LCM Virus Research, Retrospect and Prospects

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To an old friend of LCM virus it is gratifying to see that a large number of scientists are now actively engaged in LCM research and that great progress has been made in recent years. In the 1930's, there was just a handful of LCM investigators scattered over a few countries. Now there are numerous groups of research workers on different continents interested in this subject. The disease, especially that in mice, appears to have obtained the reputation of being a good research model for several basic problems.

INTRODUCTION

For those in the audience who are not very familiar with LCM, I would like to make a few introductory remarks. LCM virus was isolated in 1933 by Armstrong and Lillie in Bethesda, Maryland, from a monkey used for passage of the St. Louis encephalitis virus. It was not certain whether the virus originated in man or monkey. Human cases of LCM were encountered soon thereafter by Armstrong and co-workers as well as by Rivers and Scott in New York. In 1934, I found the virus in the mouse colony of the Princeton Section of the Rockefeller Institute. A causal relationship was established between the infection in mice and one of the human cases of Rivers and Scott. More human infections were reported by several investigators in the following years but, on the whole, the incidence of the disease in man has remained low. A number of human laboratory infections with LCM virus, few of them fatal, have been reported.

A search for the virus in trapped wild mice did not give conclusive results at Princeton, but Armstrong and Sweet were able in 1939 to detect LCM virus in gray house mice and to correlate the murine infection with human cases of LCM occurring on the same premises. This geographic relationship between murine and human infections was later confirmed by numerous investigators, notably by Scheid and his colleagues at the Universitäts-Nervenklinik, Cologne, and wild house mice are now generally regarded as the main reservoir in nature of LCM virus. For this, mice are admirably equipped, as I will point out later.

It is not yet clear how the virus is transmitted from mice to man. Some investigators consider it as an arthropod-borne agent, since experimental transmission from animal to animal was successful with a variety of arthropods, such as mosquitoes, bed bugs, and ticks, and since besides certain arboviruses only LCM virus could be shown by Rehácek in 1965 to grow in tick cells in vitro. Positive results were also obtained by others with larvae of Trichinella spiralis. However,

none of these experimental vectors have thus far been shown to play a role in nature.

Besides man and mice the natural host spectrum of LCM virus at present includes hamsters and possibly monkeys. Experimental infections were successful with several other animal species, including guinea-pigs, rats, cotton rats, rabbits, dogs, and chick embryos.

LCM VIRUS

Compared with the large amount of work done on pathogenetic, immunological and epidemiological aspects of LCM, the study of the virus itself was somewhat neglected in the past. According to present knowledge, the LCM agent is a medium-sized RNA virus. Electron micrographs show virus particles budding from the cell membrane. Virions contain several electron-dense, sand grain-like particles, presumably consisting of RNA. For this and other reasons, LCM virus, Tacaribe, Machupo, and Junin viruses from South America as well as Lassa virus from Africa, which show similar ultramicroscopic structures, were included in a new taxonomic group, the arenaviruses, in which the LCM virus represents the prototype. LCM virus is ether-sensitive and generally very labile. Specific antigen(s) demonstrable by complement fixation and by fluorescent staining occur in infected cells and tissues. An interesting feature of infected cells are clusters of ribosomes which were shown by Abelson and co-workers to contain specific antigen.

LCM virus appears to be quite stable serologically but variable with regard to its pathogenic properties. Consequently, it is often not possible to repeat experimental results exactly with other virus strains. Since different strains of laboratory mice also vary in their behavior towards this virus, the duplication of experimental work becomes even more difficult.

In cultures, LCM virus was found to grow in a large variety of mammalian cells. As a rule, little or no CPE is produced, and this is a handicap for titrating the virus. No cell type fully suitable for plaque assay is thus far known. This difficulty has been circumvented by Lehmann-Grube and his colleagues and by Oldstone and Dixon who based infectivity assays on demonstrating the appearance of CF antigen or immunofluorescing antigen, respectively, in infected cell cultures.

MURINE LCM

I shall now turn to the disease in mice which has been one of my main fields of interest for many years. Unfortunately, the work was repeatedly interrupted for long periods of time by circumstances beyond my control.

Studies conducted from 1935 to 1939 showed that there are 2 main types of virus-host relationship in LCM virus-infected mice, depending on the age at the time of infection: 1) Lifelong persistent infection present in mice infected congenitally. Such animals show no signs of disease for many months. They have a lifelong solid resistance against intracerebral infection. In spite of this, no neutralizing antibody was demonstrable in their blood, although CF antibody at very low levels was occasionally found. 2) Acute adult infection causing disease, followed by relatively rapid elimination of infectious virus in survivors. CF antibody was readily demonstrable in the sera of such

animals, but the presence of neutralizing antibody was questionable for a long time. The cerebral immunity was of relatively short duration in such cases.

These results, which showed the LCM virus to be capable of persistently infecting mice following vertical transmission, were later confirmed and extended by Haas. They formed, together with Owen's work on erythrocyte chimerism in bovine twins published in 1945, the basis for Burnet and Fenner's thoughts on self-recognition which were to develop later into the concept of immunological tolerance. Since then, much more basic work on the pathogenesis and the immunology of murine LCM has been reported by a number of investigators so that today a reasonably clear picture exists of the events taking place after chronic congenital and acute adult infection in mice.

I will now try to give a brief description of the two conditions.

Mice Infected Congenitally

Mice infected congenitally, that is, in the mother's body, become permanent virus carriers. They show what Hotchin and colleagues have called a persistent tolerant infection. Their immunity was designated as "tolerant immunity" in contrast to the "active immunity" present in adult mice after acute infection.

In the colony with infected mice at Princeton, where about 50% of the breeding mice were infected or immune when the disease was recognized in 1934, congenital infection became the only mode of virus transmission in the course of 2 years, and this no doubt is important in wild mice as well. It contributes effectively to the maintenance of the virus in nature.

Congenitally infected mice look like normal individuals for many months, most of them for their entire life, in spite of the fact that they carry large amounts of virus in their organs and blood and discharge virus continuously in their nasal secretions, urine, feces, milk, and sperm. They have no effective mechanism for virus clearance. Leukocyte count is essentially normal. In contrast to mice with acute adult infection, they can readily pass the virus to normal mice by contact (nose to nose), with the milk or by sexual intercourse. In tolerant females every successive litter becomes infected congenitally, no matter whether the animal was mated with a normal male or a tolerant one. All embryos in each successive litter are infected. There is evidence for virus transmission via the ovum, which comes from a heavily infected milieu. Normal females mated with tolerant males do not always produce infected litters, and in infected litters viral antigen is often not demonstrable in all embryos. We have had cases in which only 1 out of 10 or 12 embryos was infected. When such females are again bred to a tolerant male, the progeny of the following litter is never infected because the female has in the meantime become actively immune. Much later, however, when its active immunity has subsided, mating with a tolerant male may again produce infected litters.

Lymph node cells taken from tolerant mice will grow normally in vitro. The growth curve of the virus in them resembles a horizontal straight line in contrast to the curve obtained from normal cells infected in vitro which shows repeated peaks and remissions for several weeks but later tends to become a straight line also. Lehmann-Grube produced persistent infection in L cell cultures in vitro and observed a simi-

lar pattern with alternating phases of high production and low production of infectious virus.

Immunity in congenitally infected mice is characterized by a lifelong absolute resistance to intracerebral infection. Inoculated virus becomes undemonstrable within a short time. Newborn mice are already fully resistant to intracerebral infection with a "neurotropic" virus strain which is virulent for normal baby mice. Since embryos and newborn mice are not known to be capable of a humoral or cellular immune response, the only plausible explanation is that inoculated virus is prevented from infecting cells by some sort of interference mechanism in the absence of demonstrable interferon. This is what I meant when I spoke of "cellular immunity" in my earlier papers on LCM (1938) and of "interference immunity" in later publications (1960-1963). The inactivation of inoculated virus may be effected by the body temperature. This is not unlikely in view of Lehmann-Grube's studies in which a half-life at 37°C of only 16 to 20 min for LCM virus was found as compared with 28 h for poliovirus type 1. The hypothetical interference or blocking mechanism or whatever you want to call it is only weakly active against other viruses in congenitally infected mice.

Neutralizing antibody has not been demonstrated in such mice, but very low levels of CF antibody were occasionally detected. Other investigators found low levels of antibody demonstrable by the fluorescence technique. Pollard and co-workers reported increased levels of γ-globulin in congenitally infected gnotobiotic mice.

The immunological tolerance present in such animals is of long duration. It is virus-specific since tolerant mice are fully capable of making antibody against other antigens. They are also capable of mounting a normal homograft response. The tolerance concept has recently been challenged by Oldstone and Dixon, but other eminent experts in the field are still in favor of it.

Results similar to the ones with congenitally infected mice have been obtained with mice infected neonatally. These were widely used in pathogenetic and immunological studies in recent years. Own work still in progress has revealed a difference between mice infected neonatally and mice infected congenitally. We found that the LCM virus strain WCC, which Dr. Hotchin would call "aggressive", is highly virulent for newborn mice when inoculated intracerebrally, whereas congenitally infected baby mice born of persistently infected females show no signs of disease in spite of the fact that they carry large amounts of WCC virus in their viscera at birth. We also noticed that very low levels of CF antibody are more frequent in neonatally infected mice than in congenitally infected individuals at a time when maternal antibody has disappeared from their blood. An interesting discussion of the "neonatal versus congenital" problem can be found in Lehmann-Grube's recent monograph.

Several investigators reported fruitless attempts to break the tolerance of persistently infected mice using different procedures. Remarkably successful were the experiments of Volkert and colleagues (1962-1965) with adoptive immunization achieved by transplanting isogeneic lymphoid cells from actively immunized donors. The transplants effected a 10,000-fold reduction of the virus titer within 5 weeks. Spleen and lymph nodes, which usually have high infectivity titers, could be completely cleared, but virus remained in the kidneys. Mice receiving the transplants developed 100-fold higher antibody titers than the cell donors. There was no parallelism between antibody formation and virus suppression. Neutralizing antibody, CF antibody, and

infectious virus were present in the serum concurrently. Cellular immunity was obviously more important for virus clearance than humoral antibody.

An interesting phenomenon of great importance in neonatally infected mice is the "late onset disease" described by Hotchin in 1962 and studied by him and Collins in 1963 and 1964. Such animals appeared healthy for about 10 months and then began to show signs of disease reminiscent of the runting syndrome in newborn mice. All of the mice finally succumbed to this condition. Chronic glomerulonephritis was found in 34% of the Albany and 16% of the Swiss mice used. This condition was believed to be due to an autoimmune process caused by a gradual waning of virus tolerance. In the affected glomeruli deposits of antibody-containing material were found which stained with fluorescent rabbit anti-mouse γ-globulin antiserum. Since the lesions are similar to those occurring in Aleutian mink disease, it is possible that virus-induced antigen-antibody complexes are responsible.

These findings qualify "late disease" as an example of a slow virus infection or, better expressed, slow virus disease, since infection with the LCM virus does not at all appear to be slow. The observations of Hotchin and Collins were confirmed by several other investigators. It seems that the lesions described develop more readily in neonatally infected mice than in those infected congenitally. In own experiments, gradual lymphoid hypertrophy with increasing age was seen in congenital carrier mice.

Acute Infection of Adult Mice

As compared with the congenital infection, the study of the acute infection in adult mice was somewhat neglected in the early years of LCM research because not much of a difference seemed to exist between the events following infection with LCM virus and those following infections with other viruses. An unusual feature was the difficulty to detect neutralizing antibody in recovered mice. It was not yet known then that such mice are fully capable of making neutralizing antibody as reported by Hotchin and co-workers and by Lehmann-Grube. Moreover, the foot pad test and modern tissue culture methods were not yet available.

The acute adult disease attracted much more interest after Rowe had shown in 1952 that pre-irradiation with x-rays could prevent symptoms and lesions in experimentally infected adult mice without depressing multiplication of the virus. This observation indicated that the virus itself is harmless for mature mice as it is for mouse embryos. Since x-irradiation with suitable doses is known to reduce the number of blood leukocytes drastically, the result suggested that these cells, especially the lymphocytes which are found in great numbers in pathological infiltrates of infected mice, might be responsible for the disease syndrome. Confirmation and extension of the findings came from several investigators. Similar results were later obtained by using chemical immunosuppressants, anti-mouse lymphocyte serum or anti-mouse thymocyte serum. Neonatal thymectomy would also prevent the acute disease, as shown by Rowe and colleagues and by Sikora in 1963.

The essence of the experiments with physical, chemical and biological immunosuppressive measures appears to be that the cellular rather than the humoral immune response causes virus elimination as well as acute disease.

In 1958, Hotchin proposed the hypothesis, widely accepted today, that the disease of the adult mouse is due to an immunological conflict resembling the homograft response. As in the latter phenomenon, migrating cells from the lymphoreticular system appear to play the dominant role. There is increasing evidence that their activity is directed against a new antigen being formed at the surface of infected cells.

Basic Mechanism of Immunity in LCM Virus-Infected Mice

Whereas sensitized lymphoid cells no doubt play an important part in the disease process and in virus clearance, they do not seem to be of primary importance for protective immunity in murine LCM for the following reasons: 1) Infected embryos in which a specific cellular immune response appears to be either missing or minimal are, with few exceptions, solidly immune at birth against intracerebral inoculation with a "neurotropic" virus strain, for instance WCC, which will produce severe disease in all newborn normal controls, killing approximately 80% of them. 2) Subcutaneously infected adult mice show cerebral immunity for a few months but, in the stage of waning immunity, many of them respond to an intracerebral virus inoculation with an "accelerated reaction". In the light of newer knowledge, sensitized lymphoid cells seem to be responsible for this reaction which I had interpreted as being an allergic phenomenon. The interesting feature is that it is usually no longer possible to demonstrate infectious virus in mice which have reached the "accelerated stage". Evidently, the cellular immune response had been suppressed in such animals by persisting virus before its concentration had fallen below a critical level. 3) "High dose immune paralysis", resembling the classical phenomenon caused in mice by large doses of pneumococcal polysaccharide, was first mentioned in 1936 in intracerebrally infected adult mice by Bengtson and Wooley and later studied more extensively by Hotchin and Benson and by Hannover Larsen. In this condition, high titers of CF antibody and antibody demonstrated by immunofluorescence but not protective antibody coexisted with virus in the blood. There were significant levels of anti-complementary activity pointing to the presence of antigen-antibody complexes in the circulation. Twenty months later, such mice had suppressed the virus and developed high titers of neutralizing antibody.

It seems likely that in mice with high dose immune paralysis more cells are infected initially than in animals receiving smaller virus doses and that the immune paralysis caused by the virus is therefore strong enough to inhibit a leukocytic immune response immediately. It is noteworthy that the phenomenon seems to be observed only with "viscerotropic" virus strains which have a greater affinity for the lymphoreticular system than "neurotropic" strains.

Thus, the basic disease-preventing mechanism appears to be the same in congenitally infected mice and in those infected as adults, namely, the inhibition of the leukocytic immune response by persisting virus. As numerous titrations have shown, small amounts of active virus persisting in the lymphoreticular system can produce the effect. This may also explain why it is so difficult or nearly impossible to immunize mice against disease with inactivated LCM virus.

The "sterile immunity" of long duration which can be produced in adult mice by repeated intracerebral inoculations of virus following, for instance, a primary subcutaneous injection is more difficult to analyze. One possible explanation is that repeated virus inoculations

may desensitize lymphoid cells so that they do no longer react with the hypothetical new antigen and thereby cause disease. Increased formation of neutralizing antibody may also play a role in such animals.

Persistent LCM Virus Infection and Leukemia

The interaction between persistent LCM virus infection and leukemia in mice and, to a lesser extent, in guinea-pigs has been the subject of numerous publications. We reported in 1941 that lymphatic leukemia was more frequent and appeared at a younger age in persistently infected mice from the Princeton colony than in LCM virus-free controls derived from the same stock. Later, leukemia was also seen in 1 of 2 wild mice persistently infected with LCM virus which Dr. Haas had sent me from the U.S.A.

Contamination of several strains of leukemia virus with LCM virus was described by other investigators. Sometimes the severity of the leukemia was markedly reduced by the contaminating LCM virus. In contrast, Hotchin reported that infection of L cells with LCM virus increased their oncogenicity in mice. In own experiments published in 1962, persistent LCM virus infection appeared both to increase the incidence of leukemia and to moderate its severity to such a degree that one was tempted to conclude that LCM virus might have caused the rather benign tumors. (I was not fully convinced of this, however, and therefore put a question mark on the title of the paper.)

More light was recently shed on the interaction of LCM and leukemia viruses by Oldstone, Aoki, and Dixon who reported stimulation of the production of Gross leukemia antigen by LCM virus. This effect was seen in inbred mice with high or low leukemia incidence as well as in cultures of embryonic cells obtained from the different mouse strains. The results indicate that the effect is independent of genetic host factors. They can explain the higher incidence of leukemia in mice with persistent LCM virus infection but not the reduction of the severity of the leukemia. The authors drew attention to the possibility that enhancement of leukemia antigen production by LCM virus may influence the incidence of autoimmune disease in mice.

CONCLUSION

In concluding, I would like to say that in spite of the vast amount of research work carried out on LCM in recent years enough stimulating problems remain for further work. For instance, the question should be settled once and for all whether the concept of self-recognition is applicable to the persistent infection of Mus musculus with LCM virus, as proposed by Burnet and Fenner, or whether Oldstone and Dixon's view is correct. Possibly both parties are right, the first as far as the symptom-free phase in persistently infected mice is concerned, the second with respect to the late phase sometimes culminating in pathologic alterations resembling autoimmune disease. The basic question of whether or not a self-replicating agent is at all capable of inducing true immunological tolerance will have to be answered. Such studies will throw more light on the mechanism by which persisting virus inhibits the cellular immune response.

Another problem of great interest concerns the antigens involved in autoimmune disease and the role which antigen-antibody complexes may play. Needless to say, more information is desirable on the virus

itself and on the epidemiology of LCM in the field. The study of the LCM-leukemia interaction has just passed into a more productive phase, and further results of scientific interest and perhaps practical applicability may be anticipated.

These are just a few of the problems which will keep the LCM wagon rolling.

LITERATURE

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