INTERFERON

Edited by

YASUITI NAGANO, M.D.
HILTON B. LEVY, M.D.

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YASUITI NAGANO, M.D.
Kitasato Institute, Tokyo
HILTON B. LEVY, M.D.
Laboratory of Viral Diseases, NIAID, National Institute of Health, Bethesda

IGAKU SHOIN Ltd. TOKYO

PREFACE

This volume presents the proceedings of a Symposium on Interferon held in Tokyo, May 26-28, 1969, under the auspices of the Japan-United States Committee on Scientific Cooperation. The respective agencies supporting the program were the United States National Science Foundation and the Japan Society for the Promotion of Science. The editors and the participants are greatly indebted to those organizations for their generosity, and for their efficient support in innumerable details. In particular, we wish to thank Dr. WALTER H. HODGE and Mr. HARUKI AMATSUCHI for the personal interest they took in the program.

While all the participants anticipated a productive meeting, the unusually high level of the presentations was particularly rewarding to the organizers. Both the American and Japanese members felt enriched by the experience, partly from the acquisition of new scientific information, but more importantly from the exchange with a group with whom there usually is inadequate contact. Several cooperative scientific programs are in development as a result of this exchange.

It has been popular to speak of differences in thinking habits between the orientals and occidentals. None was apparent to the participants in this program. While language was something of a barrier, the simultaneous translation kept these difficulties at minimum. A particular debt is owed to Professor MATUMOTO of the University of Tokyo for his aid in the preparation of this book. Without his help the translation into English of the Japanese texts and discussions would have been impossible.

Y. NAGANO and H. B. LEVY

PARTICIPANTS

Dr. AZUMA, MASANOBU:
Dept. of Bacteriology, School of Medicine, Hokkaido University, Sapporo, Hokkaido

Dr. BARON, SAMUEL:
Laboratory of Biology of Viruses, NIAID, National Institutes of Health, Bethesda, Maryland 20014

Dr. DE SOMER, P.:
Rega Institute, University of Louven, Minderbroedersstraat 10 Leuven, Belgium

Dr. FRIEDMAN-KIEN, ALVIN E.:
New York University, School of Medicine, New York, N.Y. 10016

Dr. FRIEDMAN, ROBERT M.:
Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Dr. FUJITA, HARUHISA:
The 2nd Dept. of Pharmaceutical Inst., School of Medicine, Keio University, Shinanomachi, Tokyo

Dr. FURUTANI, TAKESHI:
National Institute of Animal Health, Kodaira, Tokyo

Dr. GINSBERG, HAROLD S.:
University of Pennsylvania, Medical School, Philadelphia, Pennsylvania 19104

Dr. GLASGOW, LOWELL A.:
Dept. of Pediatrics, University of Rochester School of Medicine, Rochester, New York 14627

Dr. GORDON, IRVING:
University of Southern California School of Medicine, Los Angeles, California 90033

Dr. HANEISHI, TATSUO:
Research Laboratory of Sankyo Co., Ltd., Tokyo

Dr. HARA, KAZUHITO:
Dept. of Microbiology, Kyoto Prefectural University of Medicine, Kyoto

Dr. HASHIMOTO, KAZUO:
Dept. of Microbiology, School of Medicine, Keio University, Shinanomachi, Tokyo

Dr. HILLEMAN, MAURICE R.:
Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486

Dr. HOSHINO, MASAO:
Yakult Research Laboratory, Kunitachi, Tokyo

Dr. INOUE, HIROO:
Dept. of Microbiology, Kyoto Prefectural University of Medicine, Kyoto

Dr. ISHIDA, NAKAO:
Dept. of Bacteriology, School of Medicine, Tohoku University, Sendai

Dr. KARAKI, TOSHIRO:
Dept. of Microbiology, Kyoto Prefectural University of Medicine, Kyoto

Dr. KASAHARA, SHIRO:
Kitasato University, Tokyo

Dr. KISHIDA, TSUNATARO:
Dept. of Microbiology, Kyoto Prefectural University of Medicine, Kyoto

Dr. KOHASE, MASAYOSHI:
National Institute of Health, Tokyo

Dr. KOHNO, SEIYA:
National Institute of Health, Tokyo

Dr. KOIDE, HIDEOKI:
National Institute of Animal Health, Kodaira, Tokyo

Dr. KOJIMA, YASUHIKO:
Kitasato Institute, Tokyo

Dr. KOMATSU, HARUKO:
Kitasato Institute, Tokyo

Dr. KONO, REISAKU:
National Institute of Health, Tokyo

Dr. KONO, YUJI:
National Institute of Animal Health, Kodaira, Tokyo

Dr. KOYAMA, HIROYUKI:
Kitasato University, Tokyo

Dr. LEVY, HILTON B.:
Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, Maryland 20014

Dr. MAEHARA, NOBUTOSHI:
Kitasato Institute, Tokyo

Dr. MATUMOTO, MINORU:
Institute of Medical Science, Tokyo University, Tokyo

Dr. MERIGAN, THOMAS C.:
Stanford University Medical School, Palo Alto, California, 94305

Dr. MORIMOTO, TOMIOKI:
National Institute of Animal Health, Kodaira, Tokyo

Dr. MARUYAMA, TAIKI:
Dept. of Botany, Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido

Dr. NAGANO, YASUITI:
Kitasato Institute, Tokyo

Dr. PAUCKER, KURT:
Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19146

Dr. SETO, YOSHIKO:
The 2nd Dept. of Pharmaceutical Inst., School of Medicine, Keio University, Shinanomachi, Tokyo

Dr. TAKEHARA, KOICHI:
Nippon Institute of Biological Science, Tachikawa, Tokyo

Dr. TOBA, MASANORI:
Institute of Medical Science, Tokyo University, Tokyo

Dr. TONEGI, HISAKO;
The 2nd Dept. of Pharmaceutical Inst., School of Medicine, Keio University, Shinanomachi, Tokyo

Dr. TOYOSHIMA, SHIGESHI:
The 2nd Dept. of Pharmaceutical Inst., School of Medicine, Keio University, Shinanomachi, Tokyo

Dr. VILČEK, JAN:
New York University School of Medicine, New York, N.Y. 10016

Dr. WATANABE, MORIMATU:
National Institute of Animal Health, Kodaira, Tokyo

Dr. YAMADA, MORIHIDE:
Dept. of Bacteriology, School of Medicine, Hokkaido University, Sapporo, Hokkaido

Dr. YOUNGNER, JULIUS S.:
University of Pittsburgh Medical School, Pittsburgh, Pennsylvania 15213

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INTERFERON, ANTIBODY AND HOST RESISTANCE

LOWELL A. GLASGOW
Department of Pediatrics, University of Rochester School of Medicine, Rochester, New York

Present concepts of host resistance suggest that interferon is a critical factor during primary infection with a viral agent and antibody is of lesser importance. The evidence supporting present concepts concerning the host defenses against viral infection has been comprehensively reviewed by BARON[1,2]. As our knowledge has grown, however, it has become increasingly apparent that host resistance is a complex phenomenon and that a single generalized concept is no longer possible.

The purpose of this paper is to consider certain aspects of the pathogenesis of and host response to virus infections. In this discussion I would like to briefly review observations from clinical medicine relating conditions characterized by an impaired immune response with increased susceptibility to certain viral infections, and then present more recent experimental data—some from our own laboratory and some from other laboratories—which are contributing to our growing understanding of the nature of the host response to an intracellular parasite.

IMMUNOSUPPRESSION AND SUSCEPTIBILITY TO VIRAL INFECTIONS

The capacity of individuals with immunoglobulin deficiencies to handle the majority of viral infections in a normal fashion has suggested that the immune response plays a relatively minor role in host resistance to viral infections. More recently, however, it has been recognized that an increasing number of clinical conditions may be associated with enhanced susceptibility to viral infections. A summary of disease states which may be manifested by decreased host resistance is presented in Table I[3-29]. A common denominator of all these situations is a depression of the immune response. For the purpose of this discussion it should be noted that alterations in host resistance have been identified both in conditions in which thymic dependent cellular immunity is impaired, and situations in which there is a decrease in circulating antibody. No natural or iatrogenic disease states have, as yet, been recognized in which decreased host resistance to viral infections has been associated with impaired phagocytosis or interferon production.

Table 1 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

VIRAL PATHOGENESIS

As background for our discussion I would like to consider the schematic illustration of viral pathogenesis presented in Fig. 1. Although the limitations

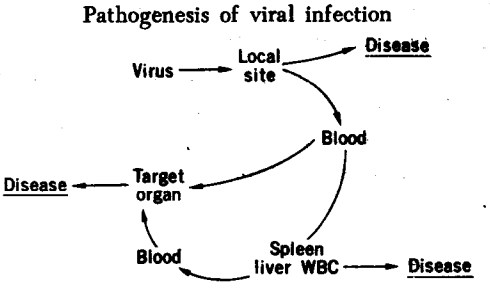

Fig. 1 A schematic diagram representing the pathogenesis of many acute viral infections.

of such a schematic outline are recognized, the concepts presented are applicable to the majority of acute viral infections. Infection is usually initiated at a local site where multiplication occurs and where the virus may produce disease. From this local site virus may spread through the blood directly to target organs or be cleared from the circulation by phagocytic cells of the reticuloendothelial system. Here it may produce disease, may be arrested, or alternately may multiply and through a secondary viremia reach target organs. MIMS[30] has reviewed the evidence that the interaction of virus with phagocytic cells may result in minimal virus replication and that further spread of the virus may be arrested at this point. We have reported the evidence that in an *in vitro* model, mouse macrophages has the capacity to control the spread of vaccinia virus in an otherwise susceptible population of cells[31].

Present concepts of host resistance suggest that early production of inter-

feron, possibly from white blood cells or reticuloendothelial system cells, may be critical in protecting target organs. Antibody production occurs after seeding of target organs and, in contrast with the importance of neutralizing antibody in resistance to reinfection, makes a relatively minor contribution to control of virus replication during primary infection.

HOST RESISTANCE TO ENCEPHALOMYOCARDITIS (EMC) VIRUS INFECTION

We have carried out a series of investigations concerning the relative role of interferon and antibody in host resistance[32-36]. As one model systemic viral infection, we have studied encephalomyocarditis (EMC) virus in mice. The pathogenesis of EMC is schematically illustrated in Fig. 2. After

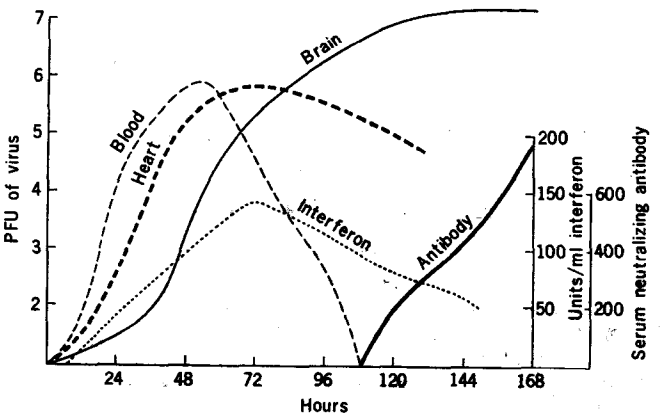

Fig. 2 A diagram of the quantity of virus in the blood, heart and brain (PFU/gram) during infection of mice with Encephalomyocarditis virus, and the levels of interferon and antibody in the serum, produced in response to infection.

intraperitoneal inoculation, primary replication occurs in lymphatic tissue with seeding of the blood followed by infection of target organs including the heart and brain. Two aspects of the host response, i.e., production of interferon and specific neutralizing antibody, are also 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 hours after infection. During the course of these studies a number of exogenous and endogenous factors have been found to modify susceptibility to this agent[32-36].

EFFECT OF X-IRRADIATION

Mice receiving whole body X-irradiation (350-650R) manifested an increased susceptibility to EMC virus[32]. Enhanced susceptibility was associated with persistent viremic phase, enhanced multiplication of virus in target organs and the failure to detect specific neutralizing antibody. The levels of virus present during the viremia in control and X-irradiated animals and

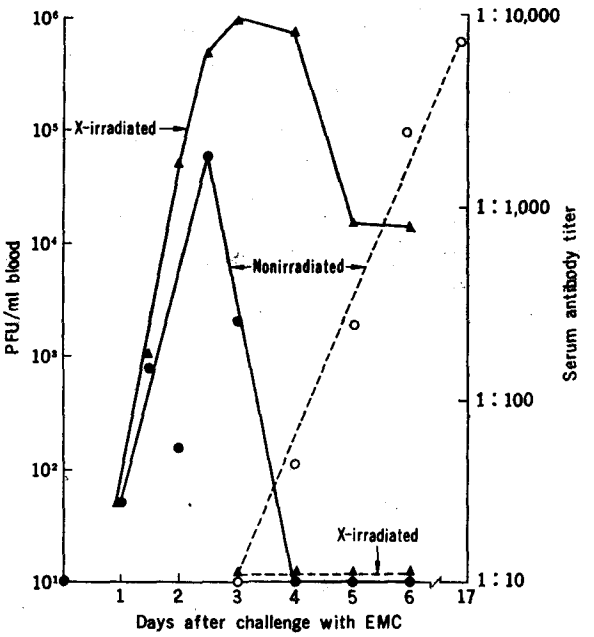

Fig. 3 Virus titer in blood, and antibody response in X-irradiated (650 R) and nonirradiated mice challenged with EMC virus. ●—●—● Virus titer in nonirradiated mice. ▲—▲—▲ Virus titer in X-irradiated mice. ○···○ ○ Antibody response in nonirradiated mice. ▲···▲···▲ Antibody response in X-irradiated mice.

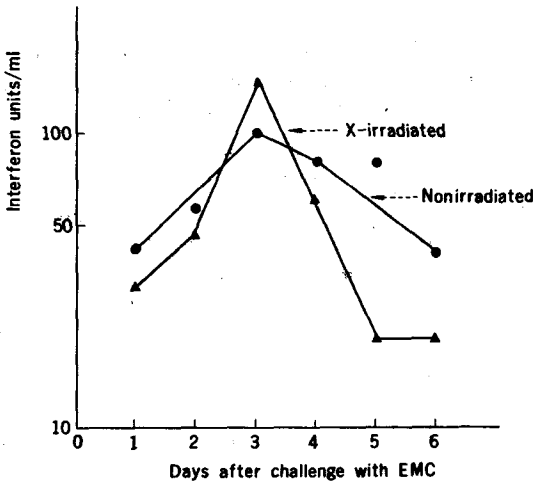

Fig. 4 Interferon response in X-irradiated (650 R) and nonirradiated mice challenged with EMC virus.

the timing and magnitude of the immune response in the non-irradiated group of mice is summarized in Fig. 3. Interferon levels were similar in control and X-irradiated animals in spite of the fact that significantly greater levels of virus were found in the blood of the X-irradiated group. These data are presented in Fig. 4. The association of enhanced susceptibility with the delay in the appearance of neutralizing antibody suggested a causal relationship. In Fig. 5 the results of the passive administration of hyperimmune

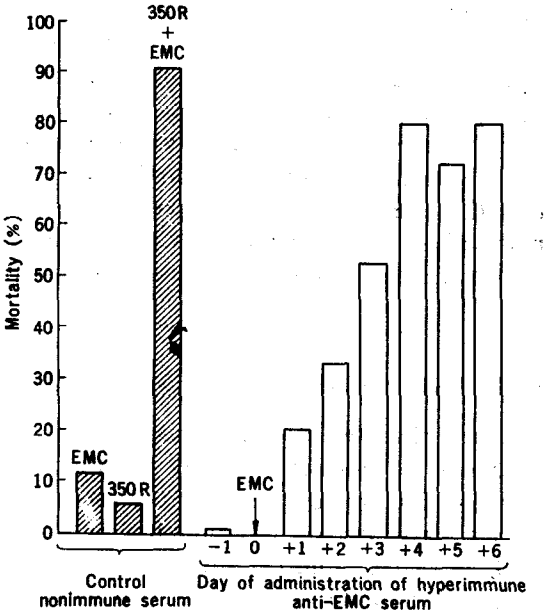

Fig. 5 The effect of time of administration of anti-EMC immune serum on the mortality of X-irradiated (350 R), EMC virus-infected mice.

antiserum on the course of EMC virus infection in X-irradiated animals is illustrated. Susceptibility of X-irradiated mice could be reversed by passive transfer of neutralizing antibody as late as 72 hours 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 the X-irradiated animal 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 considering these data, it should be recognized that these specific interpretations are clearly limited by the non-specific effect of X-irradiation on the host.

EFFECT OF IMMUNOSUPPRESSIVE DRUGS

Similar results have been obtained in a study of EMC virus infection in animals treated with cyclophosphamide (Cytoxan) and thioguanine[33]. Animals receiving immunosuppressive therapy also manifested a delay in the

appearance of neutralizing antibody. This delay was associated with persistence of the viremia, increased seeding of target organs, and an enhanced susceptibility to infection. The interferon response in the serum of the control and treated animals was again identical. These results were felt to be compatible with the interpretation of the data from the X-irradiation study and to implicate antibody in host resistance to EMC virus.

Dr. HEATH and colleagues[37] have recently reported the results of studies with Sendai virus infection in mice. They found that mice infected with Sendai virus and treated with Cytoxan were shown to have: (a) an increased incidence of lesions in the lung; (b) an increased duration of virus infection; (c) a significant decrease in the levels of antibody in the serum; and, (d) an interferon response of equal magnitude with that of controls.

Drs. COLE and NATHANSON have similarly reported evidence[38] that the outcome of two experimental arbovirus (Dengue and West Nile) infections was strikingly altered by treatment of host animals with cyclophosphamide (Cytoxan). In both cases, enhancement of virus replication in the central nervous system of experimental animals was associated with immunosuppression.

INTERFERON AND ANTIBODY IN EMC VIRUS INFECTION

We have proposed that the decreased capacity of X-irradiated or immunosuppressed animals to produce antibody resulted in increased viremia with increased seeding of target organs and increased mortality. In this experimental model we have viewed the function of antibody, schematically illustrated in Fig. 6, as a factor limiting the spread of virus to target organs.

To further test this hypothesis we developed a formalinized EMC vaccine. This vaccine does not contain infectious virus particles, does not re-

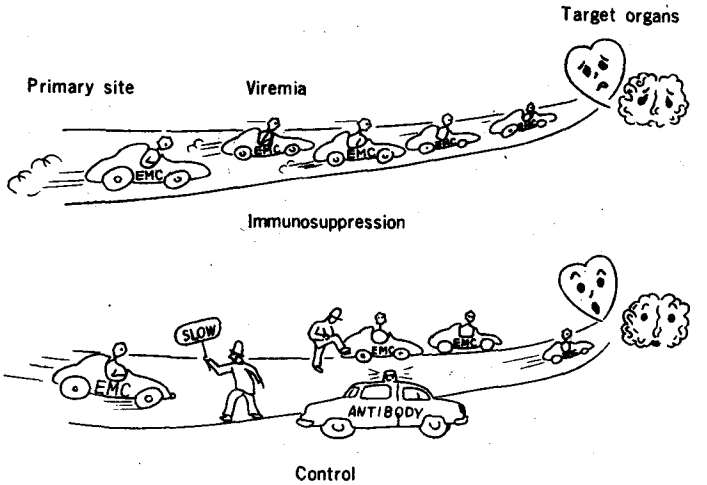

Fig. 6 A schematic illustration of the role of antibody in decreasing the viremia, thus reducing the size of the inoculum reaching target organs during the viremic phase.

plicate in tissue culture or in X-irradiated animals, and does not induce interferon. Administration of the vaccine induced the production of anti-EMC antibody within 72 hours in the CD-1 strain of mice. In a study carried out with Dr. FRIEDMAN[35], we have found that male animals are significantly more susceptible to EMC virus than females. If the enhanced susceptibility of male animals is a function of a relative deficiency in the immune response, then the inoculation of the killed vaccine 36 hours prior to virus infection might be expected to stimulate the production of antibody earlier during the viremic phase of the infection. If the earlier appearance of antibody shortens the viremia, seeding of target organs should be decreased and the mortality rate should be lower. The data presented in Fig. 7 demonstrate that the administration of vaccine to male or female animals 36 hours prior to infection with EMC virus resulted in a significant degree of protection in the more susceptible males. We have interpreted these data to support the concept that an earlier antibody response results in shortening the viremic phase, decreasing seeding of target organs, and increasing survival of vaccinated animals.

We recently observed that the C3H strain of mice are significantly more susceptible to EMC virus, yet produce significantly greater levels of interferon as compared with the CD-1 strain (the standard mouse strain in which most of our EMC virus studies have been carried out). The data presented in Fig. 8 summarize the LD_{50} and the serum interferon levels in the two strains of mice.

The LD_{50} for C3H mice was 8 PFU, while that of CD-1 mice was 40 PFU. In contrast, peak serum interferon levels in the C3H strain were ten-

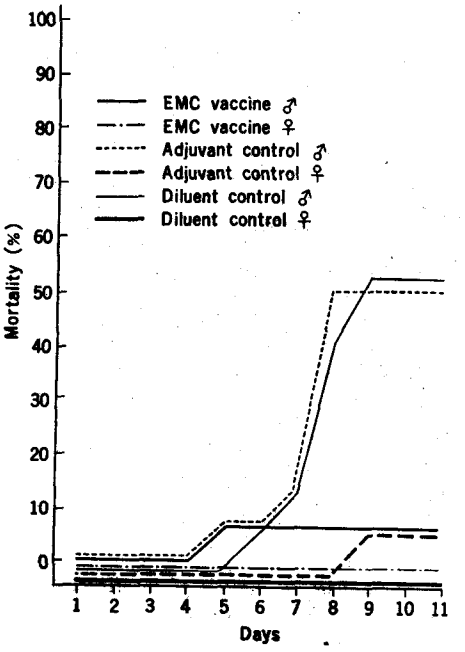

Fig. 7 The mortality rate in male and female mice infected with encephalomyocarditis (EMC) virus 36 hours after receiving a formalinized EMC virus vaccine or a control inoculation (adjuvant or balanced salt solution).

fold greater (2000–4000 versus 150–500) than in the more resistant CD-1 animals. Thus, there was a negative correlation between host resistance and interferon production.

Throughout the course of these studies we have characterized EMC as a relatively poor inducer of interferon. However, when animal were inoculated with a high dose (2.5×10^6 PFU) of a strain of EMC virus which was lethal in 48–72 hours, a more rapid and much greater interferon response was observed, approximately 13,000 units per ml at 12 hours after infection. In contrast, a lower inoculum (2.5×10^3 PFU) induced the expected levels of approximately 150 units of interferon at 24–48 hours. These results are illustrated in Fig. 9. It should be noted that the lower inoculum represents

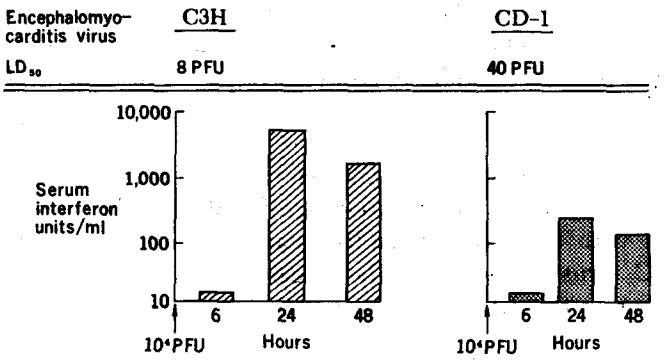

Fig. 8 The LD_{50} (in PFU) of C3H and CD-1 strains of mice infected with encephalomyocarditis virus is presented in the top half of the figure. Below, the levels of interferon in both strains infected with a lethal dose (10^4 PFU) of virus is represented in units per ml of serum.

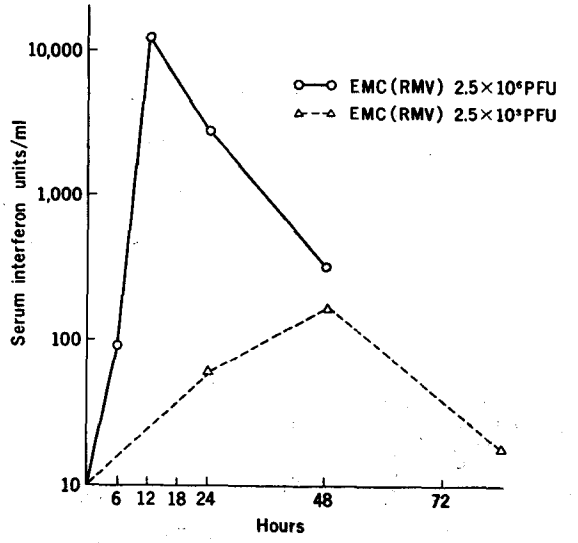

Fig. 9 Levels of interferon in the serum of CD-1 mice infected with 2.5×10^6 and 2.5×10^3 PFU of encephalomyocarditis virus.

a lethal dose and that at the time that 150 units are present in the serum there is 10^5–10^6 PFU of EMC virus in the blood. The development of the high levels of interferon (13,000 units/ml) was not associated with resistance —the mortality was 100%. We have interpreted this observation as a second negative correlation between interferon production and host resistance in this virus-host model.

SUMMARY

In Fig. 10 we have added the dimensions of the host response to our schematic illustration of the pathogenesis of a viral infection (Fig. 1). Interferon may be produced early, both at a local site of infection, following uptake of virus by white blood or phagocytic cells, or in the target organ

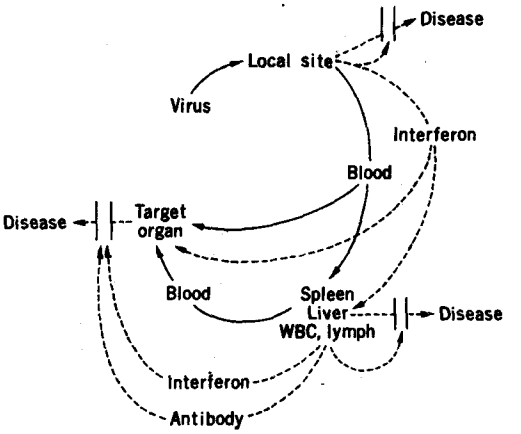

Fig. 10 Pathogenesis-host response viral infections.

itself. The early production of interferon in high quantities during the course of an infection with an interferon-susceptible virus would probably characterize a system in which interferon represents a critical determinant of host resistance. In contrast, infection with a poor interferon inducer, or with a relatively less interferon-sensitive agent, and a rapidly fatal infection (perhaps the situation with the infection described above where mice were inoculated with a high inoculum of EMC virus) may be examples in which interferon is of lesser importance in a virus-host interaction.

Antibody production, although undoubtedly occurring earlier than previously was thought, is usually produced later than interferon and lacks the capacity to act as an intracellular site to block virus replication. Antibody probably acts to decrease spread of virus via the blood and diminish the spread of cytolytic viruses within target organs. The role of neutralizing antibody is probably limited in infections where transmission of virus occurs directly cell to cell.

Interaction of virus with macrophages or lymphocytes may involve both immune (cellular and antibody) and interferon responses. The virus-macrophage interaction may also be a critical determinant of host resistance for a

number of virus infections[80]. Failure of a virus to multiply in reticulo-endothelial system cells following phagocytosis, or the production of significant levels of interferon as a result of this interaction both appear to be factors in the function of the macrophage in host resistance. We should, perhaps, note here that the evidence from the EMC-mouse system suggests minimal importance of the macrophage in the pathogenesis of EMC virus infection. The virulent variant of EMC is cleared extremely slowly from the blood in the absence of neutralizing antibody and appears to lack the capacity to multiply in macrophages, at least *in vitro*. Alternatively, the thymic dependent or cellular aspect of the immune response may be an important determinant in other viral infections as evidenced by the clinical situations in which depression of cellular immunity have been associated with varicella or vaccinia or herpesvirus infection. More recent experimental evidence utilizing antilymphocyte or antithymocyte serum has supported this concept.

In conclusion, host resistance is a complex phenomenon. Like the six blind men in the children's fable illustrated in Fig. 11, we have defined many

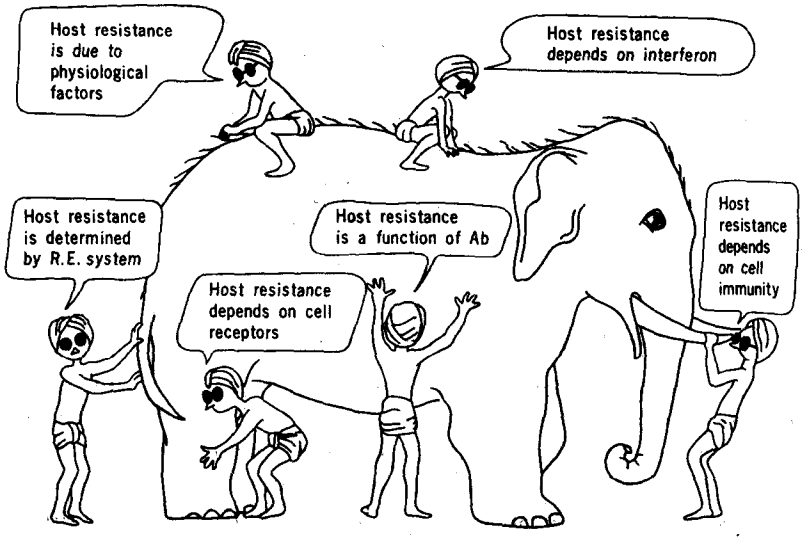

Fig. 11

individual aspects of host resistance, but I do not think we have defined host resistance in its entirety. It is apparent, however, that host resistance is no longer definable by characterizing one or two parameters. Interferon, the virus-cell interaction, circulating antibody, cellular immunity and other physiologic factors undoubtedly make different contributions and assume differing degrees of importance in different virus-host interactions.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Mrs. PATRICIA DWYER for her excellent technical assistance, and to Mrs. MARILYN GRAVER for the preparation of the manuscript.