National Institute of Allergy and Infectious Diseases

BIODEFENSE WORKSHOP SUMMARY
ANTIVIRAL INNATE IMMUNITY: RECOGNITION, DEFENSES, EVASION, AND BIODEFENSE STRATEGIES

June 24-25, 2003

Bethesda Marriott Hotel
Bethesda, Maryland

Abstract

NIAID convened a workshop June 24-25, 2003 on antiviral innate immunity and its importance for biodefense applications. Participants* presented research findings and discussed the current state of the art and recommendations in the following areas:

- Viral recognition and innate cell activation
- Innate cytokine and chemokine responses to viral infections
- Viral evasion of innate immunity
- Diagnostics, vaccines and antiviral therapies in the context of understanding of innate immunity

Discussion included the key function of Toll-like receptors in recognition of viral molecules, the role of natural killer cells in controlling specific viruses, the triggering of interferon and other cytokine responses, and viral countermeasures. Functional genomics research was discussed as an approach to address complex human innate immune responses to diverse viruses for diagnostics and the development of novel therapeutics. The participants identified priority areas that need continued support in order to continue advancing in this field. These areas include: the need to train new scientists, develop improved genetic models, develop and distribute expression vectors and virus-specific specific reagents for the research community, access to appropriate biocontainment facilities, and support of non-hypothesis driven research on basic immunology and potential products as priorities to continue advancing the field.

Introduction

Few other immune system challenges compare with the high stakes associated with early immune responses to viral infection. The initial virus foothold, established in hours or

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days, often before any disease is evident, critically determines whether severe illness or death may ensue. Certain viruses can become rapidly entrenched, for example, in the nervous system, liver, or cells of the immune system itself, as in HIV/AIDS. Unless the individual had been previously vaccinated against the specific virus, the critical days needed to ramp up the antibody levels and T cell responses of adaptive immunity could potentially leave viruses unchecked and lethal. Responsibility for immediate defenses resides in the innate immune system, the inborn system of defenses that senses invaders and takes immediate action against them. Innate immunity is based upon cells located throughout the various tissues that possess "pattern recognition" receptors that detect molecular structures found on microbes but not in the host. Bacteria contain an abundance of such patterns, including cell wall and cell membrane proteins, lipids, and polysaccharides; nucleic acid regions containing specific motifs, and flagella. Viruses, in contrast, are much less complex and contain many fewer possible signposts. A recent meeting convened by NIAID focused on antiviral innate immune detection, defenses, evasion, and possible novel approaches to control viruses based on the strategies of innate immunity.

Viral recognition and innate cell activation

Only few molecular structures unique to viruses are known that can broadly signal innate immune system activation. The most ubiquitous is double-stranded ribonucleic acid (dsRNA), which occurs in many viruses as their genomic configuration or occurring temporarily during viral replication as full or partial structures. The mammalian receptor for dsRNA is Toll-like receptor 3 (TLR3) a member of the TLR family of 10 transmembrane molecules that initiate innate immune responses to specific microbial structures. As with most of the TLRs, there is incomplete knowledge of whether the interaction between TLR3 receptor and ligand is direct or indirect. Similarly, TLR9 responds to nucleic acids having unmethylated CpG motifs. Dr. Akiko Iwasaki reported that, in a mouse model of vaginal herpes simplex virus type 2 (HSV2), only certain cells, the submucosal dendritic cells, presented HSV2 antigens to T cells. Although the receptor responsible for the interaction of submucosal dendritic cells with HSV2 is not yet known, Dr. Iwasaki found that HSV2 interaction with another dendritic cell subset, plasmacytoid dendritic cells, occurs via TLR9. CpG motifs are known to be present in herpes DNA, and may be another mechanism of broad viral recognition. The recognition of herpes virions by TLR9 is much more efficient than recognition of extracted herpes DNA. Dr. Genhong Cheng showed that TLR signaling triggered distinct but overlapping cellular responses. For example, TLR3 stimulation leads predominately to production of interferon beta plus chemokines, while TLR4 leads to interferon beta, activated phagocytosis, and inflammation. Such efficiency in TLR triggering may be the result of recognition occurring within cellular uptake vesicles, and also likely by contributions from other associated proteins that facilitate recognition of foreign molecules. Other research has shown that lectins, such as surfactants and mannose binding proteins, and IgM antibodies plus complement, discussed by Dr. Michael Diamond, can facilitate innate immune responses to viruses, possibly by focusing viruses to cellular vesicles for efficient recognition by innate immune receptors.

Other recognition receptors involved in innate immune responses to viruses appear to lack the broad capacity of TLR3 and TLR9, and may reflect the virus "choosing" to bind to certain receptors that happen to be involved in innate immune function. Respiratory syncytial virus (RSV) recognition involves the RSV F-protein (Dr. Ralph Tripp) binding to TLR4 in a CD14-dependent manner, as does bacterial lipopolysaccharide (LPS), but is not identical in all details to the LPS recognition process. Murine cytomegalovirus (MCMV), discussed by Dr. Wayne Yokoyama, activates natural killer (NK) cells essential in defense against CMV by one of its proteins, M157, interacting with the activating NK cell receptor complex Ly49H-DAP12.

Why would viruses elect to activate via receptors that may contribute to their own demise? One possibility is an evolving virus-host interaction process where advantages for the virus may include uptake into specific cells and/or activation of cells to facilitate viral replication. However, the host species may counter by selective advantages arising from genetic variants in the population, that, for example, may trigger interferon-mediated responses more effectively, or that lead to expression of the receptor on cells other than those preferred by the virus for replication.

Innate cytokine and chemokine responses to viral infections

Different viruses elicit distinctive cytokine and chemokine responses. Dr. Christine Biron presented research that MCMV elicits large amounts of interferon gamma and interleukin 12 (IL-12). In contrast, lymphocytic choriomeningitis virus (LCMV) triggers mainly a type I interferon alpha/beta response with little IL-12. For MCMV, the early triggering of NK cells seems to underlie the rich interferon gamma and IL-12 responses. LCMV infection eventually leads to interferon gamma release when CD8 T cells of the adaptive immune response develop. These model viral systems may be useful in deciding what types of interleukins would be useful in treating human infections that resemble MCMV versus LCMV in cytokine response characteristics.

Virus interaction with the chemokine system may greatly influence pathogenesis. Dr. Ralph Tripp reported that the G-protein of RSV caused a decline in certain chemokines (monocyte chemotactic proteins and interferon-inducible protein 10) that are typically secreted in response to viral infection. Specific mutation of a motif in the RSV G-protein abolished this effect, revealing that the G protein normally binds to a specific chemokine receptor, the fractalkine receptor, thereby affecting responses. This characteristic appears to underlie many features of the pulmonary disease caused by RSV.

The interferons are well recognized as carrying out major defensive functions in antiviral innate immunity. Searching the human genome for possible new molecules that matched known interferons, Dr. Sergei Kotenko and independent groups identified three novel molecules that represent a new category of interferons, called lambda interferons. These lambda interferons were induced in human cells by viral infection and their activity led to antiviral protective responses in many cell types. The newly discovered interferons also use a novel receptor molecule distinct from that used by other interferons. The discovery of these broadly expressed antiviral molecules adds to the understanding of the complex

immune responses to viral infection, and may eventually be used to treat viral infections. Currently, alpha- and beta-type interferons are licensed as therapeutics, and future studies should address whether interferon lambdas may synergize with other interferons and lead to more effective control of viral infections.

Viral evasion of innate immunity

Viruses evolve countermeasures to evade innate immunity, leading to the complex virus-host interactions seen especially for viruses that have a long history of infecting a given species. Nearly one-half of the almost 200 genes of vaccinia virus are non-essential in culture, but function in some way in the host infection cycle, including evasion of innate immunity (Dr. Geoffrey Smith). Among the known vaccinia evasion mechanisms are those that defeat IL-1, IL-18, the interferons, and steroid synthesis. The human poxvirus molluscum contagiosum possesses evasion mechanisms for IL-18, antagonists for chemokines, and a molecule that blocks an element of the apoptosis pathway. Because of their large genomes, poxviruses can utilize many different immune evasion pathways, which may account for their high infectivity and host-specific disease characteristics.

Dr. Peter Palese discussed the ways that different viruses have evolved proteins to evade the three essential parts of the interferon response pathway: interferon induction; Jak/Stat signaling molecule activity; and antiviral gene product synthesis. For example, interferon induction is affected by influenza NS-1 protein, ebola VP 35, and bunyavirus NSs. Jak/Stat signaling is impeded by mumps V-protein, adenovirus E1A, also by ebola VP 35, and by simian virus 5 V-protein. Antiviral gene product synthesis is inhibited by HIV TAT, adenovirus VA1, and also by influenza NS-1. Viruses that block interferon beta affect NK cell activation, T cell survival, and the direct antiviral benefits of the cytokine (Dr. Carl Ware). Together, these studies demonstrate the complexity of viral evasion systems, which reflect complex host innate immune responses to viruses.

Diagnostics, vaccines and antiviral therapies in context of understanding of innate immunity

Understanding the complex human innate immune responses to viral infection will enable better design of diagnostics, vaccines and therapeutics. High-throughput methods of functional genomics were described by Dr. Michael Katze, including the use of nucleic acid microarrays and appropriate bioinformatics approaches to define characteristic host "signatures" observed in different viral infections. By assembling a compendium of such responses, a comprehensive understanding of the pathways involved and affected by viral infection can be applied to more rapid diagnostics, used to determine treatment strategies, and to devise antiviral therapeutics based on novel targets.

Dr. Palese reported that intentional deletion of a portion of the influenza virus NS1 protein resulted in an attenuated virus that still retained its immunogenicity, potentially providing a new, rationally based approach for attenuation applicable to many viruses. Because attenuated viruses can elicit the wide range of cellular and antibody-based that

are often absent in inactivated virus vaccines, this approach potentially could lead to highly effective vaccines for diseases that currently lack them.

Dr. Jianzhu Chen outlined a developing approach to antiviral therapeutics based upon inhibition of viral gene function early in infection. He employed a type of RNA, called small interfering RNA (siRNA), that complexes with viral RNA in infected cells and, by a enzyme-catalyzed process, prevents their translation into proteins. The siRNA is designed to target specific regions of viral genes, and, when introduced by a carrier molecule into cells, transiently disrupt viral function. Preliminary data showed that siRNAs to two protein-encoding genes of influenza virus reduced the amount of influenza virus in the lungs of treated animals. This highly promising result needs to be further evaluated and developed before it can be tested in humans, but it may be an important therapeutic approach applicable to many viral infections.

General recommendations

In addition to support for research on the major topics outlined in this workshop, additional general recommendations for advancing the field of antiviral innate immunity were discussed. These include:

- Training: Emphasis on practical education and recruitment of new scientists in an area that bridges virology and immunology;
- Facilities: improved access to BSL-3 and other biocontainment facilities to enable studies with hazardous viruses;
- Tools: Availability of individual gene expression vectors for all open reading frames of key viruses, enabling studies of individual proteins of many viruses without the hazard of deriving the genes from the intact virus;
- Models: Development and access to altered viral genomes that are safer to work with and to better animal models, including gene knockouts and transgenic animals useful in dissecting viral responses to viral infections;
- Non-hypothesis-driven studies: Facilitate the development of genomic and proteomic approaches in order to establish a comprehensive picture of innate immune responses to viruses and to develop novel diagnostics, vaccines, and therapies.