable of cross-reaction with self epitopes as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that these mechanisms are only theoretical.

Bystander effects. Pathogenic microbes causing vaccine-preventable human diseases are acquired at mucosal surfaces, where they sufficiently evade the innate immunity of local mucosa so that they can replicate, locally or at other sites, before antigen-specific immune responses are induced. Most vaccines, however, are administered by injection. Vaccines are also complex biological products that may contain, in addition to the immunogenic microbial components, alum, stabilizers, small amounts of components carried forward from the preparation of the microbial antigens (e.g., bovine serum, egg or human cellular constituents), and, until recently, the preservative thimerosal. Theoretically, the mechanism of acquisition of vaccine antigens—by injection rather than the mucosal route—or the presence of alum or microbial constituents with adjuvant properties, could alter the quality or magnitude of the immune response compared with a wild-type infection and thereby favor the development of an autoimmune response.

Injection versus mucosal exposure. In principle, bypassing the mucosal surfaces through injection eliminates the mucosal immune response and induces solely systemic immunity. The most obvious consequence is that mucosal antibody responses leading to the production of secretory IgA are absent or less robust following vaccine injection than with wild-type infection (reviewed in Nagler-Anderson, 2001; Neutra et al., 2001). Also in principle, the circulation pattern of the memory T and B lymphocytes induced by an injected vaccine may also differ, as they would be unlikely to home to mucosal-associated lymphoid tissues. This effect does not appear to be critical to protection from disease for which vaccines are currently in use. It may, however, limit the extent to which mucosal colonization and transmission of pathogens are reduced, particularly for polio virus, which is acquired via the intestinal mucosa.

Shifting the primary site of antigen delivery from a mucosal surface to an extremity could, in theory, bypass immunoregulatory mechanisms, such as the production of immunoregulatory cytokines (transforming growth factor-β [TGF-β] or IL-10) and local regulatory T cells, that might modulate or attenuate the immune response and thereby impede the activation of potentially self-reactive cells capable of inducing autoimmunity (Maloy and Powrie, 2001; Neutra et al., 2001; Wills-Karp et al., 2001). There is, however, no experimental evidence that this occurs for current vaccines. Vaccines, in contrast to the infections they are designed to prevent, are instead a less potent stimulus of the immune response.

(The immune response to tetanus and diphtheria toxins presented by their vaccines is an exception. Tetanus and diphtheria are diseases of toxin production rather than invasive infection; such minute amounts of toxin are produced during infection that the immune response is not effectively stimulated.) Thus the bystander effects of vaccines on the systemic immune responses, which in theory might induce autoimmunity, are less robust than those resulting from wild-type infection.

Coadministration of vaccines. Another theoretical possibility is that c o-administration of multiple vaccines (particularly if given in the same extremity so that they would drain to the same regional lymph nodes) might produce an additive bystander effect, augmenting the magnitude or altering the quality of the response to the individual vaccine components. Such an effect is consistent with the findings by Ota et al. that BCG, a live mycobacterial vaccine, augmented the magnitude of the T cell response to other coadministered vaccines (Ota et al., 2002). This effect could also potentially increase the risk for activation of self-reactive T cells (Marchant et al., 1999; Vekemans et al., 2001). However, although BCG induced a potent Th1-type response to mycobacterial antigens, it promoted the production of both Th1- and Th2-type cytokines in response to unrelated vaccines. Thus, BCG is likely to impact immune response to unrelated antigens in early life through its influence on the maturation of dendritic cells, and not by shifting Th2 responses towards Th1 (Ota et al., 2002).

A reciprocal inhibitory effect of BCG vaccine on Th2 responses and allergic disease has been proposed on the basis of animal studies and ecological studies in humans (Aaby et al., 2000; Erb et al., 1998; Herz et al., 1998; Shirakawa et al., 1997), but the data regarding this effect in humans are conflicting (Alm et al., 1997; Gruber et al., 2001a; Strachan, 2000; Wills-Karp et al., 2001). In human infants in The Gambia, BCG induced a strong Th1 T cell response to mycobacterial antigens (Marchant et al., 1999; Vekemans et al., 2001) and enhanced the magnitude of the response to coadministered vaccine antigens (e.g., hepatitis B, tetanus toxoid), but the Th1–Th2 balance of the T cell response to these coadministered vaccines was not affected (Ota et al., 2002). Similarly, prior BCG immunization showed no effect on the development of IgE antibodies (an index of Th2 dependent responses) to tetanus and diphtheria toxoids following subsequent immunization with DT or DTP (Gruber et al., 2001b). Other vaccines that induce IFN-_ responses in human infants to the homologous disease antigens (i.e., live viral vaccines) (Arvin et al., 2001; Gans et al., 2001) are theoretically capable of enhancing a bystander Th1 response to heterologous antigens. The potency of these vaccines is almost certainly less than that of BCG, suggesting that if such an effect were to occur, it would likely be minor.

Th1 versus Th2 response. Autoimmunity is generally associated with Th1 responses, whereas allergy is associated with Th2 responses. DTwP vaccine includes whole formalin-inactivated *B. pertussis* cells, which contain endotoxin and bacterial cell membrane structures that activate the innate immune system

and favor a Th1 response and production of IgG1 antibodies (but not IgG4 antibodies). The vaccine also contains inactivated pertussis toxin and alum, which are adjuvants that favor a Th2 response and production of IgE and IgG4 antibodies (reviewed in Gruber et al., 2001). Some human studies have been interpreted as showing that DTwP, and pertussis infection itself, induced the development of IgE antibodies to vaccine antigens and might have a similar effect on responses to environmental antigens, thereby predisposing to allergy and presumably impeding risk of autoimmunity (Farooqi and Hopkin, 1998; Nilsson et al., 1998; Odelram et al., 1994; Odent et al., 1994; Pershagen, 2000).

More recent studies suggest that the antibody responses to tetanus and diphtheria antigens in children given DTwP vaccine are similar for IgG but signifycantly lower for IgE and IgG4 (a correlate in humans of Th2 responses) when compared with responses in children given DT vaccine (Gruber et al., 2001b). There was no significant effect on IgE antibody response to environmental antigens, suggesting that DTwP may shift the Th1–Th2 balance modestly in the Th1 direction, but only for coadministered antigens (Gruber et al., 2001a). The relative contribution of alum to the Th1–Th2 balance in response to immunization with DT seems to be minor, as the amounts of IgE induced in those given DT with or without alum were similar in one study (Mark et al., 1995). It is important to note, such results do not necessarily mean this would apply to all of the other vaccines in which alum is employed as an adjuvant (e.g., hepatitis B, various conjugate vaccines).

These studies, in which the outcome measure was IgE antibodies, parallel the findings of T-cell responses in infants with pertussis infection or in those who have received DTwP: the T cell response is dominated by the Th1 cytokine interferon-γ, with very weak production of Th2 cytokines, such as IL-5. Conversely, DTaP, which contains acellular pertussis antigens, including inactivated pertussis toxin, but not the Th1-inducing components of *B. pertussis* whole cells, induces a mixed Th1–Th2 response. The Th2 response is more prominent and persistent in children with a family history of allergy (Ausiello et al., 1997; Rowe et al., 2001; Ryan et al., 1997a, 1997b, 1998).

Thus there is some evidence of a bystander effect associated with vaccines, but this effect is relatively modest, most evident with coadministered vaccine antigens rather than other environmental antigens or infections, and inconsistently shown. Current vaccines have, on balance, weak or no Th1-inducing activities. BCG appears to demonstrate the principle for co-administered antigens. However, BCG is not used in the United States, so the relevance for this mechanism in the effects of the U.S. recommended schedule is not demonstrated. Viral vaccines carry some potential for bystander activation, but likely would have a small effect, if it occurs at all. The data on DTaP vaccine indicates that Th1 dominance is not prominent. There is also no evidence in humans that vaccine antigens lead to the pathophysiological disease state. The limited evidence from humans that does exist regards surrogates of the disease process, that is, just

some components of the events that would need to take place for the appearance of clinically relevant pathophysiology. Given the dominant Th1 nature of type 1 diabetes, MS, and most other autoimmune diseases, the prediction is that even if multiple immunizations had a cumulative bystander effect on potentially autoreactive T cells, the current vaccine program would be biased against the generation of Th1-dominated responses. And it is noted that this bias would be stronger now than at any time in the past.

The committee concludes that there is weak evidence for bystander activation, alone or in concert with molecular mimicry, as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity. Moreover, the current routine childhood immunization schedule in the United States appears even less likely to act as an initiator or facilitator of autoimmunity than in the past.

Multiple Immunizations and Infectious Diseases That Protect from Autoimmunity

The mechanisms described above posit that immunizations play a direct role in the initiation or amplification of autoimmune processes. An alternative hypothesis is that immunizations increase the risk of autoimmunity by preventing infectious diseases that have protective effects. The theoretical deleterious effect might be specific to the infection prevented by the vaccine (a homologous effect), or result from a non-antigen-specific effect on the overall nature of the immune response (a heterologous effect). In either case, the notion is that a heretofore protective effect of infection has been lost by immunization.

Homologous effects. The nature of a homologous effect can be illustrated by changes in the epidemiology of poliomyelitis, although this example does not involve autoimmune disease or vaccine-induced effects. The poliomyelitis epidemics that commenced in developed countries near the end of the 19th century are thought to have followed improvements in hygiene that postponed exposure to the virus beyond infancy (Plotkin and Orenstein, 1999; Wilson and Marcuse, 2001). Previously, wild-type poliovirus exposure most commonly occurred in infancy, at a time when passive maternally derived antibody provided partial protection. Thus, a child was first exposed under conditions in which acute paralytic disease was blocked but infection sufficient to immunize against disease on subsequent encounters resulted. Improved hygiene is believed to have reduced circulation of wild-type poliovirus sufficiently to delay exposure in many children until after passively acquired antibody was lost and they were fully susceptible. Further, reduced circulation of wild-type poliovirus would be predicted to result in lower levels of maternal antibody because of less frequent boosting, so that the magnitude of passive antibody transferred and duration of passive protection would be shorter (Zinkernagel, 2001). An immunization program might also be expected to leave infants more susceptible if

vaccine-induced immunity did not induce protective levels of passive maternal antibody and herd immunity sufficient to reduce wild-type virus spread. This potential problem was overcome by actively immunizing infants.

A similar situation may apply to other vaccine-preventable diseases. In the case of measles, mumps, and rubella, the amount of passively-acquired antibody in infants born to mothers whose immunity is due to vaccination is less than and falls more quickly than in infants born to mothers whose immunity is due to wild-type virus infection. As a result, the age at which immunization induces protective antibody responses to measles is younger now than before the widespread use of MMR vaccine (Gans et al., 2001; Redd et al., 1999).

For loss of homologous protection that results from multiple immunizations to be a factor in autoimmunity, a vaccine-preventable infection must cause autoimmune disease. The relative risk will also be affected by the extent to which wild-type infection still occurs in the community; herd immunity will reduce the risk of infection and therefore increase the risk of autoimmune disease. Of the vaccine-preventable diseases, only congenital rubella, which has been noted as inducing type 1 diabetes in about 20 percent of affected individuals, has been causally linked with a chronic autoimmune disorder. Mumps virus infection has been linked to type 1 diabetes in rare cases (IOM, 1994), but causality has not been established. Even though waning of immunity following immunization may delay the age of onset of vaccine-preventable infections, and thus may be a factor in the chronic rubella viral arthritis in adolescent females, there is no evidence that waning immunity and delayed infection increases the potential for induction or acceleration of type 1 diabetes by wild-type rubella or mumps virus. Furthermore, the incidence of type 1 diabetes has increased most in children in the youngest age group (age 0–4 years) (Karvonen et al., 1999b; Podar et al., 2001), arguing against waning immunity as the basis for the increased incidence. The same theoretical considerations apply to the vaccine-preventable diseases that are capable of inducing acute neurological autoimmune injury, including ADEM and Guillain-Barré syndrome.

In the absence of experimental or human evidence regarding loss of protection against a homologous infection as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.

Heterologous effects and the hygiene hypothesis. Possible heterologous effects of vaccination on autoimmunity can be considered in the context of the larger theoretical construct of the hygiene hypothesis, introduced earlier in the report. As noted previously, this hypothesis was first developed as a model to explain the rising increase in asthma and allergic diseases in the developed world (Strachan, 2000), and has been broadened recently to address the apparently

parallel increase in certain autoimmune diseases, including type 1 diabetes and MS (Rook, 2000; Rook and Stanford, 1998; reviewed in Wills-Karp et al., 2001).

The hygiene hypothesis. The hygiene hypothesis is based on the notion that the human immune system (and that of other mammals) evolved in concert with constant exposure to a diverse and changing array of nonpathogenic environmental and commensal microbes, as well as under the persistent threat of lethal infectious diseases caused by microbial pathogens. This microbial exposure is thought to have conditioned the human immune system to respond vigorously to pathogenic microbes but not to harmless environmental antigens (allergens), normal microbial flora and environmental commensals, or self-antigens. Because birth is associated with a rapid switch from a sterile environment to a microbe-rich environment, to which the immune system must learn to respond properly, a component of the hypothesis is that the immune system may be particularly dependent on receiving appropriate conditioning through microbial exposure in early childhood.

During the past century, the developed world has seen improvements in hygiene, the development of effective immunizations, and the advent of antibiotics. These changes have altered the relationships between humans and microbes and, by inference, the challenges that the immune system must meet to provide protection from infection. The magnitude of the change in these relationships over the past century is arguably greater than the total change over all previous millennia of human existence.

The original hygiene hypothesis, developed as a model to explain the rising incidence of asthma and allergy, relied on the then recently elucidated Th1–Th2 paradigm that describes the ability of T cells to differentiate into cells with divergent effector functions. Th1 T cells produce interferon-γ and mediate or regulate cellular immunity and protection against viruses, bacteria, and invasive protozoans. Th2 T cells produce IL-4, IL-5, and IL-13, induce B cells to secrete IgE antibodies, and promote the development of eosinophils, which together mediate allergy and immunity to worms or helminthic parasites. As noted above, a number of microbial components stimulate cells of the innate immune system to produce IL-12 and other cytokines that cause T cells to differentiate into Th1 T cells and inhibit the development of Th2 T cells.

The logic of the hygiene hypothesis is that constant microbial exposure has a bystander effect that impedes the development of Th2 T cells and allergic responses to harmless environmental antigens such as foods and pollens. Therefore the reduced microbial exposure that infants now experience is associated with reduced constraints on the development of Th2 T cells. The hygiene hypothesis also fits well with the notion (Adkins, 2000; Prescott et al., 1998; Rowe et al., 2001; Siegrist, 2001), not yet firmly established as fact (Delespesse et al., 1998; Hassan and Reen, 2000; Lewis and Wilson, 2001; Marchant et al., 1999), that T cell responses of the human fetus and young infant are Th2-biased and

gradually switch to a more balanced pattern over the first year or two of life in nonallergic children.

Although these mechanisms were consistent with observed increases in allergic diseases, they ran counter to two other observations. First, the incidence of autoimmune diseases, particularly type 1 diabetes and MS, appears to have increased in the same or overlapping populations. These diseases, however, are characterized primarily by Th1 T cell responses. Second, although worms or helminthic parasites induce robust Th2 and IgE responses, children in the developing world with large worm burdens have a lower incidence of asthma or allergic diseases (van den Biggelaar et al., 2000). Moreover, in certain mouse models intestinal worms can protect from allergic diseases (Wang et al., 2001), and conversely Th1 promoters can exacerbate allergic disease, depending on the context in which they are administered (Bryan et al., 2000; Hansen et al., 1999).

To address these apparent inconsistencies, modifications of the hygiene hypothesis posit that microbial exposure primarily acts not by deviating the immune response from Th2 to Th1, but by inducing the production of immunoregulatory cytokines (including IL-10 and TGF-β) and T cells that dampen the immune response broadly, including both Th1 and Th2 responses (Rook et al., 2000; Wills-Karp et al., 2001). Many microbes, including worms or helminthic parasites, bacteria, and viruses, induce IL-10 and/or TGF-β production (Letterio and Roberts, 1998; Moore et al., 2001; Rook et al., 2000; Wills-Karp et al., 2001). For example, increased IL-10 production in response to chronic parasitic infection with *Schistosoma haematobium* has been correlated with reduced evidence of allergic sensitization (van den Biggelaar et al., 2000). IL-10 and TGF-β can impede antigen-specific T cell responses directly, by impairing antigen presenting cell function, or by the induction of anergic or regulatory T cells. When stimulated via the T-cell receptor, regulatory T cells suppress the responses of other T cells in a nonspecific manner by contact-dependent mechanisms and by production of IL-10 or TGF-β. Mice that lack these regulatory cytokines or regulatory T cells develop inflammatory bowel disease or inflammatory or autoimmune disease in other tissues (Ermann and Fathman, 2001; Maloy and Powrie, 2001; Roncarolo and Levings, 2000; Rook et al., 2000; Shevach, 2000; Singh, 2000; Wills-Karp et al., 2001; Zhang et al., 2001).

Various findings suggest that that the human neonate can produce regulatory cytokines and T cells, supporting the notion that the neonate's immune system has the requisite immunoregulatory potential if the proper environmental signals are provided. Regulatory T cells are present and inducible in the blood of neonates (Roncarolo and Levings, 2000). Although the conditions required for their induction may differ somewhat from those in adults, it is not known at present if these differences are reproducible or biologically important. In addition, peripheral blood mononuclear cells and monocytes from the blood of human neonates can produce IL-10 and TGF-β in response to microbes or their components (reviewed in Lewis and Wilson, 2001). Although the magnitude of the

response may be reduced compared with that in adults, the production of pro-inflammatory cytokines is also reduced in neonates, suggesting no imbalance in the production of pro- and anti-inflammatory cytokines.

Thus the extent, nature, and timing of contact with microbes are proposed to play an important role in establishing a proper balance in the immune response in early childhood and in maintaining this balance thereafter. A balanced immune response fosters the development of protective immune responses against pathogenic microbes, while preventing both a deleterious Th1 response to self antigens or harmless commensal microbes and a Th2-mediated allergic response to harmless environmental antigens. The type of microbial exposure that is important in establishing this balance is not currently known. Proposed candidates include various gut microbial commensals, chronic infections with intestinal worms or helminthic parasites or *Mycobacterium tuberculosis*, frequent exposure to environmental mycobacteria or other soil organisms, and the timing, number, and nature of acute infections. Factors such as breast feeding, number of siblings and birth order, day care attendance, contact with animals, antibiotic use, and the timing and nature of immunizations have been proposed to affect risk for autoimmune (or allergic) diseases through their effects on the extent and nature of microbial contact (Rook, 2001; Rook and Stanford, 1998; Singh, 2000; Strachan, 2000; Wills-Karp et al., 2001). Whether the sum of all microbial exposure, some specific combination of exposures, or one particular type of exposure is the important factor, or whether these are surrogates for an as yet undefined factor that is important, is uncertain.

Possible impact of vaccines on autoimmunity. On a numerical basis, vaccine-preventable infections represent a minute fraction of the overall infectious and microbial exposure in childhood. For immunization to have an impact on autoimmunity under the hygiene hypothesis, it would be necessary for one or more vaccine-preventable diseases to be particularly important for conditioning immunoregulatory immune responses. The gastrointestinal tract is proposed to play a particularly critical role in this process, so it would follow that immunizations that affect infection or colonization of the gut would be good candidates, but none of the childhood vaccines currently in use do so. (The rotavirus vaccine, which did affect the gut, was in use for too short a time to influence rates of autoimmunity.)

Data from animal models suggest that no one infection is likely to be key, but, rather, a global reduction in microbial contact could be a factor. For example, prior exposure to or infection with a variety of microbes can prevent type 1 diabetes in non-obese diabetic (NOD) mice, as can certain vaccines (reviewed in Bach, 2001; Bach and Chatenoud, 2001; Hiltunen et al., 1999; Singh, 2000). The role of infection is complex in the EAE model. The potentially protective effect of prior mycobacterial or *B. pertussis* exposure (Bach, 2001; Ben-Nun et al., 1993, 1997; Hempel et al., 1985; Mostaricka-Stojkovic et al., 1988) may be related directly to their use as adjuvants at the time of subsequent immunization

with myelin proteins; thus the effect is not generalizable to autoimmune disease developing through more natural mechanisms, as in humans with MS. Nonetheless, if prior infection with one or both of these agents is assumed to be particularly important in establishing protection from autoimmune disease, the immunization schedule in the United States would have had no effect; use of BCG vaccine has never been recommended and, by analogy to the studies of EAE, administration of whole-cell *B. pertussis* vaccine—even as given in alum along with DT (Hempel et al., 1985)—should have been protective. It is possible that the acellular pertussis vaccines might lack the key components needed to provide protection against human autoimmune diseases, but even if this is so, the apparent increase in autoimmune disease began much earlier than the use of the acellular vaccine.

If immunoregulatory cytokines and regulatory T cells play an essential role in impeding the untoward inflammatory responses to normal microbial flora that result in autoimmunity and allergy, and their generation or function depends on microbial contact, it follows that the necessary microbial cues must be established early in postnatal life, in parallel with the development of effector T and B cell responses. Furthermore, such cues must either be persistent or sufficiently frequent to maintain these protective immunoregulatory mechanisms. Because these mechanisms have presumably been operative in all human populations for millennia and only recently perturbed, the associated microbial exposure must be both universally present and long established. None of the diseases prevented by the current U.S. immunization program meets those conditions, nor does tuberculosis or measles, the two candidates proposed from studies of heterologous vaccine-induced protection in Africa (Kristensen et al., 2000). Although tuberculosis may induce persistent infection, fulfilling one requirement, it has not been endemic worldwide until the relatively recent past, nor has measles (Cherry, 1998; Daniel et al., 1994). The more likely candidates are commensal bacteria and ubiquitous environmental microbes, the richness and diversity of which are reduced in hygienic urban environments, or the cumulative exposure to these nonpathogenic microbes and to various invasive infections rather than any specific infection.

The hygiene hypothesis is a model originally proposed based on epidemiological data. The biological mechanisms by which this model could explain an increase in incidence of autoimmune (or allergic) disease are substantial, and the biological evidence in support of the model is moderate to strong. However, the potential contribution of vaccine-preventable diseases as part of this model is minimal. **In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.**

In theory, molecular mimicry, bystander activation, and impaired immunoregulatory mechanisms might act in an additive or synergistic manner to affect the risk of autoimmunity. **Considering molecular mimicry, bystander activation, and impaired immunoregulation collectively rather than individually, the committee concludes that there is weak evidence for these mechanisms as means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.**

Multiple Immunizations and Allergy

Allergic responses are directed against environmental antigens rather than self-antigens as in autoimmunity. In allergic disease, harmless environmental agents evoke Th2 responses and IgE antibody, which otherwise mediate useful protective responses to infections with worms or helminthic parasites. Allergic responses, such as anaphylaxis, are known to occur following vaccination and are reactions either to the vaccine antigens themselves or to other vaccine components. However, the major concern addressed here is whether there are biologically plausible mechanisms by which multiple immunizations might increase the risk of allergic responses to environmental antigens other than those contained in the vaccines—that is, *heterologous* allergic responses.

By analogy to the theoretical frameworks in which the potential effects on autoimmunity were considered, multiple immunizations might influence heterologous allergic responses through a bystander mechanism that modifies the magnitude or quality of the immune response to environmental antigens, or they might prevent infectious diseases that do so.

Bystander Effects

The elimination of smallpox vaccine in 1972 and the substitution of DTaP (containing acellular pertussis vaccine) for DTwP (containing formalin-inactivated *B. pertussis* whole cells) in the 1990s removed two vaccine-based sources of microbial signals favoring Th1 and opposing Th2 responses (Ausiello et al., 1997; Rowe et al., 2001; Ryan et al., 1998). In theory, the replacement of the oral polio vaccine with an injected vaccine given in alum, along with the addition of other vaccines given in alum and administered as early as birth, favors the development of Th2 responses relative to Th1 responses. In mouse neonates (which have a developmentally less mature immune system than that of the human neonate), such a Th2 bias has clearly been shown in response to antigens in alum compared with antigens administered with microbial adjuvants (Adkins, 2000; Barrios et al., 1996; Siegrist, 2001). Alum also induces IL-4 production

from human mononuclear cells (Ulanova et al., 2001). However, except for evidence supporting a more Th2-directed immune response to components of DTaP than to DTwP (both of which are administered in alum), direct evidence is lacking that alum-containing vaccines deviate the immune response of human infants to environmental antigens toward Th2 responses. (For more details regarding this point, see the discussion of Th1 versus Th2 responses related to bystander effects of immunization and autoimmunity.) If such a deviation were to occur, whether it would be sufficient to result in clinically manifest allergy to these antigens would depend on other factors that are as yet incompletely elucidated.

Nonetheless, the biological mechanisms by which immunizations that contain microbial stimuli favor Th1 responses and immunizations containing alum favor Th2 responses are well established. Although the impact of immunization on heterologous allergic responses is unknown, on balance the current routine childhood immunization schedule in the United States is less likely to favor Th1 responses to heterologous antigens and more likely to favor Th2 responses.

The committee concludes that there is weak evidence for bystander activation as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

Prevention of Protective Infections: The Hygiene Hypothesis.

The hygiene hypothesis is discussed in detail in the above autoimmunity section on "Heterologous Effects and the Hygiene Hypothesis". In the context of allergic diseases, either a shift of the Th1–Th2 balance, a loss of immunoregulatory mechanisms that block untoward immune responses to environmental antigens, or both, could result in an increase in allergy. All but the first of these mechanisms are compatible with a parallel increase in allergic and autoimmune diseases. The same reasoning applied to the potential role of vaccine-preventable diseases in reducing risk of autoimmunity can be applied to the question of allergy, with one modification. If a specific type of infection or microbial exposure impaired heterologous Th2 responses, even if it did not play an important role in the generation of immunoregulatory cytokines or regulatory T cells, a vaccine that prevented the disease but was not an effective surrogate for the infection could contribute to the increased incidence of allergy. It does not appear that this occurs. Tuberculosis and measles infection have been proposed as agents that impair heterologous Th2 responses, although, unlike tuberculosis which is a strong Th1-inducing microbe, measles virus is unlikely to have such an effect (Wills-Karp et al., 2001) and neither disease meets the requirement of having been a long-time, ubiquitous infection of all human populations. Although the evidence is conflicting, it has also been proposed that the vaccines against these two diseases are effective surrogates for the infections in the prevention of allergy.

The hygiene hypothesis is a theoretical model, originally proposed on the basis of epidemiological data. The biological mechanisms by which this model could explain an increase in incidence of allergic diseases are substantial, and the model is considered to be moderately to strongly plausible. However, the potential contribution of vaccine-preventable diseases as part of this theory is minimal. **In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy, the committee concludes that this mechanism is only theoretical.**

The committee concludes that there is weak evidence for the existence of any biological mechanisms, collectively or individually, by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

Multiple Immunizations and Heterologous Infections

Simultaneous or sequential infection or immunization with multiple vaccines or antigens can, through various mechanisms, influence the magnitude and/or quality of the immune response to individual antigens, either impeding or enhancing the immune response to one or the other, thereby affecting immune-mediated resolution of an infection and/or the development of protective immunity. There are several potential mechanisms by which this can occur, which vary with the nature of the antigen/agent and with the component of the immune response being evaluated or most important for providing protection. These include immune interference, T cell cross-reactivity, carrier-induced epitope suppression, and competition for antigen presentation (peptide competition for binding to MHC molecules or competition between T cells for the same antigen presenting cells). These mechanisms have been discussed in earlier reports from this committee (IOM, 2001a), and some have also been referred to in the preceding sections of this report. There is experimental animal evidence for each of these mechanisms in certain contexts. For the latter two mechanisms, there is also evidence from human studies that are relevant to the possible effects of multiple immunizations on risk for heterologous infections, which is briefly reviewed here.

Carrier-Induced Epitope Suppression

This process was first described in model systems, but has become clinically important in the context of conjugate vaccines. In such systems, the carrier is a protein antigen, to which is conjugated (covalently linked to create a single molecule) a non-protein antigen. In clinical practice, conjugation of a bacterial non-protein antigen to a protein carrier has been used to convert a

T cell-independent antigen (e.g., the type b capsular polysaccharide of *Haemophilus influenzae* or pneumococcal capsular polysaccharides) into a T cell-dependent antigen. Such conjugate vaccines are immunogenic in infants, inducing high-affinity IgG antibody and long-term immunological memory, whereas none of these features occurs when the polysaccharide alone is used as a vaccine. The basis for this is that the polysaccharide-protein conjugate will bind to and partially activate B cells that are specific for the polysaccharide, which internalize the conjugate, then process and present peptides from the protein component to $CD4^+$ (helper) T cells specific for these peptides. This leads to activation of the T cells, that in turn help the B cells to produce high-affinity antibodies to the linked polysaccharide and mature into long-term memory B cells. In competition with these polysaccharide-specific B cells, are other B cells specific for the protein component of the conjugate. These B cells also present peptides to $CD4^+$ T cells and in turn receive second signals allowing them to produce antibodies to the protein component of the conjugate vaccine. If the numbers of $CD4^+$ T cells specific for the protein are limiting, then B cells specific for the polysaccharide component and B cells specific for the protein component of the conjugate are in competition with each other for limited numbers of $CD4^+$ T cells capable of providing help (Insel, 1995). A variant of this can occur if multiple different carbohydrate antigens are conjugated to the same protein. In this case, the B cells specific for different carbohydrates may compete with each other for limited numbers of $CD4^+$ T cells. The latter situation may account in part for reduced responses seen when multivalent pneumococcal-tetanus toxoid conjugate vaccine was given along with *H. influenzae* type b-tetanus toxoid conjugate vaccine (Dagan et al., 1998).

Competition for Antigen Presentation

This describes a situation in which T cells responding to one antigen or infection compete with other T cells that are responding during the same time frame to other antigens or another infection, and one of the responses has a head start—it precedes the other by a few days or weeks. This gives the response to the earlier challenge a competitive advantage, such that it dominates and impedes the response to the delayed antigenic challenge or infection. Such competition is most readily observed in the context of strong CD8 T cell responses to viral infections (Chen, HD, 2001; Selin et al., 1998, 1999) or artificially manipulated immune responses (Kedl, 2000) in experimental animals. Bystander effects, including viral immune interference (see IOM, 2001a for more details) rather than competition for antigen presentation may affect responses to heterologous viral infections. An example of a heterologous effect in humans is the recent findings related to the timing of administration of MMR vaccine and varicella vaccine. If MMR and varicella vaccine are given at the same time or an interval of 30 or more days elapses between the administration of MMR and varicella vaccines, MMR and

varicella vaccine efficacy is not compromised. However, if varicella vaccine is given after but within 30 days of MMR the relative risk for later development of breakthrough varicella, defined as cases of varicella that occur following exposure to wild-type virus >42 days after varicella vaccine administration, is 2.5 (1.3–4.9). The risk for breakthrough varicella is not increased when varicella vaccine is given within 30 days after DTP, Hib, OPV, IPV, or hepatitis B vaccines. These findings parallel those in earlier reports that had shown a reduction in responsiveness to smallpox vaccine following measles vaccine (MMWR, 2001c).

The committee concludes that there is strong evidence for the existence of biological mechanisms by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk for heterologous infections.

SIGNIFICANCE ASSESSMENT

The charge to the Immunization Safety Review committee includes consideration of the public health response to the immunization safety concerns it examines. Most previous IOM immunization-safety studies by contrast, were limited to conclusions from causality assessments and to recommendations for future research. The public health response to an immunization safety concern potentially encompasses a broad range of activities, including policy reviews, new research directions, and changes in communication to the public and health care providers about issues of immunization safety. In formulating the breadth and direction of the recommended public health response, the committee considers not only its conclusions regarding causality and biological mechanisms, but also the significance of the immunization safety issues for society—the context in which policy decisions must be made.

Public concerns about immunization safety must be examined carefully because most vaccines are given to healthy children not only for their direct protection but also to help protect others in the population. In fact, to achieve this broader level of protection, certain vaccines are mandatory in all 50 states for school and day-care entry. Exemptions on medical grounds (contraindications) are allowed, although they are considered too limited by some (Fisher, 2001a). Exemptions are also allowed on religious grounds in 48 states and on philosophic grounds in 15 states (Evans, 1999). Such exemptions are rare, however, and it is argued that these public health mandates, because they are imposed on healthy children, place a special responsibility on the government for rigorous attention to safety issues, even for rare adverse outcomes.

In the present case, the committee considers the possibility that the exposure of infants to multiple immunizations might increase risks of immune dysfunction. This issue has gained attention because of indications that autoimmune and allergic diseases are increasingly common in children and because of the likelihood that yet more vaccines will be added to the recommended schedule of childhood immunizations. As part of the committee's assessment of the

significance of this issue, the disease burden (e.g., seriousness, treatment, complications) associated with autoimmune and allergic diseases, especially type 1 diabetes and asthma, is reviewed here. Also discussed are indications of public concern about the safety of multiple immunizations and ideas that have been put forward about alternative approaches to the formulation of immunization policy.

Disease Burden

Autoimmune Disorders: Type 1 Diabetes

As noted, diseases of autoimmunity affect 3 to 5 percent of the U.S. population (Jacobson et al., 1997), which translates into as many as 14 million people in 2001. From 500,000 to 1,000,000 people in the United States are thought to have type 1 diabetes, based on estimates that this form of the disease accounts for 5 to 10 percent of the roughly 10 million diagnosed diabetes cases (NIH, 1999). No national surveillance system exists to provide data on the incidence of type 1 diabetes. Rates from local diabetes registries and research projects suggest that about 30,000 new cases develop each year in the United States (LaPorte et al., 1995). Internationally, various registries indicate an average incidence increase of 3% per year (Onkamo et al., 1999). The disease can develop at any age, but incidence rates are higher in children and young adults. Moreover, among children under age 16, the incidence of type 1 diabetes is higher than that of other chronic illnesses, including all forms of cancer combined (Libman et al., 1993).

In type 1 diabetes, the destruction of insulin-producing beta cells in the pancreas prevents proper metabolism of glucose. If not treated, the disease is fatal. Administration of insulin one or more times each day helps compensate for the loss of the beta cells, but the dosage must be calibrated to account for food intake and exercise levels. Children and adults with type 1 diabetes must monitor their blood sugar levels regularly. If blood sugar is not maintained at appropriate levels, there is risk of acute complications, particularly coma. Ketoacidotic coma occurs if insulin administration is inadequate, resulting in hyperglycemia and ketone production. Hypoglycemic coma results if insulin administration is excessive for the blood glucose level.

Type 1 diabetes is associated with many serious long-term complications (Harris, 1995). Mortality rates are elevated at all ages, especially for women and girls, and life expectancy may be reduced by as much as 15 years. Acute coma is the greatest mortality risk during the first years with the disease, replaced over time by renal disease. Among persons who have had Type 1 diabetes for 30 years or more, cardiovascular disease accounts for most deaths. Several chronic complications are also common, and they are more likely to occur if blood sugar levels are poorly controlled. One such complication, diabetic retinopathy, is a leading cause of blindness in the United States. People with diabetes are also at risk of kidney damage that can progress to end-stage renal disease. Neuropathies

are common, and along with peripheral vascular disease, can result in damage to lower extremities (e.g., ulcers, infections) that necessitates amputations. Infection rates are higher, as are periodontal disease rates. Women with type 1 diabetes who become pregnant may see a worsening of any existing eye or kidney damage, and they are at increased risk of spontaneous abortion, preterm delivery, and birth defects.

The financial cost of type 1 diabetes is high. People with this disease are more likely to experience work disability, and they make greater than average use of health care services. A 1988 paper estimated treatment cost for a patient through age 40 at $40,000 (LaPorte et al., 1995). Health expenses and office visits for diabetics exceed those of nondiabetics.[7] For example, the estimated annual cost of physician visits for a diabetic was nearly twice that for a nondiabetic ($1,045 versus $554). Costs of prescriptions and medical supplies are over five times as expensive for diabetics as for nondiabetics ($1,056 versus $201). Increased costs in overall health expenses also affect diabetics far more than nondiabetics ($11,157 versus $2,604 annually) (Javitt and Chiang, 1995).

Allergic Disorders: Asthma

Allergic disorders—including asthma, rhinitis, and dermatitis—are the sixth most common chronic disease in the United States. Together, they result in $18 billion in health care costs annually (AAAAI, 2000). For this report the committee focused on asthma.

A serious allergic disease, asthma was estimated to have affected 14.6 million adults in 2000 (CDC, 2001b) and about 4 million children in 1998 (CDC, 2001a). The prevalence rates of self-reported asthma appear to have increased overall by 74 percent between 1980 and 1994 (Mannino et al., 1998). For children age 0 to 4 years, rates increased by 159 percent during this period (from 22.0 per 1,000 to 57.4 per 1,000). Increases in asthma prevalence were seen in all race, sex, age, and regional groups. The reasons for the increasing prevalence are unclear, although changes in environmental or behavioral factors are considered likely (IOM, 2000).

Symptoms of asthma include shortness of breath, coughing, wheezing, and chest tightness. Some people experience these symptoms only occasionally, but in the most severe cases, symptoms are continuous. Even when the general level of disease is mild or moderate, individual episodes can be severe and may require hospital or emergency department care. Without proper treatment, severe episodes can be life-threatening. Management of the disease includes limiting exposure to environmental triggers (e.g., allergens, tobacco smoke, exercise, viral infections) and appropriate use of medications in response to the underlying level of symptoms and any acute changes. Daily use of medications may be

[7] Most estimates of treatment cost in the medical literature are for both type 1 and 2 diabetes.

necessary. In addition to disease-related complications, children with asthma are more susceptible to comorbid upper and lower respiratory conditions (Weiss and Sullivan, 2001).

The societal impact of asthma is reflected in over 10 million missed school days and 100 million days of restricted activity (AAAAI, 2000). In 1998, asthma was also responsible for almost 13.9 million office visits, 2.0 million emergency department visits, and 423,000 hospitalizations (CDC, 2001a). In 1998, asthma-related costs were estimated at $12.7 billion, attributable to medications and healthcare (Weiss and Sullivan, 2001). Disease severity affects the cost of treatment. Malone and colleagues (2000) found that fewer than 20 percent of asthma patients accounted for more than 80 percent of treatment costs. This high-cost minority was composed of individuals who reported their health as poor or fair, and used four or more different asthma medications.

Attitudes Toward Multiple Immunizations and Vaccine Safety

As this report reflects, there are concerns that the growing number of immunizations routinely given to young children could be a contributing factor to increases in rates of some allergic and autoimmune conditions. The extent of this concern among parents with young children is suggested by a national telephone survey conducted in spring 1999 (Gellin et al., 2000). Among these parents of children under age 6 or expectant parents, 87 percent of the respondents rated immunization as extremely important. But 25 percent agreed with the statement that they were concerned that the immune system could be weakened by too many immunizations, and 23 percent agreed with the statement that children receive more immunizations than are good for them.

Gellin and colleagues (2000) note that levels of concern about immunization safety might now be even higher because of events following the survey. In July 1999, the American Academy of Pediatrics and the U.S. Public Health Service called for the removal of thimerosal, a mercury-based preservative, from vaccines (CDC, 1999a); and in October 1999, the rotavirus vaccine was withdrawn from the childhood immunization schedule because of its association with increased reports of intussusception.[8] Most recently, the threat of bioterrorism has led to discussion of vaccines against smallpox and anthrax, focusing attention on the benefits as well as the risks of using those vaccines.

Without direct evidence, however, it is hard to know what effect such events have on beliefs and perceptions regarding vaccine safety, including any concerns regarding administration of multiple vaccines. Moreover, interpretations of an event can vary. For example, some may view the withdrawal of rotavirus vaccine

[8] Based on reports of intussusception to VAERS, the CDC recommended in July 1999 that r o-tavirus vaccination be postponed, and in October 1999, the ACIP recommended rotavirus vaccine not be given to infants (CDC, 1999b).

as an indication of inadequate pre-licensing testing. Others, however, may view the withdrawal as an indication of the successful use of VAERS as a warning system and appropriate responsiveness of immunization policymakers.

A fundamental concern for immunization policymakers, discussed in previous reports from this committee (IOM, 2001a,b), is that apprehensions about the safety of vaccines will lead to lower rates of vaccination and increases in serious morbidity and mortality from vaccine-preventable disease, as experienced recently in the United Kingdom (Communicable Disease Report, 2001). Gellin and colleagues (2000) called for periodic assessments of parental attitudes toward vaccines and immunization policy so that clinicians, researchers, and policymakers will have a better understanding of concerns about immunization and can develop more effective responses. But a better understanding of how such concerns affect decisions about immunization will also be needed.

Considering Alternative Approaches to Immunization Policy

The increasing number of vaccines in the childhood immunization schedule—and the anticipated addition of still more vaccines—is raising questions not only about the safety of multiple immunizations but also about the adequacy of the current approach to immunization policy-making, which emphasizes national recommendations and state mandates for universal immunization. For example, the public may perceive new vaccines as less compelling if an assessment of these vaccines are based on their cost-benefit, not their public health benefit. Immunization policies must, implicitly or explicitly, make tradeoffs among a variety of factors, including disease risks, the efficacy and safety of vaccines, the financial costs of disease and vaccines, and the differing perspectives of individuals and society.

A recent paper by Feudtner and Marcuse (2001) argued for greater attention to ethical considerations in developing immunization policies and explores some of the complexities that should be addressed in evaluating policy alternatives. The authors propose a policy framework that explicitly incorporates ethical considerations along with the epidemiological and economic considerations that dominate current decisionmaking. Such a framework should guide both the articulation of policy objectives and the evaluation of policy options to achieve those objectives. In particular, they emphasize the importance of considering matters of personal liberty and equity in the distribution of the benefits and burdens of immunization. For instance, there may be benefits to individuals who have philosophical reasons to refuse immunization. However, there also may be an increased burden on those individuals should they be affected by a vaccine-preventable illness, and the burden would extend to the caretakers of those individuals and to society at large.

Feudtner and Marcuse (2001) also proposed consideration of a broader range of policy options to accommodate a greater degree of autonomy in immunization decisions. The current emphasis on universal immunization recommendations and state mandates may not be appropriate or necessary. The experience of the 15 states that allow philosophic exemptions to required immunization illustrates that the availability of exemptions does not appear to be directly related to levels of immunization coverage. In 2000, although some states that allow philosophic exemptions had some of the lowest immunization rates, other states offering exemptions had some of the highest rates (Marcuse, 2001). An alternative approach might allow for a range of priorities (e.g., mandatory, recommended, or elective), based on an evaluation of the immunization objectives and tradeoffs associated with specific vaccines.

Feudtner and Marcuse (2001) acknowledged the challenges of reaching consensus regarding immunization policies with their broader approach to these issues, but they argued that more explicit attention to a wider range of conflicting views and values is needed to maintain public trust in immunization and other public health programs.

Conclusions

The committee's assessment of the significance of concerns about possible immune system dysfunctions as a result of multiple immunizations took several factors into account: the burden of the possible adverse outcomes of autoimmune diseases such as type 1 diabetes and allergic diseases such as asthma; indications of the extent of the concern about multiple immunizations; and views regarding the framework for immunization policy-making.

Although parents appear to value immunization, a substantial minority believes that multiple immunizations could be harmful. Autoimmune and allergic diseases are common in the United States, after all, and the incidence of these conditions appears to be increasing. As represented by type 1 diabetes and asthma, these conditions are life-threatening if not adequately treated and are associated with substantial health care costs. Given also the prevalence of allergic diseases, specifically asthma, a relatively small increase in risk may lead to a significant public health impact.

A better understanding of parents' perceptions of risk and decision-making may be necessary to prevent decreases in immunization rates and increases in vaccine-preventable disease. Current approaches to immunization policy-making emphasize epidemiological and economic considerations, but may benefit from greater attention to ethical issues, including personal liberty and equity in allocation of the benefits and burdens of immunization. With new vaccines in development and discussions of the wider use of existing vaccines, more flexible approaches to immunization policies—especially regarding priorities—may be needed. Thus, **the committee concludes that concern about multiple**

immunizations has been, and could continue to be, of societal significance in terms of parental worries, potential health burdens, and future challenges for immunization policy-making.

RECOMMENDATIONS FOR PUBLIC HEALTH RESPONSE

With government and professional recommendations calling for young children to receive increasing numbers of immunizations, it is important to respond to concerns about possible increases in risk of allergic or autoimmune diseases. Although the committee's review favors rejection of a causal association between multiple immunizations and type 1 diabetes or risk of infection, and the review is inconclusive for asthma, the biological evidence does provide weak support for increased risk of allergy and for autoimmunity and strong support for increased risk of infection (see Table 6 for a summary). The committee was not able to address more than one autoimmune disease and one specific allergic disease in this report. The generalizability of the epidemiological evidence and the causality assessments to every possible type of exposure to multiple immunizations and every type of immune dysfunction is not clear. In addition, the burden of autoimmune and allergic diseases is great. Investigating whether associations indeed exist poses difficult scientific challenges, and relevant epidemiological evidence remains limited. Several important scientific and policy issues, therefore, deserve further public health attention.

Policy Review

The nature of the childhood immunization schedule is likely to change in response to such factors as the development of new vaccines and utilization of novel delivery systems. Changing perceptions of disease risks—derived from antibiotic resistance, threats of bioterrorism, or (re)emerging infectious diseases—could also lead to wider use of existing vaccines not currently included in the immunization schedule. As the array of available vaccines and disease targets expands, the current emphasis on universal recommendations and state mandates for vaccine use should be reassessed (Feudtner and Marcuse, 2001). **The committee recommends that state and federal vaccine policymakers consider a broader and more explicit strategy for developing recommendations for the use of vaccines.**

Such a strategy should include consideration of a range of perspectives (e.g., those of individuals, families, organizations, society) regarding the benefits, risks, and ethical implications of vaccine use and immunization policies. Priorities can be expected to differ among those diverse perspectives, and policymakers must consider how to achieve an equitable balance (Feudtner and Marcuse, 2001).

As part of that exercise, the committee also encourages state and federal immunization policymakers to include a discussion of state mandates for vaccine use. The committee is encouraged by an activity, tentatively called the "Workgroup on Public Health Options for Implementing Vaccine Recommendations," currently underway by the National Vaccine Advisory Committee (NVAC) of the National Vaccine Program Office (NVPO). The exact nature of that activity—its scope, timetable, and authority to initiate action—are not clear, but it appears to be an important first step toward this dialogue. The committee hopes that this important activity remains a priority for NVPO and NVAC, even as other timely vaccine-related issues influence the agenda. Such issues require long-term planning and evaluation; a reactive response to the next schedule addition will be much less effective than a proactive assessment and strategy development across the board.

As part of this overall effort, the committee encourages an exploration of the merits of accommodating requests for alternative vaccine dosing schedules and the development of appropriate clinical guidance for any such alternatives. A more flexible schedule might allow for a reduction in the number of vaccines administered at one time. Such a change would respond to some concerns about multiple immunizations; but it could also have disadvantages, such as requiring more health care visits, that might contribute to lower rates of immunization coverage in the population and consequent increases in morbidity and mortality. In addition, such a change would require extensive communication with healthcare providers and health plans in order that appropriate immunizations occur and are compensated as much as they are for the "traditional" schedule. A more flexible schedule might also permit innovative epidemiological research that currently is difficult because of the homogenous immunization schedules now extant in the United States. If more flexible schedules do gain acceptance, policymakers must ensure that those options are equally available to children who receive immunizations in public clinics and those who are served by private providers.

By issuing the recommendation listed above, the committee does not intend to signal concern about health consequences of the multiple immunizations in the recommended childhood immunization schedule. In fact, **the committee does not recommend a policy review—by the CDC's Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics' Committee on Infectious Diseases, and the American Academy of Family Physicians—of the current recommended childhood immunization schedule on the basis of concerns about immune system dysfunction.**

Similarly, the committee does not recommend a policy review by the Food and Drug Administration's Vaccines and Related Biologic Products Advisory Committee of any currently licensed vaccines on the basis of concerns about immune system dysfunction.

The committee's review of evidence regarding multiple immunizations and immune system dysfunction provides no basis for recommending

reconsideration at this time of the current childhood immunization schedule or of any specific vaccine.

Research

The committee concluded that the findings available from epidemiological sources and consideration of possible biological mechanisms—which were deemed weak—do not at this time warrant specialized studies of possible associations between multiple immunizations and immune system dysfunction. Instead, the committee encourages epidemiological studies conducted within the framework of ongoing research and surveillance programs on allergy, autoimmune disease, and vaccine safety; it also encourages additional basic research on the immune system and on allergy and autoimmune diseases.

Epidemiological Studies

The committee emphasizes the need for continuing surveillance of vaccine recipients and possible adverse events. Changes in the immunization schedule may present opportunities to study whether or not the incidence of adverse health outcomes also changes. However, one of the challenges in addressing concerns about multiple immunizations is identification of appropriate and adequately sized study populations; allergy or autoimmune diseases have complex risk factors and potentially long intervals between vaccine exposure and diagnosis.

Several vaccine-related data resources already exist, including the Vaccine Adverse Event Reporting System (VAERS), the Vaccine Safety Datalink (VSD), and state and local immunization registries. **The committee recommends exploring the feasibility of using existing vaccine surveillance systems, alone or in combination, to study safety questions related to asthma and other important allergic disorders, as well as to study type 1 diabetes and other important autoimmune diseases.**

In addition, surveillance of autoimmune diseases and allergic disorders should be strengthened. Despite the routine diagnosis of asthma, finding a widely accepted definition for the disease has been problematic (Samet 1987, Toelle et al., 1997). The absence of a universally accepted definition of asthma makes it difficult to determine a consistent operational definition for epidemiological studies (IOM, 2000).

Disease registries and long-term research programs that identify individuals with these diseases, or with known genetic risk factors, could be an efficient means of finding subjects for either retrospective or prospective studies of possible vaccine-related risks. **The committee recommends exploring the use of such cohorts for research on possible vaccine-related disease risks. Furthermore, the committee recommends that disease registries and research programs for autoimmune and allergic disorders routinely collect**

immunization histories as part of their study protocols. The committee is encouraged to see that the Diabetes Autoimmunity Study in the Young (DAISY), which includes cohorts drawn from the general population and from siblings and offspring of persons with IDDM, is already including immunizations as a routinely monitored variable.

Basic and Clinical Science

Research on the developing human immune system, especially in relation to vaccines, is limited. Studies of animal models are essential to advancing knowledge of the immune system, but those studies have limits because of important differences between humans and animals. Thus, **the committee recommends continued research on the development of the human infant immune system.** A better understanding of the development of the human immune system is needed as a basis for improved understanding of infants' response to vaccines and other environmental exposures. In addition, the committee encourages collaborative activities, such as NIAID/NICHD workshops and initiatives, that help the research community synthesize the results of individual research efforts. The inclusion of vaccinologists and vaccine safety researchers in these efforts is encouraged.

Genetic factors are known to be an important source of variability in the responses of the human immune system and in the risks of allergic or autoimmune disease. But understanding of the complex interactions among genetic variables, as well as of the interactions between those variables and environmental exposures (including vaccines and wild-type viral and bacterial agents), remains incomplete. **The committee endorses current research efforts aimed at identifying genetic variability in human immune system development and immune system responsiveness as a way to gain a better understanding of genetic susceptibility to vaccine-based adverse events.**

For some autoimmune and allergic disorders, surrogate biological markers of disease or disease risk have been identified. In particular, in individuals at risk for type I diabetes, the development of multiple autoantibodies to GAD65 (glutamic acid decarboxylase), IA-2 (protein tyrosine phosphatase-like molecule), and insulin correlate strongly with later development of overt type I diabetes (Notkins and Lernmark, 2001). However, there are to date no other surrogate markers that have sufficient predictive power to be useful in monitoring risk for other autoimmune diseases in children receiving routine immunizations (Leslie et al., 2001). For allergic disorders, the clinical history of allergic diseases should be collected in follow-up evaluations, and the feasibility of specific tests for atopy considered. Studies of the normal development of the immune system in conjunction with surrogate markers of autoimmune and allergic disease and a cohort analysis of autoimmune and allergic disease could be carried out not only in the United States but in infants in a less developed country. Such a compari-

son might more clearly define how an earlier and more intense exposure to microbes might influence the maturation process and alter the proposed impact of immunizations on allergy and autoimmune disease. In theory, collecting data on known markers in the course of vaccine research and testing would present an opportunity to study the prevalence of such markers before and after immunization. Similarly, it might also be possible to study whether the prior presence of a marker was associated with differences in the response to a vaccine. **The committee recommends exploring the feasibility of collecting data on surrogate markers for type I diabetes and clinical history of allergic diseases in the vaccine testing and licensing process.** Such data might also be useful in vaccine-related studies in high-risk cohorts, such as those in the DAISY study. **The committee recommends exploring surrogates for type I diabetes and clinical history of allergic diseases in existing cohort studies of variations in the immunization schedule.**

Communication

Along with the increasingly complex immunization schedule has come a dramatic increase in the complexity of vaccine safety issues, and it appears that some people have redefined their conceptions of the related risks and benefits. The focus seems to have shifted from whether children will get a disease if they are not vaccinated to whether children will experience temporary or potentially longer-term adverse events if they *are* vaccinated (McPhilips and Marcuse, 2001).

The committee is not convinced, however, that available reports on such attitudes provide an adequate scientific basis for understanding either these changes in perception or the groups that are experiencing them. Reports from population-based telephone surveys, for example, typically provide information about what people think, but such surveys rarely can probe adequately about why respondents think the way they do. More information is needed in order to develop effective risk-benefit communication strategies on immunization and vaccine safety.

A deeper understanding of why and how people make decisions as they do is needed, but relying on impressions, assumptions, or any single research method (e.g., survey, focus group, mental modeling, decision analysis) will be too limited. Therefore, **the committee recommends that an appropriate panel of multidisciplinary experts be convened by the Department of Health and Human Services. It would develop a comprehensive research strategy for knowledge leading to the optimal design and evaluation of vaccine risk-benefit communication approaches.** By communication approaches, the committee is not referring to communication tools, such as vaccine information statements, lists of frequently asked questions (FAQs), or websites. Instead, the committee intends that this panel consider a larger definition of risk-benefit communication goals and strategies. In addition, this multidisciplinary panel

may wish to explore the assessment and characterization of these risks. Finally, it must be emphasized that the Immunization Safety Review Committee is not the panel being recommended. A new panel with specialized expertise related to communication issues is necessary.

SUMMARY

A substantial minority of parents (23–25%) participating in a recent survey agreed with the statement that getting too many vaccines is not good for a baby and can weaken the immune system (Gellin, 2000). But a review of the possible biological mechanisms for any adverse effects of multiple immunization on immune function suggests that the infant immune system is inherently capable of handling the numbers of antigens presented during routine immunization.

A review of the clinical and epidemiological literature favors rejection of a causal relationship between multiple immunizations and risk of infection and type 1 diabetes. The evidence was inadequate to accept or reject a causal relationship. Meanwhile, the biological evidence that immunization might lead to infection, autoimmune disease, or allergy is more than only theoretical. This literature base is somewhat limited, however.

Therefore, the committee recommends limited but continued public health attention to this issue in terms of capitalizing on current research efforts. No recommendations for policy review is made, but the committee does recommend an analysis of new frameworks for immunization policy, particularly as the number of licensed vaccines increases.

TABLE 6 Biological Mechanisms for the Possible Role of Immunizations in Increasing the Risk of Immune Dysfunction

Adverse Health Outcome	**Mechanism**	**Committee Conclusion About the Weight of the Biological Evidence**
Autoimmune disease	Molecular mimicry	Theoretical only
	Bystander effect	Weak
	Loss of protection induced by homologous infection	Theoretical only
	Via the hygiene hypothesis	Theoretical only
	Collective mechanistic possibilities	Weak
Allergic disease	Bystander effect	Weak
	Via the hygiene hypothesis	Theoretical only
	Collective mechanistic possibilities	Weak
Heterologous Infections	Carrier-induced epitope suppression	Strong
	Competition for antigen presentation	

BOX 1 Committee Conclusions and Recommendations

SCIENTIFIC ASSESSMENT

Causality Conclusions

The committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of heterologous infections.

The committee concludes that the epidemiological and clinical evidence favors rejection of a causal relationship between multiple immunizations and an increased risk of type 1 diabetes.

The committee concludes that the epidemiological and clinical evidence is inadequate to accept or reject a causal relationship between multiple immunizations and an increased risk of allergic disease, particularly asthma.

Biological Mechanisms Conclusions

Autoimmune Disease

In the absence of experimental or human evidence regarding molecular mimicry or mercury-induced modification of any vaccine component to create an antigenic epitope capable of cross-reaction with self epitopes as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that these mechanisms are only theoretical.

The committee concludes that there is weak evidence for bystander activation, alone or in concert with molecular mimicry, as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.

In the absence of experimental or human evidence regarding loss of protection against a homologous infection as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.

In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity, the committee concludes that this mechanism is only theoretical.

Considering molecular mimicry, bystander activation, and impaired immunoregulation collectively rather than individually, the committee concludes that there is weak evidence for these mechanisms as means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of autoimmunity.

Allergic Disease

The committee concludes that there is weak evidence for bystander activation as a mechanism by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

In the absence of experimental or human evidence regarding mechanisms related to the hygiene hypothesis as a means by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy, the committee concludes that this mechanism is only theoretical.

The committee concludes that there is weak evidence for the existence of any biological mechanisms, collectively or individually, by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk of allergy.

Heterologous Infection

The committee concludes that there is strong evidence for the existence of biological mechanisms by which multiple immunizations under the U.S. infant immunization schedule could possibly influence an individual's risk for heterologous infections.

SIGNIFICANCE ASSESSMENT

Conclusions

The committee concludes that concern about multiple immunizations has been, and could continue to be, of societal significance in terms of parental worries, potential health burdens, and future challenges for immunization policy-making.

PUBLIC HEALTH RESPONSE RECOMMENDATIONS

Policy Review

The committee recommends that state and federal vaccine policymakers consider a broader and more explicit strategy for developing recommendations for the use of vaccines.

The committee does not recommend a policy review—by the CDC's Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics' Committee on Infectious Diseases, and the American Academy of Family Physicians—of the current recommended childhood immunization schedule on the basis of concerns about immune system dysfunction.

The committee does not recommend a policy review by the Food and Drug Administration's Vaccines and Related Biologic Products Advisory

Committee of any currently licensed vaccines on the basis of concerns about immune system dysfunction.

Research

Epidemiological Research

The committee recommends exploring the feasibility of using existing vaccine surveillance systems, alone or in combination, to study safety questions related to asthma and other important allergic disorders, as well as to study type 1 diabetes and other important autoimmune diseases.

The committee recommends exploring the use of cohorts for research on possible vaccine-related disease risks. Furthermore, the committee recommends that disease registries and research programs for autoimmune and allergic disorders routinely collect immunization histories as part of their study protocol.

Basic Science and Clinical Research

The committee recommends continued research on the development of the human infant immune system.

The committee endorses current research efforts aimed at identifying genetic variability in human immune system development and immune system responsiveness as a way to gain a better understanding of genetic susceptibility to vaccine-based adverse events.

The committee recommends exploring the feasibility of collecting data on surrogate markers for type I diabetes and clinical history of allergic diseases in the vaccine testing and licensing process.

The committee recommends exploring surrogates for type I diabetes and clinical history of allergic diseases in existing cohort studies of variations in the immunization schedule.

Communication

The committee recommends that an appropriate panel of multidisciplinary experts be convened by the Department of Health and Human Services. It would develop a comprehensive research strategy for knowledge leading to the optimal design and evaluation of vaccine risk-benefit communication approaches.

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Appendix A

Chronology of Important Events Regarding Vaccine Safety

Year	Vaccine Licensure	Legislation and/or Policy Statements	IOM Reports on Vaccine Safety
1955	Inactivated poliomyelitis vaccine (IPV) available		
1963	Oral poliomyelitis vaccine (OPV) available, replaces IPV		
	Measles vaccine available		
1967	Mumps vaccine available		
1969	Rubella vaccine available		
1971	Measles-Mumps-Rubella (MMR) vaccine available		
1977		Mumps vaccination recommended	*Evaluation of Poliomyelitis Vaccines*
1979	Current formulation of Rubella vaccine available, replaces earlier versions		
1982	Plasma-derived hepatitis B vaccine available		

Continued

Year	Vaccine Licensure	Legislation and/or Policy Statements	IOM Reports on Vaccine Safety
1985	Hib vaccine licensed for children >15 months		
1986		Congress passes Public Law 99-660, the National Childhood Vaccine Injury Act (introduced in 1984) calls for: est. of NVPO est. of NVAC est. of VICP est. of ACCV IOM review of 1) pertussis and rubella, 2) routine child vaccines	
1988			*Evaluation of Poliomyelitis Vaccine Policy Options*
1990	2 Hib conjugate vaccines licensed for use beginning at 2 months		
1991	Acellular pertussis component licensed for the 4th and 5th doses of the 5-part DTP series in ACEL-IMUNE	Hepatitis B recommended by ACIP for addition to childhood immunization schedule ACIP recommends Hib be added to childhood immunization schedule	*Adverse Effects of Pertussis and Rubella Vaccines*
1992	Acellular pertussis component licensed for the 4th and 5th doses of the 5-part DTP series in Tripedia	Hepatitis B vaccine: Added universal vaccination for all infants, high-risk adolescents (e.g., IV drug users, persons with multiple sex partners)	
1993	Combined DTP and Hib vaccine (Tetramune) licensed		
1994			*Adverse Events Associated with Childhood Vaccines: Evidence Bearing on Causality* *DPT and Chronic Nervous System Dysfunction: A New Analysis*

Year	Vaccine Licensure	Legislation and/or Policy Statements	IOM Reports on Vaccine Safety
1995	Varicella virus vaccine available (Varivax)		
1996	DTaP vaccine (Tripedia and ACEL-IMUNE licensed for complete 5-dose series	ACIP recommends using IPV for the first 2 polio vaccinations, followed by OPV for remaining doses. Intended to be a transitional schedule for 3–5 years until an all -IPV series is available ACIP recommends children 12months – 12 years receive Varicella vaccin	*Options for Poliomyelitis Vaccinations in the United States: Workshop Summary*
1997	Additional DTaP vaccine (Infanrix) licensed for first 4 doses of 5-part series	ACIP recommends DTaP in place of DTP	*Vaccine Safety Forum: Summary of Two Workshops* *Risk Communication and Vaccination: Workshop Summary*
1998	Additional DTaP vaccine (Certiva) licensed for first 4 doses of 5-part series	ACIP updates MMR recommendation, encouraging use of the combined MMR vaccine	
1999		ACIP updates varicella vaccine recommendation, requiring immunity for child care and school entry ACIP recommends an all-IPV schedule begin January 2000 to prevent cases of vaccine-associated paralytic polio AAP and PHS recommend removal of thimerosal from vaccines Also recommended postponement of hepatitis B vaccine from birth to 2–6 months for infants of hepatitis B surface antigen-negative mothers	
	Additional supply of thimerosal-free hepatitis B vaccine made available	MMWR notifies readers of the availability of a thimerosal-free hepatitis B vaccine, enabling the resumption of the birth dose	

Continued

Year	Vaccine Licensure	Legislation and/or Policy Statements	IOM Reports on Vaccine Safety
2000	Pneumococcal vaccine for infants and young children licensed (Prevnar)	ACIP recommends pneumococcal vaccination for all children 2–23 months, and at-risk children 24–59 months (e.g., immunocompromised)	
2001		October: ACIP drafts statement expressing a preference for use of thimerosal-free DtaP, Hib, and Hep B vaccines by March 2002	*Immunization Safety Review: Measles-Mumps-Rubella Vaccine and Autism* *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders*

Appendix B

Committee Conclusions and Recommendations from Previous Reports

MEASLES-MUMPS-RUBELLA VACCINE AND AUTISM

Conclusions

The committee concludes that the evidence favors rejection of a causal relationship at the population level between measles-mumps-rubella (MMR) vaccine and autistic spectrum disorders (ASD). However, this conclusion does not exclude the possibility that MMR vaccine could contribute to ASD in a small number of children.

The committee concludes that further research on the possible occurrence of ASD in a small number of children subsequent to MMR vaccination is warranted, and it has identified targeted research opportunities that could lead to firmer understanding of the relationship.

Recommendations

Public Health Response

The committee recommends that the relationship between the MMR vaccine and autistic spectrum disorders receive continued attention.

Policy Review

The committee does not recommend a policy review at this time of the licensure of MMR vaccine or of the current schedule and recommendations for administration of MMR vaccine.

Research Regarding MMR and ASD

The committee recommends the use of accepted and consistent case definitions and assessment protocols for ASD in order to enhance the precision and comparability of results from surveillance, epidemiological, and biologic investigations.

The committee recommends the exploration of whether exposure to MMR vaccine is a risk factor for autistic spectrum disorder in a small number of children.

The committee recommends the development of targeted investigations of whether or not measles vaccine-strain virus is present in the intestines of some children with ASD.

The committee encourages all who submit reports to VAERS of any diagnosis of ASD thought to be related to MMR vaccine to provide as much detail and as much documentation as possible.

The committee recommends studying the possible effects of different MMR immunization exposures.

The committee recommends conducting further clinical and epidemiological studies of sufficient rigor to identify risk factors and biological markers of ASD in order to better understand genetic or environmental causes.

Communications

The committee recommends that government agencies and professional organizations, CDC and the Food and Drug Administration (FDA) in particular, review some of the most prominent forms of communication regarding the hypothesized relationship between MMR vaccine and ASD, including information they provide via the Internet and the ease with which Internet information can be accessed.

THIMEROSAL-CONTAINING VACCINES AND NEURODEVELOPMENTAL DISORDERS

Conclusions

The committee concludes that although the hypothesis that exposure to thimerosal-containing vaccines could be associated with neurodevelopmental disorders is not established and rests on indirect and incomplete information, primarily from analogies with methylmercury and levels of maximum mercury exposure from vaccines given in children, the hypothesis is biologically plausible.

The committee also concludes that the evidence is inadequate to accept or reject a causal relationship between thimerosal exposures from childhood vaccines and the neurodevelopmental disorders of autism, ADHD, and speech or language delay.

Public Health Response Recommendations

Policy Review and Analysis

The committee recommends the use of the thimerosal-free DTaP, Hib, and hepatitis B vaccines in the United States, despite the fact that there might be remaining supplies of thimerosal-containing vaccine available.

The committee recommends that full consideration be given by appropriate professional societies and government agencies to removing thimerosal from vaccines administered to infants, children, or pregnant women in the United States.

The committee recommends that appropriate professional societies and government agencies review their policies about the non-vaccine biological and pharmaceutical products that contain thimerosal and are used by infants, children, and pregnant women in the United States.

The committee recommends that policy analyses be conducted that will inform these discussions in the future.

The committee recommends a review and assessment of how public health policy decisions are made under uncertainty.

The committee recommends a review of the strategies used to communicate rapid changes in vaccine policy, and it recommends research on how to improve those strategies.

Public Health and Biomedical Research

The committee recommends a diverse public health and biomedical research portfolio.

Epidemiological Research

The committee recommends case-control studies examining the potential link between neurodevelopmental disorders and thimerosal-containing vaccines.

The committee recommends further analysis of neurodevelopmental disorders in cohorts of children who did not receive thimerosal-containing doses as part of a clinical trial of DTaP vaccine.

The committee recommends conducting epidemiological studies that compare the incidence and prevalence of neurodevelopmental disorders before and after the removal of thimerosal from vaccines.

The committee recommends an increased effort to identify the primary sources and levels of prenatal and postnatal background exposure to thimerosal (e.g., Rho (D) Immune Globulin) and other forms of mercury (e.g., maternal consumption of fish) in infants, children, and pregnant women.

Clinical Research

The committee recommends research on how children, including those diagnosed with neurodevelopmental disorders, metabolize and excrete metals—particularly mercury.

The committee recommends continued research on theoretical modeling of ethylmercury exposures, including the incremental burden of thimerosal with background mercury exposure from other sources.

The committee recommends careful, rigorous, and scientific investigations of chelation when used in children with neurodevelopmental disorders, especially autism.

Basic Science Research

The committee recommends research to identify a safe, effective, and inexpensive alternative to thimerosal for countries that decide they need to switch from using thimerosal as a preservative.

The committee recommends research in appropriate animal models on the neurodevelopmental effects of ethylmercury.

Appendix C

Immunization Safety Review Committee

Multiple Immunizations in Newborns and Infants and Immune System Dysfunction

Public Meeting
Monday, November 12, 2001

Aljoya Conference Center
Seattle, Washington

AGENDA

8:00–8:15 a.m. **Welcome and Introductions**
Marie McCormick, M.D., Sc.D.
Chair, Immunization Safety Review Committee

Additional Comments
Christopher Wilson, M.D.
Member, Immunization Safety Review Committee

8:15–9:00 a.m. **Immunological Competition and the Infant Immune Response to Vaccines**
Richard Insel, M.D.
University of Rochester School of Medicine

9:00–9:20 a.m. **T Cell Immunity in Infants and Immune System Overload Hypothesis**
Tobias Kollman, M.D.
University of Washington

9:20–10:00 a.m. **Hygiene Hypothesis**
Graham Rook, M.D.
Royal Free and University College Medical School, London

10:00–10:30 a.m. **Discussion**

10:30–10:45 a.m. **Break**

10:45–11:30 a.m. **Immunizations and Autoimmunity: Mechanisms, Plausibility, and Genetic Susceptibility**
Gerald Nepom, M.D., Ph.D.
University of Washington

11:30a.m.–
12:15 p.m. **Diabetes: Incidence and Possible Triggers**
George Eisenbarth, M.D., Ph.D.
University of Colorado Health Sciences Center

12:15–12:45 p.m. **Discussion**

12:45–1:45 p.m. **Lunch**

1:45–2:30 p.m. **Genetic Screening and Diabetes: A New Prospective Study**
Marian Rewers, M.D., Ph.D.
University of Colorado Health Sciences Center

2:30–3:15 p.m. **Autoimmunity and the Central Nervous System**
Olaf Stuve, M.D.
University of California at San Francisco

3:15–3:45 p.m. **Research Strategies**
Ronald Kennedy, Ph.D.
Texas Tech University Health Sciences Center

3:45–4:00 p.m. **Break**

4:00–4:45 p.m. **Immunization Hesitancy**
Edgar Marcuse, M.D., M.P.H.
University of Washington

4:45–5:15 p.m. **Discussion**

5:15–5:30 p.m. **Public Comment**

5:30 p.m. **Adjourn**

Appendix D

Methods of Identifying the Literature for the Causality Assessment

To evaluate the hypothesis on multiple immunizations and immune system dysfunction, the committee collected information from several sources. At an open scientific meeting in November 2001, academic researchers gave presentations on specific scientific issues germane to the topic. All information presented to the committee at that meeting can be viewed on the project website (www.iom.edu/imsafety). An extensive review was performed of the published, peer-reviewed scientific and medical literature pertinent to the hypothesis.

Search Strategy for the Causality Assessment

To inform the committee's causality assessments, the following searches were performed. As epidemiological studies carry the most weight in a causality assessment, all searches were limited to human subjects.

Diabetes A search for relevant articles was conducted on PubMed using the MeSH term "diabetes mellitus, insulin dependent" with the terms "vaccin*" or "immunization*" in the MeSH field. The search was further limited to articles in English.

Asthma A search was conducted on PubMed. The terms "vaccines" OR "immunizations" were combined with the term "hypersensitivity" in the MeSH field. This search was then combined with NOT "cancer" OR "occupational diseases,"also in the MeSH field. The search was further narrowed by combining the results with "epidemiologic methods" in the MeSH field, and limiting it to studies in English. A total of 363 articles were found.

Heterologous Infection To examine the heterologous effects of vaccines, several searches were conducted on PubMed. In the first, the terms "vaccines/adverse effects" were combined with "viruses/etiology" in the MeSH field. The search was further limited to studies in which "vaccines/adverse effects" had to appear as a MeSH major topic—a MeSH term that is one of the main topics discussed in the article.

A second search for articles pertaining to heterologous effects of vaccines combined "pneumococcal OR polio OR diphtheria OR tetanus vaccine" with "invasive bacterial disease." A similar search combined "vaccine/adverse ef-

fects" with "bacterial infections/epidemiology," limited to MeSH terms. The final two searches combined "vaccines/adverse effects" with "morbidity" OR "mortality" in the MeSH field. The search was further limited to articles in which "vaccines/adverse effects" was a MeSH major topic.