VIRUSES AND IMMUNITY

Toward Understanding Viral Immunology and Immunopathology

Edited by

Claude Koprowski **Hilary Koprowski**

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PREFACE

In the explosive field of immunology it has been difficult to keep abreast of developments in viral immunology and viral immunopathology. Part of the problem stems from the small number of researchers specializing in this area; part from the limited training that has been available in the field; and part from the circumscribed coverage given it in general texts.

It was with these thoughts in mind that The Wistar Institute of Anatomy and Biology, Philadelphia, and the Graduate Group in Immunology at the University of Pennsylvania organized a series of interinstitutional seminars. Their goal was to survey the current state of knowledge in viral immunology and immunopathology and to discuss selected *basic* findings which could serve as an ancilla for further understanding and future experimental work.

These seminars met with such interest that it was decided to organize the material presented for publication. Although the seminars were given in 1973, the principal participants had the opportunity to revise their contributions just prior to publication so the material covered is as recent as production schedules would allow.

Viruses and Immunity: Toward Understanding Viral Immunology and Immunopathology covers a number of basic concepts dealing with immunodeficiency states and experimental immunosuppression. It looks at the immune responses in tumor growth and the role of immune complexes and autoimmune phenomena in virus infections. Finally, it covers current concepts of viral immunoprophylaxis and immunotherapy.

No attempt has been made to treat the subject matter panoramically in textbook fashion. We feel that in a field which, like *Six Characters in Search of an Author,* is still awaiting a unifying genius, an attempt at a uniform view would be contrary to the reader's best interests. Rather, the participants and editors have attempted to examine highly selected areas in depth. The reader will note that authors offer a rich diversity of views, some of them treating similar experimental data from highly varied perspectives with equally varied interpretations.

Although the authors cover a number of basic ideas, this book is not aimed at readers without a general grasp of immunology and virology. Those who gleaned the most from the seminars were junior and senior medical students,

clinicians who have kept abreast of general developments in immunology and for whom clinical examples have been included wherever practical, and graduate students of microbiology and immunology. As the text was designed for fairly rapid reading, abundant references have been included for those who wish to pursue the matter covered either more universally or in greater depth.

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INTRODUCTORY REMARKS

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Over the last five years there has been a renewal of interest in the manner by which the host defends itself against viral infections. This stems from several important developments. First, it has been shown that a number of viruses can persist in the host for months or years, despite the presence of neutralizing antibody. Examples of persistent infections in man are herpes simplex virus (HSV), cytomegalovirus, Epstein-Barr virus, hepatitis B virus, wart virus, and the agents that produce subacute sclerosing panencephalitis and progressive multifocal leukoencephalopathy. In animals, viruses such as lactic dehydrogenase, lymphocytic choriomeningitis (LCM) murine leukemia, Aleutian disease, equine infectious anemia, Marek's disease and SV-40 can all produce persistent infections. Second, there is now good evidence indicating that cellular immunity may be a crucial determinant in stopping the spread of certain viral infections such as HSV. Third, experiments from a number of laboratories have shown that under certain circumstances the immune response to a viral infection, rather than the destructive capacity of the virus itself, may be responsible for the clinical and pathologic manifestations of the infection.

How the immune response of the host stops viral infections appears to be intimately related to the mode by which the virus spreads. Basically viruses can spread by three different routes; extracellularly (Type I spread); from cell-to-contiguous cell (Type II spread); or from parent to progeny cell during cell division (Type III spread). An example of Type I spread is poliovirus; Type II, HSV; and Type III, the murine leukemia viruses or SV-40. A number of viruses, however, can spread by more than one route; HSV, for example, spreads by both the Type I and II routes.

It is becoming quite clear that different immunological processes are required to stop the different modes of viral spread. Type I spread is usually stopped by the neutralization of extracellular virus by antiviral antibody. The initial step in neutralization is the attachment of antibody to the virus, but if the amount of antibody bound to the virus is small or if the antibody is not attached to "critical sites" the result may not be neutralization, but the formation of infectious virus-antibody complexes. Only when larger amounts of antibody attach to the virus, and attach in the right place, does neutralization occur. Neutralization may occur either before the virus reaches the cell or at the level of the cell itself. Before the virus reaches the cell, antibody may neutralize the virus either by aggregating virus particles and thereby reducing the number of infectious units or by lysing the virion with the aid of complement. At the level of the cell, antibody may neutralize the virus by covering the surface of the virion thereby preventing attachment, penetration or uncoating. If antibody alone fails to neutralize the virus, the host has available to it certain accessory factors such as complement and rheumatoid factor, which may enhance the amount of neutralization produced by the antibody.

The class of the immunoglobulin involved in viral neutralization depends on the site of the infection. IgA antibody secreted by the mucous surfaces of the body is the first line of defense localizing the infection to the epithelial surfaces. IgA is therefore particularly important in combating infections of the respiratory and digestive tracts. If the virus gets into the blood, the predominant immunoglobulins in the blood, IgG and IgM, come into action, neutralizing the virus with or without the help of complement and other accessory factors.

How the host stops Type II spread is even more complex. In this case the virus is protected from neutralizing antibody because it is able to spread from cell-to-contiguous cell as a result of virus-induced cell fusion. If, however, the virus induces new antigens on the surface of the infected cell, recognition of these antigens by specific antiviral antibody and complement or immune lymphocytes can result in cell destruction. This would stop the spread of the virus provided the destruction of the infected cell occurred before the virus had spread to adjacent uninfected cells. If, however, the infected cell was destroyed after the virus had spread, the infection would not be stopped. Under these conditions, the host might still stop the spread of the infection by intervening at the junction between infected and uninfected cells or by acting on uninfected cells. For example, inflammatory cells attracted to the site of the infection might be "toxic" on a nonspecific basis to both infected and uninfected cells and thereby hinder virus-induced cell fusion. This could convert a Type II into a Type I spread; the latter can be effectively handled by the simple neutralization of extracellular virus. Alternatively, immune lymphocytes stimulated by viral antigens can release readily diffusable biological mediators such as chemotactic factors, lymphotoxin, and interferon. The latter could prevent uninfected cells at the site of the lesion from becoming infected. From *in vitro* studies, it is known that interferon released from a relatively small number of lymphocytes can protect a large number of target cells. Thus, cell-mediated immunity appears to operate, in part, through the release of interferon. Although most cells of the body are capable of making interferon, certain viruses are poor interferon inducers. The cell-mediated immune response may be particularly important against these viruses in that it amplifies the host's interferon-producing capacity at the site of the local infection.

How the host stops Type III spread is even less clearly understood. Viruses that spread by this route are often nonlytic; the viral genome may be integrated into the host-cell genome and during cell division the viral genome is passed on to progeny cells. To stop Type III spread it is necessary either to destroy the infected cell or to stop cell division. Whether the immune response is successful in destroying the infected cell depends in part upon whether or not the virus induces a sufficient amount of new antigens on the surface of the cell so that the cell is recognized as foreign by immune lymphocytes or by

antibody and complement.

Information as to the relative importance of humoral versus cellular immunity in combating a particular viral infection has been obtained from patients with specific immunological deficiencies and from animals by (*a*) selective suppression of either the cellular or humoral components of the immune system(e. g., thymectomy or bursectomy); or (*b*) passive administration of immune cells or serum to virus-infected animals. Although data are still far from complete, these studies support the idea that humoral immunity is needed to stop those viruses which spread by the extracellular route (Type I), whereas cell-mediated immunity plays an important role in stopping viruses that spread from cell-to-contiguous cell (Type II). When, however, the immunologically uncompromised host is infected, the whole army of weapons appears to be mobilized, i.e., antibody is made and lymphocytes are stimulated. Which defense mechanism actually stops the infection then depends on the mode of viral spread. The various weapons are mobilized because the host does not know which type of spread the virus will actually use and it tries to prepare itself for all of them.

The immune response to a viral infection, however, is not always beneficial to the host. In the case of acute LCM infection in adult mice, the virus itself produces little injury. It is the host's immune response to virus-infected cells which is responsible for cell destruction and the subsequent death of the animal. In the case of chronic LCM infection, the interaction of antibody with viral antigens leads to the deposition of virus-antibody complexes in the kidneys, and this results in the development of glomerulonephritis. The possibility that immune complexes of viral origin may be involved in the pathogenesis of human glomerulonephritis is now being investigated in several laboratories.

Why so many viruses persist in the presence of immunity remains a challenging and still unresolved problem. A number of reasons can be cited and others can be postulated. In the case of HSV, for example, the virus remains latent in sensory ganglia for years where it appears to be antigenically invisible, but capable of being reactivated by a number of factors including nerve injury. In certain infections, such as with lactic dehydrogenase virus, the interaction of antibody with the virus may fail to neutralize the virus, resulting in the formation of infectious virus-antibody complexes. Other viruses, such as the leukemia viruses can infect the cells of the immune system and thereby depress the immune response of the host. In still other cases, the destruction of virus-infected cells by specifically immune lymphocytes can sometimes be blocked at the level of the target cells by the binding of anti-viral antibody or at the level of the lymphocyte by immune complexes.

Persistent viral infections cause considerable morbidity and mortality in both animals and man. Is it going to be possible to develop effective vaccines against viruses that produce persistent infections? Is there any way of curing

individuals once they become chronically infected? If, for example, lack of antigenic expression on the cell surface underlies the persistence of certain viral infections, then attempts to stimulate the immune system may not be useful. Under these circumstances nonimmunological factors, such as chemotherapy or interferon, may be required. In still other cases, where the immune response to the virus is responsible for the disease, immunosuppression may prove to be the treatment of choice. Methods of preventing and treating persistent viral infections appear to represent one of the great challenges of the future in the field of viral immunology.

CONTENTS

1

Immunodeficiency States and Natural Resistance

Osias Stutman

We assume that natural resistance to the "infectious" world within and around us is mediated by immune mechanisms. Thus, any deficiency of the immune system will lead to a decrease of natural resistance to infections. The validity of such an assumption is based on the increased susceptibility to infections observed in animals and man as a result of spontaneous or induced immune deficiencies.

Immunological reactions are complex mixtures of specific and non-specific effects interacting in a delicate balance. These components are: 1. antibody-mediated reactions; 2. cell-mediated reactions (mainly thymus-dependent) and 3. other effector mechanisms which include phagocytosis by granular and mononuclear phagocytes (leukocytes, circulating and fixed histiocytes, etc.) and the small vessel reactions mediated by smooth muscle contraction, inflammation and blood coagulation. There are also biological amplification systems which include: a. the classical activation of the complement system by the interaction of antigen with antibody; b. the alternate pathway of complement activation by a wide variety of mechanisms (endotoxin, proteolytic enzymes, coagulation factors, etc); c. the kinin system of vasoactive substances and d. the production by lymphocytes and other cells of a large number of biologically active substances (lymphokines) capable of acting on mitotic rates, cell metabolism, cell motility and cell function. From the interactions of these factors, integrated responses occur, which most probably represent our basic defense mechanisms against infections (see reference 1 for a review of these interactions). We will concentrate our discussion on analyzing the role of some of such factors (antibodies, cell-mediated

immunity with special emphasis on the role of thymus) and the overall resistance-susceptibility to infections and malignancy.

IMMUNODEFICIENCY STATES AND INFECTIONS

The definition and characterization of different immunodeficiency syndromes in man has permitted an interesting approach to the study of infections. All forms of primary immunodeficiency in man are accompanied by an increased susceptibility to infections (2). The interesting fact is that the pathogenic agents are quite different when the various syndromes are compared (2). In the humoral immune deficiency syndrome, of which the sex-linked recessive form of agammaglobulinemia (Bruton's) is the prototype (3), the most common infections are pneumonitis, meningitis, otitis, etc. The common agents in these cases are mainly the high-grade encapsulated pyogenic pathogens (pneumococcus, streptococcus, meningococcus) as well as haemophilus influenza and pseudomonas aeruginosa (2,4). Agammaglobulinemic patients have also an increased susceptibility to hepatitis, developing a fulminating type of infection (5). In the cell-mediated immune deficiency syndrome, of which the congenital absence of the thymus and parathyroids (DiGeorge's syndrome) is the prototype (6,7) as well as in the combined deficiencies of which the thymic displasia or Swiss-type lymphopenic agammaglobulinemia (8) and the sex-linked lymphopenic immunologic deficiency (9,10) are the best examples, the infectious picture is quite different (2). These patients suffer severe and often lethal viral infections (rubeola, varicella, cytomegalic inclusion virus) and smallpox vaccination may lead to progressive vaccinia (2,11). They also suffer from severe fungal infections especially with candida albicans and histoplasma capsulatum (2) and they may have fatal generalized reactions to Bacillus Calmette-Gerin (BCG) vaccination (2,12). Also chronic pulmonary infections with low pathogenic agents such as Pneumocystis Carinii are common complications (13). Many of these infections are life threatening and represent the major clinical problem in the management of these patients (2,10). Similarly, these types of infections are common complications of induced immunosuppression in patients treated for kidney transplants (14,15). In the 9th Report of the Human Renal Transplantation Registry, sepsis accounted for the highest cause of death (30%) in renal allograft recipients (16). It is apparent that immunologic deficiencies in man whether spontaneous or induced, whether partial or total, are accompanied by an inordinate frequency of infections (2).

Isolated IgA deficiency is associated with a high incidence of sinopulmonary infections, intestinal malabsorption and/or sprue-like syndromes and a variety of autoimmune diseases (17). The Wiskott-Aldrich syndrome is a complex disease that includes abnormalities of platelet function as well as immune inadequacy, especially deficient in responses to polysaccharide antigens (18,19). These children also suffer from recurring, life threatening infections of lungs, skin and other tissues and may have overwhelming disease with herpes simplex, varicella or pneumocystis carinii (18). Similarly, patients with ataxia-telangiectasia, which is a complex disease that includes a combined immune deficiency of both cellular and humoral responses, are also plagued with multiple infections, especially sinopulmonary infections (20). The late-onset (acquired) hypogammaglobulinemia, which includes a number of different diseases such as sporadic agammaglobulinemia, the various types of dysgammaglobulinemias, the so-called acquired agammaglobulinemias and the primary immunologic deficiency of adults, are the most common types of immune deficiency in man (21). These diseases are also characterized by a high incidence of infections especially with encapsulated pyogenic extracellular pathogens (21) and by an inordinate incidence of autoimmune diseases (22). For a more detailed analysis of the association of autoimmunity with immune deficiency diseases see reference 23 and Chapter 8.

Deficiencies of the amplification systems or of leukocyte bactericidal capacity are also associated with an increased incidence of infections. A few examples of this are: a) deficiencies related to C3, a complement component, are associated with recurrent pneumonias (24); b) a deficit of C1r, another complement component, leads to increased frequency of infections and vasculitis (25); c) similarly a deficiency of C2 is accompanied by an increased incidence of infections (26); (for a complete review of complement deficiencies in humans and increased incidence of infections, kidney disease and autoimmunity see reference 27); d) the defect known as chronic granulomatous disease in which leukocytes are capable of phagocytosis although they cannot destroy the ingested bacteria (28,29) is also accompanied by an increased susceptibility to infections.

Therefore, it is quite apparent from the clinical data that a close link exists between an intact immune system and the capacity to deal effectively with bacterial, viral or fungal infections.

The experimental evidence between the association of an intact immune system with susceptibility to infections is compelling. For example, neonatally thymectomized mice are more susceptible than sham-operated controls to infections with hepatotropic viruses (30,31), herpes simplex virus (32), mycobacterium leprae (33), Candida albicans (34), the endotoxins of Escherichia Coli and Salmonella Typhosa (34), to infections with Leishmania (35), Trypanosoma (36), Plasmodium (37), other nematodes in rats (38), schistosoma (39), etc. These effects have been described not only in mice and rats but also in other species such as chickens and rabbits.

In some instances, the effects of thymectomy are somewhat paradoxical since by eliminating the cell-mediated response they do eliminate a pathogenic factor of the clinical disease, and as is the case with the lympho-chorio-meningitis virus, the actual cause of death (40). A somewhat similar reaction occurs with Leishmania infections in mice, in which there is a delay in the appearance of the lesions, although the infection is more severe (35).

Two anecdotes help show the link between an intact immune system and susceptibility to infections:

In his original description of the nude trait in mice, Flanagan defined the genetics of such a trait and had to accept that it was a single locus producing multiple genetic defects which included poor hair development and a liver disease which caused early death (41). Flanagan indicated that the hepatic lesions could be metabolic but that all the nude mice showed granulomas suggesting Toxoplasma Gondii infection (41). Approximately two years later it was discovered that the nude mice also had an absence of thymic development (42), which explained the liver lesion only in the homozygote nu/nu and not in the heterozygote nu/+ (which have a normal thymus) as a result of the presence of Toxoplasma as an endemic parasite in the colony associated with the profound immune deficiency in such mice.

In 1896 Abelous and Billard thymectomized frogs and observed that all the animals died by the 14th day after the operation with a disease characterized by muscular weakness and later paralysis, loss of normal skin color, skin ulcers, progressive anemia, leucocytosis, hemorraghic lesions, edema and death (43). If only the thymic lobe was removed, such symptoms did not occur and blood from such sick animals could produce the same symptoms when injected into normal animals (43). The authors interpreted their results as an indication that the thymus elaborates substances which neutralize toxins formed during normal metabolism and that the thymus is "an essential organ for life" (43). Three years

later, Ver Eecke thymectomized frogs and observed that the symptoms reported by Abelous and Billard did not occur if the water in the tanks was changed frequently, but developed only when the water was allowed to stagnate (44). Ver Eecke's conclusions were that the pathologic condition observed after thymectomy resulted from infections fostered by contaminated water and not from absence of the thymus (44). In 1905 Hammar was unable to reproduce Abelous and Billard experiments, using only clean animals (45). Finally, Pari isolated an organism, a bacillus resembling the one producing gangrenous septicemia, from the blood and organs of thymectomized frogs that were sick after confinement in unchanged waters (46,47). He succeeded in producing the disease in both thymectomized and normal frogs by direct inoculation of the agent; however, he observed that the disease was severe, generalized and lethal in the thymectomized frogs while it was usually localized to the injection site and rarely fatal in the sham-thymectomized controls (46,47). Pari's interpretation was that the loss of the thymus rendered the animals more susceptible to the particular infection described and "probably to all infections" (46,47). This was, indeed, the first statement on a direct relationship between thymus function and immunity. This lead was not followed, and for the next 50 years the work on the thymus followed the conventional endocrinologic pathway, attempting to demonstrate all kinds of hormonal functions. Incidentally, thymectomy of frog larvae produces the same immune deficiencies as those observed in mammals (48).

Other fragments of evidence from the clinic also indicate that the spleen is important in resistance to infections: splenectomized children and those with congenital asplenia show an undue susceptibility to severe and frequently fatal pneumococcal sepsis (49), and splenectomized rats have impaired clearing of pneumococcus from blood (50).

It is apparent that an intact immune system is a requirement for adequate handling of a wide variety of infectious agents. It is in this context that Ehrlich wrote in 1909 that "Fortunately, these germs (keime) remain inactive in the majority of the people because of the immune system" (51). In that same article Ehrlich added that: "If this self protection did not exist we could expect that carcinomas would appear with overwhelming frequency" (51), which brings us directly to our next subject.