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Multiplication of LCM Virus in Lymph Node and Embryo Cells from Non-tolerant and Tolerant Mice

By

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With 4 Figures

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It was reported in 1938 (17) that mice infected *in utero* with the virus of lymphocytic choriomeningitis (LCM) will carry this agent indefinitely without showing signs of disease, while mature mice surviving experimental infection have a mechanism enabling them to clear their organs more or less rapidly from detectable amounts of infectious virus. Since then, much evidence has accumulated (18, 24, 7, 20, 22, 8, 25) which favours the interpretation (3) that the carrier state is due to immunological tolerance. In later studies on tolerance by different groups of immunologists little or no attention has been paid to murine LCM, presumably, because the antigen involved is a selfreplicating agent.

To obtain more information on its fundamental mechanism, the tolerance of mice towards LCM virus was investigated at the cellular level using lymph node and embryo cells from non-tolerant and tolerant mice. The results obtained suggest that the tolerant state is primarily a cellular phenomenon. The report includes an incidental observation concerning the effect of lymph node extracts on cultures of murine lymph node cells.

Materials and Methods

LCM virus: Strain W, isolated from a naturally infected wild house mouse (22), was used throughout. It was obtained as needed from members of our colony of congenitally infected (tolerant) white mice in which all animals, young or old, are carriers of this virus.

Sources of tissue: Tolerant infected cells were cultivated from lymph nodes of mice from the infected stock. Since lymphomatosis occurs with high frequency in a line derived from this colony, cultures were also made from the tumorous lymph nodes of leukemic animals of this line. Such lymph nodes

contained considerable amounts of LCM virus and, without exception, furnished well-growing tolerant cells.

Non-tolerant lymph node cells were obtained from normal 5-week-old mice of the colony from which the infected stock originated (22). Since normal lymph node cells would often grow rather poorly, we also prepared cultures from lymph nodes of mice suffering from spontaneous lymphatic leukemia (without LCM virus). Such cases were occasionally found among old animals of the normal colony. Lymph node cells cultivated from these mice grew more abundantly than normal lymph node cells.

Further cultures were made from lymph nodes of mature mice which had been infected by subcutaneous (s. c.) inoculation of LCM virus some time previously.

Embryo cells were obtained from pregnant mice of the LCM-infected and normal colonies. The mothers were sacrificed at the end of the first or in the second week of pregnancy.

Preparation of tissue cultures: We used, with minor modifications, the technique outlined by *Schwöbel* (16) for swine kidney cells. Lymph nodes or embryos were removed aseptically, washed with PBS (4), finely minced with scissors and subjected to tryptic digestion. The dispersed cells were filtered through sterile cotton or gauze, sedimented at 2000 r. p. m. for 6 minutes and washed once or twice with cold PBS. The growth and maintenance media consisted of Earle's solution (60 per cent), inactivated normal horse serum (30 per cent), 5 per cent solution of lactalbumin hydrolysate in Hanks' saline without $NaHCO_3$ (10 per cent), phenol red (0.001 per cent), penicillin (100 units/ml.) and streptomycin (0.1 mgms/ml.). The cultures were set up in test tubes and/or Kolle flasks and incubated in a stationary position at 36.5° C, changing media routinely every 3 days. Since the cells under study did not grow equally well in all tubes and flasks, we always prepared a series of cultures of the same kind. In experiments with non-tolerant cells, some uninfected cultures were included as a check for a possible specific cytopathogenic effect in the cultures infected *in vitro*.

In cultures with good cell growth the pH of the media dropped from about 7.7 to 7.2 or 7.3 during 3 days of incubation. Metabolically active cells from embryos or tumorous lymph nodes would lower the pH more rapidly. In such cases it was adjusted to about 7.4 by adding n/10 HCl (one or two drops per tube culture) to the media on the second day after change of medium. Conversely, slow-growing cultures often needed pH-adjustment in the opposite direction using n/10 NaOH.

In several experiments, subcultures were made from primary cultures after at least 7 days of cultivation. The cells were removed from the glass for transfer by treatment with 0.125 per cent trypsin (Difco) and 0.03 per cent versene at pH 7.2. Subculturing was not always successful, however. In positive cases, pure cultures of a cell type which we consider as primitive reticular cells (see below) were obtained. These cells often grew better and were more stable in subcultures than in primary cultures.

Infectivity tests: Since LCM virus has no definite cytopathogenic effect in murine lymph node or embryo cells, the infectivity of the culture media was titrated intracerebrally (i. c.) in mice using three 4 to 5 week-old animals per decimal dilution. The infectivity titers were calculated according to the *Reed-Muench* (14) formula and expressed in the graphs as the negative $\log_{10}$ of the dilutions causing definite symptoms in 50 per cent of the injected mice [SD_{50} according to *Rowe* (15)].

Experimental

Cytological observations

Several types of cells were present in primary cultures prepared from murine lymph nodes after 4 to 7 days cultivation: round cells (lymphocytes), spindle-shaped (endothelial) cells and stellate forms as well as fibroblast-like cells. The latter were seen either as single cells or growing out of clumps of ill-defined cellular material attached to the glass. In many primary cultures there was evidence of organization (formation of threads of endothelial cells and of rudimentary capillaries) as well as lymphocyte production, in which the endothelial cells and the fibroblast-like cells appeared to participate. The latter seemed to multiply by amitotic division and gradually became the dominating cell type forming a loose network of cells and connecting fibrous processes. The network and the different cell types could be well recognized in test-tube cultures stained according to Pappenheim's method at a slightly acid pH.

The classification of the fibroblast-like cells still is a problem. On account of the fact that they are capable of forming a peculiar network of cells and (reticulin ?) fibers and apparently take part in the formation of lymphocytes, we are inclined to consider them as primitive reticular cells and shall tentatively designate them as such. They are able to store india ink particles but less so than the other cell types present in primary cultures including the round cells.

In the majority of the subcultures obtained from primary cultures by treatment with trypsin and versene the reticulocytes were predominating. After a few changes of medium they often were the only cell type present. In subcultures, too, they failed to form cellular sheets like fibroblasts. Their tendency to reticulation was just as evident as in primary cultures. Instead of single complete lymphocytes or clusters of such cells material looking like nuclear debris was produced, which still had a marked affinity for india ink particles.

Subcultures representing pure cultures of reticular cells in general produced at least as much LCM virus as primary cultures containing the other cell types also. They proved highly susceptible to *in vitro* infection with this virus but were not visibly damaged by it. The cells remained viable for long periods of time and generally were more stable than the reticulocytes in primary cultures.

Cultures of lymph node cells subjected to a single trypsination only sometimes were lost after a few weeks of cultivation because the great majority of the cells was transformed into round cells, which detached from the glass and were removed with the culture fluid. This happened especially with cultures in which lymphocyte formation had been very marked from the start. We at first suspected a peculiar cytopathogenic

effect of the virus under study but soon became convinced that this was not the correct explanation. A similar, but more rapid change of this kind was noted in cultures of lymph node cells to which lymph node extract had been added in an effort to stimulate cell growth.

In these experiments, 5 per cent lymph node extracts from normal mice or animals suffering from lymphatic leukemia were added to 1 to 3 week-old primary cultures. The deep-frozen lymph nodes were ground with sand, extracted with culture medium and the extracts filtered through Berkefeld-N candles.

The effect was adverse, but nevertheless impressive and rapid. In the course of 24 to 48 hours, nearly all cells present in the cultures, including the reticulocytes, changed into round cells which developed nuclear necrosis and gradually detached from the glass. In LCM-infected cultures treated with lymph node extract viral growth ceased as the cells disappeared. Extracts of tumorous lymph nodes from leukemic mice were more active than normal murine lymph node extracts, while lymph node extract from a cow suffering from severe lymphomatosis showed hardly any effect.

Primary cultures of murine embryo cells contained several cell types which were not identified. Subcultures were not made.

Growth of LCM virus in lymph node cells from non-tolerant mice with or without lymphomatosis

In the experiments recorded graphically in Fig. 1 the multiplication of LCM virus was investigated in cultures of lymph node cells from LCM-free leukemic mice of the normal colony (curves 1—4) and from normal mice (curves 5 and 6).

Curves 1, 2, 3, 4, and 6 were obtained from subcultures of well-grown primary cultures, curve 5 from primary cultures consisting almost exclusively of reticular cells. Prior to *in vitro* infection the cells had been under cultivation for 42 days (curve 1), 81 days (curve 2), 16 days (curve 3), 28 days (curve 4) or 10 days (curves 5 and 6). In subcultures, the intervals between the first and second trypsination varied between 7 and 34 days.

The cultures were infected either with cell-free serum of tolerant mice or, more frequently, with Berkefeld-N filtrates of infectious culture fluid from parallel cultures of tolerant lymph node cells (see following section). The culture media were changed every 3 days, exceptionally every 2 or 4 days. The amounts of virus released by the cells into the culture fluid were measured roughly by i. c. titration in mice as described above. The intervals between the titrations are indicated by dots in the curves. It should be noted that the cultures represented by curves 1 and 2, curves 3 and 4, and by curves 5 and 6, respectively, originated from the same leukemic or normal mice. The repeat tests (curves 2, 4 and 6) with parallel cultures were made to confirm the reproducibility of the results.

As can be seen from Fig. 1, the curves uniformly have a peak about the fourth day after infection followed by a low about the 14th day. This appears to be characteristic for non-tolerant lymph node cells.

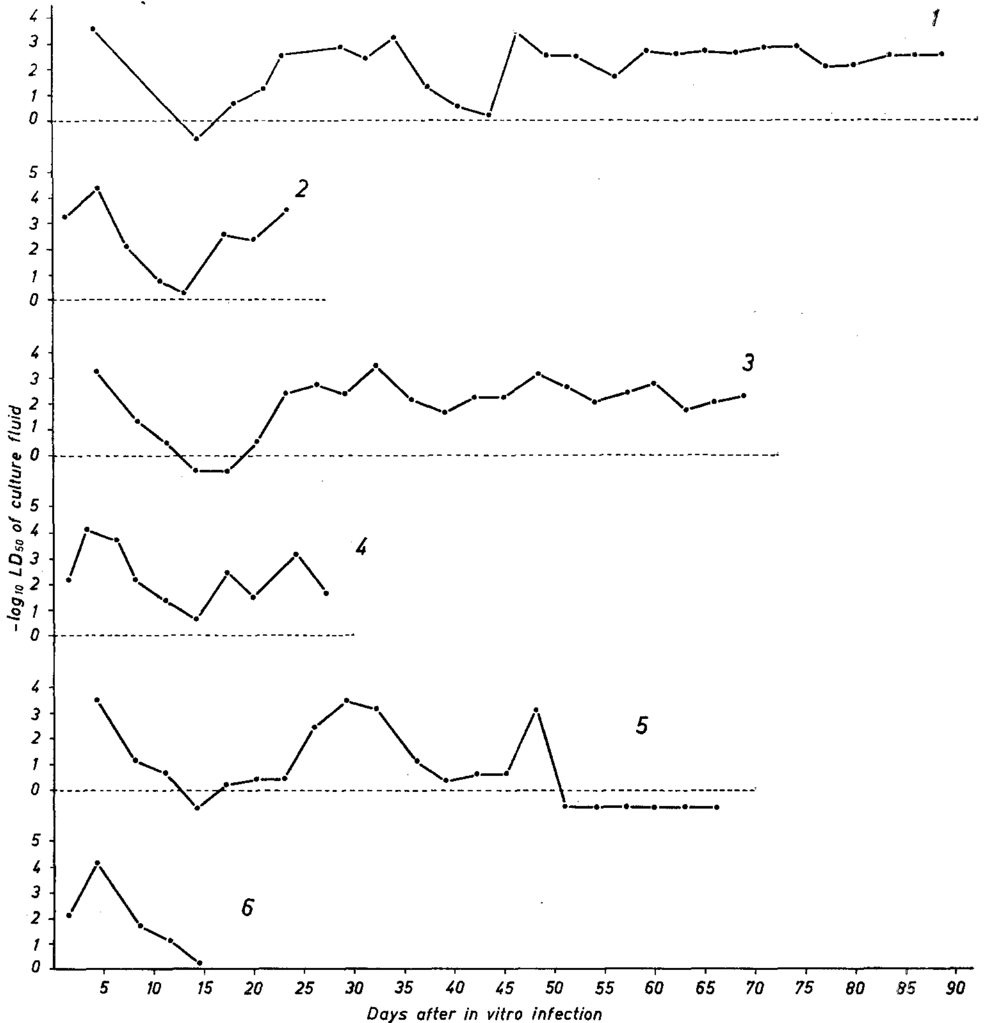

Fig. 1. Multiplication of LCM virus in non-tolerant lymph node cells infected in vitro. Cells from leukemic mice: Nos. 1–4; cells from normal mice: Nos. 5 and 6. Primary culture: No. 5; subcultures: Nos. 1–4 and 6

Curves 1, 3, and 5 concern cultures observed for long periods of time. They show several peaks indicating cyclical viral growth.

A tendency of the cells to become tolerant in the course of time, that is, to support continuous viral growth at about the same level, is evident

from curves 1 and 3 obtained from leukemic cells. The result shown by curve 1 is particularly convincing because the cell population did not noticeably decrease in the respective cultures during an observation period of about 3 months after *in vitro* infection. These cells had a remarkable vitality. This was different in the cultures which furnished curve 3. In them, the cell population steadily declined in the course of time. When stained on the 69th day after *in vitro* infection, there were only a few reticular cells left, embedded in a network of filaments and coarser fibers. This is the reason for the slow decrease in titer towards the end of the period of observation. In contrast to leukemic cells, normal cells (curve 5) failed to become tolerant. In these cultures, the virus was lost for an unknown reason between the 48th and the 52nd day after infection. Microscopical examination on the 66th day revealed numerous healthy-looking reticulocytes. We therefore tend to the conclusion that a rather sudden antiviral reaction of the cells, in combination with the harmful effect of the incubator temperature, was responsible for the inactivation of the virus. It should be recalled that the heat resistance of LCM virus is very low. When incubated in culture medium without cells it failed to survive for 2 days.

During the first four weeks after infection the mode of multiplication of the virus was similar in leukemic and normal cells. In the former, there was no evidence of interference by a leukemia agent with the multiplication of LCM virus. The age of the cultures at the time of *in vitro* infection did not appear to influence the results.

Growth of LCM virus in lymph node cells from tolerant mice with or without lymphomatosis

The growth curves shown in Fig. 2 were obtained from lymph node cells of congenitally infected tolerant mice (19). The cells had become infected in the donor animals and thus did not require infection *in vitro*. Curves 1—3 concern cultures from three different cases of typical lymphomatosis originating from the high incidence line mentioned above. Curves 4 and 5 refer to cells from healthy-looking tolerant animals from the infected stock.

The technique used was the same as that described in the preceding section for non-tolerant cells. Curves 1, 2, and 4 were obtained from primary cultures, curves 3 and 5 from subcultures. The latter were prepared from well-grown primary cultures on the 11th day of cultivation and titrated for the first time on the 15th day.

Fig. 2 shows that the multiplication of LCM virus in tolerant cells proceeded at about the same level for weeks. Some curves show minor waves, but the marked fluctuations characteristic for non-tolerant cells are missing. It is noteworthy that the cultures furnishing curve 3 showed

good cell growth for about 2 months and nevertheless produced only small quantities of virus. The decline at the right end of the curve is due to a non-specific decrease of the cell population which, at the end of the period of observation, consisted of very few reticular cells only.

Growth of LCM virus in lymph node cells from mature mice immunized by subcutaneous inoculation of LCM virus some time previously

Mice infected s. c. with strain W at the age of 5 weeks invariably show swollen lymph nodes from the 5th to about the 12th day after inoculation with a maximum about the 7th day. The lymph nodes are frequently as large as in animals sacrificed at an early stage of lymphatic leukemia. Mortality from serous pleuritis amounts to about 10 per cent.

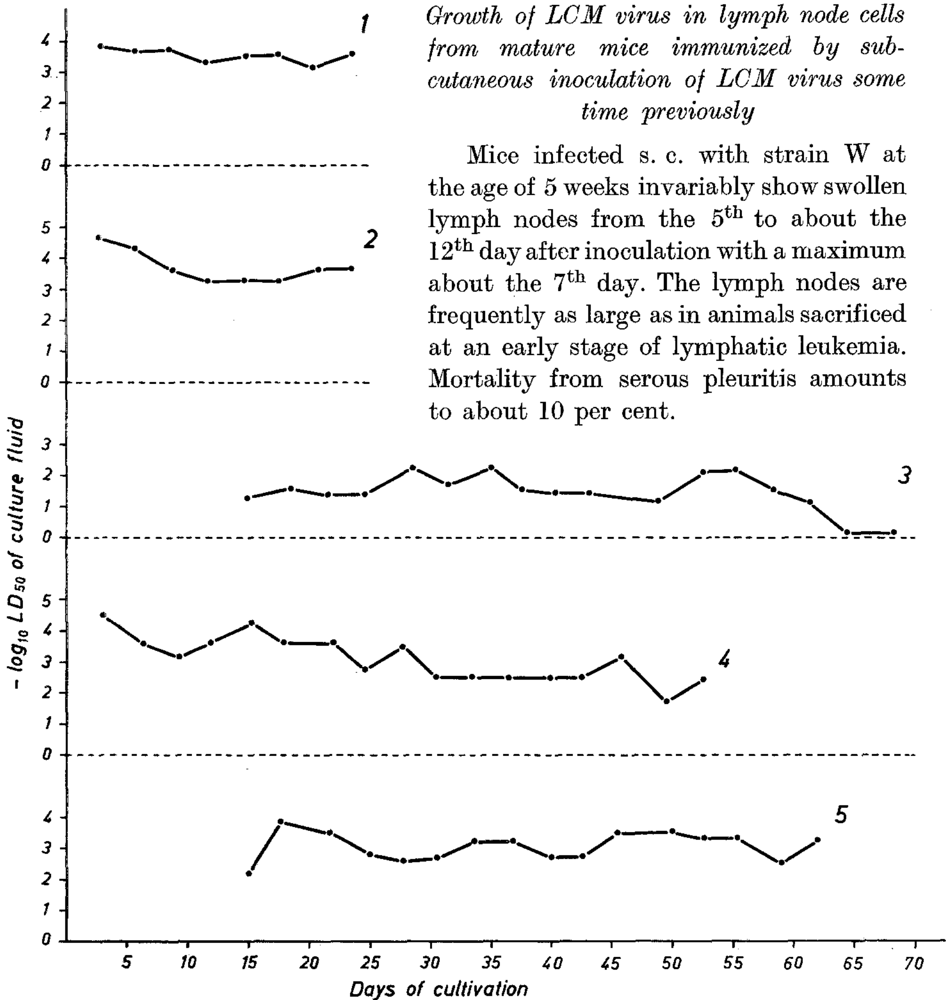

Fig. 2. Multiplication of LCM virus in tolerant lymph node cells from virus carriers infected in utero. Cells from leukemic mice: 1, 2, and 3. Cells from non-leukemic mice: 4 and 5. Primary cultures: 1, 2, and 4. Subcultures: 3 and 5

Several unsuccessful attempts were made to cultivate reticular cells from swollen lymph nodes of s. c. infected mice during the acute stage of the disease, that is, from the 5th to the 8th day after inoculation. It appears that the cells are temporarily affected by LCM virus in such

a way that they do not grow in tissue culture. Cultures prepared after the subsidence of the lymph node swelling, e. g., 2 or 4 weeks after s. c. inoculation, showed some growth of reticular cells and supported viral multiplication (see Fig. 3). On the other hand, cultures of lymph node cells from tolerant mice of the infected stock, which usually present slight lymph node swelling only, invariably showed better growth of reticulocytes than cultures made from normal lymph nodes. It appears that, in this case, LCM virus stimulated the growth of these cells.

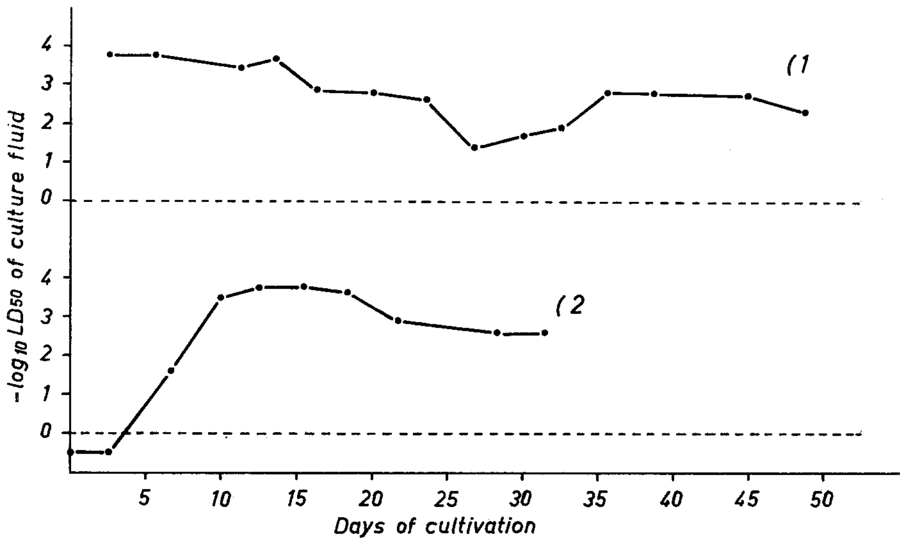

Fig. 3. Multiplication of LCM virus in Lymph node cells from mature mice infected s. c. 2 to 4 weeks before removal of tissue. (1) Cells from leukemic mature mouse infected s. c. 2 weeks previously. (2) Cells from non-leukemic mature mouse infected s. c. 30 days previously.

In an experiment, which in part is recorded graphically in Fig. 3, cultures of lymph node cells were prepared:

(1) from an adult mouse of the normal colony suffering from spontaneous lymphomatosis. (This animal was inoculated s. c. with infectious serum from tolerant mice and sacrificed 2 weeks later. It was very ill 6 to 10 days after inoculation. Its condition had slightly improved by the 14th day. Typical lymphomatosis was evident at autopsy.);

(2) from 9 mice of the normal colony inoculated s. c. with serum from tolerant mice at the age of 4 to 5 weeks and sacrificed 30 days later;

(3) from 9 mice treated in the same manner and killed 41 days later.

The aim of the experiment was to cultivate LCM virus from the lymph nodes of immunized mature mice and to investigate, with regard to tolerance, the status of the cells supporting viral growth.

In interpreting curves 1 and 2 in Fig. 3 it should be realized that, in these cases, the first reaction between virus and cells had taken place in the organs of the donor animals some time before the cells were removed. Consequently, one could not expect a sharp drop of the infectivity titers, as it occurs in cultures of non-tolerant cells between the 4th and the 14th day after *in vitro* infection. There is a certain similarity, however, between curves 1 and 2 and some of the later segments of the curves in Fig. 1. When removed from the animal, the cells may already have been on their way to the tolerant state. The relatively late appearance

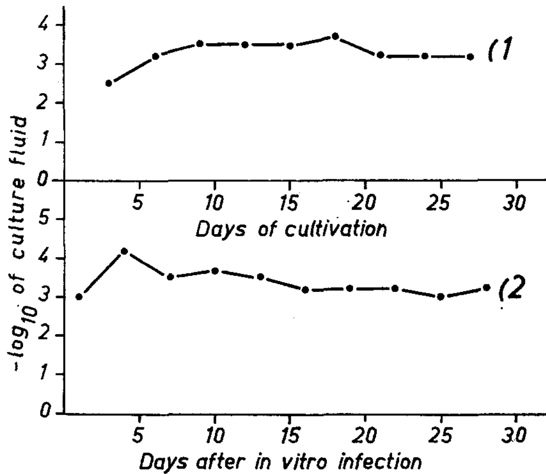

Fig. 4. Multiplication of LCM virus in embryo cells from tolerant (1) and normal mothers (2)

of the virus in the fluid portion of the cultures represented by curve 2 indicates that only a trace of active virus was left in the respective lymph nodes, which had been non-infectious upon direct i. c. inoculation in mice. In addition, the initial multiplication of the virus may have been inhibited to some extent by specific antibodies.

In the cultures obtained from the mice of group 3 LCM virus could not be demonstrated during a cultivation period of about 4 weeks*.

* Some cultures of this series were infected *in vitro* with cell-free filtrate of infectious culture fluid on the 20th day of cultivation. The viral growth curve obtained did not show the sharp drop to zero or nearly zero characteristic for non-tolerant cells. The titers recorded were: 4.5 on the 2nd day after infection, 2.4 on the 5th day, 3.2 on the 8th, 2.7 on the 11th day, and 2.7 on the 15th day. The course of this curve is similar to the viral growth curves in normal embryo cells. It suggests that, through contact with LCM virus, lymph node cells of mature mice may acquire the character of tolerant cells *in vivo* as well as *in vitro* (see Fig. 1). The percentage of the cells becoming tolerant remains to be determined in both cases.

Growth of LCM virus in embryo cells from tolerant and normal mice

In the tolerance of mice towards LCM virus the age factor plays an important role (17). Animals infected *in utero* all become tolerant and virus carriers (17, 5, 20) and so do many mice infected either by contact (17) or experimentally shortly after birth. The percentage of tolerant animals decreases very rapidly with increasing age at the time of infection (22).

In the following experiments (Fig. 4) the multiplication of LCM virus was studied in embryo cells from tolerant (curve 1) and normal mice (curve 2). The tolerant cells had become infected *in vivo*. The normal cells were infected *in vitro* by adding a cell-free Berkefeld-N filtrate of culture fluid from tolerant embryo cells to the media on the 6th day of cultivation. Here again, the LCM-infected tolerant cells grew better than the normal cells.

As can be seen in Fig. 4, the viral growth curve obtained from tolerant embryo cells was similar to those obtained from lymph node cells of tolerant mice (cf. Fig. 2). In the cultures of normal embryo cells the infectivity titer of the culture fluid rose sharply up to the fourth day after infection and then dropped only slightly. A similar slight decrease in titer with a maximum of 1 log occurred in two other experiments, while it was completely missing in a fourth experiment in which the dispersed cells had been infected artificially before incubation. In all cases, the sharp drop of the infectivity titer characteristic for non-tolerant cells (Fig. 1) was lacking and the growth curves showed a nearly linear horizontal course during the following weeks.

In general, cultures of embryo cells were less stable than cultures of lymph node cells, especially pure cultures of reticulocytes.

Discussion

It is clear from the results presented in Figures 1, 2, and 4 that tolerant and non-tolerant cells react differently towards LCM virus. Whereas tolerant cells support continuous multiplication of the virus at a fairly constant level for weeks, the growth curve of the virus in non-tolerant cells shows marked fluctuations.

Upon prolonged cultivation, however, lymph node cells from non-tolerant leukemic mice infected *in vitro* may acquire the character of tolerant cells. Further experiments will have to show whether this is peculiar to leukemic cells. It is not known whether the non-tolerant cells gradually changed their reactivity towards LCM virus or whether they were progressively replaced by tolerant cells, which may have been present in small numbers from the very start. This possibility cannot be ruled out in spite of the fact that there was no evidence of specific cell destruction in such cultures.

The mechanism of the antiviral reaction of non-tolerant cells is not known. In view of the observations made with other viruses in tissue cultures, it is not unlikely that the marked initial fluctuations in virus production are due to periodical formation of interferon (9). The significance of minor fluctuations shown by curves obtained from tolerant cells (see Fig. 2) cannot be proved. However, it should be mentioned in this connection that a similar degree of interference with EEE virus has been demonstrated in the brains of both tolerant and non-tolerant LCM-immune mice (23). It is not unlikely, therefore, that interferon is produced in both cases.

It is now possible to explain the different virus-host relationships prevailing in murine LCM (17, 6) somewhat more precisely. It has been shown that mouse embryo cells are tolerant towards LCM virus to begin with. Natural infection induces them to maintain this property and, as the experiments with lymph node cells from adult tolerant mice prove, to transmit the virus and the tolerant state to their progeny in successive generations. Apparently, such cells do not undergo the change from *Burnet's* (2) hypothetical grade 0 to grades 1 and 2 of physiological reactivity. However, they remain tolerant for LCM virus only and not for other viruses, against which tolerant mature mice can form antibodies much like normal individuals (6, 22). Further experiments will have to show how this situation can be reconciled with the "one cell, one antibody" theory (for references see 13).

In the tolerant mature mouse the production of LCM virus appears to proceed for months, if not for life, at about the same rate as in infected embryos. It is quite likely that viral multiplication occurs in the stem cells of the immunological system themselves, which may be continuously loaded with viral antigen (22) and thus not get a chance to mature to a higher grade of physiological reactivity towards LCM virus. In principle, the situation may be the same as in the tolerance of the body for autologous antigens. One may assume that they also clog the immunological apparatus from its earliest stage throughout life and prevent the maturation of the cells competent immunologically for the respective antigens. An exception is made by those substances which never normally reach centers of immunological response, e. g., antigens of the lens, of spermatozoa, and of the central nervous system as well as thyroglobulin (1). Continuous multiplication of LCM virus does not harm the animal because its susceptible cells are tolerant and there are no antigen-antibody reactions which may be harmful to the host (7, 21).

If intrauterine infection does not take place, the reactivity of the cells towards LCM virus changes shortly after parturition (20). They now react against *in vitro* infection in the manner shown in Fig. 1. This appears to be a consequence of normal cell maturation.

The reaction of mature mice infected experimentally is somewhat similar to that of non-tolerant cells in tissue cultures. There is rapid multiplication of the virus on the first four days after inoculation. The effect upon the host varies depending to a great extent on the route of injection of the virus: tremors and fatal convulsions following intracerebral inoculation, serous pleuritis and peritonitis resulting from intraperitoneal infection, and a marked reaction of the lymph nodes after s. c. injection. Irrespective of the mode of inoculation, the incubation period is about 6 days in all cases.

It is not yet clear if, and to which extent, the peculiar reactivity of non-tolerant cells plays a part in the different disease syndromes, which *Hotchin* (7) tends to attribute to an immunological reaction. The peak of viral multiplication on, or about the fourth day after inoculation is followed by a slow decline of the infectivity titer of the organs. Complement-fixing antigen disappears from the lymph nodes within about two weeks after inoculation (22), a period strikingly similar to that required by the viral growth curve in non-tolerant lymph node cells to reach its first low (see Fig. 1). The rate of elimination of infectious virus and viral antigen may therefore be correlated with the reactivity of the cells, to which *Rowe* (15) has hypothetically attributed an important role in the cerebral immunity of mice against LCM virus.

Although infectious virus may persist in the lymph nodes of non-tolerant mice for some time after s. c. infection (see Fig. 3), new waves of viral multiplication, as they occur in cultures of non-tolerant cells, have not been observed in such animals. We are inclined to attribute this to the presence of specific antibodies (15, 21).

Even though LCM virus has no definite cytopathogenic effect in cultures of non-tolerant murine lymph node cells, the growth capacity of the reticulocytes appears to be greatly diminished during the acute stage of the disease resulting from s. c. infection. On the other hand, the definite impression has been gained that LCM virus has a growth-promoting effect in tolerant lymph node and embryo cells from congenitally infected mice.

The marked effect of lymph node extracts, especially those from leukemic mice, on cultures of murine lymph node cells deserves further attention with regard to an eventual trial of the substance involved, or possible antagonists, in the treatment of lymphatic leukemia. There is no indication of its nature as yet, but it should be possible to learn more about it because a simple tissue culture test is available.

Some similarity exists between the behavior of LCM virus in non-tolerant cells and the course of equine infectious anemia, which has been puzzling virologists for a long time. It may be recalled that equines infected with this virus show repeated febrile attacks which, in surviving

animals, slowly decrease in number and intensity and finally stop. Active virus, however, persists in the organs and blood for extremely long periods of time. The available evidence suggests that antibody formation is very poor or missing in such cases. One may speculate that the affected cells are non-tolerant at first but gradually become tolerant and then do no longer react to the virus. If this interpretation is correct, the periodical febrile attacks, which are apparently preceded by viral multiplication, are not in favour of intrauterine transmission of the causative agent. However, the possibility should not be overlooked that, besides the animals showing a characteristic febrile response, there may be tolerant cases which are not recognized because they never show signs of disease.

Other viruses to be considered here comparatively are those causing ring zone formation under natural or experimental conditions (11). This phenomenon has been studied experimentally with certain pox viruses by *Mayr* and coworkers (12, 10). Even though the amounts of infectious virus produced in the different concentric zones have not been measured on account of technical difficulties, it does not appear unlikely that the phenomenon is similar in principle to the mode of reaction of non-tolerant lymph node cells to LCM virus.

Summary and Conclusions

In a study of the tolerance of mice towards LCM virus at the cellular level different viral growth curves were obtained from lymph node cells of tolerant and non-tolerant mice, the former approaching horizontal straight lines, the latter showing marked fluctuations especially during the first six weeks of cultivation. This reproducible difference allows a distinction between tolerant and non-tolerant cells. Upon prolonged cultivation, the non-tolerant cells in some cultures acquired the character of tolerant cells. The results obtained suggest that murine tolerance towards LCM virus is primarily a cellular phenomenon.

Further experiments have led to the following conclusions: Normal mouse embryo cells are tolerant for LCM virus to begin with. If intrauterine infection does not take place, they or their descendants lose this property shortly after parturition. Embryo cells infected naturally are also tolerant. In contrast to uninfected embryonic cells, they remain so and transmit the virus and the tolerant state to their progeny in successive generations. Infection with LCM virus appears to prevent embryonic cells from reaching a higher level of physiological reactivity, but only with regard to the virus under study.

Lymph nodes from mature mice infected subcutaneously furnished infected cells 14 and 30 days after inoculation, while cells obtained 41 days after injection were no longer infected.

The impression has been gained that LCM virus promotes the growth of tolerant cells. On the other hand, it appears to inhibit the growth of non-tolerant cells during the acute stage of the disease resulting from subcutaneous infection.

Lymph node extracts, especially those from leukemic mice, were found to contain a substance capable of transforming all cell types present in cultures of murine lymph node cells, including the reticulocytes, into round cells and to hasten their maturation and decay.

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