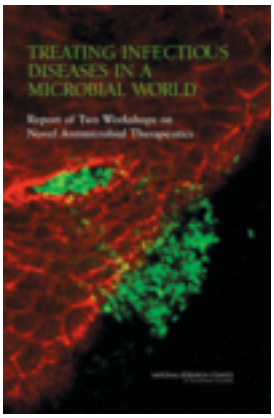

Treating Infectious Diseases in a Microbial World: Report of Two Workshops on Novel Antimicrobial Therapeutics

Committee on New Directions in the Study of Antimicrobial Therapeutics: New Classes of Antimicrobials, Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation, National Research Council

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TREATING INFECTIOUS DISEASES IN A MICROBIAL WORLD

Report of Two Workshops on Novel Antimicrobial Therapeutics

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Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation

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Cover image: Confocal micrograph depicting a common behavior of bacteria (green) gathering in mucus shed by host epithelial cells (red). Such behavior has been noted in both beneficial and pathogenic associations with microbial partners. This image shows the gathering of the microbial symbiont *Vibrio fischeri* during its colonization of tissues of the host squid *Euprymna scolopes*. As described in the report, models such as the squid-vibrio system promise to provide insight into the mechanisms underlying the reciprocal dialogue between the hosts and their microbial partners, whether the relationship results in health or disease. Image courtesy of Laura Sycuro and Margaret McFall-Ngai.

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Acknowledgments

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The immunomodulation committee would also like to acknowledge David Schneider, Stanford University, for preparing the figure highlighting the complexity of the immune system, and Fiona Roche, Simon Fraser University, for preparing the Cytoscape figure of the TLR4 pathway.

These reports have been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published reports as sound as possible and to ensure that the reports meet institutional standards of 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 these reports:

Christine A. Biron, Brown University
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Although the reviewers listed above have provided constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final drafts of the reports before their release. The review of these reports was overseen by **Elaine L. Larson**, Columbia University, and **Leslie Z. Benet**, University of California, San Francisco. Appointed by the National Research Council, they were responsible for making certain that an independent examination of these reports was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of these reports rests entirely with the authoring committees and the institution.

Contents

Appendixes

Summary

At the request of the National Institute of Allergy and Infectious Diseases, two committees established by the National Research Council organized workshops to identify promising new approaches to the development of antimicrobial therapeutics (Appendix A). One workshop focused on potential new classes of antibiotics, while the other explored the possibility of treating infectious diseases by modulating the immune system. The need for new antimicrobial therapeutics is acute because of growing resistance to available antibiotics, the emergence of new infectious diseases like SARS and West Nile virus, and the risk of bioterrorist attacks using infectious agents that may not be immediately identifiable. From one point of view, these are all manifestations of a single problem—human vulnerability to microbial disease—and therefore subject to one solution—a single drug that can protect against any infectious agent. Attractive as the idea of a "gorillacillin" superdrug might be in the abstract, discussions at both workshops made it clear that a point of view pitting human against microorganism is at best limited and at worst seriously flawed.

Through research in fields as diverse as evolutionary biology, bacteriology, ecology, immunology and developmental biology, a much more complex viewpoint is emerging—a perspective recognizing that humans exist as part of an environment full of microorganisms and that these microorganisms have been evolving, coexisting, and competing with each other for millions of years. Most of the antimicrobial agents that have revolutionized

the treatment of infectious diseases in the past several decades are derived from bacterial products that have been used as weapons against other bacteria for millions of years, and the ability of bacteria to develop resistance to them is an ancient evolutionary defense tactic. Similarly, the human immune system has evolved in the midst of this microbial world to provide highly nuanced and carefully regulated responses to the myriad microorganisms it encounters. Perhaps most discordant with the "human versus microorganism" point of view is the increasing realization that all humans live in intimate community with thousands of microbial species—the natural microbiota of our skins, guts and oral cavities—and that these microorganisms affect human health in many positive ways from development, to nutrition, to susceptibility to disease. In short, humans have evolved in and exist now in a world overwhelmingly dominated by microorganisms, the vast majority of which do not cause disease.

In such a world, the idea of developing a "gorillacillin" becomes hopelessly complicated. How would such a drug distinguish microbial friend from foe? How would it simultaneously outwit the varied defense tactics developed over millions of years by thousands of microorganisms? How could a single drug improve the performance of the highly complicated and already extremely effective human immune system? These questions are daunting, even discouraging. At the same time, antibiotics have saved millions of lives and interventions exploiting the human immune system—especially immunization—have vastly reduced human vulnerability to infectious disease. If "gorillacillin" is an unrealistic—perhaps even an undesirable—goal, it is nevertheless clear that effective antimicrobial therapeutics have been and can again be developed.

Both workshops focused on generating ideas for innovative research approaches that would contribute to the development of new antimicrobial therapeutics. There was widespread recognition, however, that the road from a brilliant idea to a clinically available treatment is long and full of pitfalls. Differing approaches to antibiotic use in different countries, declining investment in antimicrobials by large pharmaceutical companies, increasing costs of clinical trials, and complicated regulatory and legal environments, are just a few of the obstacles to bringing new compounds rapidly from the laboratory to the clinic. Interesting and important as these issues are, the workshops were not designed to address them because the committees were specifically charged to focus on the scientific possibilities. The interested reader is referred to a recent report by the Infectious Diseases Society of America, *Bad Bugs, No Drugs: As Antibiotic Discovery Stag-*

nates . . . A Public Health Crisis Brews,[1] for a useful description of these challenges specifically as they affect antibiotic development. Several reports issued by the National Academies have also addressed some of these issues, including *Discovery of Antivirals Against Smallpox: Executive Summary* by the Committee on Transforming Biological Information into New Therapies: A Strategy for Developing Antiviral Drugs for Smallpox (2004), *Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance* by the Committee on the Economics of Antimalarial Drugs (2004), and *Making Better Drugs for Children with Cancer* by the Committee on Shortening the Time Line for New Cancer Treatments (2005).

The two workshops held for this report approached the challenge by looking at the current state of knowledge, identifying the approaches that have been successful in the past, and brainstorming about ways in which new areas of research could revolutionize the treatment of infectious disease. The recommendations put forward by each committee emerged independently from their respective workshop discussions and are organized according to the topics that emerged as most promising. An overview of the recommendations of both committees, however, suggests that they are of three types.

Some of the recommendations reflect ways in which current approaches to developing antibiotics and immunomodulators could be improved. Implementation of recommendations of this type is most likely to provide improved therapeutics in the short term. For example,

- Many successful antibiotics have been discovered by studying natural products. The field of metagenomics offers the possibility of discovering gene products with antibiotic activity without having to culture individual organisms. (Antimicrobial workshop recommendation A-6.2)
- Generating slight chemical variations of compounds with promising activity frequently results in more effective drugs; new chemical synthesis approaches that allow the rapid synthesis of more varied structures could speed this process. (Antimicrobial workshop recommendations A-7.1, A-7.2, A-7.3)
- Immunization, both passive and active, has been hugely successful, but could be improved with enhanced understanding of exactly how different antibody isotypes function and how they interact with the innate immune system. (Immunomodulation workshop recommendation I-3.1)

[1]http://www.idsociety.org/badbugsnodrugs

Other recommendations reflect the committees' judgments as to which areas of basic research are most likely to lead to genuinely novel approaches to infectious disease treatment. Since the outcome of basic research is difficult to predict, these approaches might be labeled "high-risk," but have the potential also to reap great reward in the long term.

- Current antibiotic development concentrates on targets that are essential for bacterial metabolism; research into how bacteria communicate with each other may allow the development of drugs that confuse rather than kill—drugs that might be less likely to provoke resistance. (Antimicrobial workshop recommendations A-3.1, A-3.2, A-3.3)
- The human immune system is constantly interacting with the thousands of bacterial species comprising the natural microbiota; understanding how the natural microbiota communicates with the immune system and how the immune system singles out harmful microorganisms could lead to drugs that help the natural microbiota outcompete pathogens. (Immunomodulation workshop recommendations I-6.1, I-6.2, I-6.3 and Antimicrobial workshop recommendation A-3.2)
- Once considered primitive, the innate immune system is increasingly being shown to be highly complex, regulated and intimately intertwined with the acquired immune system and the nervous system. Understanding innate immune system regulatory pathways and active molecules may lead to drugs that are effective against a wide array of infectious agents. (Immunomodulation workshop recommendations I-1.1, I-1.2, I-1.3, I-1.4)

A third group of recommendations reflects cross-cutting issues. In particular, both workshops highlighted the value of improved diagnostics.

- Rapid diagnostic tools to allow identification of the disease-causing agent and its resistance profile would make it possible for physicians to reduce the use of broad-spectrum antibiotics and encourage the development of narrowly-targeted therapeutics. (Antimicrobial workshop recommendation A-1.2)
- Diagnostic profiles that describe the immune status of the patient could make it possible to predict how effective different treatments will be and target immunomodulatory drugs to the right patients at the right time. (Immunomodulation workshop recommendation I-7.2)

Both workshops also suggested that many of the techniques currently used to evaluate antimicrobial compounds are imperfect. Testing antimicrobial compounds against pure cultures under ideal laboratory growth conditions does not reflect the reality of pathogens competing against the natural microbiota in human tissue and under pressure from the immune system. Mice are imperfect models of humans, but developing and validating alternative animal models is difficult and expensive. It is also difficult to design and evaluate clinical trials of compounds that affect the highly complex and individually variable immune system.

Finally, both committees recognized that dividing the task into antimicrobial versus immunomodulatory approaches made it difficult to discuss some very promising ideas. For example, the immunomodulation committee noted that many immunomodulators may not be able to cure disease directly, but could be effective in combination with traditional antimicrobials. Participants in the antimicrobial workshop explored the idea of developing antibiotics that would be selectively activated through interaction with the compounds used by the immune system to signal damage. Future discussions of infectious disease treatments that target the disease-causing agent and enhance the immune response at the same time could generate even more promising ideas.

Challenges for the Development of New Antimicrobials—Rethinking the Approaches: Report of a Workshop

Committee on New Directions in the Study of Antimicrobial Therapeutics: New Classes of Antimicrobials

INTRODUCTION

In 1974, Lewis Thomas described the highest form of medical technology as "the kind that is so effective that it seems to attract the least public notice; it has come to be taken for granted . . . [and is] exemplified best by . . . the contemporary use of antibiotics and chemotherapy for bacterial infections . . . [which] comes as the result of a genuine understanding of disease mechanisms" (Thomas 1974, pp. 34-35). This pronouncement was overoptimistic and premature. Our understanding of the ability of microorganisms to evade modern chemotherapy and to evolve strategies for inactivating our most potent antibiotics was in fact rudimentary, and we are now faced with substantial infectious-disease challenges. In the face of newly emerging infectious organisms, the global crisis in antibiotic resistance, and the threat of bioterrorism, there is a need to invigorate the basic science and technology of anti-infective chemotherapy. To do so, the mechanisms of infectious disease must be better understood, based on a deeper appreciation of microbial physiology, a comprehensive understanding of antibiotic resistance, and a renewed commitment to the discovery of novel antimicrobial molecules and therapies.

There are several indications that new approaches are required to combat emerging infections and the global spread of drug-resistant bacterial pathogens. One is the pattern in rates of death from infectious disease in the 20th century: from 1900 to 1980, the rate dropped from 797 per

100,000 people to 36 per 100,000 people, a reduction by a factor of more than 20 and a testament in part to the efficacy of antibiotics (Armstrong et al. 1999). However, from 1980 to 2000, that rate doubled, largely because of HIV but also due to the spread of drug-resistant bacterial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, multiple-drug-resistant gram-negative bacteria, and multiple-drug-resistant tuberculosis (Cohen 2000). While the rise in mortality is due partly to infection in more seriously ill or immunocompromised patients, there is no doubting the need for new strategies and new molecules to treat pathogens that are resistant to nearly the full array of contemporary antibiotics. We are at a critical point, not seen since the pre-antibiotic era, at which infections caused by some bacterial pathogens are untreatable.

A second indication of the need for novel antibacterial therapeutics is the almost 40-year innovation gap between introductions of new molecular classes of antibiotics: fluoroquinolones in 1962 and the oxazolidinone linezolid in 2000 (Walsh 2003a,b). A third indication is the recent trend by several large pharmaceutical companies to leave the antibacterial and antifungal therapeutic arenas, suggesting a future decrease in scientific expertise in antibacterial-drug discovery and development skills (Projan 2003; Shlaes 2003). A technology gap is developing and widening, as research on and development of new antimicrobial agents are being de-emphasized or abandoned by many pharmaceutical companies.

Treatment of microbial infections—bacterial, fungal, and viral—selects for the emergence of resistant organisms that may be rare in the initial population but become increasingly prevalent under selective drug pressure. In fact, the presence of an antibiotic can accelerate mutation and recombination in bacterial populations and contribute directly to its own obsolescence (Cirz et al. 2005). This is in addition to resistance that may develop outside of the clinical setting; for example, resistance to penicillin had been documented even before its first widespread clinical use (Abraham and Chain 1988). Resistance is prevalent, heritable, and ancient.

The need for new generations of anti-infective agents, and in particular new antibacterial agents, is constant, as the emergence of resistance is largely a question of when and not if. Medicinal chemists have been highly successful over the last 50 years in reshaping the scaffolds of earlier antibiotics, both natural and synthetic; for example, current antibiotics include the fourth generation of beta lactams and the third generation of

macrolides. However, significantly new approaches and strategies for breakthrough molecules have not been forthcoming.

Antibiotic resistance affects more than one or a few patients: the global accumulation of resistant bacteria threatens everyone's health. Once a problem associated only with the sickest patients in intensive-care wards, antibiotic-resistant bacteria have become widespread in communities throughout the world. Resistance genes are not distributed randomly in bacterial populations but are commonly clustered in multiple-drug-resistant strains with resistance spread together. The frequency of international travel, combined with the lack of worldwide standards of antibiotic use, exacerbates the problem. The result is an acceleration of the spread of resistance around the globe and in every environment. All stakeholders recognize that the current antibiotic-resistance crisis is associated with a predictable, inexorable loss of efficacy of our current antimicrobial arsenal, but substantial economic, regulatory, and scientific barriers to the development of new antimicrobial agents and therapies persist (Nathan 2004).

This report arises from extensive discussions at a brainstorming workshop organized by the National Research Council of the National Academies under the sponsorship of the National Institute of Allergy and Infectious Diseases. This workshop was planned by the Committee on New Directions in the Study of Antimicrobial Therapeutics: New Classes of Antimicrobials (see Appendix B). Some 40 persons attended the workshop, held on May 23-24, 2005, in Washington, DC, to address strategies for new generations of antimicrobials (see Appendix C for a workshop agenda and participant list). The committee felt that identification of a class of antimicrobials that would be effective in the treatment of a full range of microorganisms—bacteria, viruses, and fungi—without also being detrimental to the host was unlikely. Thus, the workshop was structured around the development of antibiotics that would be effective against bacteria. However, several of the ideas described in this report (e.g., employing natural microbiota to combat pathogens) might also be applicable as treatment strategies against viruses and fungi. The accompanying report on immunomodulation offers additional discussion on treatments that might be effective against the wider range of microorganisms.

This report has four major sections: a discussion of the challenge of antibiotic resistance at the population and molecular levels, the importance of understanding bacterial communities and resident microbiota for the discovery of new antimicrobial therapies, consideration of biological processes that can guide strategic approaches to antibiotic development, and

strategies for discovering new natural and synthetic molecules, including novel screening approaches to bacterial targets. It is hoped that his report will help guide the next decade of antimicrobial research and development.

ANTIBIOTIC RESISTANCE

Antibiotic Resistance Is Inevitable

The goal of the workshop was to identify novel approaches to the development of antimicrobial therapeutics. However, workshop discussions made it clear that even the most innovative antibiotics will be made obsolete, at some point, by the inevitable emergence of resistance. Therefore, the committee concluded that it is worthwhile to identify research that would help to surmount the problem of resistance or at least slow its emergence. The recommendations in this section, although they do not lead directly to the development of novel antibiotics, could be important in increasing the useful lifespan of current and future antibiotics.

Bacteria predate humans by billions of years and have evolved a complex series of coping mechanisms that enable them to survive under harsh conditions and in the presence of numerous toxic metabolites. Most antibiotics discovered during the golden age of antibiotics (about 1945-1960) are natural products, produced for the most part by bacteria themselves (Clardy and Walsh 2004). These bioactive secondary metabolites—so called because they are not essential for cell growth or reproduction—may be produced by bacteria to provide a competitive growth advantage by killing susceptible neighbors in the environment, or they may be signaling molecules that have other functions and whose antibiotic activity at high dosages is a side effect. In either case, organisms that produce antibiotics—and organisms that have evolved to live near them—harbor specific and potent resistance strategies that inactivate or otherwise protect them from the antibiotics' toxic effects.

Synthetic antibiotics, such as the fluoroquinolones, would appear to be less susceptible to causing resistance, given that bacteria would not have had millennia of exposure to them. Thus, evolved mechanisms of resistance would be less likely to exist. However, ubiquitous and promiscuous efflux systems have evolved to protect microorganisms from diverse toxic small molecules of natural origin, and these systems often provide cross-protection against such non-natural products. As a result, genes that encode resis-

tance elements are embedded in the genomes of virtually all bacteria; these hard-wired resistance genes are inherited in vertical fashion, providing continuous protection against toxic agents in a bacterial species even in the absence of prior exposure.

Antibiotic-resistance genes, however, are not confined to bacterial genomes. They are also frequently found on mobile genetic elements (plasmids, transposons, and integrons) that readily pass horizontally from organism to organism, even across species boundaries, thereby circumventing the standard parent-to-progeny route of genetic flow (Levy and Marshall 2004). The frequency of selection for such events and for the acquisition of genetic elements increases with increased exposure to antibiotics. It is therefore not surprising that locales in which antibiotic use is rampant—such as hospitals, farms, and child-care settings—are prime sources of organisms that harbor these genetic vehicles. Furthermore, genetic elements passed between organisms in this way often collect several resistance genes; this process is, again, selected for by increased antibiotic use that has given rise to multiple-drug-resistant (MDR) organisms, some of which are untreatable—or nearly so—with the current arsenal of approved drugs. MDR organisms have changed from being primarily a health-care sector problem to being a source of community-acquired disease as patients return to their homes earlier than previously, often remaining on courses of antibiotics.

Antibiotic Resistance Is Manageable

1. *Predicting Resistance*

The inevitability of resistance is well accepted by researchers in the field, but there are barriers to collecting and sharing data on resistance among diverse geographic centers and among individual health-care settings within a single geographic region. Efforts to overcome technical and jurisdictional obstacles will improve the ability to monitor resistance, anticipate its spread, and inform health-care practitioners of its existence in the area.

Antimicrobial resistance grows as strains of bacteria that carry and exchange resistance genes spread throughout a population or region. Knowledge of resistance in bacteria from prior infections thus helps to target both treatment of new infections and efforts to contain resistance locally and globally as information about recent infections can anticipate antimicrobial

resistance in new situations. Tens of thousands of clinical and basic research laboratories throughout the world generate resistance data. But very few labs submit these data to appropriate databases that could allow local analysis or linking with a surveillance network.

The effectiveness of surveillance data can be enhanced by integrating with other types of information. For instance, molecular studies of resistance can help explain observed resistance phenotypes. Comparison with data on antibiotic usage allows estimation of and potential for the management of antibiotic selection. Data on resistance in non-pathogenic organisms, a potential reservoir of new resistance genes, could help anticipate the emergence of new resistance and to develop predictive diagnostics prospectively.

Surveillance of resistance can and should build on existing resources. Clinical laboratories in more than eighty countries have begun to build databases and link them into international networks using free software (WHONET) downloadable from a World Health Organization Web site (O'Brien et al. 2001).[1] This type of surveillance data can be complemented and cross-validated by data on isolates collected from clinical laboratories for selected studies in public health reference laboratories or in those supported by pharmaceutical companies. Several companies now collaborate with the Alliance for the Prudent Use of Antibiotics (APUA) to merge their data for these types of meta-analyses (Stelling et al. 2005). Obviously, data in such a database should be as up-to-date as possible and thus able to identify pockets of resistance as they occur.

Surveillance needs to be implemented on a grand scale and over a long period to identify trends and provide data for population studies. The surveillance network should not only be nationwide, but linked to international efforts to integrate worldwide data seamlessly. Surveillance should not be limited to the health-care sector. Mechanisms of resistance to any new antibiotic may already exist in nature, so any resistance encountered in nonpathogenic organisms in the environment or antibiotic producers should also be entered into the database. Clinicians and developers of diagnostics would then be aware of resistance mechanisms that may be encountered in the clinic. Such an integrated database would greatly enhance the ability to develop predictive diagnostics that could be rapidly brought on line as resistance elements move around the globe.

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[1]Available at <http://www.who.int/drugresistance/whonetsoftware> at the time of publication.

2. Detecting Resistance

Detection of resistance is rooted largely in the century-old technology of growth susceptibility. As a whole, medical microbiology has not adopted state-of-the-art molecular diagnostic measures, and the barriers to gene- or protein-based diagnostics have been substantial. First, in non-sterile sites, such as skin and the gastrointestinal tract, identifying the specific agent causing disease is difficult; even in sterile tissues, such as blood, present-day detection methods are often not sensitive enough to detect disease-causing organisms.

Second, the cost associated with molecular tests is often prohibitive. Nevertheless, improved diagnostics could have a revolutionary effect. For example, if a physician could know at the bedside which organism is causing a particular infection and whether that organism is resistant to common antibiotics, treatment could be tailored appropriately. Consequently, antibiotics would be used in a specific fashion, selecting only those likely to be effective; this procedure of judicious and specific antibiotic use would thus help extend the useful lifetime of new antibiotics.

Diagnostics able to identify the etiology and antimicrobial susceptibility of all infections could target therapy precisely and eliminate the use of antibacterial agents in patients who do not even have a bacterial infection. If they are done early, such tests could avoid untargeted therapy during the days needed by current diagnostics. In a recent study, polymerase-chain-reaction testing took 6 hours to identify the etiology of 76% of community-acquired pneumonia cases, while older tests took several days to identify 49.5% (Templeton et al. 2005).

Specifically, research into diagnostic tests that can reliably and quickly identify pathogenic organisms and their resistance profiles should be encouraged. Development of such tests may be difficult but could lead to significant advancement in the treatment of infectious disease. To effect the greatest reduction in inappropriate antibiotic use, such tests would need to be so rapid and reliable that clinicians would be comfortable waiting for the results before beginning antibiotic treatment. Determining the necessary degree of reliability for these diagnoses is an open challenge as the tests must not only lead to successful diagnosis of the pathogen, but also have the confidence of clinicians.

A major issue in resistance is that not only disease-causing organisms, but also other resident organisms and the host itself are exposed to an antibiotic. Minimizing exposure through precise choice of antibiotic is critical

in preventing the emergence of resistance by reducing selection in off-target organisms. That is, the use of a narrow-spectrum antimicrobial agent optimized for use against the disease-causing organism would be less likely to select for resistance in non-targeted microorganisms. Advanced diagnostics discussed above will facilitate tight targeting of pathogens and thereby enable the productive exploration of target-specific antibiotics. The advances could include selective interruption of organism-specific processes, such as virulence mechanisms, adhesion of surface antigens, and resistance mechanisms. Enhancing the host response at the site of infection is a potential creative approach to activating toxic molecules where they are needed. New tissue-specific delivery vehicles would greatly help to decrease the exposure of non-target species to antibiotics. However, it should be noted that these strategies will not eliminate resistance as resistant microorganisms also arise from the use of antibiotics in non-clinical settings such as agricultural use.

3. Deterring Resistance

How resistance elements are selected and spread throughout microbial communities is largely unknown. Understanding the fundamental principles underlying how pathogenic organisms and normal microbiota communicate and exchange genetic information is a key to the ability to manage the spread of resistance.

Research on the molecular mechanisms that facilitate resistance is also warranted. For example, do some antimicrobial agents inherently activate mutagenic pathways that can lead to resistance (e.g., Cirz et al. 2005)? If so, are there classes of molecules that are less susceptible to this action? Similarly, are there antimicrobial targets that are less tolerant of mutations selected by the presence of antibiotics?

Furthermore, how such issues as antibiotic dosage and scheduling, antibiotic mixtures, and interactions with other drugs affect the emergence of resistance is not well understood. The example of amoxicillin/clavulanate potassium (Augmentin), a highly successful combination of an antibiotic and an inhibitor of resistance, should be emulated (Matti et al. 1998). Combination therapy to inhibit the emergence of resistance has also been used in the treatment of HIV (HAART therapy) and tuberculosis (isoniazid, rifampin, and pyrazinamide) (Finch et al. 2003). Leveraging knowledge of molecular mechanisms of resistance in the development of selective inhibitors has the potential to rescue the activity of proven antibiotics that

have well-established pharmacological and disease profiles (Wright 2000). Extending the clinical lifetimes of proven antibiotics in this fashion holds great promise. The challenge will be to selectively target the most important resistance mechanisms.

Recommendations on Resistance

To respond to issues of resistance to antimicrobials, the committee recommends the following research directions and action items:

- **A-1.1 Establishment of a simple and readily searchable antibiotic resistance database into which participating institutions would upload resistance data in real time.**
- **A-1.2 New rapid diagnostics to detect pathogens and their resistance to inform therapy in real time.**
- **A-1.3 Development of strategies that will selectively target pathogenic organisms while avoiding targeting the host and beneficial or benign organisms.**
- **A-1.4 Identifying the sources of resistance mechanisms, their evolution, and the ways in which they are spread in microbial communities, to elucidate the various ways in which resistance can be manifest.**
- **A-1.5 Development of strategies that target and selectively block antibiotic resistance mechanisms to rescue antibiotic activity.**
- **A-1.6 Exploration of the effect of antibiotic usage, alone and in combination, on the development of resistance.**

A MICROBIAL COMMUNITY APPROACH TO NEW ANTIBACTERIALS

Characterization of Communities of Microbiota

There is growing evidence of the important role played by resident microbiota in offering protection from infectious disease. Rather than continuing the traditional approach of killing bacteria wherever they occur, there is a need to develop new antimicrobial strategies aimed at subtle manipulation of bacterial behavior. Such therapies would favor natural host defenses and the maintenance of the normal microbiota to keep growth of

pathogenic species in check. At the outset, design of strategies for novel antibiotics should include exploration of strategies for exploiting beneficial and commensal bacteria in fighting infections in sites where normal microbiota reside.

To develop such therapies, a deeper understanding of the diversity and ecology of the normal human microbiota and how these communities are established and stably maintained is needed. At present, understanding of human microbiota communities and their true diversity and ecology is limited (Eckburg et al. 2005; Hooper and Gordon 2001; Wilson 2005; Nataro et al. 2005). Precise definition of these associations in human health and in disease will allow the development of nontraditional therapeutics aimed at manipulating bacteria and their environment to enhance the maintenance and proliferation of the normal microbiota and inhibit the growth of pathogens.

The committee recommends the following to deepen understanding of natural microbiota:[2]

- **A-2.1 Characterization and enumeration of the normal resident microbiota in human hosts.**
- **A-2.2 Understanding the relationship between resident microbiota populations and human health.**

Manipulating Bacterial Signaling and Communication

The last decade has taught that bacteria do not live independent lives but, rather, communicate within and among species by using a variety of secreted signal molecules (Miller and Bassler 2001). Production, detection and response to these molecules allow bacteria to take a census of the population and synchronize behavior on a population-wide scale. This process, called quorum sensing, is critical for many pathogens because expressing virulence genes as a group ensures that pathogenicity factors are released only when bacterial numbers are sufficient to guarantee success against the host (Donabedian 2003; Williams 2002). More complete understanding of the chemicals that bacteria use for signaling and how bacteria integrate and interpret chemical information in their environment would allow investigation of their use in antibacterial treatments.

[2]These recommendations are similar to those in the accompanying report on immunomodulation (e.g., recommendation I-6.1).

At present, only four predominant classes of molecules used for communication are known: acylhomoserine lactones in gram-negative bacteria (Parsek et al. 1999), oligopeptides in gram-positive bacteria (Lazazzera and Grossman 1998), γ-butyrolactones in the streptomycete subset of gram-positive bacteria (Chater and Horinouchi 2003), and a furanone called AI-2 that is used for signaling in diverse bacterial species (Miller et al. 2004; Chen et al. 2002). The chemical lexicon is probably much larger than is currently recognized, and a continued study of cell-cell signaling with an emphasis on further definition of the chemical moieties used should reveal new classes of molecules that convey information about the community.

Manipulation of bacterial cell-cell signaling systems has potential use in novel antimicrobial therapies (Williams 2002; Dong et al. 2001). Enhancing growth-promotion signals of the normal microbiota at the expense of non-indigenous species might restore the normal microbial balanced state. Alternatively, specifically interrupting signaling between pathogens or giving improper signals might cripple the pathogens and make them easier to kill with standard antibiotics or by the immune system. Chemical communication between bacteria is critical for establishing and maintaining complex structured communities, such as biofilms (Davis et al. 1998; Costerton et al. 1994). Disruption of cell-cell signaling systems might provide novel opportunities for antibiotic therapy (Hentzer et al. 2003; Merritt et al. 2003; Ren et al. 2002). Furthermore, it is possible that the host recognizes and responds to bacterial signaling molecules (Chun et al. 2004), and understanding whether and how this occurs could lead to therapies for priming or boosting host defenses.

Beyond chemicals used for quorum-sensing cell-cell communication, bacteria make and release a rich variety of compounds, and enormous amounts of information could be encoded in these molecules. Bacteria probably interpret these compounds for important information about the species composition of the environment, the growth conditions, the vitality of the community, and so on. Streptomycetes are known to produce an extraordinary collection of so-called secondary metabolites (Bibb 2005). These chemicals have been and continue to be mined for those with desired activities, such as anticancer properties. The compounds are viewed as a rich storehouse of novel pharmaceuticals, but why are the bacteria making and releasing them? How do the bacteria recognize and respond to the information encoded in these chemicals? Normal human microbiota might not be as prolific in chemical production and release as the streptomycetes, but they also release complex chemical mixtures, and current understand-

ing of these molecules is inadequate. Identification and characterization of the molecules present and knowledge of how they can affect bacteria may allow researchers to begin to manipulate the chemical environment of bacteria. Such manipulation could trigger the growth of indigenous species for repopulation during or after infection or inhibit the growth or modulate the behavior of pathogens.

The committee recommends several research areas related to bacterial communication:

- **A-3.1 Identification of the signals and signal-transduction systems in bacterial communication.**
- **A-3.2 Determination of whether and how the resident microbiota and pathogens communicate with host cells and respond to the immune system.**
- **A-3.3 Development of strategies to manipulate chemical signaling in the host microbiota and pathogens.**

Probiotic Therapies

Anecdotal information suggests that the microbiota plays an active role in defense against pathogens. For example, treatment of *Salmonella* infections with antibiotics that kill both salmonellae and the normal microbiota increases the longevity of the carrier state. This indicates that the normal microbiota participates in eliminating salmonellae (Neill et al. 1991). Unfortunately, as discussed above, the composition of the human normal microbiota is poorly defined (Hooper and Gordon 2001). In fact, the vast majority of the species present in normal human microbiota have never been cultivated. Enhanced knowledge of normal microbiota could be used to invent ecologically-based therapies that favor proliferation of beneficial organisms and limitation of pathogens (Baker 2005). Probiotic therapies that enhance the metabolic and signaling activities of beneficial bacteria need to be rigorously studied and tested.

Probiotic strategies aimed at ecological control, rather than at killing bacteria, could have the added benefit of lowering the spread of community-acquired drug-resistant bacteria. For example, the spread of MRSA is a major problem in communities of people who come into contact with others who are being aggressively treated for the resistant organism (Saravolatz et al. 1982; CDC 1981; Goetz et al. 1999). It can be envisioned that not

only the patients treated with traditional antibiotic therapies be followed up with probiotic therapies, but also targeted, healthy human populations. The purpose would be to reduce the distribution of antibiotic-resistant bacteria in communities that are at risk for the spread of drug-resistant bacteria (such as sports teams, jail populations, and nurses). Such probiotic therapies could extend the lifespan of traditional broad-spectrum antibiotics because their use in the healthy community essentially converts broad-spectrum drugs into narrow-spectrum ones. It should be noted that immunocompromised patients are likely to have a different response to probiotic therapies than healthy individuals, so the needs of different populations and individuals should be considered.

The committee recommends the following research areas in the use of probiotic therapies:

- **A-4.1 Research to determine which bacteria should be used in probiotic therapies.**
- **A-4.2 Research to identify delivery mechanisms that would be most effective for probiotic therapies.**

UNDERSTANDING BIOLOGICAL PROCESSES TO DEVISE NEW ANTIBIOTICS

Routes to anti-infective research and development can differ dramatically depending on how the goals are articulated. At first glance, there would seem to be only one criterion for a successful anti-infective chemotherapeutic: an agent with excellent pharmacologic properties that kills or inhibits disease-causing microorganisms without harm to the host. Traditionally, this has meant a broad-spectrum drug that kills a wide array of pathogens. This criterion has limited the number of suitable drugs and the strategies used to discover them.

Advances in molecular diagnostics may support greater reliance on narrow-spectrum antimicrobials and allow clinicians to make a specific diagnosis of infection before choosing a therapy. Perhaps even more important, it may be possible to explicitly exclude specific etiologic agents and obviate the use of broad-spectrum treatments in case the cause of the infection was not as originally suspected. Such technology is likely to be available for hospitalized patients thought to have infections of body compartments that

are normally sterile, such as blood and cerebrospinal fluid. The technical challenges are greater in working with samples that are normally polymicrobial, such as sputum.

The aims of new criteria for successful anti-infective chemotherapeutics are to preserve the efficacy of each agent as long as possible by delaying the emergence of drug resistance and to spare the normal microbiota as much as possible. The normal microbiota is viewed as containing invaluable allies in combating microbial pathogenesis by protecting niches against new microbial competitors and sustaining the species diversity that impedes virulence (Foster 2005) and by helping to preserve the integrity and function of epithelia and the immune system (Rakoff-Nahoum et al. 2004; Hooper et al. 2001).

With these considerations in mind, the following additional criteria for successful anti-infective chemotherapy are offered. Some agents should have a broad spectrum and others a moderate or narrow spectrum, but in each case, their use should be restricted to specifically defined human populations so that they target as few microbial species as the clinical situation warrants. Combination therapy should be encouraged, but each drug in a combination need not be required to kill microorganisms on its own.

Biological Understanding Required to Intervene in Microbial Pathogenesis

Antibiotic development has focused on the identification of "essential" targets whose inhibition is lethal under conditions of maximal microbial proliferation. A fresh approach would be to revise the operational definition of essentiality so that it more accurately reflects the biological reality: Which genes are essential to the pathogen in vitro under conditions that are relevant in the host? Which genes are essential to the pathogen in specific host environments, including polymicrobial communities on epithelial surfaces, where the microorganism of interest may represent a relatively minor planktonic population; in monomicrobial populations deep in tissues, where the pathogen may attain high population densities; and in biofilms in either kind of site? (If genes expressed in a pathogen only when it is the sole microbial species yield products that are needed for pathogenesis, targeting their products could spare the normal microbiota.) Which microbial genes are essential in combination, in such a way that the joint inhibition of their products produces synthetic lethality?

An even more drastic departure from convention is to set aside the

issue of essentiality and ask if and how bacterial entrance to or withdrawal from the cell cycle, production of virulence factors and toxins, exchange or mutation of DNA, and activation of programmed cell death can be affected. These questions should be answered in the context of understanding how bacteria communicate with others that occupy the same environment and are of the same and other species. Of particular interest are microbial decision pathways that have evolved over long periods under high selective pressures. The biology of stress responses and the DNA exchange and mutagenic DNA repair processes that contribute to antibiotic resistance may be instructive (Cirz et al. 2005). A better understanding of microbial regulatory pathways, including identification of global regulators and natural chemical signals that activate them, would also be helpful. When antibiotic producers and resisters compete in natural environments, what controls the dynamic of the relationship so that neither prevails? Finally, attention needs to be paid not just to microorganisms themselves, but also their "mobilomes"—the genomes and transcriptomes of their phages, plasmids, and integrons.

Tools needed to study these questions include in vitro culture systems with pathophysiologically-relevant concentrations of oxygen, iron, and carbon sources; biologically-relevant growth surfaces; and, in some cases, mixed microbial populations with systems-biological approaches to the analysis of prokaryotes in microbial communities.

Alternatives to Direct Killing of Microorganisms

The standard goal of eliminating disease-causing microorganisms without harming the host can be complemented by an alternative goal of simply suppressing their pathogenic behavior. In immunocompetent hosts, the latter goal may be enough to allow the immune system to control less serious infections, which account for most antibiotic prescriptions. In immunocompromised hosts or those with life-threatening infections, it may be optimal to combine a bactericidal antibiotic and an inhibitor of bacterial regulatory pathways that controls stress responses.

In any case, killing all pathogens with an extrinsic antimicrobial agent may not be necessary or even desirable. For example, the eponymous gold pigment of *Staphylococcus aureus* is a carotenoid that protects the bacterium from oxidative injury by host immune cells. Interruption of bacterial pigment synthesis makes the pathogen much easier for host cells to kill but does not impair bacterial growth in vitro (Liu et al. 2005). Simply limiting

the spread of the pathogen may be enough for the host microbiota to outcompete the invader.

An anti-infective agent that does not kill is less likely to select for drug resistance as the selection pressure is less intense. Identification of such agents is difficult, however, because such a target would be missed by conventional strategies that seek inhibitors of enzymes that are essential for survival of diverse species of pathogens in vitro. For example, identifying a potential new target for antibiotic development such as the synthesis of the staphylococcal pigment mentioned above that is involved solely in (a) the virulent behavior of (b) a single bacterial species (c) in vivo would probably be missed by conventional strategies that seek inhibitors of enzymes that are (a) essential for survival of (b) diverse species of pathogens (c) in vitro.

Rather than seeking inhibitors, one could seek to develop chemical agents that mimic a natural signal activating a regulatory pathway counterproductive to the pathogen. For example, an agent that selectively triggers a program of replication in a pathogen might be used in conjunction with an antibiotic that inhibits microbial protein synthesis. Protein-synthesis inhibitors are generally bacteriostatic but might be selectively bactericidal to a species whose proliferative program is induced. Combating infectious disease by interfering with microbial signaling mimics a major strategy used by the immune system.

Targeting bacterial invasion or colonization rather than bacterial survival may be a valid strategy for prophylactic intervention in specific host populations, such as those in intensive-care units and burn wards rife with drug-resistant bacteria and those in dormitories or barracks during outbreaks of meningococcal meningitis.

It is futile to target some aspects of microbial pathogenesis that are manifest only in early stages of disease, such as colonization and transepithelial invasion, if the patient is not diagnosed until the time of advanced disease.

Most traditional anti-infectives work by blocking enzymes, but we may also be able to inhibit bacterial decision pathways controlled by transcription factors that mediate protein-protein and protein-nucleic acid interactions. For example, it may be possible to exploit the dynamic turnover critical to such functions by identifying compounds that *stabilize* such interactions.

Strategic Approaches to Discovery of Anti-infectives for Use in Combinations

Broad new classes of targets for antibiotics have been described elsewhere (Nathan 2004). Some are enzymes involved in the synthesis of products not targeted by antibiotics in current use, such as isoprenoids (Walsh 2003a) or those involved in ATP generation (Andries et al. 2005). Others dispense with inhibition of biosynthesis altogether and focus on other aspects of the life cycle of macromolecules, such as their repair and degradation (Nathan 2004) or secretion (Brown and Wright 2005). Here, we focus on an even broader question: how to exploit synthetic lethality through combination chemotherapy. A striking example was recently reported in *Mycobacterium tuberculosis* through the joint disruption of two isocitrate lyases (Muñoz-Elías and McKinney 2005).

One may create a library of mutants of the pathogen in which each gene has been conditionally disrupted. Each clone in this library can be subjected to saturation signature-tagged transposon mutagenesis (Hensel et al. 1995) to generate a sublibrary. Microarray hybridization techniques can be used to identify the genes in the sublibrary that are essential for the microorganism to survive. This determination can be made in vitro under diverse conditions and ex vivo after recovery of surviving elements of the sublibrary from experimentally infected animals (Sassetti and Rubin 2003).

Another approach is to seek synthetic lethality in the disruption of a given gene in the presence of an antibiotic. For example, a workshop participant noted that there are transposon-induced null mutations in bacterial genes that impart a counterintuitive fitness *increase* in the presence of antibiotics. These mutations may impair a programmed bacterial cell-death response to the antibiotics. Such mutations can be introduced into the parental strain, and the new mutant can be used for saturation transposon mutagenesis to discover the genes that, when disabled, convert the antibiotic-resistant strain into an antibiotic-sensitive one.

Still another approach to synthetic lethality is to use one molecule that is already known to be an antibiotic and to screen for another that inhibits the efflux pumps that transport the antibiotic out of the cell. Such pumps limit the effectiveness of the antibiotic, thereby anticipating a possible mechanism of resistance. It may even be practical to target the small number of ATP synthases that power the pumps rather than attempt to block the pumps directly. At the same time, chemoinformatic tools are needed to

identify features of compounds that improve their likelihood of accumulating in bacteria through improved entry and diminished efflux.

In some organisms, such as *M. tuberculosis*, antibiotic resistance is thought to arise exclusively from heritable mutation of chromosomal genes, not from lateral gene transfer. Even in pathogens that are adept at lateral gene transfer, such as *E. coli*, diverse antibiotics may induce resistance to others by activating a stress response that includes error-prone DNA repair and leads to chromosomal mutagenesis (Cirz et al. 2005). Combination chemotherapy in such cases could include an inhibitor of the critical error-prone translesional DNA polymerase with dual goals: to kill the pathogen more effectively and to block emergence of drug resistance in surviving cells (Boshoff et al. 2003).

Most radically, it may sometimes be possible to include the product of a host gene as one target in an effective combination therapy. Host-gene products serve as receptors for microorganisms and their toxins, for the trafficking of intracellular pathogens to replicative niches, and other processes that contribute to pathogenesis. What makes the inclusion of a temporarily nonessential host-gene product attractive as a target for combination therapy is that, unlike the microorganism, the host will not mutate to a resistant state. Examples of targets of host rather than microbial origin are the receptors for toxins of *Bacillus anthracis*; POSH, a ubiquitin protein ligase (Alroy et al. 2005) and ATM, a protein kinase (Lau et al. 2005), both required for replication of HIV; CCR5/CXCR4 antagonists to limit the replication of HIV (Princen et al. 2004); ErbB, a protein kinase required for replication of vaccinia virus (Yang et al. 2005); and Abl, a protein kinase required for dissemination of vaccinia virus (Reeves et al. 2005).

In the area of identifying targets, the committee recommends the following:

- **A-5.1 Screening for antibiotics in vitro under conditions that are relevant in the host.**
- **A-5.2 Searching for agents that exhibit synthetic lethality.**
- **A-5.3 Learning enough about bacterial behavior to influence actions crucial to disease and drug resistance, for example, by mimicking or blocking bacterial signals.**
- **A-5.4 Exploring inhibition of host targets to thwart infectious disease.**

NEED FOR NEW MOLECULES

New Molecules: Natural and Synthetic

Given the long gap in the introduction of new structural classes of antibiotics—38 years between streptogramins in 1962 and linezolid in 2000 (Walsh 2003a,b)—and the inexorable development of resistance to a given antibiotic once it is in widespread clinical use, there is a pressing and recurrent need for new molecules with antibiotic properties. Historically, two lines of discovery have been fruitful: natural products with antibiotic activity and synthetic antibacterial agents. Penicillins, cephalosporins, vancomycin, tetracycline, and aminoglycosides are in the first category, and fluoroquinolones, sulfonamides, and oxazolidinones are in the second. Natural and synthetic molecules are both likely to remain important sources for new antibiotics but offer distinct challenges.

Mining the Natural World: Discover, Diversify, and Deliver

Microorganisms themselves have been the richest source of antibiotics; with 99% of the known microbial species as yet uncultured, these clearly are untapped sources of novel molecules. Realization of their potential requires attention to new techniques for microbial cultivation—including consortia—and exploration of new biological niches of microorganisms. Each time a new set of biological microenvironments has been accessed by chemists, new classes of bioactive natural products have been isolated and structures determined (Koehn and Carter 2005; Clardy and Walsh 2004). A recent example is the isolation of abyssomicin C, which inhibits folate biosynthesis in MRSA; the molecule is produced by the rare actinomycete *Verrucosispora* collected in a sediment sample from the Japanese sea at a depth of 867 ft (Bister et al. 2004). The emerging field of metagenomics, in which the pooled genetic material of a bacterial community is sequenced without cultivating each individual member, offers the possibility of identifying novel product and biosynthetic pathways (Handelsman 2005).

Although currently unknown or unculturable bacteria are likely to be the source of novel bioactive molecules, even bacteria that are routinely grown in the laboratory may have additional capabilities that are not expressed under standard laboratory conditions. There should be an emphasis on deciphering previously unrecognized secondary metabolite pathways in bacterial genomes and in eliciting the production of end products from

metabolic pathways that are cryptic when producer organisms are grown in standard culture conditions. For example, only three of the predicted 25 polyketide synthase biosynthetic gene clusters in *Streptomyces avermitilis* have been shown to be active (Omura et al. 2001). Similarly, several sets of antibiotic gene clusters in *Bacillus subtilis* are expressed only in starvation-induced conditions not generally present in laboratory cultures (Stein 2005).

Continued, even increased, attention should be paid to natural strategies used by prokaryotes and eukaryotes against microorganisms. Hosts respond to microbial infection by secretion of peptidic molecules, such as defensins, to act locally. Narrow-spectrum protein toxins (bacteriocins) are a predominant strategy in natural microbial communities for killing neighboring strains; for example, bacteriocidic microcins secreted by one strain of *E. coli* kill neighboring strains selectively but have minimal effect on the microbial community. This effect—selectively removing pathogens while leaving commensal bacteria unharmed—would be a desired feature of antibiotics. Efforts should be focused on delivery strategies for peptides, bacteriocins, and phage-based lytic proteins (Yoong et al. 2004).

Bacteria produce bioactive molecules through a series of biosynthetic steps. The idea behind combinatorial biosynthesis is to break the biosynthetic pathways down into modules and combine the modules in a well-characterized host to generate novel end products. The ability to express biosynthetic-pathway genes in heterologous hosts will be required for efficient combinatorial biosynthesis. Ultimately, full realization of the potential of combinatorial biosynthesis will require engineering bacteria that can make the monomeric building blocks that are required for running the assembly lines and expressing a full range of post-assembly-line tailoring enzymes. Ideally, the ability to shuffle protein domains and modules in the polyketide synthase and nonribosomal peptide synthetase pathways and to engineer intersections with terpenoid and other pathways to merge other chemical frameworks onto polyketide and peptide scaffolds will be needed for maximizing natural diversity (Walsh 2004).

Finally, further investigation of the molecular biology underlying bacterial cell death is needed. In most cases, there is little understanding of why bacteria that are susceptible to particular antibiotics die or which traditional targets hold more promise for the development of bactericidal antibiotics. Bacteria, like eukaryotes, may have mechanisms of programmed cell death. If so, the genetic regulatory programs and the biochemical processes associated with the triggering of cell death could provide new targets

for antibiotic development (Engelberg-Kulka et al. 2004). Current understanding of bacterial cell death lags behind understanding of this process in higher organisms.

To maximize the discovery of novel natural molecules and to increase the ability to generate variations on natural molecules, the committee recommends the following:

- **A-6.1 Increased sampling in diverse environments and increased application of the techniques of metagenomics to identify bioactive compounds produced by currently unknown and uncultured microorganisms.**
- **A-6.2 The development of novel and varied culture conditions to identify cryptic metabolic pathways in currently cultivated strains.**
- **A-6.3 Increased research on the role of host-derived antimicrobial peptides, phage lytic proteins, and bacteriocins in the ecology of host-bacteria interactions to improve delivery strategies for these natural products.**
- **A-6.4 Increased research on combinatorial biosynthesis to allow the most varied possible uses of the novel biosynthetic pathways found in known and unknown organisms.**
- **A-6.5 Increased research on bacterial cell death, including investigation of programmed cell death and how antibacterials kill to exploit new strategies for the elimination of pathogens.**

Developing Synthetic Molecules: Diversity, Bioactivity, and Specificity

In addition to discovering and elaborating on the bioactive compounds made by bacteria themselves, the design of synthetic antibiotic molecules should also be pursued. The committee identified three kinds of research that would contribute to greater success in the design of synthetic antibiotics: developing techniques that make it easier to generate diversity in synthetic molecules, increasing understanding of the characteristics that allow molecules to enter and remain in cells, and developing the ability to move beyond using growth inhibition as the measure of a compound's activity and achieving a more sophisticated understanding of how compounds affect metabolism.

One of the principles of synthetic-molecule construction is modularity, with variable shape and architecture of modular cores; linkers or con-

nectors that are also variable in shape, length, and polarity; and surface functional groups in each module that allow rapid elaboration. A goal of such modularity is to build on initial leads and elaborate them in any direction for optimization. A further goal would be to minimize blunt ends—scaffold elements that prevent expansion—in any modular array of synthetic molecules. Two promising techniques for generating greater diversity in synthetic-molecular construction are "click chemistry" and programming small molecules genetically.

The "click chemistry" paradigm elaborated by Sharpless and colleagues (Kolb et al. 2001)—such as the coupling of azides and alkynes with copper catalysis in aqueous solution under mild conditions—is a leading example of rapid modular combinatorial chemistry. In favorable cases, the shape of the binding pocket in a target protein can guide covalent couplings into complementarily shaped molecules (Manetsch et al. 2004). The binding of small molecule fragments capable of self-assembly in cavities of target proteins may become a generalizable strategy to produce small-molecule architecture complementary to and with high affinity for target bacterial proteins.

Another promising direction for antimicrobial chemical libraries is genetically programmable small molecules (Li and Liu 2004; Halpin et al. 2004; Halpin and Harbury 2004a,b). DNA tethering can allow enhanced adjacency to promote new, high-yield chemistry and the creation of large libraries from which molecules can be selected for function. In a screen for nanomolar binders to a target bacterial protein, 10^5 promising molecules could be obtained from very large (for example, 10^{12} entries) programmable libraries; these 100,000 winners could be put through a further series of functional screens (such as for whole-cell activity) with the prospect that there would be many with high activity for further structural optimization. A key advantage of the DNA-directed programmable approach is the opportunity to evolve molecules to optimize a selected function.

However, the ability to generate diverse molecules will not be enough. A major bottleneck in drug design and evaluation is the optimization process for turning hits into molecules that will work in the host. What is needed is the ability to move smoothly back and forth between changing chemical structure and activity in the host; computational prediction and rapid preparation of related families of molecules must be integrated with pharmacokinetic measurements. Computational biology and systems biology must become central to the evaluation of new molecular scaffolds in infected animals and humans to predict the safety and efficacy of new classes of molecules.

To increase the ability to generate synthetic molecules that are not only diverse but also bioactive in a predictable way, the committee recommends the following:

- **A-7.1 Development of small-molecule libraries customized for bacterial targets.**
- **A-7.2 Increased research on DNA-directed synthesis of diverse collections of small molecules for screening and selection against bacterial targets.**
- **A-7.3 Greater emphasis on projects that systematically relate chemical structure to biological activity.**

Mining Historical Knowledge

Many pharmaceutical and biotechnology companies that have a history of pursuing the identification of new antibiotics probably have files on the development and testing of molecules that could be mined for promising leads. It might also be valuable to interview those who did the work. The workshop and committee members' experiences have brought forth anecdotes about valuable drugs nearly abandoned because of shifts in corporate policy but then saved by the conviction or special insight of one investigator. Interviews and data mining of now-ignored records might reveal promising molecules on which much work has already been conducted, but that were dropped for reasons unrelated to their efficacy. Other molecules may have encountered obstacles that can now be circumvented by new technology or understanding. Such a historical and biographical approach is unusual in drug development and in biomedical research, but may reinvigorate now-ignored research that showed promise.

Screening Issues: How to Find Functional Properties in Candidate Molecules

If it becomes possible to generate a multitude of diverse molecules, the next challenge is to improve the ability to screen them for antibiotic potential. Cell-based screening, in which the ability of compounds to kill bacteria growing in ideal, monoculture conditions is tested, has the advantage of identifying compounds with the right physical properties to penetrate and persist in cells (and affect their growth). However, because the targets are unknown, it is difficult to predict and test how the activity of the compounds could be enhanced. In contrast, target-based screening identifies

compounds that bind to or inhibit a bacterial target that is believed to be essential for bacterial survival. It has proved challenging to endow compounds that have the desired activity with the ability to penetrate cells and reach their targets. Both target- and cell-based approaches have value, but fresh approaches are needed because of the weaknesses inherent in each (Brown and Wright 2005).

For example, in cell-based screens, it is important to collect more information beyond the single criterion of growth inhibition so that it will be possible to characterize a molecule's activity and narrow its possible targets (Schreiber 2005). Screens that provide detailed information about intermediate states of bacterial cell perturbation, including gene chip arrays and metabolome profiling, would be valuable. Validation of these screens on the dozens of existing classes of antibiotics would provide the beginning of a comprehensive database. Inclusion of both pathogenic and commensal bacteria in high-density screening arrays would lend a systems-biology perspective and build the detailed resolution to identify sites of compound action. A few hundred antimicrobial small molecules would become standard probes to provide response patterns with which new candidate molecules could be compared.

In target-based screens, greater emphasis needs to be placed on increasing the likelihood of success in cell penetration and persistence of active molecules. The physical-chemical characteristics of cell permeability are poorly understood. Further research is needed to characterize the functional properties of molecules that minimize interaction with bacterial-membrane efflux pumps and allow penetration and persistent accumulation in pathogenic bacteria. The high-density screening techniques developed to improve cell-based screening could also be used to screen for these characteristics. Such efforts may help augment traditional and current chemical libraries to have a greater representation of molecules likely to be successful antibiotics and provide the data to optimize antibiotic-like molecules in future libraries.

As an example of the value of combining cell- and target-based approaches in the development of novel antimicrobials, a narrow-spectrum diarylquinolone, acting against mycobacteria by inhibition of the F0 subunit of ATP synthase (Andries et al. 2005), has recently been discovered by medicinal-chemistry optimization in whole-cell killing assays that used the fast-growing *M. smegmatis* as an initial *M. tuberculosis* surrogate. The mode of action was determined by whole-genome sequencing of resistant organ-

isms and then pharmacokinetics in mice optimized before an initial phase I trial for safety and tolerability in humans. For microbial cell-based killing assays as the starting point, this study may become a paradigm of strategic execution.

Both screening approaches would benefit from a publicly available collection of molecules that have been shown to have antimicrobial activity. Many such molecules have been reported over the last 6 decades of antibiotic research but may be languishing in the private coffers of companies not actively developing antimicrobials. Molecules could be collected from those companies or resynthesized. Another avenue worth pursuing would be donation of such molecules, especially naturally occurring ones, by pharmaceutical or biotechnology companies. The establishment of a collection of active antimicrobial compounds, numbering in the thousands, would represent a precious archive available to the research community for information-rich screens. Such a collection might also serve as small molecule-based microarrays for target-based screening.

Finally, measurements of the efficacy of novel therapeutics must use assays that closely mimic in vivo conditions. Traditional tests for antibiotic effectiveness rely on in vitro assays typically of single-species bacterial cultures grown under standardized conditions in defined laboratory media. These research assays do not effectively mimic the environments that bacteria experience in a host and although positive outcomes reliably occur in the laboratory, they are not always good predictors of outcomes in vivo. Treatment failure due to phenotypic tolerance needs to be examined so that therapies that avoid problems associated with noninherited resistance can be developed.

To improve the identification and characterization of bioactive compounds, the committee recommends the following:

- **A-8.1 Development of cell-based screening techniques that collect detailed information on cell metabolism through gene arrays, metabolome profiling, and other measurements.**
- **A-8.2 Increased research on the chemical properties necessary for cell permeability and retention.**
- **A-8.3 Establishment of a publicly available collection of molecules that have antibiotic activity.**
- **A-8.4 Development of new assays that mimic in vivo conditions.**

HOW CAN THIS WORK BE CARRIED OUT?

Not only new scientific approaches, but also legislative and institutional actions may be required to improve the prospects for development of new antimicrobial agents and prolongation of their efficacy. The introduction of three bills in the U.S. Congress bearing on antibiotic use and development suggests that major changes in society's approach to antibiotics may be on the horizon (Nathan and Goldberg 2005). While it was beyond the scope of the committee to consider non-scientific matters, the participants in the workshop noted that the scientific, regulatory and economic aspects of the development of antimicrobials are extensively interconnected (Nathan 2004).

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Promising Approaches to the Development of Immunomodulation for the Treatment of Infectious Diseases: Report of a Workshop

Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation

INTRODUCTION

The human immune system is equipped to fight a wide array of potential pathogens. Infection leads to disease only when the interaction between the host and the microorganism results in damage sufficient to disrupt homeostasis. For the last 70 years, the effort to prevent or treat infectious diseases has relied heavily on targeting microorganisms themselves with antimicrobial drugs. That approach has been extraordinarily successful, especially against bacterial infections. As described in the previous report, it has also resulted in the generation of multiple-drug-resistant (MDR) microorganisms that are threatening to become untreatable. The success and low cost of broad-spectrum antibiotics have reduced the incentive to develop alternative antimicrobial strategies, such as augmenting host responses during infectious disease.

The National Institute of Allergy and Infectious Diseases asked the National Research Council to convene a committee of experts, the Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation, to organize a brainstorming workshop to explore novel ways of modulating the host immune system to treat infectious disease (biographical sketches of the committee members are found in Appendix D). Therapeutic strategies based on modulating the immune response have several potential advantages over the use of traditional antimicrobials. First, because immunomodulators do not act on microorganisms directly,

they may circumvent the problem of rapid emergence of resistance. Second, immunomodulators may expand treatment options for immunocompromised patients, in whom traditional antimicrobials often work poorly. Third, they offer the potential of a broad spectrum of activity against viral and fungal, as well as bacterial, diseases and may provide nonspecific emergency-treatment options in the event of the emergence of a novel pathogen or a biowarfare attack.

The 2-day workshop, held on April 29-30, 2005, was attended by 33 invited participants, who had a wide array of expertise in molecular and cellular biology, chemistry, ecology, microbiology, immunology, and infectious disease. An agenda and a roster of participants and speakers can be found in Appendix E. Six speakers provided the participants with an overview of immune-system function, potential mechanisms of immunomodulation, and possible obstacles to the development of new immunomodulators. At the end of the first day, the participants formed four discussion groups. Each group identified a number of potential therapeutics and defined background research that would be necessary to make their development possible. The workshop concluded with a plenary discussion during which the breakout groups' conclusions were presented and discussed. This report describes the most interesting insights that came out of the workshop and the most promising avenues for future research on and development of immunomodulators to treat infectious diseases.

An Informed Choice of Goals

The objective of developing a single immunomodulatory agent that is effective against all infectious agents in all patients was generally considered to be unrealistic or even foolhardy. A clear theme emerging from the workshop was that immunomodulatory interventions will probably be most effective in a tailored role: against particular agents, in subsets of patients, at critical points in the course of an infectious disease, or most promisingly as adjuncts to therapeutics—such as antibiotics and antivirals—that target the microorganism directly. Because so many of the potential immunomodulatory therapies discussed at the workshop would have narrow applications, an overarching conclusion of the discussions was the importance of developing rapid and specific diagnostics both to identify the disease-causing agent and to define the patient's immune status and stage of infection. Improved ability to identify the patients most likely to require or respond to particular immunomodulators will greatly increase the likelihood of suc-

cess in clinical trials, another area identified during the workshop as a critical obstacle to the development of immunomodulators.

An infectious disease can occur only in a susceptible host, with susceptibility being a function of the effectiveness of the immune response. Damage to the host may be caused directly by microbial factors, can result from host factors—such as inflammatory responses—or both (Casadevall and Pirofski 2003). Some microbial diseases cause damage to the host because an overly vigorous immune response leads to excessive inflammation; others occur because immune responses are insufficient and require bolstering (Janeway et al. 2001). Host damage as a function of the immune response can be represented by a parabolic damage-response curve (Figure 1). The goal of therapeutic immunomodulation is to reduce host damage by shifting the damage-response curve to a point that is more beneficial to the host. This represents a shift from the more conventional therapeutic goal of killing the infectious organism.

Therefore, a fully stocked immunomodulation toolbox will require the development of both agents that stimulate and agents that suppress the immune response. Furthermore, optimal use of any immunomodulator will

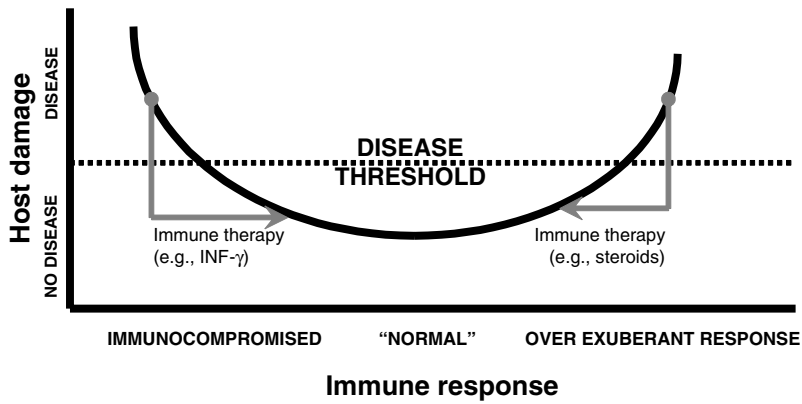

FIGURE 1 Host damage is a function of the quantitative and qualitative aspects of the host response to a microorganism. Damage can originate in microbial effects on host tissues, the immune response, or both. For most microorganism-host interactions, clinically apparent damage (disease) occurs at the extremes of the immune response. Conceptually, immunomodulator-based therapies can be considered interventions that attempt to reduce host damage by either enhancing or suppressing the immune response. For a more detailed discussion of the "damage-response framework," see Casadevall and Pirofski (2003).

require new tools to measure host damage because the type of intervention needed could depend on where the host is on the curve of damage vs. immune response. The availability of a variety of agents with the ability to fine-tune the immune response would provide clinicians with many new options in the treatment of infectious diseases. The development of more sensitive tools to determine immune status and host response will allow greater predictability of the effects of immunomodulators on different groups of patients, such as the immunocompromised.

Mining the Immune System for New Therapies

The major advantage of developing interventions for infectious diseases based on immune modulation is that it recruits and engages a system that has evolved to protect against microorganism-related diseases. Experience with vaccination and with passive antibody and cytokine therapy has shown that immune modulation can be effective and safe. However, one difficulty in developing new strategies to augment the host immune system to prevent or treat infectious diseases lies in the nature of the immune system itself. It is a complex network of intricately related, overlapping subsystems that function together to maintain immune homeostasis. It must recognize a full array of microorganisms and react to those that cause damage in such a way that they are eliminated without undue damage to the host. In general, this network responds to microbial agents in a rapid, efficient, and self-limiting manner. Appropriate stimulation of immune pathways is crucial to successful resolution of infection. Turning the immune response off after an infection is resolved is equally important, in that overstimulation of the immune system can lead to uncontrolled inflammation.

A Web, Not a Firetruck

Current understanding of the immune system divides it into two systems: innate immunity, which reacts quickly and nonspecifically to any pathogen; and acquired immunity, which generates a specific response and remembers interactions with microbial agents. Increasingly, research suggests that this simple model is inadequate. For example, vaccines and passive antibody agents that target a specific microbial agent can stimulate innate immune mechanisms (Binder et al. 2005), and the interaction of particular microbial components with receptors of the innate immune sys-

tem can exhibit some specificity (Choe et al. 2005; O'Neill 2005). Coordination of the innate and acquired responses requires constant communication among a series of interlocking feedback loops, further blurring the distinction between the two systems (Dower and Qwarnstrom 2003; Smith and Bolouri 2005). Exact prediction of how a particular immunomodulator will affect this complex network is not yet feasible. Furthermore, it is increasingly clear that factors in addition to the innate and acquired immune systems affect a person's total immune response. Some of those factors are shown in Figure 2.

We Are Not Alone

Biologists estimate that humans form integrated relationships with thousands of microbial species, collectively known as the resident microbiota (Wilson 2005; Nataro et al. 2005). These dynamic alliances begin to form immediately at birth, and undergo an ecological succession over the first few years of life that leads to the establishment of a mature set of communities at the age of about 4 years. The assemblages occur on the skin and along the mucosal surfaces of the body. They make a critical contribution to human health by providing nutrients and mediating normal development and function of host tissues, including those of the immune system (Noverr and Huffnagle 2004). The resident microbiota form the first line of defense against invasion of the human host by new pathogenic microorganisms (Figure 2, panel A). There is considerable evidence that the dynamics of the resident microbial communities are managed by their interactions with the host immune system and that a breakdown in the normal communication between the microbiota and the immune system can promote microbial disease (Kelly et al. 2005). However, the precise mechanisms that tune the interactions are poorly understood, and how the host distinguishes microbial friend from foe remains largely enigmatic.

Every Case Is Different

Developing immunomodulators is complicated by the fact that people have varied susceptibility and responses to microbial agents (Figure 2, panel E), and that the immune response changes during the course of a host-microorganism interaction. Infection with agents like HIV or immuno-suppressive treatments can result in varying degrees of immunocompromise. A person's genetic background also affects his or her response

FIGURE 2 Overview of immune system. Courtesy of David Schneider, Stanford University.

to any given agent; for example, some alleles of the CCR5 receptor confer resistance to infection with HIV (de Silva and Stumpf 2004), whereas variation along a particular innate immune pathway could dampen or stimulate an individual's reaction to particular microorganisms (Figure 2, panel B). Furthermore, each person has a unique history of exposure to microbial agents and thus has varied susceptibility to newly encountered microorganisms (Figure 2, panel C). The immune response is constantly fine-tuned during the course of a host-microorganism interaction, rather than being fixed or static, so the same immunomodulator could have different activities at different times during this response. Finally, response to a microorganism depends on communication between the immune system and other systems that contribute to homeostasis, for example, the nervous system (Tracey et al. 2001) (Figure 2, panel D); thus, therapeutics that work through the nervous system could have immunomodulatory effects and be effective in treating infectious diseases.

Increasing awareness of the complexity of the human immune response may make the process of developing and testing immunomodulatory therapeutics daunting. However, basic research continues to reveal potential immunomodulatory compounds and intervention points. Furthermore, highly successful immunomodulatory strategies, such as vaccination and passive antibody therapy, have been developed and deployed without full understanding of their modes of action. Therefore, the committee, after considering the many ideas discussed during the workshop, recommends a number of approaches as most likely to result in the development of safe and effective immunomodulators for use in the treatment of infectious disease. The committee also included recommendations for research that could bring greater scientific rigor to innovation in already established immunomodulatory therapeutics like immunization. The committee organized its recommendations into four parts: the first two discuss the most promising interventions based on modulating innate and acquired immunity, the third presents ways in which better understanding of the resident microbiota could lead to novel immunomodulators and the fourth discusses cross-cutting research needed to advance the field of immunomodulation in general.

MODULATING INNATE IMMUNITY

Innate immune responses are highly conserved across evolution (Abreu and Arditi 2004; Beutler 2004). Until recently, the innate immune system

was viewed as rather primitive, ringing the same alarm bell for every attacker. Recent research, however, has revealed that the innate immune system encompasses a complex set of receptors, signaling peptides, and cytokines linked in a web of interlocking feedback loops (O'Neill 2005). Innate immunity shares with conventional antimicrobial therapy a rapid onset of action (within minutes to hours) and a relatively low specificity: once activated, it can act on diverse microorganisms. Modulators of innate immunity may be less likely than conventional antimicrobial therapy to elicit resistance because immune modulators do not disable a specific microbial target and their mechanisms of action involve multiple effector cells and mediators.

Some microorganisms damage the host through virulence factors that subvert or mute the innate immune response (Jarva et al. 2003; Fournier and Philpott 2005). In other cases, an excessive immune response or one that fails to turn off after the threat has passed is responsible for host damage (Casadevall and Pirofski 2003; Polderman and Girbes 2004). Therefore, therapeutics based on modulating innate immunity will require both positive and negative modulators and the ability to distinguish which will be beneficial at a particular point in a given infection. Many modulators of innate immunity may prove unable to cure disease on their own but will be of particular use as adjuncts to conventional antibiotic therapies. The timing of intervention is likely to be critical because an intervention that protects if given before infection may increase the likelihood of host damage if administered after infection has occurred. Indeed, it is possible that both positive and negative immunomodulators could be useful at different stages of the same disease. The committee considered separately the most promising approaches to boosting and suppressing innate immunity.

Boosting Innate Immunity

Research in the last 10 years has revealed that the human innate immune system includes a large array of pattern-recognition receptors (PRRs), including scavenger receptors, complement receptor 3, mannose receptor, Nucleotide-binding and Oligomerization Domain-containing (NOD) proteins, and at least 10 Toll-like receptors (TLRs; originally discovered in *Drosophila*) that react to different classes of microbial signals. Each PRR then stimulates overlapping but not identical signal-transduction pathways leading to the production of a cascade of cytokines. Many of the same cytokine pathways are triggered by different PRRs, but the pattern of stimu-

lation initiated by each PRR is somewhat tailored to the class of microorganism it detects. The discovery of PRRs has stimulated research into what triggers them (agonists), the resulting signaling cascade, and the complex interactions between the events set off by PRR activation and the rest of the immune system. The list of potential molecular targets for modulators of innate immunity is extensive (Beutler 2004; Germain 2004).

The Cytoscape image shown in Figure 3 includes some of the known genes that interconnect TLR4 (which detects lipopolysaccharides (LPS), a component of bacterial outer membranes) with genes that are regulated by the NFκB transcription factor. Clearly there are numerous potential intervention points even in this small part of the innate immune system, and hints that the process is highly orchestrated (regulated) are starting to be obtained.

The committee identified three potential boosters of innate immunity as having particular promise: TLR agonists and agents that modulate the TLR response pathway, cationic host-defense peptides, and direct expansion of the effector cells normally activated by the innate immune system.

TLR agonists have already shown promise as immunomodulators. For example, species-specific CpG oligonucleotides (representing a signature bacterial DNA sequence), acting through TLR9, can enhance host resistance to bacterial and viral infections without overtly causing adverse effects through overproduction of proinflammatory cytokines (Klinman 2004). CpGs are effective in experimental infection models against a variety of pathogens—including *Francisella tularensis*, *Listeria monocytogenes*, and *Cryptococcus neoformans*—and in models of polymicrobial intra-abdominal sepsis (Ito et al. 2005; Rice et al. 2005; Krieg 2002). Other modulators acting through PRRs include the peptidoglycan subunit muramyl dipeptide, LPS-derived monophosphyl lipid A, fungal cell wall β-glucans, and various synthetic agonists of TLRs (O'Neill 2003).

Alternatively, cationic-host defense (antimicrobial) peptides can modulate innate immunity and protect against infection without inducing inflammation or even while suppressing it (Bowdish et al. 2005; Finlay and Hancock 2004). Peptides in this diverse family, produced by phagocytes and epithelial cells and present in mucus and other fluid interfaces between the host and the environment, can act against microorganisms directly when they are present in high concentrations (for example, in the granules of phagocytes); in lower concentrations (for example, at mucosal surfaces), they fulfill a still largely uncharacterized regulatory role in the innate im-

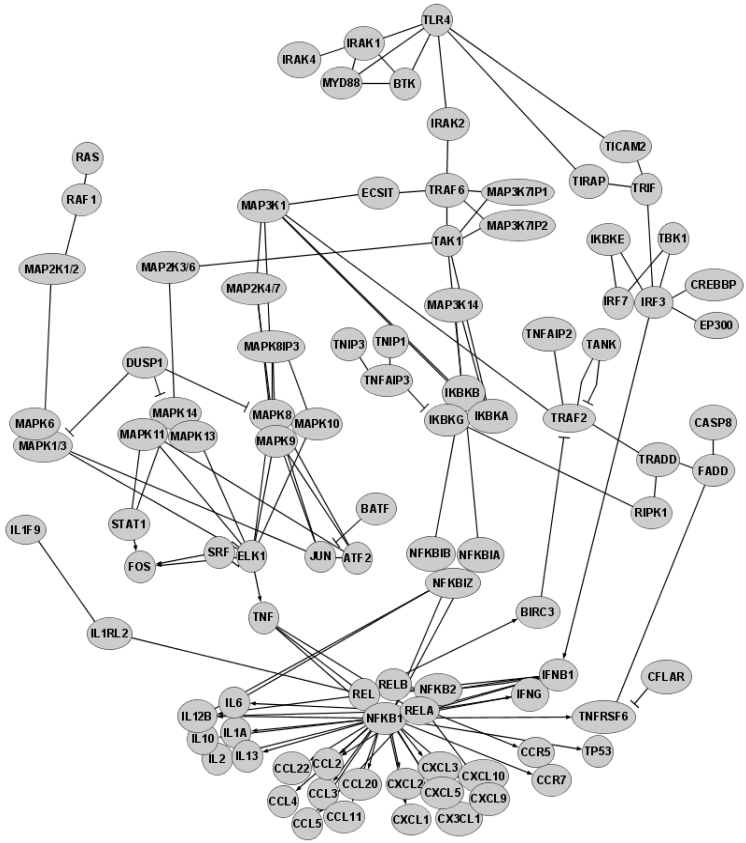

FIGURE 3 View of the TLR4 pathway with the Cytoscape program. Each circle represents a particular protein, and the lines connecting them reflect known interactions (signal transduction, gene regulation, or physical protein-protein interaction). The net effect of engagement of the TLR4 receptor by LPS is migration of transcription factor NFκB (indicated by its five known subunits which operate in pairs, NFκB1 (p50), RelA, RelB, C-Rel, and NFκB2) into the nucleus, which leads to upregulation of transcription of many genes (signified by arrows). Negative regulatory events are indicated by lines with perpendicular intersections (⊥). We believe that this network, although complicated, is much simpler than the actual case. Constructed and provided with permission by Fiona Roche, Simon Fraser University.

mune system. Derivatives without direct antimicrobial activity can also protect against infections (Leist and Jaattela 2002).

A downstream result of activation of innate immune sentinels, such as TLRs, is the expansion of various effector cells of both the acquired and innate immune systems. Therefore, another potential means of modulating innate immunity is to develop therapeutics that would increase the appropriate cell populations either indirectly by use of appropriate colony-stimulating factors (such as G-CSF and GM-CSF) or by improving technologies for white blood cell transfusions, boosting the lifespan of normal cells (for example, by controlling apoptosis), using ex vivo educated autologous effector cells (host effector cells that have been removed from the host and modified), or using synthetic effector cells (effector cells from a non-host source).

Compounds that modulate innate immunity could have unpredictable or even conflicting effects on the host response, depending on whether they counteract or augment the natural response induced by the host-microorganism interaction. The numerous interlocking regulatory mechanisms, which ensure that the immune response is sufficient to clear the disease-causing microorganism and is then promptly turned off, are not well enough understood to support prediction of the activity of innate immune modulators. Experience indicates that attempts to modulate innate immunity can have unwelcome results. Agonists of TLRs can trigger autoimmune diseases in mice that are genetically predisposed (Lang et al. 2005). Repetitive administration of CpG-containing oligonucleotide agonists of TLR9 can lead to massive lymphocyte depletion in mice (Heikenwalder et al. 2004). Administration of self-proteins such as antimicrobial proteins and CSFs, especially recombinant versions, occasionally elicits an antibody response (Reumaux et al. 2004). Therefore, the development of safe and effective innate immune modulators will require substantial basic research.

Research characterizing the intricacies of the innate immune system will facilitate the harnessing of these potential therapeutic approaches. In particular, the committee recommends basic research in the following areas to support the development of therapeutics to boost the innate immune system:

- **I-1.1 Characterization of PRR agonists, their downstream signaling pathways, and effector mechanisms.**

- **I-1.2 Characterization of elements that regulate the balance between proinflammatory and anti-inflammatory pathways in innate immunity.**
- **I-1.3 Characterization of the selective immunomodulatory effects of cationic host-defense peptides.**
- **I-1.4 Identification of biomarkers of innate immune status.**

Suppressing Inflammation

Excessive stimulation of innate immunity by some host-microorganism interactions can produce proinflammatory mediators, such as TNF-α, that promote host damage. Sepsis-like syndromes characterized by hemodynamic instability and metabolic abnormalities affect more than 750,000 people in North America annually and cause some 215,000 deaths (Angus et al. 2001). Therapies designed to treat sepsis by down-modulating immune responses have been largely unsuccessful (Opal 2003). The failures may be due, at least in part, to the heterogeneity of the syndrome and the late stage at which treatment is usually initiated. In addition, many of the candidate compounds that have been tested act specifically on a single step in the complex pathways that regulate the inflammatory process. Given the complex dynamics of the inflammatory response, the window of opportunity for such specific immunomodulators is likely to be narrow.

Many of the antisepsis agents that have had disappointing clinical results target individual mediators of inflammation. Agents acting at critical checkpoints may be more effective. Examples of promising targets that appear to amplify multiple inflammatory mediators are High Mobility Group Box-1 (HMGB1), Triggering Receptor Expressed on Myeloid Cells-1 (TREM-1), Macrophage Migration Inhibitory Factor (MIF) (Andersson and Tracey 2003; Bouchon et al. 2001; Bucala 1994) and TLR4 antagonists like eritoran and TAK-242 (Rossignol and Lynn 2005). Strategies to limit inflammation associated with infection include enhancing T-regulatory cell activity in vivo. These cells suppress a variety of inflammatory tissue disorders—such as inflammatory bowel disease, fibrosis, and asthma—and it is conceivable that harnessing their activity in a controlled manner during infection will be beneficial (Belkaid and Rouse 2005).

Control of neutrophil activation and trafficking may also be useful in this regard. Neutrophils are critically important in the innate immune response to infection, but neutrophil persistence at the site of infection can

lead to unmitigated local and systemic tissue damage. Dysregulated apoptosis, cytokine signaling, and delayed mononuclear cell infiltration to clear polymorphonuclear leukocytes contribute to the inflammatory process (McLoughlin et al. 2003); studies of this phenomenon are recommended with the goal of identifying therapeutics that target T-regulatory cell and neutrophil activation and trafficking. Alternative approaches would depend on a more profound understanding of the regulation of proinflammatory cytokines. For example, it is known that the human host-defense peptide LL-37 is one of the most effective antiendotoxins in mouse sepsis models (Klinman 2004) because of its ability to suppress the induction of the proinflammatory cytokine TNF-α by bacterial LPS; nevertheless, despite suppressing this component of innate immune responses, LL-37 can also protect against infections (Leist and Jaattela 2002). This led the committee to suggest that a better understanding of innate immunity will yield other approaches to suppressing sepsis selectively.

The disappointing results of interventions that target a single step in the inflammatory pathway may have been caused by the extremely narrow window of efficacy of such agents. The agents may be much more effective if given to the right patient at the right time. Therefore, the committee recommends prospective studies that allow for stratification of sepsis patients into distinct categories (Polderman and Girbes 2004). Advances in genomics and proteomics could provide opportunities for such stratification. In particular, gene-array technologies could lead to the discovery of novel biomarkers that can rapidly identify patients at the earliest stages of sepsis and group sepsis patients on the basis of their particular response patterns. The rapid advancement of proteomics technology and its application to sepsis could yield similar rewards. Prospective clinical studies of hospital inpatients would facilitate the identification of candidate biomarkers for assay development. Such studies should include collection and analysis of samples (for example, of blood and urine) and archiving of sample components such as serum and white cells with sophisticated database support. The development of a high-content database would allow correlation of the results of sample analysis with clinical course to identify candidate biomarkers. The archived samples could then be used for testing and validation. Eventually, the biomarkers would be used to stratify patients and guide treatment.

The committee therefore recommends two promising approaches to the development of agents to suppress innate immunity:

• I-2.1 Development of therapeutics that can modulate regulation of the inflammatory network, especially those that harness T-regulatory cell function, the TLR signaling pathways, and neutrophil activity and trafficking.

• I-2.2 Prospective studies with gene array and proteomic technology to allow the stratification of sepsis patients into distinct categories.

MODULATING ACQUIRED IMMUNITY

The exquisite specificity, long memory, and powerful effector mechanisms of the acquired immune system are its great strengths. Dramatic examples of the power of interventions targeting the acquired immune system are the eradication of epidemic smallpox by vaccination (Henderson 1976) and the development of passive antibody therapy as the first antimicrobial strategy for diverse infectious diseases from pneumococcal pneumonia to rabies (Buchwald and Pirofski 2003). Applying passive antibody therapy and vaccination to the treatment of infectious diseases is not a novel idea, but the committee identified several kinds of research that could allow these strategies to be used more widely and with greater effectiveness.

Passive Antibody Therapy

Passive antibody therapy is proven and immediately available for the treatment of infectious diseases. It involves the administration of an immunoglobulin molecule, or fraction thereof, to prevent or treat an infectious disease. Passive immunization by administration of preformed antibodies is generally well tolerated and may be particularly effective in immunocompromised people who are unable to mount a sufficient response to a vaccine. Several polyclonal antibody preparations and one monoclonal antibody, Respigam®, have been licensed for use against infectious diseases (Atkins et al. 2000). Despite the safety and efficacy of passive antibody therapy, further development is hampered by the specificity of antibody reagents, which limits each one's use to relatively few patients and consequently affects the economics of development; the need for specific microbiological diagnosis before use; and the short half-lives of immunoglobulin fragments, which must have sufficient time to counteract the targeted microorganism before being cleared by the immune system.

Each of these limitations could be addressed in further research. Although specificity is a defining characteristic of antibodies, the recognition of new mechanisms of antibody action, including mediation of damage control and other immunomodulatory functions, suggests that broad-spectrum antibody reagents could be developed, which could act on diverse microorganisms (Casadevall et al. 2004). Availability of rapid and specific diagnostics for identification of microorganisms and of biomarkers of immune status would allow more effective deployment of passive antibody therapy.

The committee identified two potential applications of passive antibody therapy as particularly promising: the development of monoclonal antibodies for use as single reagents or cocktails to address the pressing problem of multiple-drug-resistant bacterial infections in hospitals and the development of antibody reagents that take advantage of normal antibody interaction with the innate immune system, for example, by inducing mediators of damage control (such as interleukin-10 and other cytokines) to serve as broad-spectrum stimulators or suppressors of immunity.

Three kinds of research are recommended that would contribute to the development and effective use of passive antibody therapy:

- **I-3.1 Understanding the relationships between antibody specificity, affinity, isotype, dose, and protective efficacy for pathogenic microorganisms would facilitate the development of antibodies that bind more effectively and are cleared more slowly. This would improve understanding of the optimal dose and timing of administration of passive antibodies.**
- **I-3.2 Identifying the specific mechanisms by which antibodies interact with the innate and cellular immune systems to mediate damage control could allow the development of antibodies that are active against multiple agents. This research would probably also identify innate immune modulators that could act as vaccine adjuvants.**
- **I-3.3 Rapid and specific diagnostics aimed at defining not only the infectious agent but also the patient's immune status could allow rapid identification of patients in whom passive antibody therapy would be effective and could be used to determine the correct dose and timing of passive antibody treatment.**

Therapeutic Vaccination

Another type of intervention that takes advantage of the acquired immune system is active immunization in the form of therapeutic vaccination. Active vaccination has been successful in preventing many viral and bacterial diseases, and it remains an attractive option. Most available vaccines are used to prevent infectious diseases. However, the rabies vaccine is given after infection and induces a protective immune response before the onset of disease. Another example of therapeutic vaccination is the prevention of herpes zoster in infected older individuals by administration of the varicella virus vaccine. The rabies and varicella vaccines illustrate the potential usefulness of therapeutic vaccination for infectious diseases.

Because of vaccination's long history of safety and effectiveness, the committee feels that high priority should be given to research that is likely to lead to more-effective vaccines. In addition to improving the design of vaccines themselves, further research in these areas is likely to contribute to the development of more-effective adjuvants (compounds that are administered with vaccines to enhance the immune response). Given that only one adjuvant (aluminum salts such as hydroxide) is currently FDA-approved, the need for different adjuvants that can drive different types of immune responses (mucosal, Th1) is pressing. The demonstration that the *Haemophilus influenzae* type B-outer-membrane-protein-complex-glycoconjugate vaccine depends on TLR2 engagement suggests that TLR agonists may be useful as adjuvants for some vaccine formulations (Latz et al. 2004).

The development of vaccination for use in chronic infectious diseases—such as those caused by protozoal parasites, mycobacteria, fungi, HIV, and other viruses—was identified as promising. In chronic disease, the natural immune response is insufficient to clear the infection, and damage is caused by microbial action on host tissues or by the inflammatory response to the persistent microorganism. The identification of antigens that elicit beneficial and harmful immune responses remains largely empirical. The availability of molecular information on the nature of B and T cell epitopes, combined with an enhanced understanding of antigen processing, presentation and the effect of co-stimulation, might allow the design of vaccines that would elicit antibodies and cell-mediated responses that are effective in clearing or controlling chronic infections.

Current vaccines generally elicit antibodies of the IgG isotype, but natural infection elicits a wide range of antibody isotypes and a long immu-

nological memory (Holmgren et al. 2005). One reason that most vaccines raise a limited range of isotypes is that the mode of administration usually bypasses the normal, mucosal site of entry of many infectious agents, where the production of other isotypes is stimulated. Similarly, because current vaccination techniques do not perfectly mimic natural infection, the protection they provide is often not as long-lasting. Dendritic cell vaccination and the development of antigens that target dendritic cells were viewed as promising approaches to enhance vaccine effectiveness because dendritic cells are critical components of innate immunity that also initiate acquired immune responses.

The relatively recent discovery of positive and negative signaling pathways involved in the activation of T cells presents the possibility of regulating T-cell function during immunization. By augmenting positive signaling pathways for T cells such as the CD40-CD40L and CD28-B7 pathways, it may be possible to enhance T-cell help and subsequent antibody production in response to some vaccines. Conversely, engagement of negative signaling pathways, such as the PD1-PDL1 pathway, to derepress T-cell function during immunization may also enhance antibody responses.

The following research was identified as most likely to contribute to improved vaccines:

- **I-4.1 Defining the molecular nature of B- and T-cell epitopes and using this information to design novel vaccines and to identify the characteristics of protective antibodies.**
- **I-4.2 Determining how to elicit protective non-IgG responses and simulate the mucosal response.**
- **I-4.3 Devising strategies that target dendritic cells and new approaches to optimize antigen delivery to dendritic cells.**
- **I-4.4 Characterizing positive and negative signaling pathways between T cells and antigen-presenting cells.**

TAKING ADVANTAGE OF THE RESIDENT MICROBIOTA

The growing recognition that most interactions between humans and bacteria are benign, or even cooperative, was a prominent theme of the workshop. Although study of the human-bacteria ecosystem is still in its early days, the committee identified it as an extremely promising avenue for the eventual development of therapeutic immunomodulators. The ma-

nipulation of normal processes, such as interactions of the immune system with the normal microbiota, to ameliorate the effects of pathogens may offer novel, noninvasive, and inexpensive therapeutic strategies.

The resident microbiota is essential to immune development in neonates and disturbances of the microbial community cause an imbalance in human health (Eckburg et al. 2005; Hooper and Gordon 2001). In collaboration with the immune system, microbial communities form the first line of defense against microorganisms that are potentially damaging. Bacteria and other constituents of the normal microbiota act in concert with the immune system in a variety of ways, including production of antimicrobials, which are harmless to the residents themselves but deter interactions with other bacteria; and modulation of the activity of both the innate and adaptive immune systems. The normal microbiota plays a role in inhibiting inflammation despite presenting high concentrations of specific microbial molecules—microorganism-associated molecular patterns (MAMPs), such as bacterial LPS and peptidoglycan—that interact with TLRs and induce inflammation when the same MAMPs are presented by microbial pathogens (Rakoff-Nahoum et al. 2004). How such seemingly opposite effects are reconciled to generate the healthy state remains to be determined, but understanding how the immune system distinguishes "good" from "bad" bacteria is an intriguing target for the development of therapeutics.

Perturbations in the development and maintenance of microbial communities are thought to render the host susceptible to disease. For example, accumulating data support the "hygiene hypothesis"—that antimicrobial hygiene, which has had an enormous beneficial effect on public health, may be contributing to the recent rise in allergy and autoimmune disorders, such as inflammatory bowel disease (Isolauri 2004). In addition, the use of antibiotics disrupts the balance of microbial communities. Imbalance can lead not only to the overgrowth of normal microbial residents to population densities that cause host damage (as in the case of *Clostridium difficile* in the intestine) but also to enhanced susceptibility to new, potentially damaging, microorganisms.

The workshop participants acknowledged that so little is known about our normal microbiota and how it affects the activity of the immune system that improved knowledge would be essential before these partnerships could to be used effectively in therapeutics. In the long term, therapeutic agents that target the resident microbiota may take many forms, but the only current interventions that capitalize on beneficial human-bacteria re-

lations are probiotics. *Probiotic* refers to the ingestion or application of live bacteria to promote human health; an example is the consumption of yogurt to reintroduce lactobacilli after antibiotic treatment. The committee identified the development of probiotics as an approach to treating infectious diseases that could yield results more quickly than other interventions based on manipulating the normal microbiota. It recommends the following three applications as particularly promising:

- **I-5.1 The displacement of pathogens from a niche, such as the skin or the oral cavity.**
- **I-5.2 The replacement of disrupted bacterial communities after antibiotic treatment.**
- **I-5.3 The engineering of probiotic bacteria that can signal the immune system to generate immunomodulatory cells or downregulate inflammatory pathways.**

The committee recommends three kinds of basic research that would contribute to more rapid progress in developing therapeutics that are based on using the resident microbiota to prevent or treat infectious disease:[1]

- **I-6.1 Defining the composition of the resident microbiota on the skin and the mucosal surfaces.**

Research would be directed at defining how microbial communities form and mature with the human body; how the microbial members of a community interact with one another, with the host, and with more pathogenic microorganisms; how community composition differs between individuals in association with age, sex, race, haplotype, ethnic background, health status, immunocompromise status or diet. Such research efforts would benefit greatly from incorporation of the expertise of engineers and computational biologists and from enhanced emphasis on methods to characterize non-culturable microorganisms.

- **I-6.2 Defining how the composition of the normal microbiota affects the establishment of the antibody and T cell repertoires.**

[1]These recommendations are similar to those in the accompanying report on new classes of antimicrobials (e.g., recommendation A-2.1).

Research would focus on how much of the antibody and T cell repertoires of a healthy person reflect interactions with the normal microbiota and how the repertoires are altered in people who experience autoimmune diseases or are immunocompromised.

• I-6.3 Identifying the mechanisms by which the microbiota signal the host to maintain homeostasis and prevent damage from host-microorganism interactions.

Research would focus on how the host prevents intolerance and generates immunoregulatory cells such as regulatory T-cells, specialized dendritic-cell populations and γ/δ T cells. Efforts should also be directed at defining the nature of the host-microorganism interaction when a potentially pathogenic microorganism is present but there is no disease. For example, some pathogenic microorganisms such as *Neisseria meningiditis*, the causative agent of bacterial meningitis, can be recovered from healthy people who show no signs of disease; in some cases, this state may confer heightened resistance to contracting the disease by stimulating an immune response (Jordens et al. 2004; Goldschneider et al. 1969a,b). Learning how interactions between pathogenic microorganisms and hosts can persist without causing disease and identifying the conditions that promote disease are critical for an improved understanding of microbial pathogenesis.

Finally, the committee highlighted the importance of using a variety of animal models to study the resident microbiota. In animal-microorganism interactions, studies with germfree and gnotobiotic mice over the last several years have greatly expanded our understanding of the dynamics of these relationships. Support for the development and maintenance of additional germfree animal facilities would hasten progress in this field. However, a single model cannot provide the entire picture, so efforts should be directed at supporting the development and exploitation of other vertebrate and invertebrate animal models to complement the existing systems. Such models should be used to characterize not only the normal interactions of animals with microorganisms, but also pathogenic ones.

CROSS-CUTTING RESEARCH NEEDS FOR THE DEVELOPMENT OF IMMUNOTHERAPY

• I-7.1 Encouraging multidisciplinary research among microbiologists, immunologists, ecologists, and clinicians.

The preceding sections of this report divided the potential novel therapeutics recommended by the committee into sections based on the part of the immune system from which they derive or that they would affect. However, workshop participants repeatedly emphasized that traditional descriptions of the immune system fail to take into account the interconnectedness of its subsystems and its complex relationship with the nervous system and the resident microbiota. Academic departments and study sections of funding agencies continue to be organized into traditional categories, so microbiologists, immunologists, ecologists, and clinicians find it difficult to interact. Many of this committee's recommendations (for example, determining the effect of the resident microbiota on the normal antibody repertoire) will require collaboration among disciplines and may fit poorly into traditional study sections. Therefore, the committee recommends that thought be given to ensuring that such studies attract broad collaboration and receive multidisciplinary review.

• I-7.2 Developing rapid diagnostics and determining markers of immune status.

Because the immune reaction is so complex, a fundamental problem in the development of immunomodulators is measuring their effect. Rapid diagnosis of the agent responsible for damage and rapid assessment of individual immune status will be critical in the development of immunotherapy. Therefore, the committee recommends that studies be undertaken to help to provide reliable measures of immune status and host damage. An additional value of the development of more sophisticated markers of immune status would be greater ability to predict the efficacy of immunomodulatory treatments in patients with various kinds of immunocompromise. Such studies could take the form of prospective sampling of patients entering hospitals to look for markers predictive of susceptibility to infectious diseases or response to treatment.

Another promising approach would be to look for patterns of immune markers that correlate with particular infectious agents, stages of infection,

or individual variations in immune response. The gene-array, proteomic, and metabolomic technologies that would make such studies successful are increasingly cost-effective. The ability to share the results of such studies in accessible, searchable databases, however, lags behind the ability to collect information.

- **I-7.3 Developing appropriate animal models.**

Animal models play a vital role in biomedical research by paving the way for the development of novel therapeutic agents. However, the predictive value of these models for the clinical situation can be highly uncertain, especially for complex diseases. Although most animal models have their limitations, infectious disease models have been particularly useful in the discovery and development of agents that eradicate pathogenic microorganisms. The predictive value of the models rests on the notion that the in vivo efficacy of an antibiotic is relatively independent of host factors. In contrast, animal models of immunomodulatory therapies, and especially therapies that address the modulation of innate immunity, are inherently less predictive, and their reliability depends on the researcher's ability to understand and manipulate the complex immune system of the host. This increases the likelihood of late-stage failure of novel immunomodulatory therapies compared with what has been observed with antibiotic therapies historically. For example, there have been many failures of immunomodulatory agents in clinical trials for multiple sclerosis despite the success of these therapies in animal models (Wiendl and Hohlfeld 2002).

Rodents, especially mice, are the most commonly used model species for studying immune responses and a variety of sophisticated tools are available for studies in these animals. However, although there are many similarities between the immune systems of mice and humans, there are also striking differences (Mestas and Hughes 2004) that make it difficult to extrapolate data from animal models into the clinical disease reliably. Nonhuman primates offer alternative models that are potentially more predictive, but experiments with them are expensive and are constrained by ethical considerations. The development of infectious disease models that use severe combined immunodeficiency (SCID) mice in which the immune system is reconstituted with human immune cells could offer an attractive alternative, although the mixing of species introduces new complexities.

Transgenic mouse models have been used extensively to dissect components of the immune system and have led to many important insights. In

addition, relatively simple model organisms with well-defined genetics and developmental pathways, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, have effective innate immune responses and can be used to study conserved host-defense pathways. The successful development and testing of immunomodulatory therapeutics in general will require alternative animal models and therefore, the committee recommends support for research that uses novel model organisms.

THE NEAR AND FAR HORIZON

Treatment regimens that modulate the immune response have the potential to revolutionize how infectious diseases are treated. Such immunomodulatory therapies would be important additions to the current arsenal, especially because they would be expected to act synergistically with conventional antimicrobial therapies that target disease-causing organisms directly (Tzianabos and Kasper 2002). Some types of immune therapies, such as antibodies and cytokines, are already in clinical use and their application can be readily expanded to treat major infectious-disease problems. Other types are likely to require more preclinical development and their ultimate utility is more difficult to predict. This is why great emphasis has been placed on the need to develop biomarkers that accurately reflect immune status so that the effects of immunomodulators can be better predicted. However, it should be remembered that history shows that it is possible to develop immunomodulatory strategies without understanding the full complexity of immunology, as evidenced by early vaccines and serum therapy. Hence, the complexity of the host-microorganism interaction and the fact that we do not yet fully understand it need not prevent us from pursuing the development of immunomodulating therapeutics.

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Appendix A

Statement of Task

To generate new thinking about developing effective antibiotics in the future, two brainstorming workshops (one on possible new classes of antibiotics; another on immune modulation) will be organized to bring experts in microbial infections and drug development together with specialists in other areas of the biological sciences, such as cell biology, immunology, genomics, and biophysics. The workshop participants will consider how the latest biological information, technology, and research approaches can be brought to bear on the development of new classes of antibiotics and immunomodulators.

The scientific issues raised during discussion in each workshop will be captured in two short consensus reports. The reports will describe the most promising biological targets for further research and development as well as higher-risk, high-reward approaches. The reports will suggest different avenues that should be pursued in the short and long term to speed the development of new antimicrobial or immunomodulator therapies.

Appendix B

New Classes of Antimicrobials Committee Biographical Sketches

Christopher T. Walsh *(Chair)* is the Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology (BCMP) at Harvard Medical School. He has had extensive experience in academic administration, including Chairmanship of the MIT Chemistry Dept (1982-1987) and the HMS Biological Chemistry & Molecular Pharmacology Dept (1987-1995) as well as serving as President and CEO of the Dana Farber Cancer Institute (1992-1995). His research has focused on enzymes and enzyme inhibitors, with recent specialization on antibiotics. He and his group have authored over 600 research papers, books on *Enzymatic Reaction Mechanisms* (1979); *Antibiotics: Origins, Actions, Resistance* (2003); *Posttranslational Modification of Proteins: Expanding Nature's Inventory* (2005). He is a member of the National Academy of Sciences, the Institute of Medicine, and the American Philosophical Society.

He has been a consultant to government and academic institutions, including NIGMS, and a Trustee of the Whitehead Institute and the Helen Hay Whitney Foundation. He has been a consultant to large pharmaceuticals (Merck, Roche, and Abbot), and been involved in scientific advisory capacity for Genzyme, Immunogen, Leukosite, Kosan Biosciences, Millennium, Versicor, Transform Pharmaceuticals, Critical Therapeutics, and MPM capital. He sits on the Board of Directors of Critical Therapeutics, Kosan, Microbia, and Vicuron. At HMS he has recently served as co-chair of a committee to review the HMS Conflict of Interest policies. He is chair of the executive committee of the Harvard Integrated Life Sciences graduate programs.

Bonnie L. Bassler is a Professor of Molecular Biology at Princeton University. She received a B.S. in Biochemistry from the University of California at Davis and a Ph.D. in Biochemistry from the Johns Hopkins University. She performed postdoctoral work in Genetics at the Agouron Institute. She joined the Princeton faculty in 1994. Her research focuses on the molecular mechanisms that bacteria use for inter-cellular communication. This process is called quorum sensing. Bassler is the Director of Graduate Studies in the Molecular Biology Department, and she teaches both undergraduate and graduate courses. She was elected to the American Academy of Microbiology in 2002, and she was elected a fellow of AAAS in 2004. She was awarded a MacArthur Foundation Fellowship in 2002, and she is the 2003 Theobald Smith Society Waksman Award recipient. Bassler received the New Jersey Thomas A. Edison Patent Award for Medical Technology in 2003 and the New York Intellectual Property Lawyer's Association chose her as the 2004 Inventor of the Year. Bassler is an editor for *Molecular Microbiology*, and on the editorial boards of the *Journal of Bacteriology*, *Genetics*, and *Molecular and Cellular Proteomics*. She serves on many grant, fellowship, and award review panels.

Carl F. Nathan is Chairman, Department of Microbiology and Immunology, and co-chairman, Graduate Program in Immunology and Microbial Pathogenesis at the Weill Medical College of Cornell University. He joined the faculty in 1985 as the Stanton Griffis Distinguished Professor of Medicine. Prior to his current appointment, he was the founding director of the Tri-Institutional M.D.-Ph.D. Program and served as Senior Associate Dean for Research and Acting Dean of Cornell University Medical College. He previously was on the faculty at the Rockefeller University. Dr. Nathan's research furnished some of the first molecular explanations for macrophage activation and antimicrobial mechanisms of macrophages. He has made several fundamental discoveries about cytokine activation of macrophages, and determined that a major mechanism of host defense is expression of inducible nitric oxide (NO) synthase (iNOS). He holds an M.D. from Harvard Medical School. After training at Massachusetts General Hospital, the National Cancer Institute and Yale, he was board-certified in internal medicine and oncology.

Thomas F. O'Brien is Senior Physician in the Division of Infectious Diseases and Medical Director of the Microbiology Laboratory at Brigham and Women's Hospital in Boston, Massachusetts, and Associate Professor

of Medicine at Harvard Medical School. Following graduation from Harvard Medical School, Dr. O'Brien was a medical resident for two years at Peter Bent Brigham Hospital, Boston, a Harvard University Mosley Fellow for one year at Cambridge University, England, an active duty Captain in the U.S. Army Medical Corps for two years, and a research fellow in the Department of Microbiology at Harvard Medical School for another two years before a final year as a senior medical resident at Peter Bent Brigham Hospital. His dual role as clinician consulting on complicated infections and director of a laboratory identifying the microbes causing the infections and measuring their resistance to antibiotics prompted Dr. O'Brien to focus on the problems of antibiotic resistance and especially their molecular basis and epidemiology. This work led to the designation of his laboratory as World Health Organization Collaborating Center for the Surveillance of Antimicrobial Resistance and the development of software called WHONET, which now links laboratories in resistance surveillance networks in more than eighty countries.

Margaret Riley is a Professor of Biology at the University of Massachusetts Amherst. She received her Ph.D. in population genetics from Harvard University and performed postdoctoral research in microbial population genetics with a Sloan Postdoctoral Fellowship in Molecular Evolution. She joined the faculty at Yale in 1991 and recently moved to UMass Amherst. She has a broad set of research interests that range from studies of experimental evolution of microbes to developing novel antimicrobials and redefining the microbial species concept. Dr. Riley studies the evolution of microbial diversity, with a particular emphasis on the ecology and evolution of microbial toxins. Her recent work has revealed that the production of toxins is a primary force in the generation and maintenance of microbial diversity. These studies led to an interest in applying ecological and evolutionary theory to the design of novel antimicrobials for use in animal and human health. She is co-founder of Origin Antimicrobials, Inc., whose mission is to discover and refine novel antimicrobials to address the challenge of antibiotic resistance. Dr. Riley is the Director of the Organismic and Evolutionary Biology Program and the Director of the Museum of Natural History at UMass Amherst. From 1999-2002 she chaired the Gordon conference on molecular evolution and from 2003-2005 she chaired the Gordon conference on microbial population biology and evolution. She is a fellow of the American Academy of Microbiologists.

Richard J. White is Executive Vice President and Chief Scientific Officer at Vicuron Pharmaceuticals, a biotechnology company specializing in antimicrobial agents. For the last thirty years he has worked in pharmaceutical research, specializing in infectious diseases and natural products-based drug discovery. His research was carried out at Glaxo, Lederle and Bristol Myers Squibb in positions of increasing responsibility. He was Vice President for Infectious Disease Drug Discovery at Bristol Myers Squibb for twelve years and was involved in the discovery and development of cefepime and cefprozil. He has published more than 60 research papers, most of which are on the discovery, mechanism of action, mechanism of resistance, and biosynthesis of antibiotics. Dr. White has been on the editorial board of the *Journal of Antibiotics* for 15 years. He obtained an undergraduate degree in Biochemistry from the University of Manchester Institute of Science and Technology and a Ph.D.in Microbial Biochemistry from the University of Oxford in 1966.

Gerard D. Wright is Professor and Chair of the Department of Biochemistry and Biomedical Sciences at McMaster University, Hamilton, Ontario, and the Director of the McMaster Antimicrobial Research Centre. He received his B.Sc. in Biochemistry (1986) and his Ph.D. in Chemistry (1990) from the University of Waterloo. He followed this up with 2 years of postdoctoral research at Harvard Medical School in Boston and joined the Department of Biochemistry at McMaster in 1993. He holds a Canada Research Chair in Antibiotic Biochemistry and has received Canadian Institutes of Health Research Scientist (2000-2005) and Medical Research Council of Canada Scholar (1995-2000), Premiers' Research Excellence (1999) and Polanyi Prize (1993) awards. He is a member of the Canadian Bacterial Diseases Network Centre of Excellence and the Director of the American Chemical Society Short Course on Antibiotics and Antibacterial Agents. Dr. Wright is co-founder, with Dr. Eric Brown, of the McMaster High Throughput Screening Facility.

Dr. Wright's laboratory conducts research on the molecular mechanisms of antibiotic resistance including resistance to aminoglycoside, glycopeptide and streptogramin families of antibiotics, on the mechanisms of antibiotic biosynthesis, and on the discovery of new antimicrobial targets, in particular antifungal agents. He is the author of over 90 published papers and book chapters.

Appendix C

New Classes of Antimicrobials Workshop

NEW DIRECTIONS IN THE STUDY OF ANTIMICROBIAL THERAPEUTICS:
NEW CLASSES OF ANTIMICROBIALS

May 23-24, 2005
Keck Center of the National Academies • Room 201
500 Fifth Street, N.W. • Washington, D.C. 20001

AGENDA

Monday, May 23, 2005

8:00 a.m. Continental Breakfast

8:30 a.m. **Opening Remarks and Introductions**
Christopher T. Walsh (Committee Chair), *Harvard Medical School*
Michael G. Kurilla, *Director, Office of Biodefense Research Affairs, National Institute of Allergy and Infectious Diseases, NIH*
Workshop Participants

9:20 a.m.	**The Clinical Impact of Antimicrobial Resistance** Robert C. Moellering, Jr., *Harvard Medical School and Beth Israel Deaconess Medical Center*
9:50 a.m.	Questions and Discussion
10:15 a.m.	**The Question of Resistance: What It Is, Why It Develops, and How to Circumvent It** Gerard D. Wright, *McMaster University*
10:45 a.m.	Questions and Discussion
11:10 a.m.	Break
11:30 a.m.	**Antibiotics: Past, Present, and Future** Christopher T. Walsh, *Harvard Medical School*
12:00 p.m.	Questions and Discussion
12:25 p.m.	Lunch
1:10 p.m.	**Discovery of Antimicrobials: From Targets to the Clinic** Molly Schmid, *Keck Graduate Institute*
1:50 p.m.	Questions and Discussion
2:15 p.m.	**Applying Ecological and Evolutionary Theory to Meet the Challenge of Antibiotic Resistance** Margaret Riley, *University of Massachusetts-Amherst*
2:45 p.m.	Questions and Discussion
3:10 p.m.	Break
3:20 p.m.	**Molecular Detection and Diagnosis: The Role of Detection and Rapid Diagnosis in Treating Infectious Disease** Francis Barany, *Weill Medical College of Cornell University*
3:50 p.m.	Questions and Discussion

4:15 p.m.	**Small group discussions to organize topics for Tuesday breakout sessions**
5:00 p.m.	**Small group reports and discussion**
5:45 p.m.	Adjourn for the day

Tuesday, May 24, 2005

8:30 a.m.	Plan of action for the day and any new issues since Monday
9:00 a.m.	**Working breakout group discussions of key areas** Identify the most promising areas, hurdles to overcome, needed research and clarification
11:00 a.m.	**Breakout group 1 report**
11:20 a.m.	Discussion
11:45 a.m.	**Breakout group 2 report**
12:05 p.m.	Discussion
12:30 p.m.	Lunch
1:00 p.m.	**Breakout group 3 report**
1:20 p.m.	Discussion
1:45 p.m.	**Breakout group 4 report**
2:05 p.m.	Discussion
2:30 p.m.	Break
2:45 p.m.	**General discussion and conclusions**
4:00 p.m.	Adjourn

Breakout Group Topics

1. **New molecules:** Where do new anti-infective chemical entities come from and what are their desired characteristics? (What do you screen?)

2. **Ecological interactions:** What are the underlying principles of ecological interactions to exploit or interrupt for the development of new therapies?

3. **Resistance:** How do we minimize it and extend the lifetime of the next generation of antibiotics, including the appropriate use of diagnostics?

4. **Biological processes:** What are the biological approaches that will inform new strategies for *finding* new antibiotics?

Participants

- Rustom Antia, *Emory University*
- Francis Barany, *Weill Medical College of Cornell University*
- Bonnie L. Bassler, *Princeton University (member of committee)*
- Carl Bergstrom, *University of Washington*
- Martin J. Blaser, *New York University*
- Eric Brown, *McMaster University*
- Richard R. Burgess, *University of Wisconsin*
- Jon Clardy, *Harvard Medical School*
- Don Clewell, *University of Michigan (retired)*
- Julian Davies, *University of British Columbia*
- Rob Dorit, *Smith College*
- Ferric C. Fang, *University of Washington and Harborview Medical Center*
- Lou Gross, *University of Tennessee*
- Pehr A.B. Harbury, *Stanford University*
- Roberto Kolter, *Harvard Medical School*
- Walter P. Lowe, *Howard University*
- Robert C. Moellering, Jr., *Harvard Medical School and Beth Israel Deaconess Medical Center*
- Carl Nathan, *Weill Medical College of Cornell University (member of committee)*

- Thomas F. O'Brien, *Brigham and Women's Hospital (member of committee)*
- John H. Rex, *AstraZeneca Pharmaceuticals*
- Margaret Riley, *University of Massachusetts-Amherst (member of committee)*
- Marty Rosenberg, *Promega Corporation*
- Molly B. Schmid, *Keck Graduate Institute*
- K. Barry Sharpless, *The Scripps Research Institute*
- Peter M. Small, *Bill and Melinda Gates Foundation*
- Anne Summers, *University of Georgia*
- Joyce Sutcliffe, *Rib-X Pharmaceuticals*
- Saeed Tavazoie, *Princeton University*
- Julie Theriot, *Stanford University*
- Christopher T. Walsh, *Harvard Medical School (chair of committee)*
- Janet Westpheling, *University of Georgia*
- Richard J. White, *Vicuron Pharmaceuticals (member of committee)*
- Gerard D. Wright, *McMaster University (member of committee)*

Observers from the National Institute of Allergy and Infectious Diseases, NIH

- Susan Daniels (microbiology)
- Judith Hewitt (animal model drug testing)
- Michael G. Kurilla (biodefense)
- N. Kent Peters (antimicrobial resistance)
- John Prakash (regulatory affairs)
- Helen Quill (immunology)
- John Rogers (parasitology)
- Katherine A. Taylor (enteric pathogens and toxins)
- David Winter (immunology)
- Lanling Zou (bacteriology)

Staff from the National Academies Board on Life Sciences

- Adam P. Fagen, *Program Officer*
- Joe Larsen, *Postdoctoral Research Associate*
- Matt McDonough, *Program Assistant*
- Ann Reid, *Program Officer*
- Robert Yuan, *Consultant; Professor of Cell Biology & Molecular Genetics, University of Maryland*

Speaker Biographical Sketches

Francis Barany received his Ph.D. in Microbiology in 1981 at The Rockefeller University with Professor Alexander Tomasz. He was a Helen Hay Whitney postdoctoral fellow with Professor Hamilton O. Smith at the Johns Hopkins University School of Medicine from 1982-1985. Upon appointment as an Assistant Professor in Microbiology at Weill Medical College of Cornell University in 1985, he was named a Cornell Scholar in Biomedical Sciences, and in 1992 received a five year Hirschl/Monique Weill-Caulier Career Scientist Award. He currently holds the rank of Full Professor in the Department of Microbiology and Program of Biochemistry and Structural Biology at Cornell/Sloan Kettering Institute. He has an adjunct appointment at The Rockefeller University in the Department of Chemistry, Biochemistry, and Structural Biology, as well as an appointment as Director of Mutation Research at the Strang Cancer Prevention Center. He is program director of two multi-center NCI and NIAID grants to develop new methods of cancer and infectious disease detection. He is best known for developing the ligase chain reaction (LCR) and ligase detection reaction (LDR) and Universal DNA arrays for detection of genetic diseases and cancer-associated mutations. He was honored as Medical Diagnostics Research leader, *Scientific American* 50, in 2004.

Robert C. Moellering, Jr., M.D., is the Herrman L. Blumgart Professor of Medicine at Harvard Medical School and Physician-in-Chief and Chairman of the Department of Medicine at the Beth Israel Deaconess Medical Center, Boston, MA.

Dr. Moellering received his medical degree cum laude from Harvard Medical School and postgraduate training at Massachusetts General Hospital where he was also a Fellow in Infectious Diseases. He served as Chairman of the Department of Medicine at the Deaconess Hospital in Boston from 1981 through 1996. Dr. Moellering is the recipient of several awards, including an honorary Doctor of Science degree from Valparaiso University, the Garrod Medal from the British Society for Antimicrobial Chemotherapy, the Feldman Award and the Finland Award from the Infectious Diseases Society of America and the Hoechst-Roussel Award from the American Academy of Microbiology.

Dr. Moellering is a Fellow of the Infectious Diseases Society of America and a Master of the American College of Physicians, is an Honorary Fellow of the Royal College of Physicians and has been elected to membership in

the American Society for Clinical Investigation and the Association of American Physicians. Dr. Moellering has authored more than 350 publications and is Editor-in-Chief Emeritus of *Antimicrobial Agents and Chemotherapy*, Editor of *Infectious Disease Clinics of North America* and Editor of the *European Journal of Clinical Microbiology and Infectious Diseases*.

Margaret Riley is a Professor of Biology at the University of Massachusetts Amherst. She received her Ph.D. in population genetics from Harvard University and performed postdoctoral research in microbial population genetics with a Sloan Postdoctoral Fellowship in Molecular Evolution. She joined the faculty at Yale in 1991 and recently moved to UMass Amherst. She has a broad set of research interests that range from studies of experimental evolution of microbes to developing novel antimicrobials and redefining the microbial species concept. Dr. Riley studies the evolution of microbial diversity, with a particular emphasis on the ecology and evolution of microbial toxins. Her recent work has revealed that the production of toxins is a primary force in the generation and maintenance of microbial diversity. These studies led to an interest in applying ecological and evolutionary theory to the design of novel antimicrobials for use in animal and human health. She is co-founder of Origin Antimicrobials, Inc., whose mission is to discover and refine novel antimicrobials to address the challenge of antibiotic resistance. Dr. Riley is the Director of the Organismic and Evolutionary Biology Program and the Director of the Museum of Natural History at UMass Amherst. From 1999-2002 she chaired the Gordon conference on molecular evolution and from 2003-2005 she chaired the Gordon conference on microbial population biology and evolution. She is a fellow of the American Academy of Microbiologists.

Molly B. Schmid, Ph.D., joined the Keck Graduate Institute (Claremont CA) in January 2005, as Jacobs Professor and Entrepreneur-in-Residence. At KGI, she teaches "Risks & Rewards in Drug Discovery & Development" and continues to explore her interests in chemical genetics and antimicrobial drug discovery. Formerly, she was Senior Vice President of Preclinical Programs at Affinium Pharmaceuticals (Toronto, ON); Senior Director, Functional Genomics & Bioinformatics at Genencor International (Palo Alto, CA); and Vice President, Research Alliances with Microcide Pharmaceuticals (Mountain View, CA). From 1986-1994, she was Assistant Professor of Molecular Biology at Princeton University where her lab investigated bacterial chromosome structure and function, and her

research group discovered Topoisomerase IV in *Salmonella typhimurium,* as well as a genetic strategy for identifying new antimicrobial targets. She is a Fellow of the American Academy of Microbiology, a Searle/Chicago Community Trust Scholar and a Damon Runyon-Walter Winchell Fellow. She received her Ph.D. in Biology from the University of Utah, and her B.S. from SUNY Albany.

Christopher T. Walsh is the Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology (BCMP) at Harvard Medical School. He has had extensive experience in academic administration, including Chairmanship of the MIT Chemistry Dept (1982-1987) and the HMS Biological Chemistry & Molecular Pharmacology Dept (1987-1995) as well as serving as President and CEO of the Dana Farber Cancer Institute (1992-1995). His research has focused on enzymes and enzyme inhibitors, with recent specialization on antibiotics. He and his group have authored over 600 research papers, books on *Enzymatic Reaction Mechanisms* (1979); *Antibiotics: Origins, Actions, Resistance* (2003); *Posttranslational Modification of Proteins: Expanding Nature's Inventory* (2005). He is a member of the National Academy of Sciences, the Institute of Medicine, and the American Philosophical Society.

He has been a consultant to government and academic institutions, including NIGMS, and a Trustee of the Whitehead Institute and the Helen Hay Whitney Foundation. He has been a consultant to large pharmaceuticals (Merck, Roche, and Abbot), and been involved in scientific advisory capacity for Genzyme, Immunogen, Leukosite, Kosan Biosciences, Millennium, Versicor, Transform Pharmaceuticals, Critical Therapeutics, and MPM capital. He sits on the Board of Directors of Critical Therapeutics, Kosan, Microbia, and Vicuron. At HMS he has recently served as co-chair of a committee to review the HMS Conflict of Interest policies. He is chair of the executive committee of the Harvard Integrated Life Sciences graduate programs.

Gerard D. Wright is Professor and Chair of the Department of Biochemistry and Biomedical Sciences at McMaster University, Hamilton, Ontario, and the Director of the McMaster Antimicrobial Research Centre. He received his B.Sc. in Biochemistry (1986) and his Ph.D. in Chemistry (1990) from the University of Waterloo. He followed this up with 2 years of postdoctoral research at Harvard Medical School in Boston and joined the De-

partment of Biochemistry at McMaster in 1993. He holds a Canada Research Chair in Antibiotic Biochemistry and has received Canadian Institutes of Health Research Scientist (2000-2005) and Medical Research Council of Canada Scholar (1995-2000), Premiers' Research Excellence (1999) and Polanyi Prize (1993) awards. He is a member of the Canadian Bacterial Diseases Network Centre of Excellence and the Director of the American Chemical Society Short Course on Antibiotics and Antibacterial Agents. Dr. Wright is co-founder, with Dr. Eric Brown, of the McMaster High Throughput Screening Facility.

Dr. Wright's laboratory conducts research on the molecular mechanisms of antibiotic resistance including resistance to aminoglycoside, glycopeptide and streptogramin families of antibiotics, on the mechanisms of antibiotic biosynthesis, and on the discovery of new antimicrobial targets, in particular antifungal agents. He is the author of over 90 published papers and book chapters.

Appendix D

Immunomodulation Committee Biographical Sketches

Arturo Casadevall *(Chair)* is the Selma and Jacques Mitrani Professor of Biomedical Research at the Albert Einstein College of Medicine. He is also the Director of the Division of Infectious Diseases at his institution. He received his B.A. from Queens College, CUNY, and M.S., M.D. and Ph.D. degrees from New York University. His laboratory is interested in the fundamental questions of how microbes cause disease and how the host protects itself against microbes. The laboratory has a multidisciplinary research program spanning several areas of basic immunology and microbiology to address these general questions, which has resulted in almost 300 publications. His work is largely focused on the fungus *Cryptococcus neoformans*, a ubiquitous environmental microbe that is a frequent cause of disease in immunocompromised individuals. He is a fellow of the American Academy of Microbiology and has been elected to the American Society for Clinical Investigation and American Association of Physicians. Dr. Casadevall has served on numerous advisory committees to the NIH including study sections, strategic planning for the NIAID and the blue ribbon panel on response to bioterrorism. He is also a member of the National Science Advisory Board for Biosecurity (NSABB). He serves on the editorial boards of several journals, was recently elected Chair of the Medical Mycology division of the American Society of Microbiology and has been the recipient of numerous awards, most recently the Solomon A. Berson Medical Alumni Achievement Award in Basic Science-NYU School of Medicine 2005.

Rita R. Colwell is Distinguished University Professor both at the University of Maryland at College Park and at Johns Hopkins University Bloomberg School of Public Health as well as Chair of Canon U.S. Life Sciences. Her interests are focused on global infectious diseases, water, and health, and she is currently developing an international network to address emerging infectious diseases and water issues, including safe drinking water for both the developed and developing world. Colwell served as the 11th Director of the National Science Foundation (NSF), 1998-2004. She has authored or co-authored 16 books and more than 700 scientific publications. She produced the award-winning film *Invisible Seas* and has served on editorial boards of numerous scientific journals. Before going to NSF, Colwell was president of the University of Maryland Biotechnology Institute and professor of Microbiology and Biotechnology at the University of Maryland. She is a member of the National Academy of Sciences, the Royal Swedish Academy of Sciences, Stockholm, the American Academy of Arts and Sciences, and the American Philosophical Society. Born in Beverly, Massachusetts, Colwell holds a B.S. in Bacteriology and an M.S. in Genetics from Purdue University, and a Ph.D. in Oceanography from the University of Washington.

R.E.W. (Bob) Hancock is a Professor of Microbiology & Immunology at the University of British Columbia and is a Canada Research Chair holder. He was the founding Scientific Director of the Canadian Bacterial Diseases Network and currently heads the UBC Centre for Microbial Diseases and Immunity Research. His research interests include antibiotic uptake and resistance, functional genomics and the development of small cationic peptides as novel antimicrobials and modulators of innate immunity. He has published more than 360 papers and reviews and has 18 patents awarded. He has won many awards, including the Canadian Society of Microbiologists Award 1986, Fellow of the Royal Society of Canada 1994, the Canada 125 Silver Medal 1995, MRC Distinguished Scientist 1995-2000, Jacob Biely Faculty Research Prize 2000, BC Biotech Alliance Innovation and Achievement Award 2001, Fellow of the American Academy of Microbiology 2002, the QEII Jubilee Medal 2002, the Aventis Pharmaceuticals Award 2003, and the Zellers Scientist award and BC Innovation Council Chairman's award 2004. In 2001 he was inducted as an Officer of the Order of Canada. He is co-Founder of Inimex Pharmaceuticals Inc., and has served as a Scientific Advisory Board Member or consultant with 17

biotech and pharmaceutical companies. A group of which he is co-Director was awarded $20 million by Genome Canada to study the Functional Pathogenomics of Mucosal Immunity.

Margaret Jean McFall-Ngai received her Ph.D. in Biology from UCLA in 1983. Following postdoctoral positions in protein biochemistry at UCLA Medical School and UC San Diego, she held a faculty position at University of Southern California, where she was awarded tenure in 1994. In 1996, she moved to the University of Hawaii, Manoa, to the Pacific Biomedical Research Center, and then on to a professorship in the Department of Medical Microbiology and Immunology at the University of Wisconsin-Madison in June 2004. Her laboratory studies the influence of beneficial bacteria on health and disease using the squid-vibrio animal model system, the development of which she has pioneered with colleagues in microbiology. She was the principal organizer for a recent meeting at the Rockefeller Conference Center in Bellagio, Italy, the proceedings of which appear in a new book, *The Influence of Cooperative Bacteria on Animal Host Biology*, (McFall-Ngai, M.J., Henderson, B., and Ruby, E.G., eds.) 2005, Cambridge University Press.

Carl F. Nathan is Chairman, Department of Microbiology and Immunology, and co-chairman, Graduate Program in Immunology and Microbial Pathogenesis at the Weill Medical College of Cornell University. He joined the faculty in 1985 as the Stanton Griffis Distinguished Professor of Medicine. Prior to his current appointment, he was the founding director of the Tri-Institutional M.D.-Ph.D. Program and served as Senior Associate Dean and Acting Dean for Research. He previously was on the faculty at the Rockefeller University. Dr. Nathan's research furnished some of the first molecular explanations for macrophage activation and antimicrobial mechanisms of macrophages. He has made several fundamental discoveries about cytokine activation of macrophages, and determined that a major mechanism of host defense is expression of inducible nitric oxide (NO) synthase (iNOS). He holds an M.D. from Harvard Medical School. After training at Massachusetts General Hospital, the National Cancer Institute and Yale, he was board-certified in internal medicine and oncology.

Liise-anne Pirofski is a Professor of Medicine and Microbiology and Immunology at the Albert Einstein College of Medicine. She received her

B.A. from the University of California at Berkeley and her M.D. from Albert Einstein College of Medicine. After residency in Internal Medicine at Bellevue Hospital and the NYU Medical Center and fellowship training in Infectious Diseases at the Albert Einstein College of Medicine and Montefiore Medical Center, she did a postdoctoral fellowship in immunology in the laboratory of Matthew Scharff. She is currently a member of the Host Interactions with Bacterial Pathogens review panel of NIAID, an Associate Editor of *Medical Mycology,* and involved in medical education as course director of the Microbiology and Infectious Diseases course at the Albert Einstein College of Medicine. Her research program is focused on immunity to encapsulated pathogens, using *Cryptococcus neoformans* and *Streptococcus pneumoniae* as examples, and the interplay between the status of the antibody repertoire and microbial factors in the pathogenesis of these microbes in immunocompromised persons. She has also written numerous reviews developing useful models for the understanding of complex issues such as the bioweapons potential of microbes, a novel damage-response model of microbial pathogenesis, and the history and future potential of antibody and vaccine based immune therapeutics.

Arthur Tzianabos is an Associate Professor of Medicine (Microbiology and Molecular Genetics) at Harvard Medical School. He joined the faculty there in 1992 and has focused his research efforts on the host response to bacterial pathogens and modulation of T cell responses to prevent deleterious host tissue disorders. His work was among the first to show that bacterial polysaccharides can elicit cell-mediated immune responses and can be used to modulate deleterious host tissue responses such as surgical adhesion formation. He has recently begun a program to develop novel conjugate vaccines for the prevention of diseases caused by *Francisella tularensis*, a Class A agent of bioterrorism. Currently, he holds two R01 NIH grants and is a project leader for New England Center for Regional Excellence for Biodefense and Emerging Infectious Diseases funded by the NIAID/NIH.

Dennis M. Zaller received his Ph.D. in Biological Sciences from Columbia University. His thesis focused on the regulation of immunoglobulin gene expression. He then spent three years at the California Institute of Technology as a postdoctoral fellow in the laboratory of Dr. Leroy Hood. His postdoctoral work involved the elucidation of the repertoire of autoreactive T cells that emerge in response to myelin basic protein, and the study of transgenic mice expressing an autoreactive T cell receptor. He

then accepted a position with Merck Research Laboratories, where he has been for 14 years. He is currently an Executive Director and Head of the Immunology Therapeutic Area at Merck. He leads a Department of approximately 80 scientists who are focused on the development of novel therapies to treat inflammation-based disorders.

Appendix E

Immunomodulation Workshop

NEW DIRECTIONS IN THE STUDY OF ANTIMICROBIAL THERAPEUTICS:
IMMUNOMODULATION

April 28-29, 2005
Keck Center of the National Academies • Rooms 201 and 109
500 Fifth Street, N.W. • Washington, D.C. 20001

AGENDA

Thursday, April 28, 2005 (Room 201)

8:00 a.m. Continental Breakfast

8:30 a.m. **Opening Remarks and Introductions**
Arturo Casadevall (Committee Chair), *Albert Einstein College of Medicine*
Michael G. Kurilla, *Director, Office of BioDefense Research Affairs, National Institute of Allergy and Infectious Diseases, NIH*
Workshop Participants

9:30 a.m.	**Bad Bugs, No Drugs: The Perfect Storm** John E. "Jack" Edwards, Jr., *David Geffen School of Medicine at UCLA and Harbor-UCLA Medical Center*
10:00 a.m.	Questions and Discussion
10:20 a.m.	**The Inflammatory Reflex: Neural Control of Lethal Immune Responses** Kevin J. Tracey, *North Shore-LIJ Research Institute and Albert Einstein College of Medicine*
10:50 a.m.	Questions and Discussion
11:10 a.m.	Break
11:30 a.m.	**Antibody-Mediated Immunity: Something Old, Something New, Something Borrowed . . .** Liise-anne Pirofski, *Albert Einstein College of Medicine*
12:00 p.m.	Questions and Discussion
12:15 p.m.	Lunch
1:00 p.m.	**Therapeutic Applications of Innate Immunity** Alan Ezekowitz, *Harvard Medical School and Massachusetts General Hospital*
1:30 p.m.	Questions and Discussion
1:50 p.m.	**Factoring in the "Normal" Condition: The Impact of the Coevolved Microbiota on the Biology of the Human Host** Margaret McFall-Ngai, *University of Wisconsin-Madison*
2:20 p.m.	Questions and Discussion
2:40 p.m.	Break

3:00 p.m. **Activation of Protective Innate Immunity via in vivo Triggering of Toll-like Receptor 9**
Arthur M. Krieg, *Coley Pharmaceutical Group*

3:30 p.m. Questions and Discussion

3:50 p.m. **Organization and focus of Friday breakout sessions**

5:30 p.m. Adjourn for the day

Friday, April 29, 2005 (Room 109)

8:30 a.m. Plan of action for the day and any new issues since Thursday

9:00 a.m. **Working breakout group discussions of key areas**
Identify the most promising areas, hurdles to overcome, needed research and clarification
Group 1 (Room 109)
Group 2 (Room 202)
Group 3 (Room 104)
Group 4 (Room 106)

11:00 a.m. **Breakout group 1 report**

11:45 a.m. **Breakout group 2 report**

12:30 p.m. Lunch

1:00 p.m. **Breakout group 3 report**

1:45 p.m. **Breakout group 4 report**

2:30 p.m. Break

2:45 p.m. **General discussion and conclusions**

4:00 p.m. Adjourn

Breakout Group Topics

1. Intervention strategies in adaptive immunity
2. Intervention strategies boosting innate immunity
3. Intervention strategies based on damage control
4. Intervention strategies based on interactions of normal microbiota with host

Participants

- Lorne A. Babiuk, *University of Saskatchewan*
- Jacques Banchereau, *Baylor University*
- Christine A. Biron, *Brown University*
- Arturo Casadevall, *Albert Einstein College of Medicine (chair of committee)*
- Yung-Chi "Tommy" Cheng, *Yale University*
- Rita R. Colwell, *University of Maryland, Johns Hopkins University, and Canon U.S. Life Sciences (member of committee)*
- John E. "Jack" Edwards, Jr., *David Geffen School of Medicine at UCLA and Harbor-UCLA Medical Center*
- R. Alan B. Ezekowitz, *Harvard Medical School and Massachusetts General Hospital*
- Danielle A. Garsin, *University of Texas Health Science Center at Houston*
- R.E.W. (Bob) Hancock, *University of British Columbia (member of committee)*
- Katherine Knight, *Loyola University Chicago Stritch School of Medicine*
- Arthur M. Krieg, *Coley Pharmaceutical Group, Inc.*
- Philip O. Livingston, *Memorial Sloan-Kettering Cancer Center*
- Elias Lolis, *Yale University*
- Richard Malley, *Harvard Medical School and Children's Hospital Boston*
- Jennifer Maynard, *University of Minnesota*
- Margaret Jean McFall-Ngai, *University of Wisconsin-Madison (member of committee)*
- Francis Michon, *BioVeris Corporation*
- Cathryn Nagler-Anderson, *Harvard Medical School and Massachusetts General Hospital*

- Carl Nathan, *Weill Medical College of Cornell University (member of committee)*
- Kenneth H. Nealson, *University of Southern California*
- William Paul, *National Institute of Allergy and Infectious Diseases, NIH*
- Liise-anne Pirofski, *Albert Einstein College of Medicine (member of committee)*
- Jane Salmon, *Weill Medical College of Cornell University and Hospital for Special Surgery*
- Monisha G. Scott, *Inimex Pharmaceuticals*
- Alan Sher, *National Institute of Allergy and Infectious Diseases, NIH*
- Brad Spellberg, *David Geffen School of Medicine at UCLA and Harbor-UCLA Medical Center*
- Roland K. Strong, *Fred Hutchinson Cancer Research Center*
- Kevin J. Tracey, *North Shore-LIJ Research Institute and Albert Einstein College of Medicine*
- Elaine Tuomanen, *St. Jude Children's Research Hospital*
- Arthur Tzianabos, *Harvard Medical School (member of committee)*
- Aaron Weinberg, *Case Western Reserve University*
- Dennis M. Zaller, *Merck Research Laboratories (member of committee)*

Observers from the National Institute of Allergy and Infectious Diseases, NIH

- Petr Bocek (immunology)
- Susan A. Daniels (microbiology)
- Alison Deckhut Augustine (immunology)
- Michael Kurilla (biodefense)
- Marshall Plaut (immunology)
- Gyan (John) Prakash (regulatory affairs)
- David Winter (immunology)

Staff from the National Academies Board on Life Sciences

- Adam P. Fagen, *Program Officer*
- Joe Larsen, *Postdoctoral Research Associate*
- Matthew McDonough, *Program Assistant*
- Ann Reid, *Program Officer*
- Fran Sharples, *Director, Board on Life Sciences*

Speaker Biographical Sketches

John E. "Jack" Edwards, Jr., is Chief of Infectious Diseases at the Harbor/UCLA Medical Center and Professor of Medicine at the David Geffen School of Medicine at UCLA. His research interests are in fungal infections in immunocompromised hosts, with a focus on disseminated candidiasis. The investigators in his group are engaged in projects related to novel ways of treating infections. They include the use of synthetic peptides, context activated peptides, the infusion of activated phagocytes in neutropenic patients, and manipulation of host iron levels during infection. Dr. Edwards is currently engaged in preclinical studies to develop a vaccine using a recombinant protein known to be an adhesion for *Candida* to human cells. This vaccine is targeted primarily for short-term use in intensive care patients and eventually in immunocompromised hosts. Dr. Edwards is also the past chairman of the Public Policy Committee of the Infectious Diseases Society of America (IDSA) and currently a member of the Antimicrobial Availability Task Force of the IDSA, which is proactively addressing the lack of development of new antiinfectives within large pharmaceutical companies. Current positions also include being the Chairman of the FDA Antiinfective Advisory Committee and a member of the Board of Scientific Councilors of the Clinical Center at NIH.

R. Alan B. Ezekowitz is The Charles Wilder Professor of Pediatrics at Harvard Medical School, Chief of the Pediatric Service as Massachusetts General Hospital, Chief of the MassGeneral Hospital for Children, and Chief of the Pediatric Service for Partners HealthCare System in Boston. He earned a D.Phil. from the University of Oxford and an M.B.Ch.B. from the University of Cape Town and has previously held appointments at the Dana Farber Cancer Institute and Children's Hospital in Boston. He serves on the Board of the Society for Leukocyte Biology, the Editorial Board on *Microbes and Infection*, and chaired the 2002 Keystone Symposium on "Innate Immunity: Evolution and Link to Adaptive Immunity." Dr. Ezekowitz chairs the Scientific Advisory Committee for Anika Therapeutics, serves on the Board of NatImmune and on the scientific advisory committees of EntoMed and Codman. Major research interests include pattern recognition molecules in innate immunity, immunodeficiency, and vascular tumors of infancy. His laboratory has focused on examining how the innate immune system distinguishes species self from nonself in both mammalian systems and insects. From mammalian systems, he has learned that subtle variations in pattern recognition molecules, like the mannose-

binding lectin (MBL) appear to alter the balance between the host and the infectious agents. In insects, the focus of the laboratory has been to explore the mechanisms of phagocytosis using *Drosophila melanogaster* as a model system.

Arthur M. Krieg is Chief Scientific Officer, Senior Vice President for Research and Development, and co-founder, Coley Pharmaceutical Group. He discovered the CpG motif in 1994, and co-founded Coley Pharmaceutical Group in 1997. In addition to responsibility for internal Coley R&D, Dr. Krieg coordinates collaborative research programs with numerous other academic groups as well as corporate partners including Pfizer and sanofi-aventis. Dr. Krieg is also the Principal Investigator for $35 million in sponsored research programs on CpG technology with NIAID and the Defense Advanced Research Projects Agency.

Dr. Krieg graduated from Haverford College in 1979, received his M.D. from Washington University in 1983, and completed a residency in Internal Medicine at the University of Minnesota in 1986. He was a Staff Fellow at the NIH in the Arthritis Institute from 1986 to 1991, when he left to become an Assistant Professor in the Department of Internal Medicine at the University of Iowa. He was promoted to full Professor in 1998, and has been on a leave of absence from the University since 2001, when he joined Coley full-time. Dr. Krieg is co-founder and co-Editor of the journal *Oligonucleotides*, and is on several other editorial boards. Dr. Krieg is a board-certified rheumatologist and a Fellow of the American College of Rheumatology. He has published more than 200 scientific papers and is co-inventor on 10 issued U.S. patents covering CpG oligos. His 1995 *Nature* paper reporting the discovery of the CpG motif has been cited more than 1200 times.

Margaret Jean McFall-Ngai received her Ph.D. in Biology from UCLA in 1983. Following postdoctoral positions in protein biochemistry at UCLA Medical School and UC San Diego, she held a faculty position at University of Southern California, where she was awarded tenure in 1994. In 1996, she moved to the University of Hawaii, Manoa, to the Pacific Biomedical Research Center, and then on to a professorship in the Department of Medical Microbiology and Immunology at the University of Wisconsin-Madison in June 2004. Her laboratory studies the influence of beneficial bacteria on health and disease using the squid-vibrio animal model system, the development of which she has pioneered with colleagues in microbiology.

Liise-anne Pirofski is a Professor of Medicine and Microbiology and Immunology at the Albert Einstein College of Medicine. She received her B.A. from the University of California at Berkeley and her M.D. from Albert Einstein College of Medicine. After residency in Internal Medicine at Bellevue Hospital and the NYU Medical Center and fellowship training in Infectious Diseases at the Albert Einstein College of Medicine and Montefiore Medical Center, she did a postdoctoral fellowship in immunology in the laboratory of Matthew Scharff. She is currently a member of the Host Interactions with Bacterial Pathogens review panel of NIAID, an Associate Editor of *Medical Mycology*, and involved in medical education as course director of the Microbiology and Infectious Diseases course at the Albert Einstein College of Medicine. Her research program is focused on immunity to encapsulated pathogens, using *Cryptococcus neoformans* and *Streptococcus pneumoniae* as examples, and the interplay between the status of the antibody repertoire and microbial factors in the pathogenesis of these microbes in immunocompromised persons. She has also written numerous reviews developing useful models for the understanding of complex issues such as the bioweapons potential of microbes, a novel damage-response model of microbial pathogenesis, and the history and future potential of antibody and vaccine based immune therapeutics.

Kevin J. Tracey is Professor and Head of the Center for Patient Oriented Research at the North Shore-LIJ Research Institute, Manhasset, NY, Program Director of the General Clinical Research Center (GCRC), and Professor of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY. He received a B.S. from Boston College, an M.D. from Boston University, and completed clinical training in neurosurgery at the New York Hospital-Cornell University Medical College. Since 1992 he has been at the North Shore-LIJ Research Institute, directing a laboratory that focuses on cytokine biology in disease pathogenesis. With his colleagues, Dr. Tracey discovered the cytokine activity of HMGB1, a protein known previously only as a transcription factor, and demonstrated that HMGB1 is an experimental therapeutic target for sepsis and arthritis. With his colleagues, he also discovered that the neural output of the vagus nerve can regulate the magnitude of the innate immune response to infection and threat. Based on this work, clinical testing of anti-HMGB1 antibodies, and specific cholinergic agonists, is anticipated to begin in 2006-7, sponsored by a biotech company (Critical Therapeutics, Inc.) that Dr. Tracey cofounded in 2000.