Immune responses to LCM virus infection *in vivo* and *in vitro*

Mechanisms of immune-mediated disease*

G. A. COLE [1] & E. D. JOHNSON [2]

Both recovery and death of mice following acute infections with lymphocytic choriomeningitis virus appear to be mediated by a population of virus-specific thymus-derived (T) effector lymphocytes that possess lytic activity in vitro *against virus-infected syngeneic fibroblasts. Whether recovery or death occurs is determined by the balance between two interdependent factors: (1) the extent of virus-induced modifications in the surfaces of cells comprising " target " tissues, and (2) the efficiency of the immune inductive process leading to the generation of effector T lymphocytes that recognize and destroy these modified cells.*

INTRODUCTION

Since its original isolation more than 30 years ago, lymphocytic choriomeningitis (LCM) virus has remained both an enigma and a delight to the numerous experimentalists who have studied the diversity of responses elicited by this agent in the mouse, its natural host, as well as in other animal species. After long remaining a singular oddity, divorced from other major families of animal viruses by its apparent lack of serological or morphological relatives, LCM virus is now recognized as the prototype of the newly emerged arenavirus group. Our present knowledge of the immunopathological phenomena that occur during naturally acquired or experimental LCM virus infections of the mouse can serve as a background against which the pathogenesis of other arenavirus infections of man can be compared.

GENERAL FEATURES OF MURINE INFECTIONS

An appreciation of the biology of LCM virus came first from the pioneering studies of Traub (1–3) followed by those of Rowe (4) and Hotchin (5). Collectively, these investigators defined most of the experimental conditions under which the administration of virus to mice could lead to a life-long carrier state, an abortive immunizing infection, or an acutely fatal disease.

The work of Rowe and his colleagues (4, 6) unequivocally established that fatal cerebral infection, or its visceral analogue, was immune-mediated and it also clarified the differences in tissue tropisms that exist between different virus strains. Thus, the nature of the acute disease produced in adult mice by viscerotropic strains depends on the dose of virus and the route by which it is given. The pathological hallmarks of infection with low doses range from destructive lesions of the lung, liver, and kidney following peripheral inoculation, to choriomeningitis following intracerebral inoculation (7). Recovery from visceral infection is accompanied by a solid immunity to a second, normally lethal, virus challenge. Infection with large doses, however, may lead to the induction of a persistent carrier state (6, 8), the immunological aspects of which have been given little attention.

On the other hand, small or large doses of brain-passaged or " neurotropic " strains replicate inefficiently in adult mouse parenchymal tissues and produce an abortive immunizing infection when administered extraneurally, yet they uniformly kill when given intracerebrally.

The inoculation of newborn mice, by any route, with all common strains of LCM virus usually results in the survival of a high proportion of animals who subsequently develop permanent carrier infections. At any time during their lifespan, high titres of virus

* From the Division of Infectious Disease, Department of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205, USA.
[1] Professor.
[2] Predoctoral fellow.

are readily demonstrable in blood and many parenchymal tissues of carrier mice, and the infection is perpetuated by congenital transmission to their progeny (3, 7, 8).

For many years, the LCM virus carrier state was considered to be the paradigm of specific immunological tolerance since, by conventional means, no virus-specific antibodies could be detected in carrier mouse sera. This notion was dispelled, however, when Oldstone & Dixon (9, 10) using specific fluoresceinated antisera to stain sections of kidneys from carrier mice, were able to demonstrate the presence of glomerular deposits containing viral antigens, IgG, and complement.

In addition, IgG eluted from carrier mouse kidneys contained virus-specific complement-fixing antibody. These observations established that LCM virus carrier infections are accompanied by a humoral response directed against one or more virus-specific determinants resulting eventually in chronic immune complex disease. In additional studies (11), Oldstone and his associates suggested that the severity of immune-mediated disease may be determined by genes that map within the H-2 gene complex. In retrospect, these findings appear to provide the basis for an explanation of the appearance of a runting syndrome in certain strains of mice with long-standing carrier infections and termed " late disease " by Hotchin (5).

THE EFFECT OF IMMUNOSUPPRESSION

The most convincing evidence for the immunopathological basis of acute LCM comes from the dramatic effect of immunosuppression on the course of the disease. Since Rowe's observation that irradiation prior to infection prevented or delayed the onset of disease (4) a number of investigators have shown that other immunosuppressive procedures are also effective to a greater or lesser degree (see review by Cole et al., 12).

One of the most efficient ways to convert a potentially lethal cerebral infection to a carrier state is by the use of cyclophosphamide (CY). A single immunosuppressive dose of this drug given to adult mice within 2–3 days after intracerebral inoculation of the neurotropic Armstrong strain of LCM virus prevents the development of immune-mediated choriomeningitis and results in the development of a chronic asymptomatic carrier infection (12). This simple procedure, combined with adoptive immunization, provided the means of determining which of the virus-induced immune response components generated during acute LCM virus infection is responsible for the elicitation of, and recovery from, disease.

REQUIREMENT FOR T LYMPHOCYTES

As first shown by studies from our laboratory (12, 13), splenic lymphocytes obtained from mice immunized by intraperitoneal injection with Armstrong virus, when adoptively transferred to syngeneic CY-induced virus carrier BALB/c mice, regularly produced an acute lethal central nervous system (CNS) disease, which, clinically and histologically, was typical of classical LCM. The administration of serum with high virus-specific complement-fixing activity was without effect. Furthermore, both CNS disease and virus clearance mediated by exogenous immune lymphoid cells required the participation of T lymphocytes (14). Depletion of this population with anti-θ serum and complement completely abrogated the ability of the transferred cells to elicit LCM, although their capacity to produce virus-specific complement-fixing antibody was retained. These findings, together with the fact that neutralizing antibody is not detected during acute infections, constitute a strong argument against any significant role of antibody in mediating acute disease.

CELLULAR IMMUNE REACTIVITY *IN VITRO*

The characteristic ability of LCM virus to establish a persistent infection in cultured mouse fibroblasts without accompanying cytopathology provides a readily obtainable population of " target " cells against which the lytic (effector) activity of lymphoid cells from infected or immune mice can be measured. When ^{51}Cr-labelled infected target cells are co-cultivated overnight with syngeneic lymphoid cells, any released radioactive label can be quantitated and directly correlated with the effector activity of the lymphoid population.

Using this procedure, we have shown that effector lymphocytes appear in the spleens of adult mice about 4 days after an immunizing infection with the Armstrong strain of LCM virus (15). The response reaches a peak after 8–10 days and then declines rapidly, becoming undetectable by 30 days. Effector activity is not displayed against uninfected targets; it is abolished by treatment with anti-θ serum and complement and is, therefore, a function of virus-specific T lymphocytes.

The generation of the effector T cell response is heralded by marked splenic lymphoproliferation, as measured by the incorporation of radioactively labelled DNA precursors in addition to direct cell counts. A sharp decline in both effector and lymphoproliferative responses is seen at about the time infectious virus in the spleen becomes undetectable (15).

THE PROTECTIVE ROLE OF EFFECTOR T LYMPHOCYTES

The temporal relationship between the kinetics of the effector cell response and the disappearance of infectious virus from the spleen suggested that recovery from infection was mediated by effector T lymphocytes. To test this supposition, two experimental approaches were used.

First, non-immune BALC/c mice were given intravenous adoptive transfers of 10^8 viable spleen cells taken from syngeneic immunized donors, either at the peak of their effector T cell response (day 8) or after its disappearance (day 30); 24 hours later, they were challenged with a normally lethal, intracerebral dose of LCM virus. As controls, groups of animals that had not been adoptively immunized, or that had received an intraperitoneal dose of virus 24 hours previously, were similarly challenged.

Only those animals that received spleen cells containing effector activity were protected against intracerebral challenge, although some exhibited clinical evidence of a transient, mild CNS disease. Treatment of these donated cells with anti-θ serum and complement eliminated their protective effect. The results of similar experiments published elsewhere (15) showed that histological choriomeningitis was attenuated in protected animals, and the maximal virus levels reached in their brains were approximately 10% of those that normally occur in control animals developing fatal LCM.

The second approach was to employ essentially the same experimental protocol, except that the recipients of the adoptive cell transfer were 8-week-old $(C_3H \times C_{57}Bl)F_1$ mice that had been thymectomized at 4 weeks of age, lethally irradiated, and then given a syngeneic bone marrow (ATxBM) graft. This procedure results in a severe depletion of T lymphocytes and renders mice incapable of mounting a cellular immune response so that they invariably become chronic carriers after LCM virus inoculation (15).

When ATxBM mice were intracerebrally challenged 24 hours after adoptive immunization with effector T lymphocytes, infection was aborted (15). These animals developed no overt disease and when killed 2–3 weeks after virus challenge, no viral antigen was detectable in their neural membranes by immunofluorescent staining. However, histologically, all presented with a mild choriomeningitis, indicating that the transferred effector cells were able to mediate some degree of pathology, apparently without the cooperative benefit of recipient T lymphocytes. In contrast, the transfer of non-immune spleen cells to ATxBM mice restored their capability to respond immunologically to virus challenge and they all developed clinical LCM between 8 and 10 days after infection (15).

Thus, it seems clear that protection by effector T lymphocytes against the development of either a normally fatal CNS disease or a chronic carrier state is accompanied by a limited choriomeningitis, which results from immune-mediated elimination of virus and/or infected cells after infection is established.

MEDIATION OF ACUTE LCM BY EFFECTOR T LYMPHOCYTES

Previous studies (14) have established that T lymphocytes are required for the production of acute LCM by adoptive immunization of CY-induced carrier mice. To determine whether CNS disease is caused specifically by T lymphocytes with effector activity, groups of CY-induced carrier mice were given, intravenously, 10^8 syngeneic splenic lymphocytes obtained from donors either 8 or 30 days after immunization. Although 90% of all recipients developed fatal LCM, the group receiving lymphocytes with effector activity died 4 days after adoptive immunization, whereas carriers given immune lymphocytes devoid of effectors died after 8 days. The increased survival time of the latter group indicated that a certain period of time was required for a population of effector T lymphocytes to be generated *in vivo* from a donated precursor cell pool, presumably by proliferation mediated by (viral) antigen and/or differentiation. By comparison, the relatively rapid production of fatal LCM by donated lymphoid cells containing an already established effector population is strong evidence that this population actually mediates immunopathological CNS disease.

DISCUSSION

On the basis of the collective observations described above and elsewhere (8, 15), it can be concluded that the functional activity of LCM virus-

induced effector T lymphocytes as expressed *in vitro*, namely, their ability to recognize and destroy infected target cells, is the same in the intact animal. Furthermore, the net result of acute infection with LCM virus is undoubtedly determined by the kinetic relationship between two interdependent variables. The first is the extent of infection in relevant target tissues, while the second is the efficiency with which the infectious process can lead to the generation of a cellular immune response. The various outcomes of murine infection can be explained in terms of these variables.

Classical LCM is the result of widespread immune-mediated damage to the meninges, choroid plexus, and ependyma. These membranes are heavily infected when the peak of the cellular immune reactivity is reached (12) and they present an extensive target for effector T lymphocytes. The fatal convulsive CNS disease that ensues is probably secondary to the severe choriomeningitis.

During primary abortive infections, the balance between infective and immune induction is shifted in favour of the host, either because of a decreased susceptibility of extraneural tissues to virus or the greater ability of these tissues to sustain a considerable degree of immune-mediated injury without fatal consequences. There is no evidence that the initial site of primary infection has any significant effect on the kinetics of immune induction.

The resistance of primed mice to reinfection can be explained by the presence of an established pool of lymphoid precursor cells from which effector T lymphocytes can be rapidly generated (15). A similar degree of resistance can be achieved in nonimmune mice by providing them with exogenously generated effector lymphoid cells.

The establishment of the LCM virus carrier state in either the neonate or the immunosuppressed adult occurs under similar conditions, namely, the unimpeded progression of infection to all tissues in the absence of a wholly functional immune system. With the eventual acquisition (or re-acquisition) of immune competence, the high levels of free and cell-associated viral antigen(s) already present probably serve as a paralytic stimulus that prevents the development of virus-specific effector T lymphocyte precursors but not, however, the development of complement-fixing antibody.

The requirements for induction of the humoral response of carrier mice have yet to be defined. Recent studies by Lehmann-Grube and his associates (unpublished) indicate that its specificity is for a subviral component found within infected cells but not expressed on their surfaces. The implication of this finding is that virus-specific antigens involved in immune complex disease are different from those recognized by effector T lymphocytes. The provocative and conceptually important findings of Zinkernagel & Doherty (16) favour this view since they show that the specificity of LCM virus-induced effector T lymphocytes is directed toward surface H-2 antigens modified by virus as a result of infection and not simply toward viral determinants alone.

The influx of monocytes and macrophages into sites of developing LCM virus-induced immunopathology has been well-documented by sequential light microscopy (13, 17) and by electron microscopy (M. del Cerro & A. A. Monjan, unpublished observations). Although macrophages are obviously responsible for eliminating virus-infected cells destroyed *in situ* during this process, it is not clear if, or how, they inactivate infectious, cell-associated virus, since this interaction has been little studied. One possibility, for which there is no direct evidence, is that the virus-induced modifications in the surface of permissive cells which render them susceptible to lysis by effector T lymphocytes occur prior to the development of infectious viral progeny. If such were the case, then no special virucidal property would be required of the macrophage. Alternatively, macrophages may acquire this property as a result of becoming activated at foci of inflammation (18).

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RÉSUMÉ

RÉPONSES IMMUNITAIRES *IN VIVO* ET *IN VITRO* À L'INFECTION PAR LE VIRUS DE LA CML: MÉCANISMES DE LA MALADIE À MÉDIATION IMMUNITAIRE

Il est possible d'établir un rapport entre la fonction lytique (fonction d'effecteur) observée *in vitro* dans une population de lymphocytes T produite dans la rate de souris atteintes d'infection aiguë par le virus de la choriomeningite lymphocytaire et la même fonction *in vivo* qui aboutit soit à la guérison, soit à la mort. L'issue est déterminée par l'équilibre entre deux facteurs interdépendants, à savoir l'étendue des modifications provoquées par le virus dans les surfaces cellulaires de tissus « cibles » et l'efficacité du processus immunitaire aboutissant à la production de lymphocytes T effecteurs qui reconnaissent et détruisent les cellules altérées.

Seuls les lymphocytes T qui lysent spécifiquement les cultures de fibroblastes infectées par le virus peuvent être utilisés 1) soit pour conférer aux souris adultes syngéniques non-immunes ou démunies de lymphocytes T une protection contre, respectivement, la chorio-méningite mortelle ou l'état chronique de porteur de virus, qui fait inévitablement suite à l'épreuve virale intracérébrale chez les animaux témoins non traités, 2) soit pour provoquer une forme rapidement accélérée de chorio-méningite lymphocytaire chez des souris devenues porteurs par l'effet de la cyclophosphamide.

La production de lymphocytes T effecteurs paraît être la voie finale commune tant pour le développement de la maladie à induction immunitaire que pour l'élimination de l'infection.

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DISCUSSION

OLDSTONE: In the experimental allergic encephalomyelitis model, if you transfer sensitized lymph node cells or effector T cells direct into the target organ (the brain), the time necessary for immunopathological lesions to appear is the same as if you transferred the cells intravenously. Do you know whether in the LCM adoptive transfer system it makes any difference if the effector cells are introduced direct into the CNS instead of being given intravenously?

COLE: We have talked about doing this experiment for years, but have never done so.

OLDSTONE: Is there any evidence that when you give an effector T cell, it goes direct to the lesion, or do you think that the T cell produces something that recruits other cells to the lesion?

COLE: This is a difficult question to answer because the only semidirect evidence comes from the work of Doherty who has done cisternal taps on mice inoculated with appropriate effector cells. He suggests that, using the right parent-F_1 combination, effector cells go direct to the target.

OLDSTONE: Have you tried radioactive labelling of the cells that you transfer?

COLE: We are doing that at present.

MIMS: In these experiments you have transferred sensitized spleen cells to your cyclophosphamide-induced carriers and produced neurological disease. With LCM, however, there is a difficulty that nobody has yet thought out. Many years ago, Dr Volkert injected sensitized cells into congenital carriers in an infected colony of mice and produced, at most, only the faintest histological evidence of lesions and no neurological disease. As far as I know, nobody has interpreted this in terms of the targets, the surfaces of infected cells offered in those animals, or other immunological inhibitors. Have you any comments on that?

COLE: I really should agree and acknowledge that we are following in the footsteps of Dr Volkert who did similar studies 10 years ago or more but who, as you mentioned, was using a congenital carrier mouse. Carrier mice are quite different because the virus load in the cyclophosphamide-induced carrier mouse is largely confined to the CNS. In fact, if the cyclophosphamide-induced carriers are kept for 50 or 60 days, one has great difficulty in killing them with CNS disease. This seems to be related to the fact that there is a gradual centripetal extension of virus to all tissues; the mice then behave biologically very much like congenital carriers. So I think that we are dealing with an antigen gradient and we should not expect sensitized cells to " swim upstream " towards a target that has no attraction for them.