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Unsolved Problems and Concluding Recommendations Regarding Cytomegalovirus[1]

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I. THE ROLE AND IDENTIFICATION OF DONORS IN THE TRANSMISSION OF CMV

Although epidemiologic data suggest that CMV is transmitted with blood products, the identification of virus carriers has been elusive. Additionally, it has been suggested that CMV may be transmitted in or with transplanted organs and other tissues. In these latter instances it is uncertain whether CMV has been transferred in endogenous cells or in circulating cells carried passively within the organ. The location of virus and identification of carriers will have to precede the application of techniques to eliminate these infections. Some specific suggested questions and topics for study are outlined below.

A. Blood

1. Do lymphocytes carry virus? Latent? Defective?
2. What characterizes the state of CMV latency?
3. Do circulating macrophages or polymorphonuclear leucocytes carry virus?
4. Are circulating antigen–antibody complexes demonstrable?
5. What is the mechanism (or mechanisms) for activating latent virus?
6. Can techniques for the demonstration of CMV be applied to the identification of carriers?

B. Kidney

1. Is CMV transmitted in renal (tubular) cells?
2. Is CMV transmitted in lymphocytes within the renal parenchyma or vascular bed?
3. Does preexisting CMV infection (latent or active) influence the retention and/or function of the graft?
4. Does the presence of CMV in the donor (latent or active) influence course of the homograft?
5. What is the impact of CMV upon the host-immune response in general?

[1]These recommendations, summarized here by Drs. Ho and Lang, represent suggestions made by the entire N.H.L.I. CMV contract group which in addition includes William L. Bayer, M.D., Milan Fiala, M.D., Eli Gold, M.D., and Joseph S. Pagano, M.D., and their associates.

While these recommendations may seem like a "shopping list" for research grants and contracts, their formulation represented the considered opinions of active, established investigators. They are included here as a means of delineating unsolved problems in the field.

C. Bone Marrow, Other

Similar questions apply to the study of marrow and other homografts.

II. CHARACTERIZATION OF THE RESPONSE OF THE RECIPIENT TO CMV INFECTION

In this program the contractors concentrated most of their efforts upon problems relevant to the transmission of CMV with blood products. Efforts were devoted to the identification of carriers, elucidation of mechanisms pertaining to transmission and activation, and epidemiologic features of CMV infection. Less attention was devoted in the present program to the identification of specific CMV-associated syndromes. Although the presence of CMV correlates with certain clinical syndromes, the widespread occurrence of this virus in the absence of recognizable disease has made difficult the general identification of the etiologic responsibilities of this virus. At the same time the long-term effects of apparently asymptomatic persistent and/or latent CMV infections remain to be assessed. Some questions appropriate for investigation are posed.

A. Infection, Exogenous

Can primary and secondary CMV infections be identified and distinguished?

What is the role of various virus strains in reinfection?

B. Infection, Reactivated

Can reactivated latent infections be distinguished from new CMV infections and reinfections?

Can techniques (some of which were explored and developed by contractors) be developed or improved which will facilitate these distinctions?

C. Disease

What proportion of CMV infections lead to disease (short-term or long-term)?

How do host factors (including genetic constitution) pertain to the response to CMV infection?

III. THE PATHOGENESIS AND IMMUNOLOGICAL PARAMETERS OF INFECTION AND DISEASE CAUSED BY CMV

Many of the problems related to CMV cannot be understood or solved without fundamental studies on how and by what mechanism infection or disease is brought about, and on the nature and efficacy of the immunologic response to such infection. A related but separate issue is the nature of modifying influence of immunologic disorders or manipulations on the susceptibility and resistance of the host. Suggested studies may be subdivided as follows.

A. Mechanism of Transmission

1. Transplacental infection.
2. Perinatal infection.
3. "Natural" infection in the child, adolescent or adult.
4. Transmission by blood and other foreign tissues.

While our contract program has concentrated on the hazards of blood and its components and to a lesser extent on the kidney as a vehicle of transmission of CMV, it becomes evident that whenever one implicates one of these factors, one must rule out "usual" or "natural" means of transmission. CMV is assumed to be transmitted

in many ways, but the precise mechanism is not known in any particular mode. How important is transmission by respiratory droplets, milk, venereal route, urine, stool, or fomites?

B. Host Factors

1. Genetic constitution.
2. Socioeconomic background.
3. Age.
4. Sex.
5. Pregnancy.

To understand the natural history of CMV infection and disease, many host susceptibility factors have been suspected, and some data are available on each of the host factors cited. We do not know how such factors would operate in different modes of transmission. For example, while the pregnant woman has been reported to have higher rates of CMV cervical infection, is that limited to certain modes of transmission? Is she also more susceptible in general (e.g., to CMV transmitted blood)?

C. Role of Humoral and Cell-Mediated Immunity

CMV is a herpes virus, and many of our concepts regarding the development and efficacy of acquired humoral and cellular immunity following CMV infection are derived from what we know about other herpes viruses. For example, we assume that acquired humoral immunity exists, but that it is not potent enough to preclude reactivation or superinfection. We don't really know how effective *specific* antibodies are in the host. In immunosuppressed patients the presence of antibodies may be more an index of potential reactivation than of protection. Cell-mediated immunity has been assumed to be operative and important, but most of the evidence is indirect and circumstantial. We don't know how long immunity lasts.

D. Mechanism of Persistence, Latency, and Reactivation

1. Site of latency (organ, tissue, cell, and subcellular structure).
2. Mechanisms of reactivation (immunosuppression, allograft reaction, etc.).

It is a truism that CMV produces chronic, persistent, and possibly latent infections which are activated under certain conditions. This important property of the virus should be studied at all levels, particularly in view of the rapid advances in the study of latency of other herpes viruses. The nature and site of infection at the subcellular, cellular, and host level and the mechanisms of reactivation at the cellular and host level should be understood.

E. The Immunological Implications of CMV Infection

It is clear now that besides stimulating classical humoral and cell-mediated immunity following infection, this virus produces other profound immunological changes in the host, and in turn immunological changes in the host produced by other methods have a profound effect on CMV infection and disease.

1. Immunological effects of CMV infection.
   a. The effect of CMV infection on specific and nonspecific immune mechanisms such as humoral, cell-mediated immunity, interferon formation, etc.
   b. Effect on resistance to other viral and nonviral infections.
   c. Effects on other aspects of the host, such as the immunological response to allograft and possibly noninfectious diseases.
2. Effects of immunosuppression on CMV infection.

a. Immunosuppressive agents (cytotoxic agents, antilymphocyte serum, steroids, etc.).

b. Physical agents (X-irradiation, etc.).

IV. METHODOLOGY

Progress in the field of CMV research depends on advances in laboratory research techniques and purely virological aspects of this agent. One such problem is the identification of viral subtypes, if indeed they exist. Immunological methods should be pursued, but they may be supplemented by newer methods such as nucleic acid hybridization. An offshoot of this problem is the specificity of serologic tests. Areas requiring special attention are listed.

A. Identification of Virus Subtypes

B. Serological Methods

1. Sensitivity and specificity.
2. Identification of specific subtypes.

C. Improved Methods of Detecting Infection

Detection of antigen in circulation or in tissues, for example.

D. Methods for Detection of Latent Virus

V. PREVENTION AND THERAPY

The contractors did not work specifically upon aspects of therapy, although some studies were pertinent to preventive measures. It is not clear at present whether immunoprophylaxis is feasible in the case of an agent which may possess a multiplicity of varients and which can establish persistent or latent infections. Studies of human and murine CMV have implied that attenuation may be achieved by *in vitro* passage. This "attenuation" may reflect the *in vitro* selection of defective-interfering CMV which in turn raises questions about potential oncogenicity. Questions posed by the contractors include:

A. Immunization

Can a killed CMV vaccine be developed?
Does CMV attenuation reflect the selection of defective virus?
Does the route of inoculation influence the host response to CMV infection?
Does "attenuation" enhance CMV (experimental) oncogenicity?

B. Chemotherapy

Can chemotherapy alter the establishment of latent or persistent CMV infections?
Are adenine arabinoside or phosphonoacetic acid applicable to the therapy of CMV infections? To the eradication of the carrier state? Latent infections?

C. Immunotherapy

Is the use of immunologic adjuvants such as BCG or *Corynebacterium parvum* applicable to the elimination of CMV persistence or latency?

D. Screening

Will the rapid recognition and identification of CMV carriers permit their elimination from pools of donors (blood, kidney, marrow, etc.)? Is this appropriate? (See IB.) Is it feasible?

E. Interruption of Transmission

If CMV is demonstrated in specific cells, it would seem appropriate to seek means to remove or eliminate these virus-carrier cells. For example, if leukocytes (? lymphocytes) or macrophages harbor latent CMV and if antigenic interactions activate these agents, the use of component transfusion might eliminate CMV from blood products.