Interrelationships of Interferon and Immunity During Viral Infections

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ABSTRACT Interferon is one determinant of host resistance. The immune responses, cellular or humoral, are other components. Cell-mediated responses appear to be involved in host resistance to certain viral infections, particularly the herpesvirus group and vaccinia virus. It is suggested that immune and interferon responses may complement one another and contribute to host resistance. The relative importance of each component depends upon the virus-host interaction. Finally, evidence has been presented which suggests that production of interferon as a result of antigen-sensitized cell interaction may further link these two components of the host response.

Host resistance to viral infections is a complex phenomenon. Following the discovery of interferon by Isaacs and Lindenmann in 1957 (1), a significant body of evidence has developed which suggests that this antiviral substance is an important determinant of host resistance (2). In another paper in this Symposium, Dr. Baron (3) has reviewed the contribution of interferon to the host's defense mechanisms.

The purpose of this report is to consider the function of interferon as it interrelates with both the humoral and cellular immune responses of the host during the course of viral infections.

VIRAL PATHOGENESIS

As background for our discussion, a schematic illustration of viral pathogenesis is presented in Fig. 1. Infection is usually initiated at a local site, e.g., the respiratory tract. Virus replication may occur at this site resulting in the production of disease. Alternatively, if the particular agent has the capacity to invade the host and produce systemic disease, virus may spread from the local site of infection through the blood to target organs; occasionally this spread may occur through lymphatics or nerves. During this primary viremic phase virus particles are exposed to leukocytes or phagocytic cells of the reticu-
loendothelial system and may or may not be cleared from the circulation by these cells, depending upon the virus and the host. If a virus is taken up by leukocytes or phagocytic cells, the outcome of this virus–cell interaction may be a critical determinant of the subsequent course of the infection. Having achieved an intracellular position, the agents may (a) fail to replicate and be controlled by the host, (b) multiply and produce disease, (c) multiply and through a secondary viremia reach target organs, or (d) be carried to distant sites of infection within the circulating leukocytes.

Mims (4) has reviewed the significant body of evidence which suggests that the interaction of virus with phagocytic cells of the reticuloendothelial system may be a critical determinant in blocking further spread of the virus to potential target organs. Work from our own laboratory (5) has indicated that in an in vitro model, mouse macrophages have the capacity to control the spread of vaccinia virus within an otherwise susceptible population of cells. In certain viral infections this interaction may be the common denominator in the initiation of both the interferon and the immune response. Present immunological concepts indicate that processing of an antigen by macrophages is the first step in initiation of the production of humoral antibody. Similarly, evidence has been presented which suggests that phagocytic cells of the reticuloendothelial system also may serve as the source for interferon present in the serum early during the course of a viremia. Thus in certain viral infections both the immune and interferon responses may have a common cell pathway in at least one phase of the host response.

HOST RESISTANCE

A wide variety of physiological factors have been implicated in the host's defenses against a virus infection. In Fig. 2 this complexity is schematically illustrated in terms of the children's fable about the six blind men attempting to define the nature of an elephant. The intent of this illustration is to point out (a) the difficulty in our attempts to define the nature of the host response...
in situations where we frequently are limited in our ability to define more than one or two parameters, and (b) the importance of directing our efforts toward examination and delineation of a more complete picture of host resistance.

INTERFERON AND THE IMMUNE RESPONSE

In discussions of host resistance frequently we have let ourselves be trapped into considering interferon "vs." antibody. This is unfortunate because it has created an "either-or" atmosphere. Rather, it would seem more reasonable to approach the problem from the point of view of trying to integrate the various factors contributing to host resistance. This leads us to consider the following question. How do both interferon and antibody contribute to host resistance? As the first step in answering this query, the nature of the antiviral action of interferon and humoral antibody are compared in Table I.

If one seeks logic in biological phenomenon these two host defenses appear to be logically complementary. Interferon may be rapidly produced and may establish its antiviral activity before the infection is established in the target organ and prior to the presence of neutralizing antibody. Interferon acts intracellularly while antibody is not able to act on a virus in an intracellular location. Finally, interferon may be produced and act at the local site of infection in contrast with the usual production of antibody at a distant site. In each of these characteristics interferon appears to complement the action of neutralizing antibody. The contribution of each of these factors obviously will not be resolved until either interferon or antibody can be selectively in-
Interrelationships of Interferon and Immunity

**Table I**

| Characteristics of Interferon and Antibody |
|--------------------------------------------|
| Interferon                                 |
| Produced by all cells                        |
| Produced at site of infection               |
| Action—intracellular                        |
| Production time—hours                       |
| Virus nonspecific (broad spectrum)          |
|                                            |
| Antibody                                   |
| Produced by specific cells                   |
| Produced at distant site                     |
| Action—extracellular                        |
| Production time—hours—days                  |
| Virus specific                              |

Host resistance to encephalomyocarditis virus infection

We have carried out a series of investigations concerning the relative role of interferon and antibody in host resistance in one model of a systemic viral infection, encephalomyocarditis virus (EMC) in mice (6–9). The pathogenesis of EMC virus is schematically illustrated in Fig. 3. After intraperitoneal inoculation, primary replication occurs in lymphatic tissue with seeding of the blood followed by infection of target organs including the heart and the brain. Two aspects of the host response, e.g. production of interferon and specific neutralizing antibody, are illustrated in this figure. We have consistently found that clearance of viremia has been associated with the appearance of neutralizing antibody. This has been observed to occur as early as 72 hr after infection. We have interpreted these data to indicate that clearance of the viremia is directly related to the production of specific antibody and that initial formation of antibody begins earlier than the time we are first able to detect it (72–120 hr).

Mice receiving whole body X-irradiation or immunosuppressive therapy with cyclophosphamide (cytoxan) and thioquanine manifested an increased susceptibility to EMC virus (7). The mortality of mice infected with EMC virus was increased from 0 in control animals to 90% in mice receiving cytoxan and thioquanine. Animals receiving immunosuppressive therapy were found to have a delay in appearance of neutralizing antibody associated with persistence of the viremia and enhanced multiplication of virus in the target organs. The levels of virus present during the viremia in control and immunosuppressed animals as well as the timing and magnitude of the immune response in experimental and control groups of mice are summarized in Fig. 4.
Interferon levels were similar in control and immunosuppressed animals in spite of the fact that significantly greater levels of virus were found in the blood of the experimental group. These data are summarized in Fig. 5. Similar results have been obtained with animals receiving whole body X-irradiation (6). Since neutralizing antibody could never be detected during the course of an experiment in mice receiving X-irradiation, the effect of administration of hyperimmune anti-EMC antibody on the course of infection is illustrated in Fig. 6. The mortality rate was increased from 10% to approximately 90% in X-irradiated animals. Only one animal died from X-irradiation alone. Hyperimmune serum was administered 24 hr prior to, simultaneously with, or at daily intervals from day 1 through 6 following infection. The purpose of this experiment was to determine whether the earlier presence of neutralizing antibody, presumably by shortening the viremic phase, would decrease seeding of target organs and protect the animal. Susceptibility of the X-irradiated mice could be reversed by passive transfer of neutralizing antibody as late as 72 hr after infection. This timing correlated with the usual time of clearance of the viremic phase in normal control animals. These data have been interpreted to indicate that the decreased capacity of immunosuppressed and X-irradiated animals to produce antibody was a critical determinant of host resistance and that the delayed appearance of antibody resulted in an enhanced viremic phase with increased seeding of target organs, greater multiplication, and death of the animal. In this experimental model we have viewed the function of antibody, as schematically illustrated in Fig. 7, as a
factor limiting the spread of virus to target organs. In interpretation of these
data it should be recognized that any specific conclusions are clearly limited
by the wide range of nonspecific effects that X-irradiation and immunosup-
pressive therapy may have on the host.

To further test this hypothesis a formalized EMC virus vaccine was pre-
pared. This vaccine was shown to contain no infectious virus particles, and
there was no evidence of replication of virus either in tissue culture or in
X-irradiated animals. Furthermore, the vaccine preparation did not induce
interferon either in tissue culture or in vivo. The administration of the vaccine
induced the production of specific neutralizing antibody within 72 hr of an
intraperitoneal inoculation. In another study carried out with Dr. Friedman,
we demonstrated that male mice are significantly more susceptible to EMC
virus than are females. If our interpretation of the role of antibody in host
resistance to EMC virus is valid then the inoculation of the vaccine prior to

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**Figure 4.** Levels of virus and neutralizing antibody in blood of mice infected with
EMC virus. Titers in animals receiving cyclophosphamide (cytoxan) and thioguanine
are compared with nontreated, control animals. *Figure reprinted by permission from the Ameri-
can Society for Microbiology (7).*
virus infection might be expected to induce the earlier formation of antibody in recipient mice. If the immune response were initiated more rapidly, then the viremic phase should be shortened, seeding to target organs decreased, and animals protected against infection. The data presented in Fig. 8 demonstrate that the administration of vaccine 36 hr prior to infection with EMC virus does, in fact, result in a significant degree of protection in the more susceptible male animals. We've interpreted these data to support the concept that neutralizing antibody affects the duration of the viremic phase, secondarily the degree of seeding of target organs and ultimately survival of the animal.

In considering EMC as a model infection we have recognized that the EMC–host relationship must be considered individually, and it is in these terms that we have developed our concept of host resistance. The individual variation in the interferon response may be illustrated by comparing the Newcastle disease virus (NDV)–host interaction with that of EMC virus. NDV induces 10,000–50,000 U/ml in the serum of mice 2–6 hr after inoculation,
Figure 6. The effect of time of administration of anti-EMC immune serum on the mortality of X-irradiated (350R), EMC virus-infected mice. Figure reprinted by permission from the American Society for Microbiology (7).

Figure 7. A schematic illustration of the role of antibody in decreasing the viremia, thus reducing the size of the inoculum reaching target organs during the viremic phase.
while EMC virus stimulates only 50–150 U with peak levels not being reached until 24–48 hr after inoculation. It seems highly unlikely that interferon could make the same contribution to host resistance in these two virus-host relationships.

**Cellular Immunity**

Dr. Baron (3) has focused principally upon the host response during primary infection; I would now like to direct our attention to an area in which interferon and immune responses of the host may be more directly related. As background we should briefly consider the cellular aspect of the immune response. Cooper and coworkers (10) have presented the concept of the two-component immune system. One population of cells derived from the thymus is related to cellular immunity; the other derived from the bursa in chickens or Peyer's patches lymphoid tissue in mammals is responsible for the humoral
antibody response. In recent years a number of clinical conditions have been recognized which are associated with enhanced susceptibility to viral infections (11–17). A common denominator of many of these situations has been impairment of the immune response. In accord with the two-component concept of the immune response, clinical conditions associated with enhanced susceptibility in Table II have been divided into the following three general groups: (a) decreased immunoglobulin production with normal thymus function, (b) normal immunoglobulin production with impaired thymus function, and (c) varying degrees of combined dysfunction of both components of the immune mechanism. Although there are some exceptions, the majority of individuals in the group with deficiency of humoral antibody formation but intact thymus function develop normal delayed hypersensitivity reactions and handle most viral infections in a relatively normal fashion. This supports the general concept presented by Dr. Baron (2, 3) of the relatively limited role

### TABLE II

CLINICAL CONDITIONS CHARACTERIZED BY IMMUNOLOGICAL DEFICIENCY AND INCREASED SUSCEPTIBILITY TO VIRAL INFECTION

| Malignancy and/or Immunosuppression |
|------------------------------------|
| 1. Varicella                         |
| 2. Vaccinia                         |
| 3. Cytomegalovirus                  |
| 4. Measles                          |
| 5. Herpes                           |

| Immunological Deficiency Diseases   |
|------------------------------------|
| A. Decreased immunoglobulin production with normal thymic function |
| 1. Poliovirus, vaccine strain       |
| 2. ECHO                             |
| 3. Serum hepatitis                  |
| 4. Cytomegalovirus                  |
| 5. Adenovirus                       |
| 6. Varicella                        |
| 7. Vaccinia                         |
| B. Normal immunoglobulin production with thymic deficiency |
| 1. Vaccinia                         |
| 2. Measles                          |
| C. Decreased immunoglobulin production with thymic dysfunction |
| 1. Vaccinia                         |
| 2. Measles                          |
| 3. Varicella                        |
| 4. Cytomegalovirus                  |
| 5. Adenovirus                       |
| D. Wiskott-Aldrich Syndrome         |
| 1. Herpes simplex                   |
| 2. Cytomegalovirus                  |
| 3. Measles                          |


of neutralizing antibody in many primary viral infections. In contrast children with various conditions involving dysfunction or aplasia of the thymus manifest a pattern of deficiency of cellular immunity as evidenced by a reduced capacity to reject homografts and failure to develop delayed hypersensitivity. It is this group of individuals who are most conspicuous in their impaired capacity to control a number of viral infections.

The following two general patterns emerge from these experiences: (a) from the point of view of the host—conditions in which cell immunity is impaired alone or in combination with other components of the host response are associated with increased susceptibility of the host to certain viral infections, particularly herpesvirus, vaccinia virus, cytomegalovirus, and perhaps measles virus; (b) from the virus aspect—cytomegalovirus, herpesvirus, vaccinia virus, are all agents in which cell-to-cell transmission of virus is characteristic of the pathogenesis of the virus. Although current knowledge does not permit an explanation of these patterns they strongly suggest a causal relationship.

IMMUNOSUPPRESSION—EXPERIMENTAL MODELS

The effect of immunosuppressive therapy on experimental infections in animals has been widely investigated and tends to support the general concept which has been presented. This interpretation, as we have indicated, must be limited by the knowledge that most methods used to produce immunosuppression have multiple sites of action and may adversely affect more than one component of host resistance.

Within these limitations, one interesting study by Fulginiti supports the theme of this discussion, namely the interrelationship between interferon and the immune response. In this study X-irradiation and antilymphocyte sera were utilized as suppressants of host resistance in monkeys infected with vaccinia virus. In animals treated with antilymphocyte sera, the vaccinia virus lesions were larger and contained more virus. Furthermore virus was disseminated with development of secondary lesions over the entire body. Delayed hypersensitivity skin tests to vaccinia virus antigen were markedly reduced in size, but serum antibody and interferon levels were comparable with control animals. Discontinuation of the administration of antilymphocyte sera was followed by a cellular response at the site of infection, development of a delayed hypersensitivity reaction at the vaccination site, and eventual clearing of all lesions. In primates receiving a lethal dose of X-irradiation, vaccinia virus rapidly became disseminated from the necrotic, nonhealing primary vaccination site. In two animals the infection with vaccinia virus was controlled. Both animals had restoration of lymphatic tissue. One of these occurred spontaneously, the other was produced experimentally by replace-

1 Fulginiti, V. A. Simian vaccinia virus infection. Presented at Society for Pediatric Research Meeting, Atlantic City, New Jersey, in May, 1969. Manuscript in preparation.
ment of bone marrow. Lymphatic restoration was associated with reappearance of lymphocytes, development of delayed hypersensitivity, and recovery of the capacity to synthesize interferon at the local site of virus infection. These data have been interpreted to indicate that the presence of cellular immunity or of at least cell-mediated responses appears to correlate with host resistance. The combined loss of capacity to produce interferon and humoral antibody in the X-irradiated animals was associated with a greater enhancement of susceptibility than occurred with the administration of antilymphocyte sera alone. Recovery of cellular response and the capability of interferon production also were associated with restoration of the host's capacity to control vaccinia virus infection. These results support the concept that lymphocytes and cell-mediated responses may be critical determinants of host resistance to vaccinia virus infections. The data also strongly suggest that host resistance is a multifactorial phenomenon and that each component, interferon and the cellular and humoral immune response, contributes to the host defense against infection with vaccinia virus. Although certainly not definitive, this study suggests the possible interrelationship between cellular immune response and interferon.

The clinical observations which were reviewed, as well as the experimental evidence of which Fulginiti's study is one example, suggest that cellular immunity contributes to host resistance. It is important to bear in mind, however, that results of immunosuppression on experimental virus infection have included the following: (a) enhanced susceptibility of the host, (b) enhanced resistance of the host, and (c) no recognizable effect. Under these conditions, it is evident that the role of the various components of host response depends upon the nature of the virus-host interaction, and that the immune response may, in certain virus infections, contribute to the virulence or pathogenesis of the virus while in others a cellular component of the immune response may also be a contributor to host resistance.

**INTERRELATIONSHIP OF INTERFERON AND CELLULAR IMMUNITY**

The complexity of altered responses or reactions of the immune cell on exposure to specific antigen has been increasingly recognized. Fig. 9 summarizes the wide spectrum of cell responses which may result from this interaction. For the purpose of this discussion attention will be focused on the evidence which suggests that induction of interferon production may be one of the altered responses of the immune cell and may provide another link between these two components of the host's defense mechanism. Several years ago we reported (18) the observation that peritoneal macrophages from mice immunized to Chikungunya virus (CV) produced increased levels of interferon on reexposure to the same agent in vitro. This reaction is specific for CV and
could not be correlated with (a) enhanced uptake of virus by immune cells, (b) the presence of cytophilic antibody, or (c) the presence of neutralizing antibody. It was postulated that the immune cell might have an altered capacity to respond on reexposure to a virus and that enhanced interferon production was one manifestation of this altered reactivity. If this phenomenon also occurs in the intact animal it will further suggest a possible mechanism for cellular immunity to viral infection in vivo and will establish a relationship between interferon and the immune response.

More recently work from a number of laboratories has confirmed and extended this concept. Similar results have been obtained by Yamada and Azuma (19) who observed that interferon production was markedly increased in macrophages harvested from the peritoneal cavity of mice immunized with NDV or Sindbis virus and exposed to the corresponding agent in tissue culture. The potential significance of these observations has been broadened by the recent studies of Green, Cooperband, and Kibrick (20) who extended the phenomenon from that of a virus-immune cell interaction to a more general interaction of antigen with immune cells. They demonstrated that human leukocytes from individuals with sensitivity to tuberculin, tetanus, or diphtheria toxin produced interferon on exposure to the respective antigens in vitro. Reaction was specific for cells from immune donors and could not be found in nonsensitized cells. Evidence that this phenomenon occurs in the intact animal as well as in tissue culture has been demonstrated by Stinebring and Absher (21). They found that mice which had been sensitized to Mycobacterium tuberculosis produced strikingly greater levels of interferon in the serum following inoculation of PPD than control, nonsensitized animals. This series of investigations supports the concept that interferon production may represent one manifestation of the altered reactivity of the immune leukocyte or macrophage on reexposure to the immunizing antigen. The data further suggest that production of interferon by the immune cell exposed to
specific antigen may have implications for the intact animal as well as tissue culture models. The question of the potential function in the intact animal remains intriguing but unanswered.

SUMMARY

Interferon is one determinant of host resistance. The immune responses, cellular or humoral, are other components. Cell-mediated responses appear to be involved in host resistance to certain viral infections, particularly the herpes-virus group and vaccinia virus. It is suggested that immune and interferon responses may compliment one another and contribute to host resistance. The relative importance of each component depends upon the virus-host interaction. Finally, evidence has been presented which suggests that production of interferon as a result of antigen-sensitized cell interaction may further link these two components of the host response.

This work was partially supported by Grant AI 06388 from the National Institutes of Health, United States Public Health Service.

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