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Immunity to viruses

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Introduction

Many common infections leading to disease are caused by viral pathogens. The host generally recognizes these viruses as foreign entities and initiates an effective immune response which can prevent entry of the virus into cells and replication and spread. The initial phase of this antiviral response is recognition of viral components, particularly peptides, as foreign in the context of the major histocompatibility complex (MHC) antigens on the surface of the antigen-presenting cells. Once this recognition event has taken place, a cascade of cellular and humoral responses to distinct viral determinants is established. These antiviral humoral and cellular reactions complement each other. For example, antibodies can neutralize free circulating virus and also destroy virus-infected cells. The cellular responses can lyse virus-infected cells as well as help initiate antibody synthesis. Soluble factors, such as tumor necrosis factors (TNFs), interleukins (ILs) and interferons (IFNs), interact with lymphocytes, either suppressing or augmenting their immune functions.

The interaction of the virus with the immune system is very important in determining the outcome for the host; the ideal result is clearance of the virus, and host recovery and survival. Many problems concerning virus–immune system interactions have been resolved over the last few years. However, several interesting questions have not been fully answered. For example, it is still not clear how certain viral pathogens such as herpes or measles viruses are able to avoid the functional cellular and humoral immune responses of the host and establish a persistent infection.

Furthermore, several mechanisms have been proposed for the initiation of autoimmune disease by viruses. A virus-induced response to viral and cross-reacting self-antigens may activate immunopathologic reactions resulting in autoimmune disease. This concept of molecular mimicry was described by Fujinami and Oldstone in 1985 (*Science* 1985, 230:1043–1045). Since then, many reports dealing with shared epitopes between viruses and host components and determinants responsible for disease initiation have been published. These have been reviewed recently (Fujinami, *Adv Neuroimmunol* 1988, 540:210–217).

The following short report is not an update on all the research into virus–immune system interactions. However, we will survey published work on lymphokines, major histocompatibility molecules, T-cell–B-cell interactions affecting antibody production, virus neutralization and neutralizing epitopes involved in pathogenesis. These studies are important in understanding interactions between viruses and the immune system.

Cytokines

Classically, IFNs are secreted glycoproteins which can be divided into three general subgroups (IFNα, IFNβ and IFNγ) depending on their sequence, physiochemcial and biological activity, as well as their cellular origin. IFNα is secreted from leukocytes, IFNβ from fibroblasts and other non-immune cells and IFNγ primarily from T lymphocytes and macrophages in response to stimuli from viruses and other antigens. Normally, the IFN-producing cells only secrete minimal amounts. Thus, in the past, large amounts of pure material were difficult to obtain. However, the cloning and expression of large quantities of highly purified IFNs now allow studies of the immunoregulatory and antiviral properties of the IFNs.

Recent published work suggests that IFNs may have various antiviral activities; they can have an antiviral effect with or without inducing antibody production. For example, Schijns *et al.* [1] concluded from their *in vivo* studies that IFNγ may directly inhibit virus replication in the cell without stimulating the immune system. The antiviral activity of recombinant rat IFNγ was also measured in immunodeficient rats: administration of recombinant rat IFNγ protected adult, immunologically immature newborn or athymic nude rats and γ-irradiated rats against a

Abbreviations

AIDS—acquired immunodeficiency syndrome; **CTL**—cytotoxic T lymphocytes; **HAV**—hepatitis A virus; **HIV**—human immunodeficiency virus; **HSV**—herpes simplex virus; **IFN**—interferon; **IL**—interleukin; **ISCOMS**—immune-stimulating complexes; **LCMV**—lymphocytic choriomeningitis virus; **mAbs**—monoclonal antibodies; **MHC**—major histocompatibility complex; **SV**—sendai virus; **TMEV**—Theiler's murine encephalomyelitis virus; **TNF**—tumor necrosis factor; **VSV**—vesicular stomatitis virus.

lethal pseudorabies infection. No detectable neutralizing antibodies were found in the sera of the surviving rats. Challenge of the surviving animals with a lethal dose of pseudorabies virus as many as 28 days after IFN treatment resulted in 60% protection and there were no detectable levels of neutralizing antibodies. IFN-induced cell-mediated processes may be of major importance in protection after late challenge. These results suggest that IFN can induce an antiviral process in the absence of neutralizing antibodies. These studies complement those of Maier *et al.* [2], whose chromium release assays using hepatitis A virus (HAV)-infected fibroblasts and peripheral blood lymphocytes from HAV-infected patients showed the induction of virus-specific T cells. These T cells were found to secrete human IFNγ and to be cytotoxic against HAV-infected fibroblasts.

The above results are further evidence of an antiviral process initiated by IFN without the stimulation of an antibody response. In contrast, in other virus systems such as vesicular stomatitis virus (VSV) infection, administration of IFNγ enhanced the production of virus-neutralizing antibodies [3]. Treatment of mice or cattle with recombinant IFNγ at the time of infection with VSV increased the levels of neutralizing antibodies after a secondary virus challenge and protected animals from VSV disease.

To define the specific functions of single IFN genes and their protein products further, two models are discussed. In the first system, Chen *et al.* [4] introduced the DNA encoding human IFNβ into the germ lines of mice, thereby creating transgenic mice; some of these mice expressed human IFNβ in their serum. This human IFNβ had protective antiviral effects: the transgenic mice had enhanced resistance to pseudorabies virus infection, and the degree of protection correlated with the IFNβ serum concentration.

In the second system, conserved areas of IFNα were studied. Several areas from the human IFNα are known to be important for antiviral activity (Camble *et al.*, *Biochem Biophys Res Commun* 1986, 134:1404–1411). The same residues or regions are also conserved in the murine IFNα. To test the significance of these conserved areas of murine IFNα for their antiviral activity in L929 cells infected with encephalomyocarditis virus, Kerry *et al.* [5] introduced single amino-acid substitutions within these conserved regions. Using oligonucleotide-directed site-specific mutagenesis, two important conserved amino-acid residues were determined. An arginine at position 33 and a tyrosine at 123 were critical in maintaining antiviral activity. Substitution of these amino acids caused a marked decrease in antiviral activity in murine L929 cells when challenged with encephalomyocarditis virus.

The induction of specific subgroups of IFN also depends on the type of virus used for infection. For instance, Goble *et al.* [6] studied the kinetics and appearance of IFNα and IFNβ messenger RNAs after *in vitro* stimulation with two different viruses, herpes simplex virus (HSV) and Sendai virus (SV). They used *in situ* hybridization with two specific probes for IFNα and IFNβ messenger RNA. It was found that HSV only induced IFNα messenger RNA in human mononuclear leukocytes, while SV induced both IFNα and IFNβ messenger RNA. This suggests that different viruses use different mechanisms to stimulate IFN production.

The above studies emphasize the importance of IFNs as antiviral response inducers. However, antiviral reactions may also arise without the aid of IFN. King and Kesson [7] reported IFN-independent antiviral responses. Infection of mouse embryo fibroblasts by flaviviruses resulted in a marked increase in MHC class I antigen expression, which was partly independent of IFNα and β secretion. Antibodies to IFNα or β inhibited the expression of the MHC class I antigen complex minimally. MHC class I expression by fibroblasts was dependent on cellular RNA synthesis, as determined by actinomycin D treatment, which blocks cellular RNA synthesis.

In addition to IFN, another group of soluble cytokines are involved in antiviral immune responses. IL-1 is produced by antigen-presenting cells and induces IL-2 production by T cells. IL-2 has a positive effect on the proliferation of T cells and previously stimulated B cells. IL-3 is one of the colony-stimulating factors. IL-2 alone, in contrast with IFN, does not have an antiviral effect *in vitro*. For example, HSV-infected neonatal mice were treated with syngeneic or allogeneic adult mouse macrophages in combination with various doses of IL-2 [8]. Both macrophages and IL-2 were required for successful protection of neonatal mice against HSV infection.

Infection of lymphoid cells can affect IL production and have severe immunological and pathological consequences. By infecting immune cells, viruses can modify the host antiviral response to their advantage; in this way the virus could avoid immune-mediated clearance and produce a persistent infection. For example, an abortive Semliki Forest virus infection of murine spleen macrophages decreases the production of IL-1 and thus indirectly decreases IL-2 levels [9]. In addition, an abortive infection of human peripheral blood mononuclear cells by cytomegalovirus results in reduced synthesis of IL-1 and IL-2 [10]. Furthermore, exogenous IL-1 and IL-2 did not restore the proliferative response of cytomegalovirus-infected lymphocytes and monocytes to mitogens such as phytohemagglutinin. Diminished levels of IL-1 and IL-2 have a severe effect on the immune response. Thus, ILs play a pivotal role in lymphocyte activation, differentiation and in the co-ordination of T-helper/B-cell interactions which lead to the production of antibodies.

It is becoming increasingly clear that another cytokine, TNF, plays an important role in immune regulation. TNFs are polypeptides synthesized primarily by macrophages, but also by B cells activated by various inducers such as lipopolysaccharide, tubercle bacilli or viral infections [11]. TNF has an antitumor effect *in vivo* and cytotoxicity *in vitro* towards many transformed cells. In addition, TNF has been reported to produce antiviral effects (Wong and Goedel, *Nature* 1986, 323:819–822). The induced antiviral effects of TNF towards several animal viruses occur either alone or in combination with other cytokines. TNF

is able to protect human WISH cells from the cytopathic effects of VSV [12] but pretreatment of cells with TNF is required for this antiviral activity. Since the response could be inhibited by antibodies to IFNβ, the authors concluded that the antiviral effect of TNF was mediated by activation of IFNβ. The activity of 2-5A synthetase, a known marker for IFN activity, was also enhanced after the addition of TNF. Thus, the protective effect of TNF in these human WISH cells is probably through stimulation of IFNβ.

Similar results have been found with TNF and IFNγ. The antiviral activity of TNF and IFN was found to be enhanced if they were used in combination [13]. Normally, human IFNγ has only a slight inhibitory effect on HSV-1 translation *in vitro*. However, the combined effects of IFNγ and TNF blocked a step before early HSV-1 gene expression in HEP-2 cells. This synergism between IFNγ and TNF may be used as a combined antiviral chemotherapy in the future.

Other functions of TNF have been described by Matsuyama *et al.* [14]. They studied the importance of TNF in human immunodeficiency virus (HIV) infections *in vitro*. In acquired immunodeficiency syndrome (AIDS) patients, a selective depletion of $CD4^+$ lymphocytes is observed but only a few cells are infected by HIV so this does not explain the global loss of $CD4^+$ lymphocytes. It has been suggested that other factors such as lymphokines, including TNF, play a role in this depletion. Matsuyama *et al.* [14] demonstrated, in an *in vitro* system, that TNF could lyse chronically HIV-infected cells. Additionally, after *in vitro* exposure to TNF, HIV replication increased as measured by the level of HIV-specific RNA. The above theories were supported by the findings of Roux-Lombard *et al.* [15] who measured the production of TNFα and IL-1 in cultured peripheral blood monocytes from AIDS patients. TNFα levels in symptomatic HIV-1-infected patients were significantly higher, while the levels of IL-1 were only slightly increased, compared with normal controls. Furthermore, Ito *et al.* [16] demonstrated that the cytopathogenicity of HIV-1 and HIV antigen expression were enhanced in TNF-treated MOLT-4 cells. In addition, Folks *et al.* [17] found that TNFα induced the expression of HIV products in a chronically infected T-cell clone, as shown by the activity of reverse transcriptase in the supernatant of these cells. Similar mechanisms may be of importance *in vivo* and may be responsible for the progression in AIDS from a chronic to a productive infection.

T cells and B cells

To trigger an immune response, T cells must first recognize the antigen as foreign. T cells recognize antigens in the presence of MHC molecules expressed on the cell surface. It is well documented that many cytotoxic T lymphocytes (CTL) are $CD8^+$ and are dependent on MHC class I recognition for their cytotoxic activity. Viral proteins are modified in the cytoplasm of infected cells and the resulting peptides transported to the cell surface together with MHC class I molecules (Townsend *et al.*, *Cell* 1986, 44:959–968). Viral epitopes important for MHC-restricted T-cell recognition can be studied either by expressing specific protein regions in the target cells or by exposing the target cells to high concentrations of synthetic peptides derived from the amino-acid sequence of the whole protein. Del Val *et al.* [18] determined the antigenicity and immunogenicity of a single domain of murine cytomegalovirus protein, pp89, cloned into recombinant vaccinia virus expression vectors. This non-structural protein has a regulatory function in the immediate-early phase of virus replication. Administration of the recombinant vaccinia virus constructs to Balb/c mice protected them against a lethal cytomegalovirus infection. By studying vaccinia virus vectors expressing the various deletion mutants of pp89 in the target cells, the authors were able to identify an epitope of 10 amino acids which was responsible for recognition by CTL in Balb/c mice.

Lymphocytic choriomeningitis virus (LCMV) infection of mice is a well established system for studying a variety of events during CTL recognition. Production of CTL specific for LCMV constitutes a major immune response in the acute disease. Oldstone *et al.* [19] defined a discrete viral protein sequence responsible for MHC-restricted CTL recognition. Target cells were exposed *in vitro* to different truncated peptides which were chosen from a region of the glycoprotein known to contain CTL epitopes (Whitton *et al.*, *Virology* 1988, 162:321). The target cells were lysed by LCMV-specific CTL clones. In this way they identified a nine-amino-acid epitope as a major determinant of the LCMV-specific $H\text{-}2D^b$-restricted CTL response but not of the $H\text{-}2K^b$-restricted response.

To study the mechanisms involved in the assembly, transport and surface expression of viral antigens in association with MHC class I molecules, Townsend *et al.* [20] used a specific cell mutant with a defect in the association of β2 microglobulin and class I MHC. These cell mutants have lost the ability to present an epitope from influenza virus nucleoprotein synthesized in the cytoplasm. However, the same cells could present the epitope on the cell surface when it was a small peptide at high concentration in the extracellular fluid. Peptides could reach intracellular compartments, such as the endoplasmic reticulum; here, they induced the heavy chain to fold and associate with β2 microglobulin. Folding of the heavy chain depended on the accessibility to peptides. These data suggest that the antigenic peptide must be associated with the binding site of class I MHC molecules for the assembly with β2 microglobulin and subsequent transport of the antigen–MHC complex to the cell surface to occur.

T_H cells are $CD4^+$, MHC class II-restricted and have a positive effect on B-cell proliferation and differentiation. Recently, MHC class II-restricted CTL clones have been reported [21]. In these experiments, in addition to having helper activity, $CD4^+$ CTL clones could lyse HSV-infected cells. This lysis was not dependent on soluble secreted factors. These $CD4^+$ CTL clones proliferated in response to stimulation with viral antigens in a MHC class II-restricted manner. Yasukawa *et al.* [21] concluded from

their findings that some $CD4^+$ T cells could simultaneously stimulate B cells to produce antibodies as well as to lyse infected cells by their cytotoxic activity. These results indicate that $CD4^+$ T cells have an important bifunctional role in antiviral protection.

Other studies using influenza virus-specific T_H clones lead to an interesting concept of of T-cell–B-cell interactions [22]. Adoptive transfer of T_H cells specific for any of the major structural influenza virus proteins, including internal viral proteins, to athymic nude mice, 1 day after infection, enhanced the antibody production. The resulting antibody response was primarily to the viral surface proteins. Postponing the transfer of T_H cells to later after infection produced a delayed antibody response to the surface proteins, and the antibody titer was reduced. Production of antibodies to the internal viral proteins was only enhanced when T_H cells specific for internal viral proteins were added. Scherle and Gerhard [22] concluded from their results that B cells recognizing viral surface antigens can receive help signals from T_H cells specific for any viral protein determinant, including internal viral proteins, whereas B cells recognizing internal proteins can only be helped by T_H cells specific for the same internally processed viral protein.

Neutralization

One of the major antiviral processes is neutralization by antibodies. Neutralizing antibodies can be induced by whole viruses, single proteins, or small peptides corresponding to an amino-acid sequence bearing neutralizing epitopes. Induced neutralizing antibodies, as well as passively transferred neutralizing antibodies, have the ability to protect the host from virus infection. For example, immunization with immune-stimulating complexes (ISCOMS) containing canine distemper virus surface proteins induced neutralizing antibodies which protected animals from a virulent challenge [23]. Furthermore, immunization with purified envelope glycoprotein of Aujeszky's disease virus produced virus-specific neutralizing antibodies and protected mice against a virus challenge [24]. In another study, small, chemically synthesized oligopeptides homologous to the external spike glycoprotein of murine hepatitis virus induced high levels of neutralizing antibodies and protected mice from a lethal virus infection [25]. These small peptides allowed the mapping of important areas for virus neutralization and the definition of protective epitopes.

An alternative method of mapping functional epitopes of infectious agents is by selecting neutralization-resistant variants. Virus variants are selected in the presence of neutralizing monoclonal antibodies (mAbs). Virus variants which escape the antibody neutralization are those which contain alterations within the epitopes that form a neutralization site or sites modifying such regions. Neutralizing epitopes of rotaviruses were determined by finding the nucleotide sequence of antigenic mutants resistant to neutralization by mAbs [26]. These nucleotide sequence analyses revealed that the observed amino-acid substitutions of the escaped rotavirus mutants clustered within two variable regions: amino acids 87–101 and 208–221 of VP-7. In a similar manner, epitopes involved in the neutralization of foot-and-mouth disease virus were studied [27]. Some of these neutralizing determinants were localized within conformational epitopes on VP-2 and VP-3.

Theiler's murine encephalomyelitis virus (TMEV) is an animal model for demyelinating diseases such as multiple sclerosis. To study the role of the viral capsid in this demyelinating disease, we selected TMEV variants that were resistant to neutralizing mAbs to an external capsid protein, VP-1 [28]. The pathology of the regular TMEV virus was compared with the disease induced by the neutralization-resistant variant. The variant virus, when injected into susceptible mice, was shown to be markedly less neurovirulent than the parental wild-type virus. These studies demonstrated one of the important biological activities of TMEV VP-1 in determining neurovirulence. More recently, we determined the exact amino-acid substitution responsible for neutralization resistance and neurovirulence, using direct RNA analysis (Zurbriggen *et al.*, *J Exp Med*, in press). The observed amino-acid change was localized on a highly exposed loop of VP-1. This area on the surface of the virus appears to be of general importance for picornaviruses. Murray *et al.* [29] described a short amino-acid sequence corresponding to the neutralization antigenic site I in another picornavirus, the mouse-adapted Lansing strain of poliovirus type 2. That this defines host range was demonstrated by the use of recombinant hybrid viruses.

Gould *et al.* [30] used neutralization-resistant virus variants to study viral morphogenesis. Ultrastructural and immunofluorescence examinations of cells infected with Bluetongue virus variants with alterations in VP-2 allowed the authors to localize VP-2 assembly to the virion. In addition, single amino-acid changes within a neutralizing epitope of VP-2 could dramatically alter viral morphogenesis. Viruses with altered morphogenesis, as demonstrated by these researchers in an *in vitro* system, may play an important role *in vivo* in avoiding immune responses and establishing persistent infection.

A delayed neutralizing antibody response combined with the development of virus variants was observed in goats infected with caprine arthritis–encephalitis virus [31]. This delayed neutralizing antibody response and the appearance of virus variants that escaped neutralization could explain caprine arthritis–encephalitis virus persistence in goats. Sustained or periodic expression by persisting virus could initiate the recurrent arthritis that is seen in this disease.

Further studies dedicated to virus–immune system interactions will provide additional insights into the understanding of mechanisms involved in MHC–antigen complex association, antigen recognition by the immune system, neutralization of viruses, the antiviral activities of cytokines, and their applicability for treatment.

Annotated references and recommended reading

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- •• Of outstanding interest

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