National Institute of Allergy and Infectious Diseases, NIH

Volume 1

Frontiers in Research
National Institute of Allergy and Infectious Diseases, NIH

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Frontiers in Research

Edited by

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Dedication

To the thousands of investigators who, for more than 50 years, have received the support of the National Institute of Allergy and Infectious Diseases (NIAID) and have dedicated their lives and careers to biomedical research.

RESEARCH IS NOT A SYSTEMATIC OCCUPATION
BUT AN INTUITIVE ARTISTIC VOCATION

Albert Szent-Györgyi
Preface

For more than 50 years, as part of the National Institutes of Health, the mission of the National Institute of Allergy and Infectious Diseases (NIAID) has been to conduct and support basic and applied research to better understand, treat, and prevent infectious, immunologic, and allergic diseases with the ultimate goal of improving the health of individuals in the United States and around the world.

In recent years, NIAID has responded to new challenges including emerging and re-emerging infectious diseases, potential bioterrorism threats, and an increase in pediatric asthma prevalence. A cornerstone of NIAID-supported research also continues to be the discovery and improvement of vaccines focused on an array of infectious diseases with global public health importance.

As part of its mission to foster biomedical discovery and to reduce the burden of human disease, NIH and NIAID in particular, are committed to encouraging the accelerated translation of biomedical discoveries into effective clinical care and public health practice throughout the world. In pursuit of this goal and its disease-specific scientific objectives, NIAID seeks to broaden research opportunities and collaborations involving scientists and institutions outside the United States.

During 2006, special emphasis was given to fostering scientific collaboration between U.S. researchers and investigators in Central and Eastern Europe, the Baltic Region, Russia, Ukraine, and other newly independent states that were formerly part of the Soviet Union. Although the countries of Central and Eastern Europe have strong traditions in biomedical research, scientists from this region have been less successful than their Western European colleagues in competing for NIAID funding and in forming partnerships with U.S. scientists. To help address this situation, NIAID convened a research conference in Opatija, Croatia (June 24–30, 2006) so that U.S. and European scientists could explore shared research interests with a focus on microbiology and infectious diseases, HIV/AIDS, and basic and clinical immunology.

In the field of microbiology and infectious diseases, major presentations at the conference focused on recent research developments in emerging and re-emerging infections (anthrax and other potential biological weapons, vector-borne infections, tuberculosis, and influenza). A number of presentations discussed ongoing research targeting the development of infectious disease prophylactics and therapeutics.

One of the most serious problems worldwide that confronts efforts to control and treat infectious diseases is the increasing resistance of some pathogens to the current armamentarium of drugs. Microorganisms belonging to all four classes of infectious agents (bacteria, viruses, parasites, and fungi) have developed resistance to previously effective chemotherapeutics, thereby becoming serious threats to individual well-being and international public health. One striking example of drug resistance is the emergence of extensively drug-resistant tuberculosis. Several conference presentations were therefore focused on drug resistance.

HIV/AIDS also remains a major infectious disease research priority and it was well addressed during the conference. Since the start of the HIV/AIDS pandemic in the early 1980s, nearly 20 million people worldwide have died of the disease. According to an estimate issued by the Joint United Nations Programme on HIV/AIDS (UNAIDS) by the end of 2003, about 38 million adults and children were living with HIV/AIDS and in many countries overall prevalence still is rising. Although much progress has been made in the treatment of AIDS and in understanding effective strategies to prevent HIV transmission, research is urgently needed on vaccines, microbicides, therapeutic agents, behavioral prevention strategies, and the management of HIV-related co-morbidities.

NIAID-funded research in basic and clinical immunology has led to significant discoveries that have guided the effective treatment of a host of immunological conditions. For example, “tolerance induction” research has enabled the selective blocking of inappropriate or destructive immune responses while leaving protective immune responses intact. Major presentations at
the conference discussed various topics in immunomodulation, autoimmunity, infections and immunity, and vaccine development.

Finally, two sessions at the research conference were designed to inform participants about NIAID’s research funding mechanisms and the NIH application process.

With more than 100 participants, the 2006 NIAID Research Conference in Croatia clearly demonstrated NIAID’s commitment to a cutting-edge scientific exchange to help generate more research cooperation. Following the meeting, numerous research collaborations have been explored and numerous joint research applications have been prepared and submitted.

NIAID is pleased to have supported this important and unusual meeting and it welcomes publication of the important scientific findings presented there. The future of science lies in cooperation across national borders. Therefore, it is particularly rewarding to see research partnerships grow between scientists from countries previously characterized by a lack of communication and mutual understanding. With a strong research base, talented investigators in the United States and abroad, and the availability of powerful new research tools, NIAID will continue to support scientists in the forefront of basic and applied infectious and immune-mediated disease research.

Vassil St. Georgiev
Bethesda, MD
We would like to express our appreciation to Ms. Caroline Manganiello and the staff of technical writers for their help in the preparation of this volume.
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Part I
Introduction
The National Institute of Allergy and Infectious Diseases (NIAID) of the U.S. National Institutes of Health (NIH) is within the U.S. Department of Health and Human Services (DHHS; Figure 1). The NIH is the DHHS agency responsible for biomedical research and research training. In the U.S. federal system, health is considered primarily a local and state responsibility, with the federal government providing support and assistance as required. Biomedical research, however, is viewed as a federal responsibility. For that reason, the NIH size and budget have resulted in its becoming the largest of the DHHS agencies.

The NIH consists of 27 institutes and centers, 24 of which carry out and fund biomedical research and three that support the NIH biomedical research endeavor (Figure 2). Each institute consists of two major components: the extramural and the intramural. Intramural programs consist of NIH scientists working in NIH government laboratories. Intramural research constitutes of about 10 to 20% of each institute’s research effort and budget. Intramural researchers select scientists to come to their laboratories for research training and conduct international research using the funding available to their laboratory. The extramural program of each institute is approximately 80 to 90% of its total funding and operates through both unsolicited and solicited research applications for grants, collaborative agreements, and contracts. Applications are submitted to the NIH Center for Scientific Review, which assigns each application to the appropriate initial review group for scientific peer review and to an institute according to the scientific content of the application and the research mission of the institute. NIH is unique among national biomedical research agencies in that nearly one-half of the intramural scientists are not U.S. citizens and that foreign scientists are eligible to apply directly or as a partner in extramural awards.

NIAID is similar in its organization to other NIH institutes in that it has three intramural divisions and five extramural divisions (Figure 3). The Division of Intramural Research heavily emphasizes basic biomedical research, while the Vaccine Research Center’s mission includes the discovery and early development of vaccine products. The Division of Clinical Research was established in 2006 to set up domestic and international sites to carry out human subject studies on new or improved diagnostic tests, drugs, vaccines and other prevention products. The Division of Microbiology and Infectious Diseases is responsible for all infectious and parasitic diseases except for the human acquired immunodeficiency syndrome (AIDS). The Division of AIDS is responsible for AIDS and related conditions. The Division of Allergy, Immunology, and Transplantation is concerned with the human immune system. The Division of Extramural Activities provides support to the other three extramural divisions through NIAID-organized initial review groups, grant and contract management, and award databases.

The NIAID mission is to understand, treat, and ultimately prevent infectious, immunological, and allergic diseases that affect or threaten U.S. populations and hundreds of millions of people worldwide. The major areas of NIAID investigation currently are (in alphabetical order): AIDS; acute respiratory infections, including influenza; antimicrobial drug resistance, asthma and allergic diseases; civilian biodefense; emerging infectious diseases; enteric infections; genetics, transplantation, and immune tolerance; immune disorders; malaria and other tropical diseases; sexually transmitted diseases; tuberculosis, and vaccine development and evaluation.

The evolution of the NIAID budget is summarized in Figure 4. Prior to the recognition of AIDS, NIAID was the seventh largest NIH Institute. As a result of its research responsibilities in infectious diseases and immunology, funding for AIDS and AIDS-related research rose to become one-half of the NIAID budget. Subsequent to the anthrax attacks in 2001, NIAID was given lead responsibility for the U.S. Civilian Biodefense Research Initiative. At the present time, NIAID is the second...
largest institute after the National Cancer Institute. NIAID research funding is approximately one-third AIDS, one-third civilian biodefense, and one-third non-AIDS/non-biodefense. Following a Congressional mandate to double the NIH budget in the 1990s, the NIH budget has been flat for the past several years, resulting in overall inflation-adjusted negative growth. During this period, NIAID funding for international research has maintained a slow and steady growth (Figure 5) so that international research now accounts for 10% of the total NIAID budget. This remarkable sustainability is due to the globalization of health problems, the relevance of health conditions globally to domestic U.S. health problems, humanitarian objectives, and the economic development, political stability, and increasing investment in international health on the part of key international partners such as Brazil, China, and India. This sustained interest and growth in international research is not seen across NIH. One major factor that fuels NIAID’s global research activities is that our mission in infectious diseases necessitates that we partner with countries that have heavier burdens of disease and/or different risk factors in the development of clinical sites and the evaluation of new or improved diagnostic tests, treatment modalities, or prevention products.

NIAID operates under five guiding principles in Global Health Research. First, every effort is made to target collaborative research efforts to the needs of the partner country or region. Second, it strives to develop collaborative relationships that begin with collaboration in basic research and discovery so that intellectual property can be shared and proceed through product development, the design of human subject studies, and the conduct of rigorous clinical trials that generate data resulting in approval of the product by regulatory agencies. Third, to achieve multidisciplinary research collaboration, research capacity must be built and sustained in the host country. Fourth, NIAID strives to stimulate scientific collaboration and global multi-sector partnerships. Finally, NIAID international collaboration must develop training, communication, and outreach programs.

NIAID uses six approaches to support its international research. The first is through the NIAID intramural research divisions for pre- and postdoctoral research training. This research training frequently results in sustained collaboration once the visiting scientists have returned to their home countries. Intramural collaboration is limited by the resources available in each laboratory but has the advantages of being...
decentralized and scientifically driven, and it provides the opportunity to establish long-term collaboration with the NIAID laboratory and other researchers who have trained there. Because about 50% of NIH intramural scientists are from outside the United States and only 10% of intramural scientists become tenured, the intramural research training experience provides an opportunity to become part of a global network linking trainees and their home institutions with NIAID-tenured scientists, U.S. scientists who take academic or private sector appointments or join other U.S. agencies, and foreign scientists who return home to continue their research careers.

Foreign investigators are encouraged to partner with U.S. extramural investigators in the submission of investigator-initiated research applications or in response to solicited program announcements (PAs) and requests for applications (RFAs). This is how NIAID supports the bulk of its international research. If the collaboration is between U.S. scientists and scientists in another industrialized country, there may be no NIAID funding involved. On the other hand, if the collaborating overseas scientist is from a middle- or lower-income country and/or does not have his or her own funding, NIAID will provide the U.S. investigator with research funds to support the overseas component.

NIH is unique among national domestic research agencies in that foreign investigators are eligible to apply directly for investigator-initiated research awards. Foreign scientists and institutions are also eligible to apply for most solicited grant and collaborative agreement solicitations. There are no international set-aside funds, and foreign investigators must compete against experienced U.S. investigators. All unsolicited foreign applications with a competitive score must also be approved by the National Allergic and Infectious Diseases Council before funding. Because of the intense competition and grantsmanship required, NIAID does not encourage foreign investigators to apply directly unless their ideas are...
truly novel and the investigator has considerable experience preparing NIAID grant applications. NIH is obligated to follow U.S. contracting laws, so that foreign institutions can be funded in response to requests for proposals only if there is a prior determination that there is no viable U.S. source, or the foreign application is clearly superior to responses from U.S. institutions.

NIAID also participates in a number of bilateral programs with foreign governments and institutions. These agreements may be developed at the Presidential, State Department, DHHS, or NIH levels in science and technology, health, or biomedical research. In the majority of cases, these agreements have no NIAID funding associated with them and collaborative activities must be undertaken with resources currently at hand in intramural laboratories or using extramural funding mechanisms.

NIH intramural scientists are encouraged to collaborate with counterparts at other U.S. government agencies such as the Centers for Disease Control and Prevention, the Food and Drug Administration, and the U.S. Army or Navy. U.S. Government scientists, however, may not compete for NIH extramural research funds. When there is mutual interest, however, NIH may negotiate interagency agreements with these and other agencies such as the State Department or the U.S. Agency for International Development that serve as contractual mechanisms to transfer funds and resources between the participating agencies.

Finally, NIAID collaborates with multilateral agencies such as the World Health Organization (WHO), the Pan American Health Organization, and the Joint United Nations Program on HIV/AIDS through consultation, serving on advisory boards, and participation in technical meetings. NIAID has provided targeted funding to the WHO/World Bank/UNDP Special Program for Research and Training in Tropical Disease Research. NIAID also has a Congressional mandate to provide funding to the Global Fund to Combat AIDS, Tuberculosis, and Malaria.

Figure 3. (See Color Plates).
Figure 5. (See Color Plates).

Figure 6. (See Color Plates).
The NIAID strategy to respond globally to new or emerging infectious diseases and scientific opportunity has been first to encourage the intramural research community to turn their talent and attention to the new or underserved research area. The second step is to encourage extramural investigators working in relevant research areas to submit supplemental research proposals. The third step is to alert the more general scientific community about NIAID’s research priorities and interests in the area through notices, PAs, and RFAs in the NIH Guide for Grants and Contracts. Foreign investigators are ordinarily eligible to partner with U.S. applicants and, if they prefer, apply directly for NIAID funding. The result of these solicitations is to increase the research, the research training base, and eventually the pool of investigators in the targeted area. NIAID fulfills the need for directed activities in support of research in the targeted area through contracts to build the infrastructure and to provide research reagents and repositories.

After a critical mass of individual extramural awards has been reached, NIAID usually puts out an RFA to establish multidisciplinary centers of excellence in the field. These centers of excellence provide further opportunities for research training of U.S. and foreign scientists. The centers of excellence are usually encouraged to engage in international research and/or carry out research training through the center award and/or independent research and research training awards. Examples of NIAID centers of excellence programs include the Sexually Transmitted Disease Research Centers, the Tropical Disease Research Units, the Centers for AIDS Research, the Tuberculosis Research Unit, the Regional Centers for Emerging Infectious Diseases, and the recently announced Centers for Influenza Research and Surveillance.

Once the domestic centers of excellence are established, the next phase is the establishment of special programs to link the domestic network to international partners. RFAs are published to solicit applications for collaboration with one or more foreign partners. This is the time when the NIH Fogarty International Center solicits applications from U.S. institutions for international research training in the targeted area. Examples of linkage programs include the International Collaboration in Infectious Disease Research Program, the HIV Vaccine Trials Network, HIV Prevention Trials Network, the NIAID International Centers of Excellence, and the International Emerging Infectious Disease Research and Training Program.

The third phase is reached when the linkage programs are mature and international partners have developed the capacity to carry out and account for their own research. NIAID develops solicitations open to foreign institutions to apply directly to NIAID in the targeted area. Examples of mechanisms to support foreign researchers include the Tropical Medicine Research Centers, the Multilateral Initiative on Malaria, the Comprehensive International Program for Research on AIDS, and the International Research in Infectious Diseases Program.

Part II
Microbiology and Infectious Diseases
Section 1
Emerging and Re-Emerging Infections
1.1 Introduction

Infectious diseases, like most human diseases, are affected by complex polymorphic and nonpolymorphic interactive traits that influence host–pathogen interactions and modulate disease phenotype. It is well established that host genetic variability strongly affects susceptibility to infectious diseases and can significantly potentiate the severity of their clinical manifestations. The same individual could be highly susceptible to a particular infection yet completely resistant to another—ultimately these complex genetic variations ensure that some of us will be selected to survive catastrophic biological threats and help protect our species from extinction. As a result of global environmental, social and political changes, we are facing real danger that could result from major natural, deliberate, or accidental biological threats. The best means of protection against these impending threats is to be better prepared. To do so, we need to gain a deeper understanding of how our genotypes modulate our susceptibility and reaction to specific infectious agents, because this information helps us to better understand disease mechanism.

Our research has been focused on linking specific genotypes to susceptibility phenotypes and delineating pathways and molecular events that modulate host resistance or susceptibility to specific infectious pathogens. Inasmuch as it is quite difficult to conduct certain infectious disease studies in humans, there has been a critical need for small animal models for infectious diseases. Appreciating the limitations of the existing models, we have developed several novel and complementary mouse models that can be used to gain a better understanding of complex disease mechanisms and reveal the interactive network(s) that lead to eradication of the infection or to serious pathology caused by our overzealous response to it.

1.1.1 A Genetically Diverse, Genomically Well Defined Reference Mouse Panel Afford an Ideal Model for a Systems Biology Approach to Infectious Diseases

Traditionally, most experimental models of infectious diseases have involved inbred rodents, including the most common 10 strains of inbred mice (i.e., A/J, BALB/c, CBA, C3H/He, C57BL/6J, DBA/2, NZB, and AKR). Whereas these models have been invaluable to scientists, their downfall is their limited genetic variability, where certain phenotypes may be suppressed or grossly exaggerated. A good analogy would be like conducting a clinical trial using the same eight people every time and expecting to generalize the results to the rest of the human population. Clearly, this is neither optimal nor representative of the variation seen in humans. For this reason, several groups have been generating panels of genetically diverse mice to study the genetics of susceptibility to various diseases. Of these, the RI mice are ideal for many reasons (1, 2).

The RI strains are generated by crossing two inbred strains followed by ≥20 consecutive generations mating among siblings (1–4) (Figure 1.1). These RI mouse strains are a powerful tool for identifying QTL and interactive gene networks...
modulating infectious disease phenotypes. The panel we have in Memphis is the advanced RI (ARI) mice derived from the parental C57BL/6J and DBA/2 strains, which are known to differ considerably in their susceptibility to a number of infectious agents. These ARI BXD strains contain roughly twice as many recombinations as standard RI strains. The BXD ARI strains can therefore be used to map QTLs with twice the positional precision as can be achieved with the original BXDs. Figure 1.1 illustrates the schema used for generating those strains.

We currently have 80 BXD strains that are being extensively phenotyped and genotyped. Each BXD strain is genetically distinct from other strains, but all members of a given BXD strain are inbred (i.e., genetically identical). Thus, studies can be repeated on the same strain (individual) at different times, for as many times, and with a large number of biological replicas, thereby providing strong statistical power for the data. Another important feature of our BXD strains is that both parental strains have been sequenced and this greatly facilitates the identification of genes within mapped QTLs.

Prior to using the ARI mice for mapping and reverse genetics studies, we spend considerable time optimizing and standardizing the infection model. Once this has been accomplished, we basically infect mice from the ARI panel with the same dose of pathogen and measure different phenotypes (e.g., survival, weight loss, pathogen load in blood and dissemination to peripheral organs, etc.). The ARI mice are then ranked relative to each other for a given phenotype. These relative phenotype values are then analyzed in the context of the mouse genotype using WebQTL tools available on www.gennetwork.com, which provides the QTL mapping for phenotypes of interest. The bioinformatics tools allow us to inspect the single nucleotide polymorphism density within the mapped loci and to examine the genes within the loci in order to narrow down the number of candidate genes that should be further interrogated. The tools also allow us to identify interactive loci, through which we can discover interactive pathways modulating the measured phenotype.

Data generated using the ARI reference population reveal polygenic and pleiotropic networks modulating disease
phenotype and thereby providing a disease roadmap that helps focus hypotheses and expedite the process of forward systems discovery and research translation. The studies described in this chapter illustrate the utility of these mice in infectious disease studies.

1.1.2 Studies on the Genetics of Susceptibility to Invasive Group A Streptococcal (GAS) Sepsis Illustrate the Utility of RI Mice in Infectious Disease Research

Severe forms of invasive GAS infections associated with high morbidity and mortality were prevalent during the 1918 flu pandemic, then virtually disappeared from 1920 to the 1980s, when suddenly severe invasive disease reemerged in many parts of the world, causing panic and leading the media to dub it “the flesh-eating disease” (6–10). The bacteria are considered an ideal model for studying the effect of host genetics on the infection outcome, because the same bacteria can cause a wide spectrum of diseases in different individuals. These diseases range from mild sore throat to deadly diseases, such as streptococcal toxic shock (STSS), necrotizing fasciitis (NF), rheumatic fever and rheumatic heart disease (RHD), glomerulonephritis, and neurological disorders. We and others have identified specific immunogenetic polymorphisms that predispose to particular forms of GAS diseases and determine the level of risk for the severe forms of these illnesses, including RHD, NF and STSS (11–13).

Our STSS susceptibility studies have been ongoing since 1992 in collaboration with Dr. Donald E. Low and the Ontario Streptococcal Study Group. GAS are the richest known bacteria in superantigens (SAgs), with more than 13 identified SAgs to date (SpeA-C, SpeF-M, SSA, and SmeZ 1-24), with different GAS strains having different SAgs repertoires. SAgs trigger excessive activation of T cells and MHC II-expressing cells, and cause massive release of inflammatory cytokines (e.g., TNF-β and IFN-γ). Responses of different individual to the same SAgs can also vary quite drastically (11–13). Besides the SAgs, GAS possess many surface and secreted proteins that interact with the immune system (immune cells and complement proteins), e.g., M protein, C5a peptidase, SIC, and many streptodornases, which are involved in degrading neutrophil extracellular traps (14).

In the first phase of our studies, we focused on genetic elements that may potentiate the host response to GAS SAgs. We identified specific HLA-II alleles and haplotypes that confer very strong resistance to STSS, and others that predispose to it. We validated our association studies, biologically, through both in vitro studies with human PBMC (different HLA types) and in HLA-tg mice carrying alleles of interest. The role of HLA-II variation in STSS susceptibility is logical because the GAS SAgs, which are piovital mediators of STSS, utilize the HLA-II molecules as receptors through which they interact with TCRVβ elements and elicit potent inflammatory responses leading to STSS in genetically predisposed high responders (Figures 1.2 and 1.3).

Thus, we hypothesize that other host genetic elements might also modulate susceptibility to severe GAS sepsis and STSS, notably in earlier stages of infection controlled by the innate immune response of the host, and we are interested in finding pathways and networks rather than individual genes that are modulated by immune cells in response to GAS.

To discover additional genetic variations and pathways that modulate the outcome of GAS sepsis, we turned to the ARI BXD mice described earlier. The data included in the following paragraph underscore the utility of these mice in the discovery process.

Our initial studies showed that DBA/2J mice are more susceptible to severe GAS sepsis than C57BL/6J. Initially, we used approximately 300 mice from 20 BXD strains to optimize the infection dose and identify confounding non- genetic factors that need to be adjusted or included as significant covariates in the final analysis. An optimal dose of 1–3 × 10^7 CFU/100 µL per mouse of a virulent MIT1 GAS clinical isolate and BXD strains ages 40 to 120 days were used in this intravenous model of GAS sepsis. As shown in Figure 1.4, several BXD strains showed phenotypes outside the ranges of the parental strains, with several significantly more susceptible or resistant than their ancestors. In nine ensuing experiments, about 360 mice from 34 strains (32 BXDs and two ancestors) were infected intravenously with the optimal dose of the bacteria and survival was monitored every eight hours for seven days. Mice were weighed every 12 hours and weight loss was calculated. Bacterial load in blood (CFU/mL) was determined for all mice at 24 hours, and a bacteremia index was determined and corrected for covariates. All mice developed bacteremia, but with considerable differences in severity and survival rates (Figure 1.4).
Survival was recorded in day/day fraction post infection, and for each experiment the data was normalized to calculate a relative survival index (RSI). RSI for each strain and for each experiment were corrected for variables (mainly age) to generate a corrected index, cRSI. Using the Data Desk statistical program, we conducted multiple regression analyses for individual as well as for all nine combined experiments. Age was confirmed as a significant determinant of survival and bacterial spread, but the strongest factor influencing survival, as expected, was the genetic background of BXD strains \( (p \leq 0.0001) \).

These studies allowed us to map a strong QTL-modulating sepsis severity to a locus on chromosome 2. We are currently fine-tuning the mapping using additional BXD strains and interrogating genes of interest within the mapped QTL. We believe it will be quite informative to acquire systems information on GAS in the BXD strains \( \textit{in vitro} \) and \( \textit{in vivo} \). We plan to compare the data to human \( \textit{in vitro} \) responses and patient’s acute and convalescent plasma. This will provide a comprehensive mouse to human \( \textit{in vitro} \) and \( \textit{in vivo} \) correlation using a very well characterized set of samples.

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