Meeting the human immunology challenge

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Although studies of the laboratory mouse model have laid the groundwork for our rich understanding of immunobiology, they have fallen short in deciphering human disease and providing much needed therapeutic modalities. Indeed, bench-to-bedside approaches have not been a particularly effective means of developing translational research.1 Recently, a symposium was held at the National Institutes of Health (NIH) entitled “Meeting the Human Immunology Challenge,” highlighting the opportunities for the new Intramural NIH Center for Human Immunology, Autoimmunity, and Inflammation (http://www.nhlbi.nih.gov/resources/chi/); among other things it has become clear that a broader definition of the human immune spectrum in health and disease is needed. The human immunology meeting was held in the Clinical Center of the National Institutes of Health, Bethesda, Maryland, on September 3 and 4, 2009.

Keywords: immunology; bench-to-bedside approaches; immunobiology

Disparities between human and mouse immunobiology

Francis Cuss (Bristol-Myers Squibb) spoke from the pharmaceutical industry’s perspective highlighting the fact that patients with autoimmune diseases now have more options than ever before, with a large number of biologics being recently approved and more are in the pipeline. Nevertheless, the last decade has witnessed a progressive decline of first-in-class drugs, despite a continuous increase in clinical drug development. Cuss highlighted opportunities to develop greater understanding of disease mechanisms and to translate this information into knowledge.

First, collaboration with academics and within the industry should help in drug development, and within pharmaceutical development, there is a clear need for better outcome measures, biomarkers, and patient stratification strategies; in addition, innovative pathways for development and regulatory approval are being forged. Within pharmaceutical research there is a need for novel therapeutic target modalities; these novel targets should be pursued in conjunction with supporting biomarkers of response and patient stratification, as well as more predictive animal models. Cuss then reviewed progress in developing biomarkers to generate hypotheses, select patients, monitor treatment and safety, and measure outcomes. In addition, he spoke of the need to expand the “druggable genome.” The current therapeutic target pool includes up to 1,500 of the 30,000 genes in the human genome; at least twice as many targets have yielded a drug lead and many more disease-modifying genes could provide additional therapeutics. Approaches to expand the
target pool are necessary, “as the low hanging fruit has already been picked.” Intracellular biologics, new drug target classes, and new chemical space are examples of new technologies and new knowledge that will be used to find and develop new gene targets.

Expanding the diversity of existing decks (platforms) like kinases, proteases, G protein-coupled receptors, and mechanism-based individual syntheses will help expand the therapeutic modalities. More recently, antibodies and antibody-type platforms have provided new drugs. Peptides, macrocycles, and siRNAs are expected to provide additional drug classes. Technical innovation should allow expansion of the definition of “druggable” and, thus, expand the pool of potential therapeutic targets. And defining disease-modifying genes and how they are active in individual diseases will expand the potential for therapeutics that may work across diseases.

In contrast, Cuss discussed how the use of animal models for predicting treatment efficacy in humans has thus far been relatively disappointing. Kinetics of disease (typically days in animals versus months to years in humans), heterogeneity of human diseases (autoantigen diversity/expression, major histocompatibility complex (MHC) associations, polymorphisms), the differential role of inflammatory mediators like cytokines (e.g., interferon-gamma therapy improved experimental autoimmune encephalomyelitis but made multiple sclerosis worse), and differences in environmental stimuli (bacteria or viruses) contribute to the failure of animal models to predict therapeutic efficacy in human disease. These shortcomings point to opportunities for improvement: the development of more “humanized” animal systems, additional “spontaneous” models of disease, and more detailed immunologic analyses on drug effects in animal models to ensure that key pathways are inhibited. Models for treating the disease should incorporate therapeutic dosing regimens as opposed to preventive regimens when appropriate. Last, in translating these models to the bedside, markers shown to be altered by the therapeutic agent in animal models of disease should be used for stratification of patients in clinical trials.

Alan Krensky (Senior Investigator, Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute) discussed the many differences between mouse and human immunobiology and how the disparities in genes and environment are ignored in model systems. To meet the challenge of providing cures for human diseases of immunologic etiology, researchers will have to study humans and take genes and environment into account.

A human immunologist, Krensky had a number of examples from his own work of important differences between mouse and human immunobiology. In addition to the human lymphocyte function-associated antigen 1 (LFA-1), he helped identify other inhibitory antibodies his group named LFA-2 and LFA-3; the latter two molecules had not been identified in the mouse. Subsequent work showed that a gene for LFA-3 (now called CD58) does not exist in mice, and that CD58 likely arose in humans by gene duplication after the two lineages split (in mice, the LFA-2 (CD2) ligand is CD48, whereas in humans both CD48 and CD58 can function as ligands for LFA-2). In human cells the distributions of CD48 and CD58 are different, and the CD2-CD48 two-dimensional affinity is 50-fold lower than for CD2-CD58 interaction. Among other things, these differences mean mouse T cells are much less dependent on CD2 interactions than are human T cells. Work in Krensky’s laboratory also identified granulysin, a cytolytic and proinflammatory molecule important in a variety of human diseases and that is not expressed in the mouse.

These are not the only examples of molecular and physiologic differences between mice and humans. Mestas and Hughes catalogued immunologic differences, including the percent of circulating neutrophils (lower in mouse than human) and lymphocytes (higher in mouse than human), CD4/CD8 ratios in adults, and polarization of T helper cells (Th cells). Additionally, human-activated T lymphocytes and endothelial cells express MHC class II antigens while the mouse counterparts do not. Mouse and human lymphocytes express different immunoglobulin (Ig) classes and have different requirements for Ig light chains. Other differences include different expression in number or type of defensins, toll-like receptors, inducible nitric oxide synthase, natural killer (NK) cell inhibitory receptors, Fc receptors, and B and T cell signaling pathways. Individual chemokines, of broad significance in immune cell trafficking, may be expressed by both mouse and human cells or by only one, or
Table 1. Comparison of the immunity of mice and men

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent neutrophils in blood</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Percent lymphocytes in blood</td>
<td>75</td>
<td>40</td>
</tr>
<tr>
<td>Percent monocytes in blood</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Percent gamma-delta in blood</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CD4:CD8 ratio in adult</td>
<td>2:1</td>
<td>3:1</td>
</tr>
<tr>
<td>Polarization of Th1/Th2</td>
<td>very strong</td>
<td>moderate</td>
</tr>
<tr>
<td>Nitric oxide production to LPS(a)</td>
<td>very strong</td>
<td>moderate-weak</td>
</tr>
<tr>
<td>ROS(b) production to LPS</td>
<td>weak</td>
<td>moderate</td>
</tr>
<tr>
<td>MHC class II expressed on T cells</td>
<td>never</td>
<td>activated</td>
</tr>
<tr>
<td>Epithelial cells present antigen</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Predominant pan T cells CD</td>
<td>3</td>
<td>2,3</td>
</tr>
<tr>
<td>Transplacental immune transfer</td>
<td>major</td>
<td>moderate</td>
</tr>
<tr>
<td>Immunoglobulin classes</td>
<td>IgG1, 2a, 2b, 3</td>
<td>IgG1, 2, 3, 4</td>
</tr>
<tr>
<td>Light chain usage</td>
<td>kappa</td>
<td>Both used</td>
</tr>
<tr>
<td>Secretory Ig</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Lymph</td>
<td>cellular</td>
<td>cellular</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

\(a\) Lipopolysaccharide
\(b\) Reactive oxygen species

they may have different functions in each species. These differences become even more complicated in multicomponent processes. For example, many chemokines interact with multiple receptors in hierarchical ways, giving rise to complexities that differ among species. Such complexities can be multiplied further in disease. In organ transplantation, for example, vascularized grafts are often tolerizing in mice but are rapidly rejected in humans; in multiple sclerosis, interferon-gamma therapy exacerbates disease, while such therapy is highly protective in the mouse model experimental autoimmune encephalomyelitis.\(^5\) And in diabetes mellitus, mouse models have proven very useful for delineating pathways of beta cell destruction and/or proof of principle for some therapeutic approaches, but have thus far failed to provide a single successful therapy for humans. Finally, even if human and mouse immunobiology were more similar, disease is largely determined by genetic difference \(within\) a population and environmental factors.

Krensky then contrasted the general aims and focus of biology and medicine along these lines: in one sense biology describes how humans are similar to other organisms; thus biological studies can provide fundamental (shared) building blocks and pathways of life. By contrast, medicine is often concerned with how individual humans (or other animals) differ (e.g., infection of a population with the same organism nearly always leads to a range of outcomes). Not surprising, many of the differences among how individuals respond to assault are accounted for by genetic and environmental differences. In order to overcome this variation in the laboratory mouse, models have been specifically developed to normalize for these differences. Laboratory mice, for example, are backcrossed, and single, genetically homogeneous strains are used to study biology on a uniform genetic background; similarly, controlled animal care facilities are designed to control the environment. However, although these constraints—genetic homogeneity and controlled environment within the population—make model studies highly reproducible on the one hand, they fail to account for some of the most important factors underlying human disease (for a list of such differences, see Table 1).

Krensky summarized by saying that although mouse models have been instrumental in describing mammalian immunobiology, new approaches will
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be needed to develop cures for human immunemediated diseases: this is especially true because mouse and human immunobiology differ, and genes and environment are of paramount importance in disease. Consequently, reallocation of resources and new approaches will be necessary to study clinical immunology of human disease.

Next, Jeffrey A. Bluestone (Director of the NIAID/JDRF-supported Immune Tolerance Network (ITN), University of California, San Francisco) spoke on “Lessons learned from the ITN,” focusing on the ITN’s efforts to establish a new paradigm for clinical research that goes beyond the traditional endpoints of safety and efficacy. With a focus on translational and “critical path” immunological research, the ITN has pioneered a model of “integrated clinical trials” that incorporate simultaneous investigations of the biological mechanisms of disease and possible therapeutic interventions. One type of intervention that the ITN has investigated is immune tolerance therapies: short-term or infrequent interventions that aim to halt problematic immune responses while leaving immune defenses intact. These emerging therapies stand in contrast to current immunosuppressive therapies that broadly and nonspecifically suppress the function of the entire immune system.

Challenges in immunology and infectious diseases

One of the greatest challenges in public health is to develop safe and effective countermeasures against human diseases caused by pathogenic microbes. Meeting this challenge requires not only an understanding of microbial biology and pathogenesis but also an appreciation of the host immune response. Most infectious agents have the genetic flexibility to avoid the human immune response, and such adaptations vary widely in kind and degree. Three well-known examples of infectious diseases with significant burdens of morbidity and mortality illustrate how different and challenging microbial adaptation to human immunity by infectious agents can be. These diseases were considered by Anthony Fauci (Director of the NIAID and Chief of the NI-AID Laboratory of Immunoregulation, NIH) during his keynote address “Challenges in Immunology and Infectious Diseases: A Tale of Three Diseases.” Specifically, Fauci discussed influenza, a prototypic acute infectious disease; AIDS, a chronic infectious disease; and malaria, an acute, chronic, and recurrent infectious disease. Each of these diseases exhibits distinct pathogenic mechanisms and elicits different host responses.

Subtypes of influenza A virus are distinguished by their surface glycoproteins, each of which “drifts” (mutates in and around several critical epitopes) during human-to-human circulation. Such drift enables viruses to escape immunity elicited by the previous years’ natural influenza infections and vaccines. Drift can occur far more rapidly than our ability to identify epidemiologically important viruses and make effective vaccines against them. Moreover, influenza viruses generally infect surface epithelial cells, and thus encounter only mucosal immune responses. The key mucosal antibodies appear to be IgG, IgM, and IgA subtypes, which exist at comparatively low levels at mucosal surfaces. Because of these and other immunologic challenges, it is difficult to make vaccines that match circulating viruses, are effective, and provide long-lasting immunity. An important research goal is to identify antigens shared by all influenza viruses and exploit them to make broadly immunogenic vaccines—sometimes referred to as “universal” vaccines.

The RNA virus HIV, the cause of AIDS, has its own reverse transcriptase enzyme that changes its RNA into DNA, which then integrates into the human chromosome where it remains “hidden” from detection by the host cell. Like the influenza A viruses, HIV has developed powerful means to avoid the human immune system. These include numerous
critical but immunologically hidden HIV-expressed epitopes that, within chronically infected hosts, often undergo antigenic change to escape neutralization. Unlike most other human viruses HIV is never fully cleared by the immune system—depriving investigators of a natural correlate of protective immunity to aim for in designing a preventive vaccine. Despite this very discouraging picture, a recent HIV vaccine trial indicating a modest level of short-term protection is being carefully examined for clues about immunologic correlates of protection that might be exploited to make better vaccines.9

Malaria is another challenging disease because the parasite goes through multiple, transient lifecycle stages, each associated with the expression of different antigens (the Plasmodium falciparum parasite produces at least 5,400 gene products). This challenging situation is further complicated by the existence of five malaria species, each of which can cause human infection and can be carried by at least 40 species of vector mosquitoes.

In natural settings, immunity to malaria builds gradually after repeated exposures, is quickly lost when exposures decrease in frequency, and is incomplete at best in preventing re-infection or severe complications. As with HIV, correlates of malaria immunity are not known, and they probably differ at different stages of the parasite lifecycle. Although these are serious challenges, hope is afforded by the large number of expressed malaria proteins that can be examined for immunogenic potential, and by the fact that the parasite’s multiple life-cycle stages offer numerous potential targets for vaccines.10

Fauci concluded with the reminder that the many microorganisms that infect humans have found a variety of ways to escape the limiting pressures of a broad natural and acquired immune repertoire. Next-generation vaccines to prevent infections by these agents will require research that leads to a better understanding of the specific immunologic interplay between these agents and their human hosts.

**Disease entities and concepts**

**Degenerative diseases with inflammatory components**

Peter Libby (Brigham and Women’s Hospital, Harvard Medical School) discussed the role of inflammation in atherosclerosis. As with so many of the degenerative diseases, atherosclerosis is now being evaluated for its immune-mediated mechanisms. A main point of Libby’s message was the growing acceptance of atherosclerosis as a chronic inflammatory disease—similar to concepts being developed in age-related macular degeneration. Chronic diseases of many organ systems involve common mechanisms of innate and adaptive immunity, a concept long applied to rheumatoid arthritis, but only more recently to atherosclerosis. While the traditional view of atherosclerosis was of the occluded vessel walls as occurring from “rust from within,” Libby highlighted instead the several features of inappropriate immune activation as being the cause of this disease: the initiation of atherogenesis begins with the attachment of monocytes to the vessel wall and subsequent penetration into the wall itself, with monocytes and macrophages present at different stages of development of the disease (and each with unique function). Mouse models have shown, for example, that inflammatory monocytes accumulate in animals with high systemic cholesterol, with Ly6C hi monocytes localizing in mouse atheromata. In fact subsets of these monocytes have been reported, each with a unique function, ranging from proteolysis, to phagocytosis, to angiogenesis, to promoting inflammation; it has also been reported that the spleen is the source for the rapid deployment of these cells. On the other hand, under hypercholesterolemic conditions CD11c+ dendritic cells mediate antigen processing and presentation, which leads to CD4+ T cell priming.

Another cell type that appears to participate in atherogenesis is the mast cell, as mast cell–deficient mice have muted atherosclerotic lesion formation, for example. Several mast cell–mediated mechanisms may mediate atherogenesis; mast cell–derived IL-6 and interferon gamma (IFN-γ) contribute to the potentiation of atherogenesis at least in mice. In addition, mast cell–mediated activation of cysteineyl proteinase cathepsins S and L may also help to promote this process.

Further links among inflammation and immunity are indicated in the thrombotic complications of atherosclerotic disease. Thrombi that cause fatal myocardial infarction in humans usually result from a physical disruption of the plaque; the structural integrity of the plaque’s overlying fibrous cap depends on the interstitial collagen fibrils synthesized by smooth muscle cells. Libby’s work has demonstrated that in humans atheromatous rather than fibrous
plaques might be prone to rupture owing to increased collagenolysis associated with macrophages: activated macrophages in plaques elaborate the interstitial collagenases MMP-1 and MMP-13, both of which have been implicated in human plaque rupture and thrombosis (incidentally, because mice do not express MMP-1, they cannot be used to model this pathogenic pathway).

A major question remains as to what triggers inflammation during atherogenesis. One important component is adipose tissue, which is a source of proinflammatory cytokines (such as IL-6), adhesion molecule expression (such as ICAM-1), and factors that can cause insulin resistance. Interferon gamma appears to play, at least in mice, a central role in adipose tissue’s induction of inflammation. Obese mice T cells produce high amounts of interferon, and interferon-gamma knockout mice have reduced adipose expression of mRNA encoding for inflammatory genes. In contrast, adiponectin appears to be an endogenous anti-inflammatory mediator.

While most of these observations have been shown to occur in mice, most need confirmation in humans. One exception to this is C-reactive protein (CRP), which is being used as a biomarker to translate inflammation biology to the clinic. CRP levels indicate increased risk for cardiovascular events beyond the traditional risk factors, underscoring the link between inflammation and cardiovascular events in humans. This inflammatory biomarker can select individuals who can benefit from otherwise unindicated preventive therapies.

Libby summarized his talk by saying that inflammation appears to drive all phases of atherosclerosis, from initiation, to progression, to even complications. Biomarkers for inflammation are now being used in the clinic to help define those at risk for cardiovascular events and target therapies. Some of the unanswered questions include, What are the antigens that drive the adaptive immune response in the atheroma? and What therapies directed selectively at innate or adaptive immunity can prevent cardiovascular events over and above existing therapies?11–15

Robert Nussenblatt (NEI) presented information concerning age-related macular degeneration (AMD). As the name implies the disease occurs in aging individuals, especially over the age of 55 years. AMD affects almost 2 million Americans, and it has been predicted that over 25% of the older population in the United States will have the disease a decade from now. AMD affects the macula and results in two types of clinical disease: dry type, where an atrophy of the macula occurs, and wet type, where there can be atrophy but choroidal neovascularization is present, causing a marked decrease in vision. One of the important hallmarks of the disease is large or giant drusen, the presence of which puts a patient at very high risk to develop this disorder (Fig. 1). Until recently the disease was considered to be a degenerative one of the aging individual. Recently several researchers have reported the association of various single nucleotide polymorphisms with AMD; almost all are associated with various immune functions. Two examples are complement factor H and HTRA1. The presence of these products has been shown in large drusen and in the surrounding tissue, including the retinal pigment epithelium. Histologic evaluations have shown the presence of macrophages at the same level of the eye, further supporting the notion of immune activation, as does the presence of activated macrophages in the peripheral blood of patients with AMD.

Animal models, either knockout or double knockout animals, do show some clinical characteristics that are seen in humans. However, none faithfully reproduce what is found in the human situation. Further, full immunological characterization of these models still needs to be done. The initial observations in the models suggest that macrophage hyporeactivity is central, while initial observations in humans would suggest the reverse.

Although animal data would suggest a strong role for complement and the innate immune system, recent work in humans supports the notion that the adaptive immune system seems to be central to the immune alterations noted in AMD. The presence of both C3a and C5a receptors on T cells are seen. Further, the enhanced presence of these receptors on circulating cells from AMD patients is associated with increased proinflammatory cytokines. Initial reports show that inflammatory cytokines can be found in the vitreous of these patients.

One important issue is not so much whether the immune system is involved in AMD but how central it is to the pathogenesis of the disease and whether it can be manipulated to possibly alter the outcome of the clinical course. Nussenblatt reported briefly on an NEI intramural randomized pilot study in which AMD patients with recurrent neovascularization...
Figure 1. Early (A) and late (B) changes in the back of the eye due to age-related macular degeneration (AMD). In the early stage of the disease, the optic nerve is seen on the left. Large drusen are strewn throughout the fundus. This finding increases dramatically the possibly of the development of AMD. The later photo shows the findings of the “wet” type of AMD. There is hemorrhage and scarring in the back of the eye that is a result of new blood vessel growth. The result is very poor vision.

were randomized to immunosuppression coupled with anti-VEGF injections (standard of therapy) or standard of therapy alone. Those receiving immunosuppressive therapy needed fewer injections of anti-VEGF than did those receiving standard of therapy. This proof-of-concept study supports the centrality of the immune system in this disorder.

The evaluation of AMD patients suggests mechanisms that may be different from the animal models described to date. Nussenblatt finished his talk by urging researchers to return to the animal model and better characterize them immunologically in order to see which ones best mimic the human situation.16,17

Autoimmune diseases

Roland Martin (Director of the Institute for Neuroimmunology and Clinical Multiple Sclerosis Research, University Medical Center, Hamburg, Germany) presented successes and challenges in translating the wealth of knowledge from the experimental autoimmune encephalomyelitis (EAE) animal model to human multiple sclerosis (MS). He reiterated the prevailing notion that the MS disease pathogenesis is analogous to EAE and is mediated by autoreactive Th1/Th17 CD4+ T cells. However, he pointed out that from the standpoint of testing prospective new therapeutics, EAE studies have been less than 100% predictive of therapeutic efficacy in MS. He has used blockade of the α4 subunit of α4β1 integrin (VLA-4) as an example of an MS therapeu- tic (natalizumab) that was successfully predicted by EAE studies.18 On the other hand, neutralization of TNF-α, which is highly effective in EAE, proved to exacerbate the MS disease process, and in fact can induce MS-like disease in patients who receive TNF-α blockers for different indications.19,20

Martin then focused on several translational stud- ies where the investigation of the effects of the applied therapy exerted on the human immune system helped to elucidate important differences between MS and EAE. The first example was use of daclizumab, a monoclonal antibody that blocks the IL-2-binding epitope on CD25, which is the high-affinity α-chain of the IL-2 receptor. Both animals and humans with the genetic deletion of CD25 develop severe autoimmunity, as high-affinity IL-2 signaling is important for the development and maintenance of regulatory FoxP3+CD4+ T cells (Treg cells).21–23 Thus, one would predict that blockade of CD25 may exacerbate, rather than treat, autoim- mune disorders. Indeed blockade of CD25 in mice
had no effect on the active induction of EAE. Nevertheless, CD25 blockade by daclizumab proved to be highly beneficial in inflammatory uveitis as well as high-inflammatory MS, despite the fact that this treatment did inhibit T<sub>reg</sub> cells. Immunological studies performed during clinical trials of daclizumab in MS demonstrated that this treatment expands immunoregulatory CD56<sup>bright</sup> NK cells, which are capable of inhibiting T cell responses by killing of autologous activated T cells. The CD56<sup>bright</sup> NK cell, or its exact counterpart, has not been identified in rodents; as such, this observation from the human system was completely unanticipated from animal studies.

A second example of the crucial difference between human and animal systems lies in the use of altered peptide ligand (APL) as a therapy of autoimmune diseases. Because EAE is most commonly induced by active immunization with myelin protein or peptide in the susceptible animal strain, the main target of the CD4<sup>+</sup> autoreactive T cells is well defined in such a system and usually represents a single immunodominant peptide/epitope. Thus, the disease can be either prevented or successfully treated by immunization with an APL, which represents the defined immunodominant epitope with substitutions in the amino acids that make contact with the T cell receptor (TCR). APLs can inhibit autoreactive T cells specific for the original immunodominant epitope by diverse mechanisms such as the induction of anergy, partial agonism, or bystander suppression, and this antigen-specific therapeutic strategy is extremely efficacious in animal models of autoimmunity, including several types of EAE. However, a clinical trial of an APL based on the immunodominant epitope of myelin basic protein (MBP<sub>83–99</sub>) in MS patients had to be terminated prematurely because of the occurrence of highly atypical MS exacerbations in three of the eight treated patients.

Associated immunological studies demonstrated the unparalleled diversity of the MHC-II and TCR repertoires in the outbred human population as compared to the inbred single animal strain, making any predictions of the TCR contact sites in a single peptide irrelevant; peptides will be presented by many different MHC-II molecules and will be able to stimulate a highly diverse polyclonal population of CD4<sup>+</sup> T cells. Indeed, that is what happened in the aforementioned clinical trial, resulting in more than a 1000-fold expansion of APL-specific CD4<sup>+</sup> T cells, some of which cross-reacted with native autoantigen, gained access into the central nervous system (CNS) and mediated severe inflammatory activity, as measured as contrast-enhancing lesions on a brain MRI. This study exemplifies the main problem between EAE and MS: in different EAE models the target of the immune response and disease-mediating population is fully defined; in MS, in contrast, the target(s) of the immune response, the pathogenic disease population(s), and the mechanism(s) by which they mediate destruction of the CNS are not currently understood.

As a final example, Martin demonstrated the importance of immunological studies accompanying therapeutic interventions in humans as a means to identify biomarkers predictive of the treatment response. He and his colleagues collected peripheral
blood mononuclear cells (PBMC) in MS patients before the initiation of interferon-beta (IFN-β) therapy. They then followed these patients for two years on therapy; and on the basis of clinical outcomes they divided them into treatment responders versus nonresponders. Using expression profiling on pre-treatment PBMC, Martin and colleagues were able to identify group differences in type I IFN signatures present already at baseline: a less inducible type I IFN pathway in monocytes was associated with lack of therapeutic response to subsequent IFN-β treatment. Such biomarkers, predictive of a treatment response, if validated, will be of great help to the clinician in selecting optimal first-line therapy for every MS patient. Martin concluded by saying that human studies in MS provide novel insights that were not predicted from animal models either because of interspecies differences in the immune system or because of true biological differences in the pathophysiology of EAE versus MS.

Richard Blumberg (Chief of Gastroenterology at Brigham and Women’s Hospital, Boston) spoke about current trends in inflammatory bowel disease (IBD) research. As defined by Blumberg, current research in the inflammatory bowel diseases (ulcerative colitis, Crohn’s disease, and other colitis) centers on studies of genetic susceptibility, immune dysregulation, the microbial flora of the intestine, and various environmental factors. These areas of research also define the major etiologic factors that interact with one another to cause these diseases.

Within the area of genetic susceptibility research, the major advance has been the identification of multiple single nucleotide polymorphisms (SNPs) associated with IBD. While most of these polymorphisms make only a small contribution to the overall genetic basis of these diseases, it is clear that they can reinforce one another to form a strong genetic complex that predisposes to intestinal inflammation. This is well supported by the fact that the polymorphisms have been found in genes that could be involved in various aspects of the mucosal inflammation underlying IBD, such as genes governing innate immunity and autophagy (NOD2, ATG16L1, and IRGM), basic adaptive immunity (IL-23R, JAK2, STAT3, PTPN2, and IL-10), downstream inflammation pathways (MST1, CCR6), and ER stress pathways affecting epithelial cell function (XBP1). The discovery of these genetic associations has led to a cornucopia of new research directions. On a basic level, these include the study of the disease mechanisms underlying polymorphisms in particular genes and the study of how these genetic abnormalities interact with each other and other factors to lead to gut inflammation. On a clinical level, new research directions include the study of how these genetic factors predict the course of disease and the response to therapy.

The systematic study of the human microbiome, the huge population of bacteria and other organisms that inhabit the bowel, is a second and newly emerging area of research into the mechanisms of IBD. This flows from the widespread acceptance of the idea that whereas in the normal gut a noninflammatory equilibrium between the microflora and the mucosal immune system prevails, in IBD the microflora is inducing pathologic immune responses leading to chronic inflammation. The gut microbiota consists of some $10^{13}$ to $10^{14}$ bacteria as well as $10^{12}$ to $10^{13}$ other microscopic life forms, and among these groups there exists a huge diversity of species. Many IBD research questions can be posed in relation to this abundant microbial life. Perhaps the most pointed is the question of whether there are dominant microbial organisms (and their associated antigens) that drive the disordered immune response of IBD and the related question of how the genetic polymorphisms associated with IBD shape the host response to commensal organisms. These and other questions will require a major shift in research emphasis in IBD toward the study of microbial characteristics and function, as well as the development of molecular techniques to evaluate microbial populations.

A third area of research in IBD, and perhaps the most productive so far in terms of therapeutic development, has involved the delineation of the immune responses that underlie mucosal inflammation. The basic kinds of T cell responses underlying Crohn’s disease (a mixed Th1/Th17 response) and ulcerative colitis (an NKT cell response productive of Th2-type cytokines) have now been defined. In addition, a great deal is now known about the regulatory responses of the mucosa involving TGF-beta/retinoic acid–induced regulatory T cells (Treg) as well as regulatory cytokines such as IL-10. Finally, it is now understood that these responses are driven or influenced by epithelial cells, dendritic cells, and other innate immune cells that are responding to the
innate and adaptive properties of antigens present in the bacterial flora.

Despite these advances in understanding IBD, many unanswered questions remain. Blumberg underscored the need for a better definition of the relation between the innate and adaptive immune systems in the establishment of mucosal tolerance, a better answer to the question of the role of epithelial cells in limiting and/or shaping mucosal responses and how these relate to the microbial milieu, and a better understanding of the possible role of regulatory cells in the treatment of mucosal inflammation (Fig. 3).

The identification of the factors underlying the immunopathology has already lead to the identification of new immune “targets” for IBD therapy, and in the near term we can look forward to effective treatments of this syndrome of diseases that are based on the blockade of newly identified immune cascades. In Crohn’s disease, for instance, the success of anti-inflammatory anti-TNF may now be supplemented or even supplanted by newer anticytokines such as anti-IL-12p40, which targets both IL-12 and IL-23. Correspondingly, in ulcerative colitis, selective adhesion molecule inhibitors, such as antibodies targeted against α4β7 or anti-IL-13, may come on line as effective therapies.

Blumberg summarized by defining the major challenges in IBD research. These included the identification of the full set of gene abnormalities involved in disease pathogenesis and the functional significance of these abnormalities. At the same time there is an urgent need to define the environmental factors such as diet, and exposure to antibiotics and pollutants that interact with genetic factors and influence the composition of the intestinal microbiota and immune response of the mucosa. Then, with regard to the latter, there is a need to better delineate the innate immune response of the gut and how these interrelate with and possibly

Figure 3. Inflammatory bowel disease is a multifactorial disorder. (Reprinted by permission from Annu. Rev. Immunol.35)
Carla Greenbaum (Director, Diabetes Program at the Benaroya Research Institute, Seattle, Washington) presented an overview of the challenges for human immunology in Type 1 diabetes (T1D). Greenbaum began her presentation with epidemiologic evidence for the rising T1D incidence worldwide, calculated at 3–5% per year, with the greatest percentage increase in incidence of T1D in the patients 1–4 years of age. Greenbaum then described the natural history of T1D with the development of autoantibodies preceding the development of clinical T1D by several years. BABYDIAB, a natural history study of children at high risk for the development of T1D, demonstrated that more than 25% of children who have either both parents or a parent and a sibling affected with T1D develop T1D-associated autoantibodies by age 6 and that the offspring who develop autoantibodies to multiple islet autoantigens have a 70% risk of developing T1D by 8 years of age. Larger studies of relatives of individuals with T1D, such as the Diabetes Prevention Trial, as well as those with no family history have confirmed that genetic, autoantibody, and metabolic testing can robustly identify individuals of high, intermediate, and lower risk, thus setting the stage for clinical trials to prevent onset of disease.

Clinical T1D is preceded by the development of insulitis and failure of beta cell function. While the progression of beta cell dysfunction has been well studied, there is limited information about the pattern and nature of the islet infiltrate over time in humans. The recent analysis of pathological specimens suggests that insulitis can persist long after disease onset in at least some individuals, and a variable number of residual beta cells in islets within each pancreas as well as between subjects. When present, the composition of the immune infiltrate includes variable numbers of CD8+ and CD4+ T cells, macrophages, and B cells. Despite the knowledge of autoantibody targets in T1D, measurement of T cell autoreactivity have proven to be much more difficult; multiple assays (T cell proliferation, cellular immunoblot, ELISPOT, and tetramer staining) reach limited positive (51–86%) and negative (50–77%) predictive values, making their utilization to distinguish individuals with and without autoimmunity unlikely. However, these tools are still expected to play a role in evaluating responses to therapy.

The enhanced knowledge about the pathogenesis of T1D is driving clinicians to test more candidate agents for immunomodulatory therapy in T1D with the aim of finding effective therapies without the adverse events seen with earlier generations of therapies (e.g., renal toxicity with the use of cyclosporine). Recent trials using many agents (e.g., anti-CD3 Ab, rituximab, etanercept, and antigen-specific immunization with alum-formulated glutamic acid decarboxylase (GAD)) have reported some success in prolonging endogenous insulin production in patients with new onset T1D. Of these studies, only the rituximab and the anti-CD3 trials were adequately powered, placebo-controlled trials that met their primary endpoint; and there were some significant adverse events in these trials; thus, while encouraging further validation of these, other approaches are needed. Ongoing and future clinical trials in T1D are trying to target the preclinical stages of T1D in high and intermediate risk populations in order to limit progression of insulitis into clinically evident T1D while at the same time developing further immunomodulatory treatments for the earliest stages of clinically apparent T1D.

Greenbaum identified, as major challenges for ongoing research, the identification of the causes for the rising incidence of T1D, and how one can effectively study interactions between genes, environment, and the ongoing processes at the level of beta cells. From the standpoint of the development of therapies, availability of several, at least partially effective, immunomodulatory therapies, is certainly a welcomed development; but one has to address the risk–benefit ratio if these therapeutic modalities offer only short-term benefit while striving to develop therapeutic strategies that prevent disease onset and prolong the endogenous production of insulin long term. Once the armamentarium of immunomodulatory strategies for T1D expands the development of predictive biomarkers indicative of treatment, response will be an essential guide for clinicians to apply these therapies most effectively.

Raphaela Goldbach-Mansky (National Institute of Arthritis and Musculoskeletal and Skin Diseases) outlined new developments and challenges in rheumatologic disease research being pursued at NIH and elsewhere. Initially, she focused...
on the rapidly developing area of autoinflammatory diseases characterized by systemic inflammation due in large measure to abnormalities of the cytosolic inflammasome, that is, the cryopyrin (NLRP3)-based immunologic mechanism that converts pro-IL-1 and pro-IL-18 into mature and secreted cytokines that induce inflammation. Most likely as a result of this inflammatory cytokine profile, the inflammation in these diseases is uniquely characterized by its highly neutrophilic character.

Abnormalities of the NLRP3 inflammasome lead to a spectrum of conditions characterized by fever, urticaria, joint symptoms, hearing loss, and conjunctivitis. The most severe is NOMID/CINCA (neonatal onset multisystem inflammatory syndrome); of intermediate severity is the Muckle-Wells syndrome, and the least severe is familial cold urticaria. The most severe is NOMID/CINCA syndrome; of intermediate severity is the Muckle-Wells syndrome, and the least severe is familial cold urticaria. The most severe is NOMID/CINCA syndrome; of intermediate severity is the Muckle-Wells syndrome, and the least severe is familial cold urticaria. (neonatal onset multisystem inflammatory syndrome); of intermediate severity is the Muckle-Wells syndrome, and the least severe is familial cold urticaria. (neonatal onset multisystem inflammatory syndrome); of intermediate severity is the Muckle-Wells syndrome, and the least severe is familial cold urticaria. (neonatal onset multisystem inflammatory syndrome); of intermediate severity is the Muckle-Wells syndrome, and the least severe is familial cold urticaria. (neonatal onset multisystem inflammatory syndrome). Recent clinical studies have shown that both the systemic and organ-specific manifestations of these conditions are highly responsive to an agent that blocks IL-1β function, namely, the interleukin receptor antagonist anakinra. These studies establish the role of IL-1 as the main cause of the inflammation in these syndromes and show that patients must be treated early to prevent irreversible organ damage.

Very recently a new autoinflammatory disease was discovered that is due to mutations in the naturally occurring IL-1 receptor antagonist (IL-1Ra) and that, like the inflammasome defects, results in excessive IL-1 function. This disease, deficiency of the IL-1 receptor antagonist (DIRA), characterized by severe bone dysplasia and other autoinflammatory manifestations but differs from NOMID by lack of hearing and vision abnormalities. It is also successfully treated with anakinra. Interestingly, the skin disease in this condition has been shown to be associated with cells that produce IL-17; this points out that IL-1β promotes the differentiation of Th17 T cells and suggests that IL-17 inhibitors will be a useful alternative or adjunctive treatment in many of these inflammatory states.

Both NOMID and DIRA share clinical features with other inflammatory diseases, such as chronic recurrent multifocal osteomyelitis/synovitis, acne, pustulosis, hyperostosis and osteitis (CRMO/SAPHO), Behcet’s syndrome, gout, Type II diabetes, and pustular psoriasis. This raises the question of whether these other diseases as well as “classical” rheumatologic diseases are also mediated by IL-1β, at least in part. Indeed, this view is favored by the fact that in some cases it has been shown that these diseases improve following treatment with anakinra. In addition, it has recently been shown that polymorphisms in the IL-1 gene cluster, and in IL1RN (the gene encoding IL-1Ra), have been associated with reduced production of IL-1R antagonists and increased severity of many inflammatory diseases. Thus, the more general principle that emerges from these studies is that treatment of inflammatory diseases will increasingly involve the identification of the cytokine “networks” responsible for the inflammation and then apply single or multiple inhibitors that block these networks.

**Cancer: inflammation and therapy**

Raymond DuBois (Provost and Executive Vice President of the MD Anderson Cancer Center, Houston, Texas) discussed several clinical and experimental lines of evidence that support a causal role for inflammation in the progression of colorectal cancer. Indeed many epidemiological studies (reviewed by DuBois) indicated that individuals using nonsteroid anti-inflammatory drugs have a significantly decreased risk of colon adenoma or invasive carcinoma. Also, the work of Galon and collaborators has shown that the presence, number, and localization of immune cells and T lymphocytes, in particular within the human colorectal tumor, predict the clinical outcome.

DuBois discussed how cyclooxygenase 2 (COX-2) is expressed at increased amounts in intestinal epithelial cells and colorectal tumors, and through its induction of prostaglandin E2 (PGE2), it controls inflammation by acting as a vasodilator and an enhancer of hematopoietic cell homing. In addition, COX-2 affects epithelial cell adhesion and apoptosis and regulates immune functions. The roles of inflammation and PGE2 in colonic tumors was extensively characterized in adenomatous polyposis coli (APC)–mutant mice that spontaneously generated polyps in the small intestine but generate a significant number of colon adenomas only when inflammation is induced by treatment with colitis-inducing agents such as dextran sulphate sodium (DSS) or by treatment with PGE2. PGE2 synthesis can be blocked by nonsteroid anti-inflammatory drugs, such as aspirin or indomethacin that inhibit both cyclooxygenases, or by specific COX-2 inhibitors such as celecoxib. The
effects of PGE2 on cell migration and proliferation are mediated in synergy with epidermal growth factor receptor (EGFR) signaling through phosphoinositide 3-kinase (PI3K). For example, polyp formation in APC-mutant mice was strongly inhibited by treatment with COX-2 inhibitors that acted in synergy with EGFR inhibitors.

Interestingly, a similar antitumor effect of COX-2 and EGFR inhibition was observed in human colorectal carcinoma xenografts in immunocompromised mice. In addition to cyclooxygenase inhibitors, the effect of PGE2 on human colon cancer could be targeted by inhibiting other PGE synthases downstream of cyclooxygenase, by blocking the EP receptors to which PGE2 binds, or by enhancing the activity of the PGE2-inactivating enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH) using EGFR tyrosine kinase inhibitors. Loss of PGDH leads to increased colonic tumor burden in APC-mutant mice, and, interestingly, loss of PGDH is often observed in human colorectal cancers. Although many studies have demonstrated that the use of anti-inflammatory agents reduces the risk of colorectal cancer, their effect after cancer diagnosis was not established. Two recent studies, however, have demonstrated that aspirin or COX-2-specific inhibitors increase overall and recurrence-free survival following surgical resection in the subset of patients with diagnosis of colorectal cancer that overexpressed COX-2 or presented three different mutations upstream of the start codon of the COX-2 gene. A large phase III trial with the COX-2 inhibitor was terminated early, however, because of the withdrawal of the drug due to its cardiac toxicity. This is obviously an unfortunate situation because the potential protective effect of the drug against colorectal cancer outweighed the risk posed by the modest incidence of cardiac complications.

The data presented by DuBois are evidence for a direct role for inflammation in the progression of a common and deadly type of human cancer. A similar situation is likely true for several other types of human cancer. In the case of colorectal cancer, good experimental models that mimic the genetic mutations known to occur in sporadic human colorectal carcinoma have allowed the investigators to test several of the mechanisms by which inflammation and PGE2 specifically affect tumor progression and to test successfully some of the therapeutic approaches that have been, at least in part, validated by clinical studies. However, even in this positive example of successful preclinical studies in the mouse, it is evident that the regulation of the inflammatory and immune response is not identical in humans and mice, and that real progress in using more specific and nontoxic therapeutic approaches will require a much better understanding of the human-specific regulation of these immune responses. Molecular and systems biology approaches aimed to characterize the modifications of the immune homeostasis induced by the presence of the cancer and by the immunomodulatory treatments will be very important to allow investigators and eventually physicians to understand the effect of the therapy on the tumors and to monitor its efficacy.

Steven Rosenberg (National Cancer Institute) presented an overview on the development and current status of cellular-based immunotherapy for cancer. Three main approaches to cancer immunotherapy have been explored over the past few decades: nonspecific stimulation of immune effectors (i.e., high-dose IL-2), active immunization to enhance antitumor reactions through the use of cancer vaccines, and the passive transfer of activated immune cells with antitumor immunity.

Rosenberg’s talk focused on clinical trials conducted within the surgery branch of the NCI that have used adoptive T cell transfer to treat cancer. For example, adoptive transfer of tumor-infiltrating lymphocytes (TIL) consists of transfer of large numbers of highly activated T cells with antitumor function; TIL are obtained from excised tumor samples and then grown in vitro in media containing IL-2 with or without irradiated feeder cells. In the non-conditioned host, adoptively transferred TIL expand only minimally in vivo.

Recently, investigators have explored the use of highly immunosuppressive conditioning regimens to lympho-deplete the patient’s own regulatory T cells and other immune populations that might either suppress the proliferation of adoptively transferred tumor-reactive T cells or compete for necessary homeostatic cytokines such as IL-7 or IL-15, which are vital for T cell survival. In their landmark study in melanoma patients, Dudley and colleagues showed that a conditioning regimen using cyclophosphamide and fludarabine followed by adoptive transfer of TIL given with IL-2 resulted in substantially improved in vivo proliferation of...
tumor-reactive T cells and a higher overall objective response rate (48%) compared to regimens using TIL without conditioning.\(^4^6\) Efforts to achieve even greater lympho-depletion by adding myeloablative doses of total body irradiation (TBI) to the conditioning regimen, followed by autologous stem cell rescue, have further improved upon those results. To date, 18/25 (72%) melanoma patients receiving 1200 cGy of TBI combined with fludarabine and cyclophosphamide have had an objective tumor response, including 7 patients who have achieved a durable ongoing complete response of 25–36 months' duration. Importantly, persistence of adoptively transferred cells, longer telomere lengths of in vitro-expanded TIL, and expression of CD27 on CD8\(^{+}\) TIL (characteristic of central memory T cells) have all correlated with an increased likelihood of cancer regression.

Rosenberg reported that investigators have recently attempted to improve cell transfer therapy using T cell-receptor (TCR) gene-modified cells. High-affinity TCRs can be identified by screening multiple antitumor TIL and peripheral blood lymphocyte clones or can be generated by immunizing transgenic mice, which may bypass tolerance to human self-peptides, or by mutagenesis of the CDR2 and CDR3 regions to increase the affinity of the TCR. The substitution of mouse constant regions for human constant regions or the addition of cystines in the alpha and beta chains to form a second disulfide bond are currently being explored as methods to enhance pairing of the introduced TCR chains to avoid mispairing of the inserted alpha and beta chains with endogenous alpha and beta chains.

The NY-ESO-1 cancer antigen, which is expressed in approximately 25% of epithelial tumors, potentially represents an excellent target for TCR-modified T cells. In a recent and ongoing surgical branch protocol, infusion of autologous peripheral blood leukocytes (PBL) transduced with a retroviral vector encoding a high-affinity NY-ESO-1 TCR has resulted in tumor regression in a number of patients with synovial sarcomas. Retroviral vectors encoding TCRs with high affinity for a variety of tumor antigens, including MART-1, P53, GP-100, and CEA, have also been developed and are currently being explored in pilot clinical cancer trials to transduce autologous PBL to express tumor-reactive TCRs for subsequent adoptive transfer in cancer patients.

Such studies have shown that cell-transfer immunotherapy can mediate the regression of metastatic cancer in humans.\(^4^7\)–\(^4^9\) Pilot clinical trials have provided preliminary evidence that autologous peripheral lymphocytes genetically modified to express antitumor T cell receptors can mediate cancer regression in vivo. The ability to genetically modify human T cells opens possibilities to improve the effectiveness of cell transfer immunotherapy and extend it to patients with common epithelial cancers.

Jacques Banchereau (Director of the Baylor Institute for Immunological Research) presented an interesting and challenging series of translational studies indicating the possibility of testing gene expression in human peripheral blood cells as a way to detect signatures of pathological conditions, identify molecular targets for therapeutic intervention, and follow disease progression and the effectiveness of the therapy. These studies were initiated on the basis of the original observation that serum from patients with systemic lupus erythematosus (SLE), a chronic inflammatory autoimmune disease that affects many different organs, contained high levels of type I interferon that could induce the differentiation of human monocytes to dendritic cells. Studies of gene expression by microarray analysis of peripheral blood cells allowed Banchereau and collaborators to detect a clear (bioinformatic) signature of type I IFN-dependent gene expression in SLE patients but not in control healthy donors. The signature in SLE patients was eliminated by therapeutic treatment with high-dose glucocorticoids but not by other conventional treatments for SLE (which only affected the signature of activated plasma cells that were also observed in these patients).

The attempt to use the same approach for another autoimmune condition, systemic onset juvenile idiopathic arthritis (SOJIA), proved more problematic because, although a blood signature clearly differentiated SOJIA patients from healthy children, it could not differentiate them from other patients with inflammatory syndromes, for example, those with infections from various pathogens. However, a more extensive analysis using not only the fold-induction of certain genes but also the statistical probability of the observed differences, allowed the investigators to identify a specific SOJIA signature that differentiated the patients with this syndrome from those with other inflammatory syndromes,
including infections and SLE. A decisive further refinement of this approach came with the identification of gene expression modules characteristic of certain biochemical responses (e.g., the IFN or the inflammation signatures) or of a specific blood cell type (e.g., B or T lymphocytes). This modular representation of the blood gene expression analysis allowed the investigators a more immediate interpretation of the data that readily identified specific pathological condition signatures. The original version of this approach included approximately 30 modules but with a more precise identification of signatures by taking into account the specific signatures identified in purified preparations of the different cell types present in the peripheral blood. The second generation of this analysis has now been extended to approximately 80 modules.

The use of this approach in patients with SOJIA has allowed Banchereau and collaborators to identify IL-1 as a potential therapeutic target on the basis of the fact that serum from the patients induce IL-1 and IL-1–dependent gene expression in PBMC from healthy donors. Proof of concept was obtained by showing clinical remission in the patients by treatment with anakinra to block IL-1 activity. The efficacy of the treatment was evaluated by sequential blood gene expression analysis that showed a major reduction of the disease signatures after one month of treatment and an almost complete “normalization” at six months. The usefulness of this approach has been demonstrated by Banchereau’s group in several other disease conditions, including the analysis of disease progression in HIV infections and in melanoma patients, as well as in the follow-up of the response to vaccination.

One of the difficulties all human immunologists face is analyzing the immune response in humans under both physiological and pathological conditions. It is not possible, for example, to routinely obtain tissue samples of the organs in which the immune or inflammatory response is taking place. But if one could demonstrate that gene expression analysis of the peripheral blood was sufficient to readily identify alterations specific to either the systemic or localized immune and inflammatory conditions, such a demonstration would clearly open new important avenues for diagnosis and follow-up of patients. As Banchereau commented, in the future it may well be that microarray analysis of blood gene expression will be a clinical assay as common as the complete blood cell count is today. Obviously, other molecular analyses at the peripheral blood level could complement the gene expression microarray; for example, a proteomic analysis, the analysis of circulating cytokines, and/or the identification of the fine antigenic specificity of circulating antibodies are possibilities. A network of comprehensive human immunology centers could initially be involved in the use of such new diagnostic tools for research in many medical fields, creating reference centers and databases for the validation and widespread clinical use of these tools, and identifying biomarker signatures that will enable clinicians to better diagnose, prognose, and make personalized treatment decisions. Also, as some of the studies of Banchereau’s group have already shown, a better understanding of human disease pathogenesis resulting from these molecular tools will undoubtedly lead to the identification of new potential therapeutic targets that could be validated by clinical trials in which the same tools could be used, in addition to the clinical response, as surrogate markers for early evaluation of the efficacy of the treatment.

The relationship between the immune system, natural resistance and adaptive immunity, and the initiation, progression, and dissemination of cancer is very complex. Depending on the country and hygienic conditions, between 8 and 15% of cancers have an infectious origin; pathogens either directly transform normal tissue cells and initiate the neoplastic process or they induce a situation of chronic inflammation and continuous tissue repair that facilitates the development of neoplastic lesions. In a larger proportion of cases, a situation of chronic inflammation (for example, due to a genetic defect or to exposure to chemical, physical, or irradiation trauma) facilitates the initiation or progression of cancer. The immune response can, however, also prevent cancer progression by eliminating or controlling early lesions (e.g., via immunosurveillance or immunoediting) or by slowing the progression of established lesions (as shown by the favorable prognostic significance of a brisk lymphocytic infiltration demonstrated in different types of cancer).50–54

Allan Hildesheim (Division of Cancer Epidemiology and Genetics, National Cancer Institute) spoke about population-based approaches to the study of immune/inflammatory mechanisms involved in cancer development and prognosis. The goal of such research is to understand how immune mechanisms
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are involved in cancer development and how immunologic markers can be used to predict cancer risk or cancer recurrence/death. Barriers to achieving these goals are several and include the fact that immune mechanisms are complex and inherently difficult to measure, and that in studies of humans it is difficult to gather the biologic specimens necessary for immune assessment.

In record linkage studies, information already present in large databases is used to evaluate how specific conditions are related to cancer risk. Two good examples of this approach are NCI’s HIV/AIDS Cancer Match Study, in which data on 600,000 HIV-infected individuals were analyzed to determine the impact of infection on cancer occurrence, and the Transplant Cancer Match Study, in which data on 400,000 transplant recipients were matched with 18 cancer registries to assess cancer risk among transplant recipients. In the HIV/AIDS match study it was determined that AIDS-related cancer in patients has declined with the use of more effective therapy, whereas non-AIDS-related cancer has held steady. However, an unexpected finding was that whereas Hodgkin’s lymphoma (an EBV-related tumor) was increased in immunosuppressed HIV and transplant-recipient patients, paradoxically, Hodgkin’s lymphoma was increased rather than decreased in HIV-infected patients on effective therapy (HAART).

In longitudinal cohort studies large populations are typically recruited, exposure and risk factor information is collected, biological specimens are collected and stored, and participants are followed for many years to ascertain disease outcomes. These studies are optimal for testing specific hypotheses and for assessing risk associated with exposures and biomarkers (including immunological markers) measured prior to disease development. While such cohort studies have been successfully used to evaluate genetic factors associated with disease risk (e.g., the NCI Consortium of Cohorts), they have not been extensively tapped for studies of immunity and cancer. As an example of the type of studies that could be developed to evaluate the role of immune response in cancer development, Hildesheim described results from one nested case-control study conducted within a large cohort in which C-reactive protein (CRP) levels were evaluated in a subset of close to 600 individuals who subsequently developed lung cancer and 670 cohort participants who did not. This study found that risk of lung cancer increased with increasing level of CRP and that the absolute risk of lung cancer development was substantial, particularly among smokers with elevated CRP levels. These results suggest that both smoking and CRP levels might be useful indicators of subsequent cancer risk.

In case-only tissue-based studies, the focus is on the evaluation of tumor-associated markers that can help better understand disease subgroups and define predictors of clinical response to treatment. This study design simplifies the acquisition of biological specimens, but suffers from the limitation that specimens are obtained at or after diagnosis and that non-diseased controls are often unavailable. In one example of this approach, the NCI Polish Breast Cancer Study of some 1250 patients, tissue microarrays were constructed and used to define tumor subtypes of etiologic and prognostic relevance.

Hildesheim concluded his talk with a discussion of the potential for a new generation of studies that incorporate longitudinal epidemiological designs with the collection of biospecimens that can be used for immunological assessment. An example of this type of study is the NCI/Costa Rica HPV Vaccine Trial involving some 7500 women in Costa Rica who were randomly administered either a human papillomavirus vaccine or a control vaccine (hepatitis A vaccine) and then followed over time. In this study, the main aim was to determine the safety and efficacy of the HPV vaccine in preventing HPV infections and cervical pre-cancers. However, the biological specimens and other risk factor information collected from participants, combined with the active follow-up of participants, will allow for further studies to understand mechanisms of vaccine protection and failure and to understand the role of immunity in the natural history of HPV infections and their associated outcomes. Overall, the NCI/Costa Rica HPV Vaccine Trial can serve as a model for future studies developed to employ powerful epidemiological and laboratory methods in order to evaluate immunologic mechanisms that influence cancer risk.

The past, present, and future of human immunology

In his keynote address Mark Davis (Stanford University) stressed that immunology was based upon empiricism and phenomenology, reviewing the
history from Jenner to Pasteur, in laying the basis for vaccination, and of Ehrlich, Koch, and Metchnikoff who provided the foundation for understanding host–microbe interactions in infectious disease. Basic mechanisms—including clonal selection of lymphocyte antigen specificities, antibody diversity and isotype switching, MHC restriction, antigen processing and presentation, and innate immunity—have all been revealed in the past 50 years by basic work largely using inbred mice. While mouse immunology has provided a great deal of insight into the immune system over the past half century, surprisingly little of the information gained has been put to use clinically, except for a few instances in defined fields such as infectious disease, rheumatology, and allergy. According to Davis, even though the immune system is central to health it is conspicuously absent from medical practice. Davis asked how we measure human immune responses and what these responses mean in health and disease.55

Defining a “normal” human immune response is hampered by the inherent diversity of human beings, fragmentation of medical specialties, ethical limitations, paperwork, the “small lab” ideal, and the overemphasis on individual achievement. For Davis, the genome project taught us that “big science,” with teamwork and economies of scale, can propel a field without damaging the culture of small, entrepreneurial laboratories; thus it is now time to organize such efforts in human immunology. The Stanford Human Immune Monitoring Center is aimed at building infrastructure to enable such an approach.

Davis reviewed the spectrum of human disease, from overactivity in autoimmunity and allergy, to underactivity in aging, drug treatment, infections, inborn errors (mutations), to what presumably lies in between: health. The Stanford group has developed a series of high-throughput assays and databases to investigate the human immune response in health and disease. Davis highlighted a multiparameter analysis of seroconversion and seroprotection after seasonal flu vaccination. Phospho-flow technology detected blunted immune responses in older subjects. New peptide-MHC tetramer approaches enabled T cell repertoire analyses indicating that the human T cell ligand repertoire is very similar to that in the mouse, despite the fact that humans typically have greater than a 1000-fold more naive CD8+ T cells. He also discussed new methods for evaluating microarray data, e.g., taking advantage of the diversity of cell types in a tissue to adjust for differential expression. In an era of high-throughput measurements, it is clear that scientific information will accumulate faster than we can turn it into “knowledge.” Thus, according to Davis, it is increasingly important that new bioinformatics tools and approaches be developed to support these endeavors.56

**Novel therapies, transplantation, and stem cells**

Carl June (Department of Pathology and Laboratory Medicine, and Abramson Family Cancer Research Institute, University of Pennsylvania) presented an overview of novel methods for adoptive T cell transfer in treating cancer and for engineering T cells to be resistant to HIV infection. June’s talk focused on adoptive T cell transfer—or “passive” immunotherapy—to bolster anticancer and antiviral immunity. Such therapy typically requires harvesting patient T cells, either from peripheral blood mononuclear cells (PBMCs) or from tumor infiltrating lymphocytes, followed by either nonspecific polyclonal stimulation over 7–10 days or repeated cycles of antigen-specific stimulation for up to 6 weeks using antigen-pulsed APCs; this in vitro stimulation results in both activation and expansion of T cells to numbers required to meet the minimal threshold necessary to render a therapeutic effect of the cells after they are adoptively transferred into a patient. A number of cell culture approaches for the expansion of human lymphocytes, including γδ T cells, NK T cells, and polyclonal CD3+ T cells, that are compliant with FDA regulations have been explored. One in vitro approach that achieves rapid polyclonal expansion of clinical grade T cells has used beads conjugated to CD3- and CD28-specific antibodies. The use of CD3/CD28-expanded, adoptively transferred T cells has been recently explored in patients with multiple myeloma undergoing autologous stem cell transplantation. Previous studies of autotransplants for myeloma provide evidence that transplants containing higher doses of infused lymphocytes are associated with superior clinical outcome.

Rapoport and colleagues recently explored a clinical trial of autologous transplantation in multiple myeloma in which patients received adoptive transfer of CD3/CD28-expanded autologous
T cells infused after myeloablative chemotherapy and stem cell transplantation.\textsuperscript{56} Recipients of adoptively transferred T cells had a shorter duration of lymphopenia, more rapid lymphocyte recovery, and more robust T cell responses to flu peptide vaccination, compared to controls undergoing conventional transplantation without adoptive T cell transfer. Homeostatic expansion of T cells was schedule-dependent, occurring when T cells were infused on day 2 after vaccination but not when infusions were delayed 12 days after vaccination. Rapoport and colleagues noted that 16% of patients developed T cell “engraftment syndrome,” characterized by diarrhea, rash, and fever (a syndrome resembling acute graft-versus-host disease), which investigators hypothesized may have occurred as a consequence of conditioning-associated eradication of regulatory T cells and thus loss of tolerance to autotantgens.

Moving from cancer to viruses, June and colleagues have also explored methods to genetically modify T cells to target HIV antigens. SL9 is an HIV-1 GAG epitope that is HLA-A2 restricted and appears to be universally expressed in HIV-infected cells. June described that an HLA-A2–restricted T cell receptor with high affinity for SL9 has recently been cloned and transduced into \textit{in vitro}-expanded T cells; a clinical trial to evaluate the safety and antiviral effects of adoptively transferred, polyclonal populations of CD8\textsuperscript{+} T cells transduced to express a high-affinity TCR for SL9 will be explored to evaluate cytotoxic T lymphocytes (CTLs) as immunotherapy to treat HIV.

An alternative approach to treating HIV involves genetic modification of T cells to confer antiviral resistance. Levine and colleagues have used a lentiviral vector encoding an antisense to the HIV envelope.\textsuperscript{57} In a clinical trial, HIV\textsuperscript{+} patients underwent an apheresis enriched for CD4\textsuperscript{+} cells expanded in number by CD3/CD28 treatment and then transduced with a lentiviral vector encoding a marker gene and an antisense RNA specific for the Env RNA sequence. Although this trial is ongoing and efficacy against HIV remains to be determined, 18 patients have received adoptive T cell transfer without toxicity and with evidence for engraftment of genetically engineered T cells in the blood and in the rectal mucosa. Analysis revealed >7,000 unique lentivirus integration sites in \textit{ex vivo}-expanded cells, with 240 unique sites from cells recovered after infusion and no evidence for insertions within 50 kb of any known proto-oncogenes.

June described another novel approach to confer T cell resistance to HIV that involves genetically silencing the expression of the chemokine receptor CCR5 using zinc finger nuclease (ZFN)–mediated genome editing. CCR5 is the primary HIV-1 coreceptor; people who are homozygous for a CCR5 delta 32 deletion (i.e., people who carry the mutation in both CCR5 alleles) are resistant to HIV infection. Therefore, disruption of CCR5 expression can be used to confer T cell resistance to HIV. Viral transduction of cells \textit{in vitro} with a ZFN-expressing virus resulted in highly efficient disruption of CCR5 expression. A phase I trial evaluating adoptive transfer of \textit{in vitro}-expanded CCR5-deleted CD4\textsuperscript{+} T cells is currently ongoing.\textsuperscript{58–60}

Richard Childs (National Heart Lung and Blood Institute, NIH) spoke on the use of allogeneic immunotherapy to treat advanced cancers, such as renal cell carcinoma, and the use of allogeneic T cells to identify novel tumor antigens. For more than 30 years allogeneic hematopoietic stem cell transplantation has been used as a clinical modality to cure patients with advanced leukemias, lymphomas, and other hematological malignancies. The graft-versus-leukemia or graft-versus-tumor (GVT) effect that occurs after allogeneic hematopoietic stem cell transplantation, which is due to immune activity of transplanted donor immune cells, is responsible for mediating the majority of these cures.

Hematological malignancies vary in their susceptibility to the GVT effect. In general, malignancies with slower growth kinetics tend to be more responsive to GVT effects, while cancers with rapid growth kinetics, such as blast crisis CML or chemotherapy refractory AML or ALL, are more resistant to GVT effects. Allogeneic T cells are the dominant immune populations mediating GVT effects, and minor histocompatibility antigens (mHAs) are the dominant targets of these T cells. As targets of T cells, mHAs expressed broadly on normal and leukemic cells can stimulate simultaneous GVT effects and graft-versus-host disease (GVHD); mHAs with expression restricted to hematopoietic cells can stimulate GVL concomitant with graft-versus-host hematopoiesis; and some patients will have a GVT reaction without clinical evidence for GVHD. In the latter cases \textit{in vitro} data suggest that donor T cells may be targeting antigens.
restricted to the leukemia, although only a handful of these antigens have yet been characterized.

Childs described a pilot trial conducted at the NHLBI in which renal cell carcinoma (RCC) was identified to be a target for a GVT effect following allogeneic hematopoietic cell transplantation (HCT). Childs reported that 30 of 76 patients (39%) with metastatic RCC had tumor regression following a milder regimen of allogeneic HCT, consistent with a GVT effect. In some cases, tumor regression occurred in the complete absence of any clinical signs of GVHD.

In an effort to maximize GVT effects while avoiding GVHD, significant research efforts have focused on methods to target the donor immune system specifically against the malignancy. Vaccinating patients following transplantation with tumor vaccines, or donors prior to stem cell harvest, theoretically could increase the frequency of tumor antigen-reactive T cells. Both strategies have been shown to boost GVT effects in animal models of HCT. The major limitation of these approaches is the requirement of identifying tumor antigens with properties that would make them good antigens to target, e.g., being highly expressed only in tumor cells. A better understanding of the tumor antigens expressed on RCC targeted by donor immune cells could lead to the development of transplant approaches that incorporate tumor vaccine strategies to augment GVT effects while avoiding GVHD.

Childs reported that they detected RCC-reactive CD8$^+$ T cells by ELISPOT analysis in the blood of several responding patients with metastatic RCC following HCT that were absent before transplantation. Using PBMC obtained from a patient who demonstrated a graft-versus-RCC effect associated with prolonged disease-free survival, CD8$^+$ T cell clones were isolated with RCC-specific tumor cytotoxicity. Using cDNA expression cloning, a 10 amino acid peptide was found to be the target antigen of RCC-specific T cells. The genes encoding this antigen were found to be derived from a human endogenous retrovirus type E (named CT-RCC HERV-E), previously unknown to be expressed in any human tissues. Using RT-PCR, Childs and colleagues showed this endogenous retrovirus was expressed in the majority (13/17) of clear cell tumors but was not expressed in normal kidney tissues or the non-clear cell histological subtypes of kidney cancer (0/16). A number of different endogenous retroviruses, recently shown to be expressed in both solid tumors and hematological malignancies, can induce cytotoxic T cell responses in vivo. However, the factors regulating transcriptional activity of most endogenous retroviruses that lead to tumor-restricted expression are poorly understood. Because the clear-cell histological subtype of RCC is characterized by inactivation of the VHL tumor-suppressor, VHL was hypothesized to regulate expression of CT-RCC HERV-E. Again using RT-PCR, Childs and colleagues showed VHL expression was absent in all clear-cell tumors expressing the CT-RCC HERV-E. Transfection of wild-type VHL into VHL-mutant clear-cell carcinomas dramatically reduced expression of HERV-E–derived transcripts. In VHL-mutant RCC cells, HIF-2α overexpression was required but not sufficient to induce HERV-E expression; siRNA inhibition of HIF-2α silenced HERV-E expression, although the provirus was only expressed in clear-cell tumors that expressed HIF-2α and had demethylated proviral HREs.

Childs concluded with the point that allogeneic stem cell transplantation can result in powerful GVT effects against hematological malignancies and certain solid tumors such as RCC. Allogeneic T cells are the principle immune effectors mediating these responses, and they can be used to identify novel tumor antigens that are the target of a GVT effect. Using allogeneic T cells, a tumor antigen derived from a HERV-E was found to be expressed in the majority of clear-cell RCC cells expressing an antigen targeted by T cells that mediated regression of metastatic kidney cancer in a patient who had undergone allogeneic HCT. Because CT-RCC HERV-E is not expressed in normal tissues, antigens derived from this provirus can serve as excellent targets for cellular immunity.

New approaches to clinical studies

Jeffrey Siegel (team leader at the FDA) reviewed the options available to investigators who are interested in studying rare diseases. Certainly the standard large, randomized placebo-controlled trial (RCT) remains the gold standard. However, for many disorders this approach is not feasible. Devising trials for new therapies in a rare disease can be a challenge, and using a placebo may not be ethical. Siegel reviewed alternative trial designs that can be considered.
There are specific challenges related to studying rare diseases, including the small number of patients, often poorly validated ways to measure disease activity, determining the clinical course, and establishing the standard of care for these patients. Choosing an appropriate endpoint can be a real challenge, and often a composite of symptoms may be better than choosing just one to determine the endpoint. An alternative is choosing a surrogate endpoint, e.g., a laboratory value that is closely associated with a symptom or the progression of the disease. This can allow for more expedient trials and, possibly, decrease the sample size. While surrogate endpoints can be objective measures, it is clearly critical that the surrogate’s reliability be proven. Siegel outlined six alternative, randomized, parallel group designs in addition to the classic RCT.

In the randomized withdrawal design participants who respond positively to a medication given in an open-label fashion are then randomized to blindly receive continued therapy or placebo. Recurrence of disease or symptoms can be a study endpoint. The advantage to this approach is that it enriches the study population for patients who respond to the study drug and lessens the time on placebo. It is also useful to evaluate the long-term effectiveness of a drug. Some of the downsides of this approach include treating patients with placebo after showing that a drug may have an effect, and the possibility of a carryover effect from the original therapy that could affect how those receiving placebo respond. A crossover design, in which subjects receive two medications in a different sequence separated by a washout period, is another alternative. This approach is attractive because each patient serves as his or her own control and fewer patients are needed. A carryover effect of the first medication is always a concern, however. And this is a difficult approach to use if the disease is unstable with a variable course. A third approach is the add-on design. Here subjects are enrolled while maintained on standard of care therapy and then randomized to either placebo or active therapy. This is a very useful approach if it is unethical to leave patients untreated. However, it does not provide information about the drug of interest as monotherapy. Furthermore, researchers need to be concerned about interactions between the test drug and therapy given as standard of care. In a dose–response design subjects are randomized into one of several different dose groups. Here a positive study would show increasing efficacy with an increasing dose. An advantage of this design is that all patients receive the active drug. However, to assure interpretable results one needs to have a clear understanding of the dose–response relationship in order to choose doses. Further, it may be very difficult to interpret the results. The placebo-phase design starts one group of subjects on the study drug immediately and other groups with varying times of delay. Patients receiving the study drug at later time points receive placebo initially in order to maintain the blind. A positive study is one in which similar efficacy in all groups is noted in a temporal manner consistent with the time they receive the active drug. This design has not been widely used to date. The three-stage design is perhaps the most complicated. In this design all eligible subjects enter into a standard RCT with a placebo arm (Stage 1); those subjects who respond to the study medication enter into a randomized withdrawal phase (Stage 2); and in Stage 3 placebo-treated patients who did not respond in Stage 1 but did respond to open-label treatment are entered into a randomized withdrawal phase. This approach reduces sample size but has not been used very often.

Siegel concluded by saying that rare diseases present real challenges regarding optimal study design. However, a variety of alternative study designs beyond the classic RCT do exist.

**Important messages from this meeting**

More intensive evaluation of the human immune system is clearly needed. Animal models, while important, will often not provide the immune community with information needed to understand and treat human disease; this realization is taking root in many centers and underlies the new Center for Human Immunology at the NIH. The human immune system is not yet fully understood, and new insights will largely depend upon discovery-driven, rather than hypothesis-driven, investigation. Although superb science is now performed by many groups, if a major goal of research is to improve the health of patients, it is clear that some of the paradigms we presently use must be changed. Innovations in deep databases, including details of normal and disease phenotype, genetics and genomics, and understanding clinical perturbations (environment, therapies, and disease) will be required; these
innovations will entail, among other things, a systems biology approach where data is integrated in ways that are just beginning to be investigated. In addition, a population biology-based approach, recognizing the differences between the population and the personal nature of disease, is required. Among the types of approaches already being examined include detailed phenotyping of normal and diseased people, defining the natural course of disease, registering more clinical trials, and collecting large interactive databases. In addition to detailed genetic and genomic information, including HLA typing, single nucleotide polymorphisms, and gene expression patterns, are the newer “omics,” including proteomics, metabolomics, and materi-omics. New environmental metrics must also be applied. Serum cytokine profiles, leukocyte functional responses (cytokines, phosphoproteins), leukocyte phenotyping (biomarkers), and RNA expression profiling are examples of standard assays being developed.

This conference highlighted the urgency of studying the human immune response with the goal of understanding immune-mediated disease and the development of effective diagnostics, biomarkers, and therapeutics.

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Conflicts of interest

The authors declare no conflicts of interest.

References

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