Immune Activation in the Pathogenesis of Treated Chronic HIV Disease: A Workshop Summary

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Abstract

With the advent of highly effective antiretroviral therapy (ART), infection with human immunodeficiency virus (HIV) has become a chronic disease rather than a death sentence. Nevertheless, effectively treated individuals have a higher than normal risk for developing noninfectious comorbidities, including cardiovascular and renal disease. Although traditional risk factors of aging as well as treatment toxicity contribute to this risk, many investigators consider chronic HIV-associated inflammation a significant factor in such end-organ disease. Despite effective viral suppression, chronic inflammation persists at levels higher than in uninfected people, yet the stimuli for the inflammation and the mechanism by which inflammation persists and promotes disease pathology remain incompletely understood. This critical gap in scientific understanding complicates and hampers effective decision making about appropriate medical intervention. To better understand the mechanism(s) of chronic immune activation in treated HIV disease, three questions need answers: (1) what is the cause of persistent immune activation during treated HIV infection, (2) what are the best surrogate markers of chronic immune activation in this setting, and (3) what therapeutic intervention(s) could prevent or reverse this process? The NIH sponsored and convened a meeting to discuss the state of knowledge concerning these questions and the best course for developing effective therapeutic strategies. This report summarizes the findings of that NIH meeting.

Introduction

THE DIVISION OF AIDS (DAIDS) in the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) partnered with the NIH Office of AIDS Research (OAR) to convene the workshop “Immune Activation in HIV Pathogenesis: Models and Targets” in Potomac, Maryland on April 21–22, 2010. The workshop brought together a wide array of investigators pursuing human immunodeficiency virus (HIV)- and simian immunodeficiency virus (SIV)-related research as well as research in non-HIV fields and was organized by a committee composed of DAIDS staff and outside experts in the HIV field. In advance of the workshop, the committee presented the speakers with questions (listed in Table 1) to be addressed during the 1.5 days of the workshop. The primary purpose of the workshop was to provide a participatory forum to explore the state of knowledge regarding the role of persistent immune activation in the pathogenesis of chronic, antiretroviral-treated HIV infection.

The fundamental pathogenic process in untreated HIV infection is the progressive loss of circulating CD4⁺ T cells, resulting in immune deficiency and susceptibility to opportunistic infections and malignancies. The exact causes of CD4 T cell loss are still unresolved, but may involve direct viral cytopathicity, bystander cell death via triggering of apoptotic pathways, and reduced T cell regeneration.^{1–3} Although progressive loss of CD4⁺ T cells and the resulting immunodeficiency are causally related to the development of many AIDS-related conditions, it has been apparent since the earliest years of the AIDS epidemic that other factors—in particular a persistent state of generalized immune activation—predict outcomes independent of the viral load and CD4⁺ T cell count.

Ascher and Sheppard first introduced the concept that chronic immune activation (referred to in a clinical context as inflammation) contributes to HIV pathogenesis.⁴ The role of generalized immune activation in HIV pathogenesis was further recognized and investigated in the first decade of the epidemic by the Miedema laboratory in the Netherlands⁵ and

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TABLE 1. OVERARCHING QUESTIONS

1. What are the inducers of chronic immune activation in the context of suppressive ART?
2. Which cell types predominantly sense and mediate these signals? Are relevant cells tissue-specific? Can they be found in blood? Which cells promote resolution of CIA? How are they affected in chronic HIV?
3. What are the regulators of these effects? How are these signals maintained and how could they be resolved?
4. At which point in the cascade would be the most feasible point for intervention (inducer of signal, sensor of signal, mediator of signal, cellular or tissue target of signal)? Can we expect that targeting one pathway will be sufficient and have equal effects on target tissues?
5. For the proposed mechanisms, what can we deduce from other models of chronic immune activation where that mechanism may be a contributor?
6. What animal models are currently being used to understand chronic immune activation, both within and outside of the HIV field? Do we need additional models? What questions can and cannot be addressed using these models? What information can be gleaned from the comparison of the natural and nonnatural NHP models?
7. Assuming that chronic immune activation in HIV infection is mediated by multiple inflammatory mechanisms, Systems Biology approaches have the potential to be informative. However, given differences in disease course, adherence, comorbidities, age, genetic diversity, and other confounding factors would these approaches be feasible in chronically infected subjects?

ART, antiretroviral therapy; CMV, cytomegalovirus; CIA, chronic immune activation; NHP, nonhuman primate.

by Grossman *et al.* at the National Institutes of Health.⁶ Dr. Janis Giorgi, a leading immunologist in the early years of the AIDS epidemic until her untimely death in 2000, advocated that immune activation played both protective and pathogenic roles in the evolution of HIV infection. She and her colleagues from the NIH-funded Multicenter AIDS Cohort Study (MACS) published a series of clinical studies on persistent immune activation^{7–9} and showed the better prognostic value of the level of cell surface expression of CD38, as an indicator of CD8 T cell activation, as compared to viral load in the evaluation of disease progression. Although the significance of these papers was not widely recognized at the time, they have since become extensively cited and continue to inspire new areas of research (in recognition of her pioneering work, this workshop was dedicated to Dr. Giorgi's memory).

The critical independent effect of persistent immune activation on disease progression probably became most evident once the virus could be controlled indefinitely by highly effective antiretroviral therapy (ART). As discussed at this workshop, although ART causes a precipitous decrease in immune activation, individuals on long-term ART generally have higher levels of immune activation than uninfected individuals^{10,11}; the abnormally high levels of immune activation independently predict progression to non-AIDS-related morbidity and mortality.¹²

Many questions remain concerning how HIV maintains immune activation, and in turn, how chronic immune activation contributes to HIV pathogenesis and the development

of comorbidities. At the symposium, speakers shared data related to these questions. The following report highlights these discussions.

Overview of the Pathogenesis of Persistent Immune Activation in HIV Infection

Immune activation in viral infection

Dr. Danny Douek opened the workshop with an overview of immune activation in response to virus infection. To successfully defend itself during an acute viral infection, the body activates the immune response, which is then regulated in a highly complex cascade of biochemical signals directed at clearing the invading organism. Eventually this response resolves to prevent immune-mediated pathology and immune exhaustion due to a persistently activated immune system. Depending on the virus, activation of the immune system may or may not result in viral clearance; for example, flu is cleared quickly, whereas HCV is not.^{13,14} In most infectious diseases, including those in which the pathogen persists indefinitely, immune activation invariably decreases dramatically after the acute phase.

In acute HIV infection, the immune system is highly activated in concert with peak viremia. As the infection enters the chronic phase, the level of viremia decreases considerably. Immune activation persists, however, which is unique to HIV infection.¹⁵ This phenomenon begs the questions that researchers discussed during the workshop: how is immune activation maintained, and how is it linked to pathogenesis?

Immune activation in HIV infection engages a range of molecular and cellular processes, spanning both the innate and adaptive arms of the immune system. On the innate side, scavenger cells such as macrophages and dendritic cells are activated, and often become refractory to stimulation *ex vivo* and indicative of chronic stimulation *in vivo*.^{16–18} From the incipient stages of HIV infection, the levels of certain circulating cytokines and chemokines are increased, including tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), and interleukin 1 beta (IL-1 β). Certain acute phase proteins such as serum amyloid A and C-reactive protein (CRP) are also elevated.^{19,20} The coagulation cascade is activated, leading to increased levels of D-dimers and tissue factor, reflective of hemostatic abnormalities such as *in vivo* clot formation and fibrinolysis. Concomitantly, the levels of matrix metalloproteinases and collagen deposition are elevated, leading eventually to fibrosis in lymphoid organs. Although the natural history of these inflammatory markers is not fully known, they clearly persist at elevated levels during the chronic phase of infection.

The adaptive immune system is also activated, producing effector as well as memory T and B lymphocytes with characteristic cell surface markers of activation.^{21,22} Both T cells and B cells have increased turnover, an altered phenotypic profile, and an altered functional profile. Hypergammaglobulinemia occurs as activated but dysfunctional B cells produce antibodies of varying specificities.²³ Immune activation also induces expression of the HIV coreceptor CCR5, which along with the cell surface receptor CD4 allows HIV entry into target cells, enhancing replication of HIV. Thus, what begins as part of a normal immunologic reaction to an acute virus infection evolves into an unregulated panoply of

immunologic effects, ultimately leading to immune dysregulation, exhaustion, and depletion of memory T and B lymphocytes.²⁴ The result is the ultimate paradox: while the hallmark of AIDS is immune deficiency, the larger part of the chronic pathology of HIV infection is founded on persistent immune hyperactivation.^{25,26}

Causes of persistent immune activation in HIV infection: studies in nonhuman primates

Dr. Guido Silvestri spoke about a number of findings from his extensive work in nonhuman primate (NHP) models of SIV infection and led a succession of speakers whose presentations focused on potential stimuli of and mechanisms for the pathologic consequences of HIV-associated persistent inflammation. Most untreated HIV-1-infected individuals and SIV-infected macaques have persistent viral replication, high-level immune activation, CD4⁺ T cell loss, and high risk for progression to AIDS. The natural hosts of SIV, sooty mangabeys and African green monkeys, however, maintain high levels of viral replication but exhibit only mild to moderate CD4⁺ T cell loss and have strong but transient increases in immune activation during acute infection. These animals do not progress to AIDS.^{27,28} Although immune activation during acute infection is present in these natural hosts, it resolves quickly, even though the virus continues to replicate at high levels. Dr. Silvestri and others have postulated that this rapid immunoregulatory response protects animals from the deleterious effects of chronic immune activation.

If this is correct, the question arises as to how and why immune activation in these natural hosts resolves despite persistently high viral loads, whereas partial control of viral replication in the postacute phase of HIV infection does not result in a concomitant decrease in immune activation. Are the innate and adaptive responses muted, or does normal response to viral infection occur, but with rapid down-regulation and return to baseline activation? Dr. Silvestri and his collaborators have tried to answer these questions by doing comparative analysis of transcriptional profiling of the NHP pathogenic model (pigtail or rhesus macaques) versus the nonpathogenic model (sooty mangabeys or African green monkeys). Experiments indicated that both models have massive activation of gene expression during the acute phase of infection, but only the pathogenic model showed large clusters of activated genes during the chronic phase.²⁹

In particular, these investigators surveyed the interferon response genes, which are very sensitive markers of *in vivo* activation of the innate immune system, especially responses mediated by plasmacytoid dendritic cells (pDCs) and TLR-7/8/9 signaling.³⁰ In the pathogenic model, there was strong and persistent up-regulation of the interferon response genes through the chronic phase of infection, while in the nonpathogenic model the activation became quiescent by the time of the chronic infection.

Dr. Silvestri's team also explored the role of low interferon-alpha (IFN- α) in chronic infection, asking if it is a consequence, cause, or marker of low immune activation. They addressed this issue by administering IFN- α to eight sooty mangabeys for approximately 4 months.³¹ IFN- α transiently lowered viral load early in the chronic infection of the nonpathogenic

model. While slight increases in T cell activation markers were observed transiently, they were down-modulated, leading to the conclusion that IFN- α alone is unlikely to induce chronic immune activation or progression to a pathogenic phenotype in the natural hosts.

Dr. Silvestri also briefly discussed recent data that showed that central memory CD4⁺ T cells (CD4⁺ TCM, defined as CD4 62L⁺ and CD95^{bright}) of SIV-infected sooty mangabeys are relatively resistant to SIV infection *in vivo* when compared to effector memory CD4⁺ T cells (CD4⁺ TEM, defined as CD62L⁻). This protection related in part to reduced expression of CCR5 on CD4⁺ TCM upon activation, although other mechanisms also appear to be involved.³² Interestingly, the level of cell-associated SIV DNA in TCMs is about one log lower in sooty mangabeys as compared to rhesus macaques while the level of infection in effector memory cells is comparable. This finding is consistent with earlier work by several groups working with the pathogenic macaque model, in which disease progression was linked to depletion of the TCM pool.³³⁻³⁵

Dr. Silvestri proposed a model in which more virus in the TCM pool causes increased immune activation in untreated animals, which will further feed viral replication. Theoretically, infection of the TCM pool during pathogenic infection may result in more immune activation by creating more pressure on the homeostatic replenishment of this compartment and by promoting damage to the architecture and function of the lymph node.³⁶ These findings raise the following questions: whether the low level of immune activation in natural hosts is due to the protection of the CD4⁺ TCM compartment and whether reversal of this process in rhesus macaques could abrogate chronic immune activation thought to contribute to the progression to AIDS.

Microbial translocation

Several studies have indicated that HIV-mediated destruction of gut mucosal integrity and the subsequent translocation of microbial products into the systemic circulation is a major cause of chronic immune activation. Drs. Douek, Silvestri, and others have shown increases in serum lipopolysaccharide (LPS, a major component of the outer membrane of Gram-negative bacteria) as well as other markers of bacterial products in both humans and macaques.^{11,37,38} In an experiment with African green monkeys, Ivona Pandrea and Christian Apetrei demonstrated that immune activation can be replicated in a nonpathogenic host by the administration of LPS. LPS administration led to a transient increase in the fraction of CD4⁺ cells expressing CCR5 and an increase in viral replication.²⁸ Thus, it is possible to generate increases in immune activation during a nonpathogenic SIV infection; it remains unclear, however, as to whether this immune activation is harmful in the long term.

Innate immunity and chronic immune activation

Considerable discussion at the workshop was focused on the role of innate signaling pathways in the maintenance of immune activation during chronic HIV disease. As the first line of defense, innate immune responses help to control viral replication early in the infection and to direct adaptive immune responses. Persistent innate immune-mediated recruitment of CD4 T cells to foci of viral replication could be

harmful, however, as it could lead to chronic immune activation, increased viral replication, and rapid loss of CD4 T cells.

Dr. Gene Shearer shared a model centered on the role that pDCs play in sensing and mediating signals that may play a role in sustaining elevated levels of immune activation. Multiple studies have shown that HIV can stimulate the activation and maturation of pDCs in both the early and chronic phases of infection, and it is thought that this may lead to constitutively elevated levels of Type I IFNs.^{39,40} These Type I IFN responses suppress T cell responses while sustaining the subpopulations of activated T cells.⁴¹ Dr. Shearer stated that the dysregulation of pDC function in HIV infection and the consequent dysregulation of adaptive immunity suggest that AIDS should really stand for “activated immune dysregulatory syndrome.”

Immune regulation

Dr. Shearer also briefly described studies performed with Dr. Genoveffa Franchini that assessed the impact of intervening in the indoleamine 2,3-dioxygenase (IDO) pathway in SIV-infected rhesus macaques. In these studies, ART-treated animals were given 1-methyl-tryptophan (D-1mT), a competitive inhibitor of IDO that catalyzes the degradation of tryptophan (Trp) and shows increased activity during HIV/SIV infection. HIV-mediated increases in pDC production of interferon (summarized above) lead to up-regulation of IDO, which in turn shifts the development of CD4 T cells into regulatory T cells (Tregs) that down-regulate other T cell responses.⁴² Following treatment with the IDO inhibitor D-1mT, the blood levels of virus became undetectable, suggesting that the chronic inflammatory response was contributing to higher levels of viral replication during partially suppressive ART.

Dr. Douglas Nixon then spoke about natural killer T (NKT) cells, a unique subset of CD1d-restricted immunoregulatory T cells that mediates both innate and adaptive immune functions. NKT also express the CCR5 coreceptor, making them a target of HIV infection. Nixon explained that NKT cells are selectively depleted during chronic HIV infection and noted that IFN- γ -producing NKT cells are severely compromised in infected patients. This reduction of IFN- γ -producing NKT cells could not be restored by ART, suggesting that NKT cells in patients on suppressive therapy remain impaired. While the consequences of this impairment are unclear, several lines of evidence in other disease models suggest that dysfunction in NKT cells may mediate disease pathogenesis.⁴³ Interestingly, Dr. Nixon also found that HIV-1 Viral Protein U (Vpu) directly affects CD1 antigen presentation, suggesting a role for NKT cells in host defense against HIV.⁴⁴

Immune exhaustion and central memory cells

Dr. Rafick-Pierre Sekaly discussed immune exhaustion in chronic HIV infection, in which T cells have a reduced capacity to produce cytokines and effector molecules as well as an impaired capacity to proliferate, and B cells have altered phenotypic and functional characteristics.⁴⁵ What are the molecular mechanisms underlying immune exhaustion and how do they relate to immune activation? Dr. Sekaly's group has shown that the transcription factor FOXO3a, a member of the forkhead protein family, controls the persistence of

memory cells in HIV infection.⁴⁶ During HIV infection, pDCs and T cells secrete inflammatory cytokines such as interferons (IFNs) that prevent the phosphorylation of FOXO3a, increasing its transcriptional activity and in turn down-regulating the development and persistence of memory cells. Additionally, the death of activated T cells is driven by the stimulation of a subset of TNF receptor family members including Fas, DR, and TRAIL.⁴⁷

Biomarkers for Immune Activation

Beyond CD4 and viral load

Drs. Alan Landay and Mario Roederer moderated the second of the three sessions concerning the search for appropriate biomarkers of immune activation that potentially could be used for prognosis of inflammation-related morbidities as well as for monitoring the effectiveness of therapeutic agents to treat inflammation. Several inflammatory and regulatory biomarkers in serum such as IL-10, TNF- α , and soluble β_2 -microglobulin appear to be prognostic of mortality and opportunistic disease in patients infected with HIV.⁴⁸ Dr. Landay posed two questions: (1) Can biomarkers be integrated with routine immunologic monitoring? (2) Can downstream pathways in TLR signaling—MyD88, NF-kappa-B, among others—be monitored as potential biomarkers?

Dr. Landay noted the importance of microbial translocation in immune activation through the TLR-4 pathway and in inducing proinflammatory cytokines.⁴⁹ He suggested the possibility of a common pathway of the proinflammatory cytokines driving immune activation characterized by HLA-DR and CD38 coexpressed on CD4 and CD8 T cells.

Several serum components that reflect microbial translocation are available for use as biomarkers. These include, among others, LPS, LPS binding protein (LBP), soluble CD14 (sCD14), IFN- α , 16SRNA, and potentially an antibody (Ab) to LPS, EndoCAb. Soluble CD14 is an important predictor of outcome and, of the aforementioned markers, is the only one correlated with all-cause mortality. CD14 is highly expressed by cells of myeloid origin, is considered a specific marker for macrophages, and is shed by activated monocytes.⁵⁰

Dr. Landay and others have developed intracellular flow assays to measure IFN- α , which is lower in HIV-positive subjects who are virologically suppressed with good CD4 T cell responses. Work from Dr. Marcus Altfeld's laboratory has shown a gender difference in the profile of interferon production, with HIV-mediated interferon induction much higher in women compared to men.⁵¹ Women also have higher immune activation than men, which increases through reproductive aging.⁵²

Many of the clinical aspects associated with aging appear to be accelerated in ART-treated HIV-infected adults. Dr. Landay outlined a number of immunologic abnormalities that are associated with aging and appear relatively more common in HIV-infected adults. This includes expansion of senescent T cells, which are generally characterized as proinflammatory, well-differentiated, and potentially apoptosis-resistant. Dr. Landay stated that senescent T cells may affect organ function; a crucial part of biomarker research is to link these markers to a functional outcome, for example, cardiovascular disease (CVD), neurocognitive changes, osteoporotic alterations, and other morbidities of aging. Latent coinfections, particularly with the herpes viruses such as

cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes-simplex virus (HSV), affect T cell function in older individuals and may provide opportunities for new biomarkers in HIV-infected adults. Dr. Landay and his collaborators are quantifying CMV levels in HIV-positive and HIV-negative patients with CVD, correlating CMV-specific immune responses for CD8, HIV antibody, and viral markers.

T cell markers of immune activation and senescence

Dr. Beth Jamieson discussed T cell surface markers of immune activation and senescence. CD38, one of the major markers of immune cell activation, is expressed on the surface of many cell types of the myeloid and lymphoid lineage, including NK, T, and B cells.⁵³ CD38 has ectoenzyme activity that mobilizes extracellular and intracellular calcium, is involved in the transendothelial migration of leukocytes, and promotes chemotaxis. When T cells become activated, surface expression of CD38 is up-regulated. Dr. Jamieson focused on the CD8⁺ T cell population, where much of the prognostic value of this marker in HIV infection lies. Her laboratory found that a threshold of about 1800 CD38 molecules on CD8⁺ T cells allowed fairly good discrimination between an at-risk individual and an HIV-infected individual.⁵⁴

CD38 up-regulation on CD8 cells is not specific to HIV infection, since acute viral infections such as EBV and CMV will also up-regulate cell surface expression of CD38. What is unique about HIV is that this activation persists. Dr. Jamieson noted that early in HIV infection CD38 is somewhat predictive for progression to AIDS, but increases in predictive value in chronic infection. Nevertheless, even during early infection, CD38 is at least as prognostic for disease progression as viral loads alone or CD4 T cell number or percentage.

Chronic immune activation drives T cell senescence, which involves changes in cell functions, the suspension of cell division due to telomere shortening, and differences in the rate of apoptosis.⁵⁵ Dr. Jamieson's team has asked if any surface markers of T cell senescence can be used to predict HIV disease progression. CD28, a T cell costimulatory receptor, is promising in this regard. The loss of CD28 is associated with T cell senescence, which is characterized by diminished proliferative capacity, cytokine suppression, decreased telomerase activity, and shortened telomeres. Dr. Jamieson suggested the need for more studies to characterize the biomarker potential of CD28 loss.

Markers in HIV-infected tissues

Dr. Jake Estes discussed markers in HIV-infected tissues, specifically related to gastrointestinal immunopathology and immune activation in SIV infection. Dr. Estes uses a classic marker of immune activation and proliferation, Ki67, which provides a generalized view of the activation state of lymphatic tissue in immunohistochemical studies.⁵⁶ The Estes group also uses the myxovirus-resistant protein A (MxA), which is tightly regulated and controlled by Type I interferons. They found a dramatic up-regulation of MxA in the lamina propria of the large bowel in both rhesus macaques and sooty mangabeys shortly after SIV infection. However, by the late acute phase, MxA was attenuated or down-regulated only in the nonpathogenic host. The sustained interferon response in rhesus macaques is very likely due to causes other than just viral replication in the colon. Dr. Estes and his col-

leagues also have noted direct evidence in SIV-infected rhesus macaques of microbial translocation from the lumen of the gut into the lamina propria and draining peripheral lymph nodes; the microbial translocation is associated with breakdown of the epithelial barrier integrity of the gastrointestinal tract.

SMART ideas about inflammation and coagulopathy

Dr. Dan Nixon discussed what the SMART study findings revealed about inflammatory and coagulopathy biomarkers. The Strategies for Management of Anti-Retroviral Therapy (SMART) trial was a large international trial comparing CD4 T cell count-guided structured interruption of ART to continuous ART. The trial showed that interrupted ART was associated with higher risk of AIDS, severe complications, or death and that risk of death was associated with elevated levels of inflammation, as indicated by the serum biomarkers IL-6 and D-dimer in particular.^{57,58} Levels of soluble CD14 (sCD14) also predicted mortality. In addition, higher levels of IL-6 and high sensitivity C-reactive protein (hsCRP) were predictive of development of opportunistic infections. Taken together, these results show a close association between levels of inflammatory biomarkers and morbidity and mortality in HIV-infected adults.

Therapeutic Interventions

Targets for Intervention

Drs. Steven Deeks and Sarah Read co-chaired the final session on therapeutic strategies to reduce immune activation, including those attempted in HIV or SIV infection as well as those proven useful in other diseases with a pathogenic inflammatory component. Dr. Deeks gave an overview of the field as it relates to clinical issues and potential targets for intervention. The overarching clinical observation in many patients with well-controlled HIV infection is that despite robust increases in CD4 T cell numbers and undetectable viral loads, normal health is not restored. They also do not live as long as their HIV-negative contemporaries and this decrease in longevity can be predicted by their levels of immune activation markers. Chronic inflammation also correlates with immunologic aging and morbidities such as cardiovascular disease and metabolic syndrome, all of which occur in individuals on long-term effective ART. Given the success of ART in most patients, novel therapeutic strategies to normalize this immune dysfunction may be needed to provide HIV-infected patients with an improved quality of life and normal lifespan.

Treatment of lymphoid fibrosis in HIV/SIV infection

Dr. Timothy Schacker discussed the role of fibrosis within lymphoid organs in the pathogenesis of HIV infection and presented results of experiments using pirfenidone to reverse the fibrotic process. He previously demonstrated that ongoing inflammation in lymphoid organs and tissues leads to fibrosis, which impairs cell movement, cytokine diffusion, and access to nutrients, ultimately resulting in the destruction of the stromal cells that comprise the reticular framework.⁵⁹ This not only impairs the immune response but inhibits reconstitution of the immune system following ART. Theoretically, if the process could be blocked and/or reversed with antifibrotic therapy, immune function and CD4 T cell

populations may be restored. Although not currently FDA approved, pirfenidone is licensed in Japan for the treatment of idiopathic pulmonary fibrosis, and animal models have indicated that pirfenidone can reverse fibrosis in the lungs, kidneys, and other organs.⁶⁰

In a pilot study of SIV-infected rhesus macaques, Dr. Schacker and his collaborators determined that pirfenidone had a protective effect on CD4 T cell populations in lymph nodes. A second pilot study looked at the effect of pirfenidone in combination with antiretroviral therapy. The team found that ART combined with pirfenidone produced a significant increase in the CD4 T cell population. The researchers concluded that administration of antifibrotic therapy appears to protect the architecture of the T cell zone and to slow the loss of CD4 T cells. They interpret their results to mean that when given with ART, antifibrotic therapy could potentially improve immune reconstitution.

Inflammation in cancer

Ongoing inflammation can enhance all steps of tumorigenesis from initiation through tumor progression and metastasis. Dr. Giorgio Trinchieri was the first of two speakers to discuss mechanisms of inflammation in cancer and potential interventions in that inflammatory process. He pointed out that tumor-promoting inflammation can include the production of chemokines with a proangiogenic role such as IL-8, and certain transcription factors downstream of inflammatory mediators. In general, alternative or M2 macrophage activation, along with factors such as IL-6 and macrophage migration inhibition factor (MIF), not only favors carcinogenesis but also exerts an immunosuppressive effect.⁶¹ Dr. Trinchieri emphasized that inflammation in the tumor microenvironment is caused by surrounding epithelial cells, fibroblasts, stromal cells, endothelial cells, as well as infiltrating innate and adaptive immune cells. These cells all communicate with each other through direct interaction and the secretion of diverse arrays of chemokines, cytokines, metalloproteases, and angiogenic factors that regulate tumor growth.

Dr. Nora Disis discussed how lessons learned from intervention strategies in cancer inflammation might be applied to HIV, diseases that share characteristics such as constant antigen stimulation, rapid T cell activation, and defects in CD4 T cell and memory populations. But in cancer, abnormal expression of self antigens stimulates the immune system rather than the foreign, viral antigens presented by HIV. The biggest problem in both diseases is how to control inflammation while trying to enhance protective immune responses.

Dr. Disis discussed her recent work on the role of B cells in sustaining chronic inflammation and suggested that therapies such as rituximab, which selectively depletes B cells, may down-regulate the chronic inflammatory environment. She also mentioned several antiproliferative chemotherapeutic agents used in cancer that have differential effects on the immune system and might be useful in immune modulation in HIV infection, including gemcitabine, cytoxan, rapamycin, and imatinib. Dr. Disis commented that the experience in cancer research in understanding the role of Treg function and the potential for therapeutically manipulating them has been mixed. She concluded with a discussion of how useful transgenic animal models of inflammatory disease have been to the cancer field and could be of value in HIV studies.

Therapeutic intervention strategies in immune inflammatory diseases

Dr. Leonard Calabrese discussed intervention strategies in immune-mediated inflammatory diseases (IMID), which share final common pathways and include autoimmune disorders. Central immune mediators such as TNF, IL-1, IL-6, and downstream cytokines clearly play cardinal roles in a variety of diseases such as rheumatoid arthritis, spondyloarthritis, and psoriasis.⁶² The available drugs that have transformed the treatment of these and other conditions include inhibitors of humoral and cell-mediated immunity and cytokine inhibitors, five of which are directed at TNF, including etanercept, infliximab, and adalimumab.

Dr. Calabrese discussed what is known about TNF and HIV, in particular the ability of HIV to utilize TNF signaling pathways and the role of TNF to augment viral production. There is limited experience in HIV infection with current TNF inhibitors in the post-HAART era. Concerns with anti-TNF treatments are increased risk of infections and the serious issue for HIV-infected patients of reactivation of granulomatous infections; a drug class warning for TNF inhibitors concerns reactivation of tuberculosis. However, anti-TNF drugs have distinct profiles and biologic mechanisms that must be considered, and etanercept may be the best choice for consideration in HIV⁺ individuals. A selective population of patients with HIV and autoimmune diseases, generally with severe forms of inflammatory arthritis, has been treated with anti-TNF therapies. Experience suggests that patients with CD4 counts greater than 200 and well-controlled viral loads can be treated successfully with this class of therapy.

Dr. Calabrese finished with a discussion of several new drugs that inhibit other cytokines and pathways. Of particular interest is the IL-6 inhibitor tocilizumab, which has been recently approved in the United States. Tocilizumab acts by inhibiting the signaling pathway of IL-6 or by binding to the soluble IL-6 receptor, which results in very broad and potent effects. Interestingly, in trials, tocilizumab dramatically reduced serum CRP levels, a well-known marker of inflammation in HIV infection.

Cytokine therapy and CCR5 antagonism

Dr. Irini Sereti discussed the other side of the inhibitory intervention strategies discussed by Dr. Calabrese, presenting her research using cytokine therapy in HIV infection. Potential goals for cytokine therapy include the restoration of a normal CD4 T cell pool and cell function, reduction in homeostatic proliferation, reestablishment of mucosal integrity, depletion of viral reservoirs, and alteration of innate immune responses, all of which may reduce chronic immune activation.

Dr. Sereti described the experience with IL-2 treatment in chronic HIV infection, more recent experience with IL-7, and how they seem to differ. The large IL-2 trials showed there was no clinical benefit in the IL-2 arms despite significant CD4 T cell increases. Dr. Sereti's research on peripheral blood T cells from the trial participants indicated that the increase in CD4 T cells was due to peripheral rather than thymic expansion and did not extend to the gut-associated lymphoid tissue (GALT). Of concern was an increase in serum markers of immune activation posttreatment. In contrast, preliminary data from an ongoing trial of IL-7 indicate T cell expansions in

naive and central memory phenotype, both CD4 and CD8 T cells, minimal T cell activation, and no release of proinflammatory cytokines. Dr. Sereti cautioned that in determining the success of cytokine therapy, the targets should be clearly defined and the timing of the administration carefully considered. Animal studies would enable researchers to obtain surrogate markers that would be useful for designing better clinical trials.

Dr. Michael Lederman spoke about CCR5 antagonists, which are a class of antiretroviral agents that allosterically inhibit the CCR5 coreceptor for HIV, preventing HIV binding and entry into target cells. The chemokine receptor 5 (CCR5) is a seven-transmembrane-spanning protein coupled to a heterotrimeric guanine nucleotide-binding protein (G protein).⁶³ Normal CCR5 functions include promoting chemotaxis as well as the induction of a variety of signaling molecules. CCR5 could also be viewed as a T cell activation marker in that it is up-regulated when cells are stimulated, acting as a homing receptor for cells to travel to sites of inflammation. Thus, CCR5 inhibitors may have multiple advantages in HIV treatment.

The use of the small molecule CCR5 inhibitor, maraviroc, results in an impressive increase in the magnitude of CD4 T cell restoration, although it is not clear if this is simply due to retention of CD4 T cells in circulation, actual CD4 T cell recovery, or decreased immune activation. There is evidence in some small clinical trials that immune activation is decreased early after initiation of maraviroc therapy. The CADIRIS trial is an ongoing, multicenter, randomized, double blind, placebo-controlled trial of the utility of maraviroc as an adjuvant to standard ART regimen that will address the role of maraviroc in decreasing immune activation and/or intravascular retention of effector cells. It is designed to look at the incidence of immune reconstitution inflammatory syndrome (IRIS) in persons with advanced HIV infection who begin ART. However, blocking lymphocyte homing, while possibly decreasing IRIS, may also impair the beneficial homing essential for an intact immune response to vaccines and the reconstitution of CD4 T cells in the GALT.

Drug development to suppress immune activation

Dr. Jeffrey Siegel discussed the development of drugs to suppress immune activation and his regulatory experience with drugs to treat autoimmune disease. Many of the drugs used to treat autoimmune disease are legacy products, small molecule pharmaceuticals, such as corticosteroids and methotrexate that are old and relatively nonspecific in their targets. In the last 10 to 15 years there has been a shift toward the development of biologic immunomodulators, such as the B cell depletion monoclonal antibody rituximab. The FDA approval process for biologics is significantly different from that for traditional, small molecule drugs.

In addition to rituximab, discussed earlier by Drs. Calabrese and Disis, a T cell costimulation blocker, abatacept, has been approved for arthritis. Several cytokine blockers also are FDA approved, including five TNF- α inhibitors, which were already discussed, and agents to block IL-1, IL-2, IL-6, IL-12, and IL-23. Two monoclonal antibodies that block immune cell trafficking are FDA-approved: natalizumab that blocks α_4 integrin and efalizumab that blocks LFA-1 (CD11a) binding to the adhesion molecule ICAM-1. These agents often have un-

anticipated mechanisms of action and toxicities, especially in combination.

With immunomodulators in autoimmune disease, the hope is that understanding the mechanism of action and the targets will help to anticipate safety concerns, although this is not always the case. Preclinical and animal studies may not fully identify all of the potential safety concerns because some risks become apparent only with longer exposure. Much critical information about the safety of these products, and indeed the basic science of the molecules in general, is through clinical trials. The FDA requires assurance that clinical trials carefully assess serious infections, opportunistic infections, induction of autoimmune diseases, and malignancies. When using a biomarker in a clinical trial, it is important to consider the marker's fitness for the particular purpose. For instance, a lower level of validation may be adequate early in drug development, whereas a higher level of validation may be required later in the process.

The FDA also considers the use of combination in immunosuppressive therapies. Synergistic toxicity may appear when two or more immunomodulators are combined, sometimes without increased benefit. With HIV, combining two different agents may be particularly important because of the background level of immunosuppression. Toxicities not appreciated in less intense regimens become apparent in the setting of more severe immunosuppression.

Workshop recommendations

Drs. Susan Plaeager and Alan Landay led the wrap-up discussion with comments about important points gleaned from the nearly 2 days of discussion. Recommendations included (1) the need for small, laboratory-intensive, exploratory clinical trials of approved antiinflammatory drugs in HIV-positive subjects that may help dissect out the various mechanisms of immune activation in long-term ART-suppressed patients; (2) pursuing parallel paths exploring the role of host response genes and immune responses using systems biology; (3) continuing work on biomarkers that can be used to assess the risk of inflammation-associated morbidities and in clinical trials of antiinflammatory agents; (4) pursuing animal model work to better define mechanisms of inflammation, including naturally SIV-infected nonhuman primates and mouse models of inflammatory disease; and (5) in the words of Dr. Douek, "collecting as many samples from as many sites, measuring as many things as possible, and remembering in the very near future we're going to be able to measure more."

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