Viral Immunity: It Takes Two to Tango

Emilio Flaño

Abstract

Viral infections were a major cause of mortality until the use of vaccination and antiviral drugs became widespread. During the last 10 years, immunologists have made tremendous progress in dissecting mechanisms that contribute to the success of vaccines, in understanding the molecular and cellular basis of innate immunity, and in recognizing the complexity of immunological memory. Virologists have also made great progress in elucidating the molecular mechanisms of how viruses evade, manipulate, and interact with host cellular processes. In spite of these advances, most of the basic mechanisms of antiviral immunity in the host are still poorly understood due to the complexity and specificity of virus-host interactions. Here we report on advances from the recent Keystone Symposia “Viral Immunity,” organized in Keystone, Colorado on January 20–25, 2008 by Jack R. Bennink, Marcia A. Blackman, and Ann B. Hill.

Introduction

VACCINES ARE ONE OF THE MOST SIGNIFICANT ADVANCES AGAINST INFECTIOUS DISEASES, but many questions still remain unanswered to make the rational design of vaccines a reality. Despite many efforts, new or better vaccines against many viruses that establish persistent infections (e.g., herpes viruses, HIV, and hepatitis C virus) or viruses with high mutation rates (e.g., influenza and HIV) are urgently needed. Using a mouse model of infection and a non-viral pathogen, John T. Harty (Iowa City, IA) showed that it is possible to accelerate the generation of “boostable” CD8 T-cell memory against malaria (*Plasmodium berghei*), and that boosted memory can confer long-term protection. It is possible that the same principles may be translated into a vaccination against viruses in model systems, or more importantly, into a human setting. One of the areas of research in vaccinology in need of substantial progress is the development of new human adjuvants. In this sense, Robert L. Coffman (Berkeley, CA) showed promising results with a CpG-based TLR-9 agonist (1018 ISS) used as an adjuvant in a hepatitis B vaccine. This approach elicited better protection, higher neutralizing antibody titers, and overcomes age-related unresponsiveness of the alum-adjuvanted vaccines currently used without inducing adverse reactions. Clearly, more studies are needed to establish proof of concept for new vaccine adjuvants that can be used in humans.

Vaccines against chronic infections have been extremely difficult to generate. Thus, immunotherapies using adoptive

cell transfers and/or antibody therapy seem to be promising alternatives. Adoptive T-cell immunotherapies have become the treatment of choice for hematopoietic stem cell posttransplant viral infections. Cliona Rooney (Houston, TX) discussed the strategies and methods for the quick and reproducible generation of multi-pathogen-specific T-cell cultures. Her laboratory uses monocyte cultures expressing virus-derived transgenes from adenovirus vectors as antigen-presenting cells combined with the selection of virus-specific T cells and the depletion of alloreactive T cells to generate multi-pathogen-specific T-cell cultures. Using the LCMV mouse model, blockade of IL-10R or PD-1L using antibody therapy enhances the clearance of persistent LCMV and induces less severe immunopathology (Matthias G. von Herrath, La Jolla, CA).

Viruses and their complex interactions with their hosts keep teaching us immunology. Membrane-associated RING-CH proteins (MARCH) are transmembrane ubiquitin ligases found in organisms ranging from viruses to eukaryotes. Cellular MARCH proteins (MARCH-1 and -8) regulate antigen presentation and other cellular processes. Viral MARCH proteins, such as those from γ -herpesviruses and poxviruses (MIR1 [K3] and MIR2 [K5] from KSHV), regulate viral immune evasion mechanisms (Klaus Frueh, Beaverton, OR). Some of these immune modulators, such as the ectromelia virus IFN- α -binding protein are key virulence factors that also can be used as targets for vaccination (Luis Sigal, Philadelphia, PA). Basic research on how myxoma virus proteins mediate cellular tropism and immune evasion has led to the

use of myxoma virus in experimental oncolytic virotherapy (Grant McFadden, Gainesville, FL). Myxoma virus can infect 70% of human tumor cells and the myxoma virus protein M-T5 is one of the proteins that regulates cellular tropism and binds and alters the host proteins Akt and cullin-1. The addition of rapamycin (which also binds Akt) increases myxoma virus replication in tumor cells, making this a promising approach to specifically eliminate cancer cells.

Persistent Virus Infections and Innate Immunity

Many important pathogens have the ability to mediate chronic, persistent, or latent infections. These infections are characterized by the persistent expression of antigens and by establishment of a delicate interplay or balance between the virus and the host. They result in high morbidity/mortality worldwide and we lack effective therapies to cure them. Ann B. Hill (Portland, OR) emphasized the co-evolution of herpesviruses (cytomegalovirus or CMV) and mammals. She highlighted the importance of viral immune evasion strategies to avoid CD8 T-cell-mediated killing and the heterogeneity of the CD8 T-cell response during CMV persistence. The establishment of lifelong latency is one of the hallmarks of herpesvirus infections. γ -Herpesvirus latency is established early after infection, is largely independent of lytic-phase replication, and is a dynamic state with continuous viral reactivation (Marcia A. Blackman, Trudeau Institute, Saranac Lake, NY).

What are the host or viral factors that play a key role in determining the outcome of infection? Barbara Rehermann (Bethesda, MD) presented functional data for differential natural killer cell responsiveness depending on killer cell immunoglobulin-like receptor/HLA (KIR/HLA) compound genotypes. These results are consistent with previous genetic studies that describe associations between distinct KIR/HLA compound genotypes and outcome of viral infections. Persistent infections may also contribute to the development of autoimmunity by molecular mimicry or by epitope spreading. In a Theiler's virus-induced model of demyelinating disease, Stephen D. Miller (Chicago, IL) showed that both innate immune and innate regulatory mechanisms (natural CD25⁺ CD4 T-regulatory cells) control the susceptibility to infection-induced autoimmunity.

The host immune system has multiple innate and adaptive mechanisms to fight viral infections. Through evolution viruses have also developed counterattack strategies to avoid or subvert those mechanisms of defense. RNA interference is a central innate immune mechanism of response to viral infection, at least in plants and invertebrates. Persistent DNA viruses have taken advantage of this mechanism and they encode microRNAs that use the cellular RNA interference machinery for their own benefit. Herpesviruses are the champions of making microRNAs, but polyoma virus and adenoviruses also use this strategy to subvert cellular responses (Bryan R. Cullen, Durham, NC). The immune response to pathogens is initiated by their triggering of pattern recognition receptors and conserved pathogen motifs. Both Anne Krug (Munich, Germany) and Evelyn Kurt-Jones (Worcester, MA) discussed the importance of pattern recognition receptors in mediating innate responses to viruses. The activation of toll-like receptor (TLR)-dependent and of TLR-independent (RNA helicases) signaling pathways by nucleic acids from dsDNA and RNA viruses triggers the ini-

tiation of the innate immune response. Not surprisingly, viruses have evolved multiple mechanisms to specifically inhibit these signaling pathways. The interaction between NK cells and dendritic cells is essential for the regulation of both innate and adaptive immune responses. Mariapia Degli-Esposti (Perth, Western Australia, Australia) showed that NK cells affect the longevity and effectiveness of the T-cell response against murine CMV. Christian Munz (New York, NY) showed that dendritic cells activate NK cells by rapid formation of an immunological synapse. This DC-mediated activation helps to stimulate preferentially NK cells that are better IFN- γ producers, and that probably contribute to limit Epstein-Barr virus spreading during the early phase of primary infection.

Antigen Presentation and Adaptive Immunity

Despite the remarkable pace of discovery in immunology, it is still uncertain where, when, and how immune responses to viruses are triggered. An important new technology to address these questions is the use of intravital microscopy to visualize activation of antiviral immunity in real time. Jon Yewdell (Bethesda, MD) presented data showing that antiviral immune responses are initiated at the lymph node periphery. Using VV and VSV models of infection, he reported that antigen and virions are transported to the lymph node in minutes where they infect macrophages and DCs localized just under the lymph node subcapsular sinus. T cells exclusively cluster, however, around infected DCs located above and between the B-cell follicles, where they become activated *in situ*. What dendritic cells are implicated in the initiation of different responses? Dendritic cells are heterogeneous and it is thought that this heterogeneity results in important functional differences. For example, dermal Langerhans cells are involved in skin tolerance, while CD8- α dendritic cells are involved in the initiation of immunity. Frank R. Carbone (Melbourne, Victoria, Australia) pointed out the role of monocyte inflammatory dendritic cells during HSV reactivation in mice. Monocyte inflammatory dendritic cells (and CD4 T cells) contribute to the CD8 T-cell expansion that during HSV reactivation occurs at the sensory ganglia. Laurence C. Eisenlohr (Philadelphia, PA) summarized several years of work in his laboratory and gave an overview of the complexity of the distinct pathways that contribute to MHC class II restricted antigen presentation. These pathways were described using epitopes from the influenza neuraminidase and hemagglutinin proteins, and they are the classical pathway (H2-M assisted), the recycling pathway (H2-M independent), and the endogenous pathway (proteasome and TAP dependent). The complexity of the class II pathways is thought to maximize the opportunities to detect foreign antigens.

B cells and antibodies are important for the control of viral infections. Molecular structural analyses are contributing to elucidate the function and the qualitative differences of distinct antibodies. The antiviral functions of IgA are less appreciated than those of IgG. The use of small angle x-ray analysis, neutron scattering studies, and surface plasmon resonance, combined with directed mutagenesis approaches have led Jenny M. Woof (Dundee, UK) to define several regions on the IgA molecule that are critical for its function. What are the factors that modulate the potency of a neutralizing antibody? Ted C. Pierson (Bethesda, MD) suggested

that antibody-mediated neutralization of West Nile virus is a "multiple hit" phenomenon that requires simultaneous binding of as many as 30 antibody molecules. Whether a particular antibody can dock on the virion with a stoichiometry that exceeds this threshold is governed by antibody affinity and epitope accessibility. However, a structural rationale to explain how antibodies neutralize infection via poorly accessible epitopes is not explained by static models of the mature flaviviruses, suggesting that dynamic aspects of the virion structure may significantly impact epitope accessibility. The extent of virion maturation may modulate epitope accessibility and the neutralization potency of some antibodies, providing functional significance for biochemical heterogeneity within populations of virions delivered through the bite of the mosquito or released from tissues *in vivo*.

The vast diversity of the T-cell repertoire makes it possible to recognize an immense array of foreign antigens. Leo Lefrancois (Farmington, CT) has developed an approach to determine the endogenous naïve CD8 T-cell precursor frequency for a given epitope. Combining dual MHC tetramer staining with magnetic bead separation, Lefrancois showed that the naïve CD8 T-cell precursor frequency varies between 100 and 800 cells per mouse. The naïve precursor frequency correlates with the kinetics of CD8 T-cell expansion after antigen stimulation, and inversely correlates with the time needed for the resulting memory population to shift towards an L-selectin high phenotype. Elucidating the molecular mechanisms that control the reprogramming of a naïve T cell during the acquisition of effector function is essential to understand the regulation of CD8 T-cell responses to viral infections. Stephen J. Turner (Melbourne, Victoria, Australia) presented results showing that different epigenetic modifications are found within the granzyme B promoter of cytotoxic CD8 T cells and helper CD4 T cells. The differences in modifications correlate with acquisition of granzyme B expression in CD8s, but not in CD4s. A key characteristic of the immune system is its capacity to remember past encounters with a pathogen and to mount a usually better response to a secondary encounter with the same pathogen. This immunological memory provides the foundation for vaccination. An exhaustive study using smallpox survivors (with and without smallpox vaccination) showed evidence that antiviral antibodies persist essentially for life, whereas CD4 and CD8 T-cell memory decline slowly over time. Interestingly, the relative levels and duration of T-cell memory appeared similar between smallpox survivors and vaccinia-immune control subjects who only received smallpox vaccination. David L. Woodland (Trudeau Institute, Saranac Lake, NY) presented results on the memory CD8 T-cell recall responses to respiratory viral infections using the Sendai virus mouse model. This recall response can be divided into two phases, early and late, which recruit different subpopulations of T cells and are differentially regulated. The early phase recruits effector-memory CD8 T cells and is mediated by CCR5, and the late phase recruits effector cells after antigen stimulation in the draining lymph nodes. The importance of the protective aspects of heterologous immunity usually goes unnoticed during the analysis of immune responses. However, cross-reactive epitopes and heterologous immunity can be important in the generation of both protective responses and unwanted immune pathological reac-

tions. Raymond M. Welsh (Worcester, MA) highlighted several examples where cross-reactive viral epitopes contribute to altered or pathogenic immune responses (e.g., dengue, infectious mononucleosis, and hepatitis C). He emphasized that defining only immunodominant epitopes during the design of a vaccine could be misleading, as the induced response could also contain "pathogenic epitopes" that should necessarily be identified in advance.

Memory CD4 T-cell responses are less well studied than those of their CD8 counterparts, among other reasons because they expand less in response to antigenic stimulation. CD4 T cells are key in providing cognate help and regulating immune responses. However, their direct role in the control of viral infections remains unclear. Donna L. Farber (Baltimore, MD) analyzed the role of heterogeneous influenza-specific memory CD4 T-cell populations during secondary responses to influenza in mice. She showed that memory CD4 T cells specific for influenza can mediate both protection and immune pathology. Experimental immune therapy using CTLA4Ig fusion molecules to block CD28 signaling reduces T-cell recruitment to the inflamed lung, resulting in decreased immune pathology and in maintenance of protection against influenza. Mariapia Degli-Esposti stressed the fundamental role of CD4 T cells in controlling virus persistence in the murine CMV model.

Summary

Viral immunity is a fast-moving field and it is hard to keep up with all the recent advances in virology, immunology, vaccinology, and infectious diseases. In this regard, the Keystone Symposia on viral immunity provides an excellent and much-needed avenue to share recent advances relevant to the field every 2–3 y. Three major themes were the highlight of this conference: (1) the analysis of responses to persistent viral infections and their interplay with the host, (2) the understanding of how immune responses are initiated and how immunological memory is maintained, and (3) recent advances in experimental therapies using vaccines and immunotherapies. Keystone provided a beautiful setting to discuss these issues, although the quality of the science and the proximity of the February 5th grant deadline did not leave much free time to enjoy the outdoors.

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Address reprint requests to:

Dr. Emilio Flaño
The Research Institute at Nationwide Children's Hospital
Department of Vaccines and Immunity
700 Children's Drive, WA4015
Columbus, Ohio 43205

E-mail: flano@ccri.net

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