TWO POPULATIONS OF T LYMPHOCYTES IMMUNE TO THE LYMPHOCYTIC CHORIOMENINGITIS VIRUS*

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The results obtained by Marker and Volkert (1) from in vitro measurements of the cell-mediated immunity to the lymphocytic choriomeningitis (LCM)[1] virus have demonstrated that the peak of cytotoxic activity of lymphoid cells from infected mice was reached about 9 days after the inoculation of virus. This activity, however, quickly vanished, and from the second or third week onward only traces of cell-mediated immunity to the virus could be demonstrated. These results agree with data obtained in in vivo experiments by Mims and Blanden (2). These authors demonstrated that lymphoid cells from infected mice 8 days after infection had a strong antiviral effect after transfer into infected recipients, whereas cells harvested late in the infection were ineffective. On the other hand, both these groups of experimental results conflict with many observations obtained by other techniques which strongly indicate that the cell-mediated immunity to the LCM virus is long lasting and plays an important role for the permanent immunity which follows the acute infection (3). However, the different results could have been dependent on the different techniques used, and what has been observed might very well be due to qualitative changes in the cell-mediated immunity during the various stages of infection. If this is so, a shift in the T-lymphocyte population responsible for the cell-mediated immunity probably also occurs. Such an idea would fit well with the evidence for the existence of a marked heterogeneity among T cells, recently obtained by workers in other fields of immunology (4–7).

It was the purpose of the experiments presented in this paper to investigate these problems. Our results suggest that the T cells functioning during the acute state of the LCM infection in mice are distinctly different from those appearing at a later stage. Thus, our data indicate the presence of two functionally different subpopulations of T cells–each of which plays its own role in combatting the viral infection.

Material and Methods

Mice.—Except for virus titration purposes highly inbred C_3H mice were used throughout. Persistent tolerant virus carrier mice (virus carriers) were produced as described previously

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[1]*Abbreviations used in this paper:* LCM, lymphocytic choriomeningitis virus; PBS, phosphate-buffered solution.

[2]Lundstedt, C. Manuscript in preparation.

[3]Monjan, A. A., and M. Volkert. Manuscript in preparation.

(8). These mice carried virus in high titers in blood and organs and had no demonstrable immunity to the virus.

Immune mice serving as donors were mice from either the early or the late state of the viral infection. The infection was initiated by i.p. inoculations of 10^3 LD_{50} virus. Lymphoid cells representing the early immunity (early immune cells) were harvested from the spleen and lymph nodes of mice infected 9 days previously, i.e., when the cytotoxic activity against infected target cells was maximal (1). Lymphoid cells representing the late immunity (late immune cells) were harvested from the same organs 30 days after the infection. At that time these cells after transfer to virus carriers could produce a strong adoptive immunization. Lymphoid cells representing the late immunity were in some cases also harvested from mothers of infected babies about 2 mo after the babies were born. These mice had undergone a natural infection and were solidly immune (9). The results obtained using the cells taken 30 days after an i.p. infection and those from the immune mothers were closely similar, and are therefore considered together.

All donor mice were female mice aged 3–5 mo. The recipients of the cell transplants and their controls were also female C_3H mice 3–5 mo old. They were chosen at random for the experimental group, and no group consisted of less than eight mice.

The LCM Virus.—The LCM virus originated from Dr. E. Traub (Ludwig-Maximilians-Universität, München, Germany). It was kept at −70°C as a 10% spleen suspension from i.p. infected C_3H mice. The virus stock contained between 10^5 and 10^6 LD_{50} virus. The virus titrations were carried out by i.c. inoculation of ordinary 12–14 g white Swiss mice. Blood and organs from experimental mice were titrated individually. Titration end points were calculated by the Kärber method (10). Titers are expressed as log 10 LD_{50}/0.03 ml i.c. dose. In the figures the data recorded are the mean log titers and the standard deviations calculated from the titration results.

Anti-Theta Serum (Aθ S).—Anti-theta serum was supplied by Dr. Rubin (Statens Seruminstitut, Copenhagen). It was produced by immunizing AKR mice with C_3H mouse thymocytes. Undiluted serum and complement killed about 35% spleen cells and 55% lymph node cells from C_3H mice but did not alter the number of plaque-forming cells in a spleen cell population containing antibody-forming cells against sheep erythrocytes. Undiluted serum in the absence of complement did not change the viability of either spleen or lymph node cells from C_3H mice. The data obtained thus strongly indicated that the cytotoxicity of the serum was T-cell specific. In all experiments described in this paper, serum dilutions of 1:2 were employed. The lymphoid cells in amounts of 2–5 $\times$ 10^8 were incubated in the serum at 4°C for 1 h. Then the cells were separated out by gentle centrifugation and resuspended in tissue culture medium containing appropriate amounts of guinea pig complement. After 45-min incubation at 37°C the cells were either transplanted or tested for cytotoxicity. Cell batches used as controls were prepared and treated in exactly the same way except that the anti-theta serum was replaced by tissue culture medium.

X-Ray Irradiation.—X-ray irradiation was administrated by a Siemens Stabilipan therapy machine operated at 200 kV and 15 mA with 0.9 mm Cu and 0.5 mm Al filtration (half-value layer equal to 1.5 mm Cu). The cells were irradiated, suspended in phosphate-buffered solution (PBS) (10 $\times$ 10^6 cells per ml), and placed in petri dishes with a diameter of 4.5 cm (4-ml suspension per dish).

Histology.—Drop-smears from suspensions of lymphoid cells were stained with May-Grünwald-Giemsa after fixation with methanol.

The complement fixation (CF) test, the cytotoxic assay, the calculation of the cytotoxic indices and the preparation of lymphoid cell suspensions were carried out by the methods described previously (1). In all experiments mixtures of spleen and lymph node cells were employed.

The adoptive immunization of virus carriers was carried out by i.p. inoculations (11). The cell doses were given in volumes of 0.5 ml. Other transplantation experiments for the

purpose of protecting infected mice were carried out by i.v. inoculation. The volumes given were 0.5 ml.

RESULTS

The Possible Role of Enhancing Substances for the Disappearance of the Cytotoxic Lymphocytes During the Acute Infection.—In Marker and Volkert's experiments the disappearance of the cytotoxic lymphocytes from the spleen and lymph nodes of infected mice coincided with the appearance of antibodies. The possibility that these antibodies or complexes of antibodies and viral antigen could function as blocking substances and thereby mask the presence of cytotoxic cells could therefore not be excluded. This type of inhibition of cytotoxicity could conceivably work in two ways: (*a*) the blocking substances could have influenced the cytotoxic cells in vivo, and (*b*) antibodies produced in vitro by the suspended spleen and lymph node cells during the 18 h test could block the receptors on the infected target cells.

To test the first possibility cytotoxically active lymphoid cells from mice infected 9 days previously were incubated with serum from immune mice. In two experiments the serum was harvested 14 days after the inoculation of virus, at a time when the antibodies had reached a titer level of 64, while the cytotoxicity of the spleen and lymph node cells had diminished considerably. In two other experiments the serum employed was harvested 28 days after the infection, i.e., at a time when the cytotoxic cell activity was zero. The serum was used in a dilution of 1:4. 1 ml of this dilution was mixed with 17.6×10^6 cells and incubated at 37°C for 30 min. After washing twice in PBS the cells were tested for cytotoxicity. Lymphoid cells incubated with normal serum and washed twice with PBS were used as controls. The results are presented in Table I. It is apparent that the treatment with immune serum did not change the cytotoxic effect.

To test the other possibility the target cells were incubated with immune serum. The serum batches used were the same as in the experiment described above. In each case the serum in a dilution of 1:4 was poured over the infected target cells which had been washed twice with PBS and were ready for the cytotoxic test. 0.4 ml of the serum dilution was employed per petri dish and the incubation took place at 37°C for 30 min. The cytotoxic assay was then carried out in the usual way using lymphoid cells from mice 9 days after the infection. The serum used for incubating was left on the cells throughout the test. Target cells incubated with normal serum in the same dilution and for the same period as used for the immune serum served as controls. The results are also recorded in Table I, and reveal that no blocking substance could be demonstrated in the immune serum.

The Size of the Spleen and Lymph Node Cells from Infected Mice.—Andersson (12) has reported that the size of cytotoxic effector cells in mice changed during the immune response to mastocytoma cells. For this reason the cell

TABLE I

Effect of Immune Serum on the Cytotoxic Reaction between Early Immune Lymphoid Cells and LCM Virus-Infected Target Cells

Cells incubated with serum	Serum used for incubation*	Cytotoxic index ± SD			
		Exp. 1	Exp. 2	Exp. 3	Exp. 4
Effector cells	Normal serum	18.5 ± 1.7	78.6 ± 4.5	40.4 ± 7.8	86.8 ± 3.4
" "	14-day serum	22.3 ± 3.6	74.1 ± 4.1		
" "	28-day serum			43.0 ± 3.2	80.5 ± 3.1
Target cells	Normal serum	24.6 ± 3.4	78.3 ± 5.2	54.9 ± 3.7	64.7 ± 3.3
" "	14-day serum	30.1 ± 2.2	75.8 ± 2.9		
" "	28-day serum			54.0 ± 6.4	66.7 ± 1.7

* Pools of sera obtained from groups of normal mice and from groups of mice inoculated i.p. with 10^3 LD_{50} of virus 14 or 28 days previously. For each of the three types of sera two different pools were tested.

size of the cells in the populations employed by us was investigated. Our findings were in agreement with Andersson. In the early immune state to the LCM infection the cell mixtures from the spleen and lymph nodes contained 30–35% large and blastlike lymphocytes, whereas the cell mixtures harvested during the late immune state contained only 10–15% of these cells.

The Change in Function of the Lymphoid Cells from Infected Mice.—

The effect on the acute infection: As mentioned in the introduction, Mims and Blanden have already shown that an antiviral effect of lymphoid cells after transfer to infected recipients could be observed with cells harvested in the early immune state. In this laboratory these observations were readily confirmed. In our experiments doses of 100×10^6 early immune cells were able to reduce the virus titers in the liver and the spleen after transfer to acutely infected recipients. Moreover, the results of transplantation experiments with cells harvested late in the infection were much less pronounced.

On the basis of these results it seemed appropriate to investigate whether transplanted lymphoid cells could influence the clinical outcome of an LCM infection. For this purpose groups of mice were infected i.c. by 10^3 LD_{50} virus and then given transplants of spleen and lymph node cells from infected mice. The experimental set up and the results are recorded in Table II. It can be seen that if 100×10^6 early immune cells are transplanted on the day of the infection, the lives of all the recipients are saved. If the cells are given 2 days later most but not all recipients survive, and if the transplantations were postponed for 4 days 19 out of 21 mice died. Moreover, a small cell dose of 25×10^6 was also effective. However, large doses of late immune cells had only a little life-saving capacity even if the cells were transplanted on the day that the recipients were infected.

The two kinds of experiments described above are in close accordance with

TABLE II

*Effect of Immune Lymphoid Cells after Transfer to Acutely Infected Mice**

Cell source	Cell dose	Time of transfer	Protection‡	
			Exp. 1	Exp. 2
—	None	—	0/10	0/12
Early imm. cells	100×10^6	Day of inf.	9/9	12/12
"	25×10^6	Day of inf.	10/10	12/12
"	100×10^6	2 days after inf.	5/8	9/11
"	100×10^6	4 days after inf.	1/9	1/12
Late imm. cells	100×10^6	Day of inf.	3/10	0/12

* Inoculated i.c. with 10^3 LD_{50} of LCM virus.

‡ Proportion of mice surviving virus inoculation (observation time 14 days).

one another. Both strongly indicate that during the infection a change occcurs in the functional capacity of the spleen and lymph node cells of the infected mice.

The effect on the persistent tolerant infection: Previous experiments by Volkert and his co-workers (9, 11, 13) have demonstrated that lymphoid cells from infected mice could also cause a violent adoptive immunization in mice with a persistent tolerant LCM viral infection. In view of the findings described above it is, however, surprising that this adoptive immunization was brought about with cell transplants originating from mice infected a month or more before the cells were harvested. Moreover, data were also obtained (11) which indicated that, with respect to their ability to confer an adoptive immunity, the cells from mice late in the infection were superior to those taken from mice in an earlier state. Because of these observations the ability of lymphoid cells to confer an adoptive immunization was investigated.

Virus carriers served as recipients of the cell transplants. Three kinds of donors were employed. The first consisted of mice at the early state of immunity, the second of mice at the late state, and the third of normal mice. The donors were grouped in accordance with their immune state and within each group suspensions of pooled spleen and lymph node cells were prepared and transplanted i.p. in amounts of 100×10^6 cells per recipient. Before the transplantation and 10 and 20 days afterwards, the blood virus titers of the transplanted mice were determined. Two experiments were carried out and the results were almost identical. The data from one of the experiments are recorded in Fig. 1. It is apparent that the lymphoid cells from mice in the late state of immunity could, as expected, confer a strong adoptive immunization, demonstrable by the pronounced antiviral effect of the transplanted cells. However, the cells from the donors at the early state of immunity had so modest an antiviral effect that it was hardly detectable. The results obtained thus once again revealed the difference between the lymphoid cells from mice

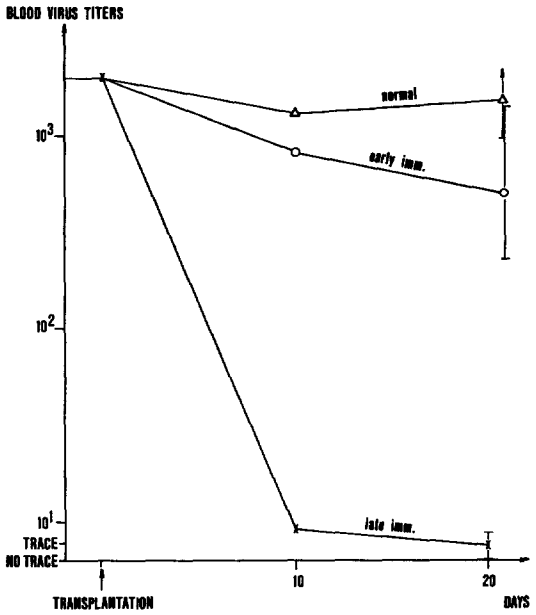

FIG. 1. The blood virus titers of virus carrier mice after receiving transplants of 100 × 10^6 early immune cells (O—O), late immune cells (X—X), or normal cells (△—△).

in the early and the late state of immunity. In this case, however, the cells from the late state were greatly superior to the others.

The effect of X irradiation: It is well known that X irradiation can cause destruction of lymphocytes or inhibition of their function. It is also known that the susceptibility to X irradiation varies considerably among the different lymphocytes. It therefore seemed possible to use X irradiation as an additional means of differentiating between the early and the late LCM immune cells. In attempts to do this, batches of early and late immune cells were irradiated with 300, 600, or 900 R. The function of the early immune cells was then tested by the cytotoxic assay and by their ability, after transfer to mice on the day of an i.c. infection, to protect against a lethal infection. The function of the late immune cells was tested in adoptive immunization experiments in virus carriers.

The effect on the early immune cells is recorded in the first part of Tables III and IV. Concerning the cytotoxic effect, it can be seen that in one experiment no reduction could be observed even after treatment with 900 R. In the other experiment 300 R caused a modest decrease but 600 R and 900 R caused no further reduction and a considerable cell activity remained. Concerning the ability to protect against a lethal infection, 300 R and 600 R had no influence and even 900 R could not completely abolish this cell function.

The effect on the late immune cells was tested in two experiments. The

TABLE III

Influence of X Irradiation and of Anti-Theta Serum Treatment on the Cytotoxic Effect of Early Immune Cells

Treatment of lymphoid cells	Lymphoid cells per target cell	Cytotoxic index	
		Exp. 1	Exp. 2
None	25	38 ± 2.1	77 ± 2.2
None	12.5	21 ± 0.3	48 ± 1.4
None	6.25	10 ± 0.2	24 ± 1.4
300 R of X rays	25	37 ± 0.2	57 ± 4.9
600 R of X rays	25	34 ± 1.1	52 ± 2.2
900 R of X rays	25	34 ± 1.9	56 ± 2.0
Incubation with anti-θ serum	25	0.9 ± 1.2	3.3 ± 0.7
Sham incubation	25	23 ± 1.4	33 ± 1.0

TABLE IV

Influence of X Irradiation and of Anti-Theta Serum Treatment on the Protective Effect of Early Immune Cells

Treatment of lymphoid cells	Cell dose	Protection*	
		Exp. 1	Exp. 2
—	None	0/10	0/10
None	100×10^6	10/10	10/10
300 R of X-rays	100×10^6	10/10	10/10
600 R of X-rays	100×10^6	10/10	10/10
900 R of X-rays	100×10^6	3/10	4/10
Incubation with anti-θ serum	100×10^6	3/10	0/10
Sham incubation	100×10^6	10/10	10/10

* Proportion of mice surviving i.c. inoculation with 10^3 LD_{50} of LCM virus. Cells were transplanted on the day of the infection.

results obtained were very similar to one another and the data from the first experiments are recorded in Fig. 2. All cells employed in each test originated from the same cell batch. The adoptive immunization with the untreated control cells was carried out with 25×10^6 cells, while the X-irradiated cells were transplanted in doses of 100×10^6. The blood virus titers of the recipients were investigated before transplantation and 10 and 20 days afterwards. The virus titer curves reveal that 100×10^6 cells irradiated with only 300 R were less effective than 25×10^6 untreated cells, i.e., the cell effect was reduced by more than 75%. The cells which had received 600 R had an antiviral activity comparable to that which had been demonstrated many times in this laboratory by doses of 10–15 $\times 10^6$ untreated cells. 900 R completely abolished the ability of the cells to influence the virus in the recipient virus carriers. The results obtained thus revealed that the early and the late immune cells also differ considerably with respect to their susceptibility to X rays.

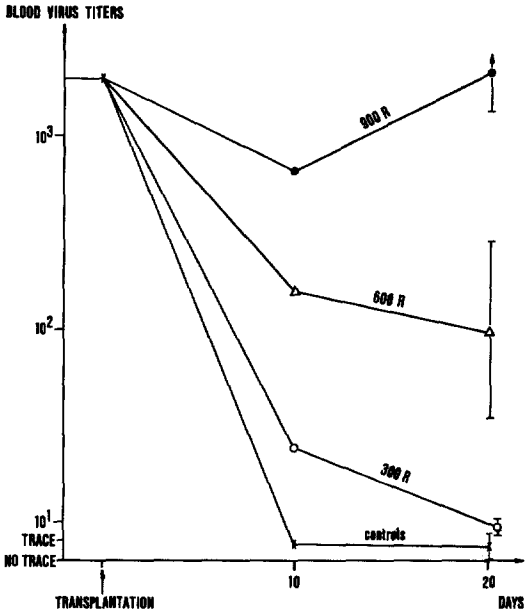

FIG. 2. The blood virus titers of virus carrier mice after receiving transplants of 100×10^6 X-irradiated late immune cells; 300 R (○—○), 600 R (△—△), 900 R (●—●). Controls received 25×10^6 nonirradiated late immune cells (X—X).

The effect of anti-theta serum treatment: As mentioned in the introduction the available data strongly indicate that cell-mediated immunity plays an important role in the early as well as in the late immunity to the LCM virus. Probably, therefore, T lymphocytes are active in both these states of immunity and what we have measured in our tests has been T-cell activity. To test this hypothesis the effect of anti-theta serum treatment was investigated. After the treatment the early immune cells were tested in the cytotoxic assay and for their ability, after transfer to mice on the day of an i.c. infection, to protect against a lethal infection. The function of the late immune cells was tested in adoptive immunization experiments on virus carriers. The effect on the early immune cells is recorded in the second part of Tables III and IV. It appears that anti-theta serum can completely abolish all cytotoxic activity and considerably reduce the protective effect against a lethal infection.

The anti-theta serum effect on the late immune cells is recorded in Fig. 3. The cells used for transplantation were all from the same cell batch. The untreated control cells were transplanted to virus carriers in amounts of 25×10^6, other control cells which had undergone the same manipulations as the experimental ones except for the omission of anti-theta serum were transplanted in amounts of 100×10^6, and the same was the case for the cells which had undergone the anti-theta serum treatment. The virus titer curves

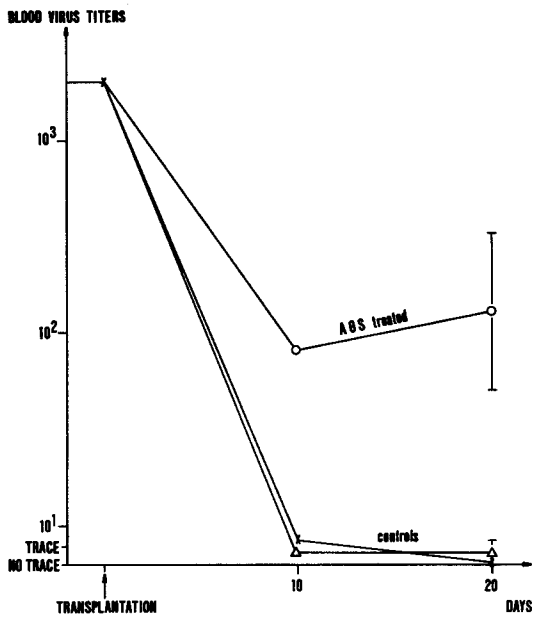

FIG. 3. The blood virus titers of virus carrier mice after receiving transplants of 100×10^6 anti-theta serum-treated (○—○) or sham-treated (△—△) late immune cells. Controls received 25×10^6 nontreated late immune cells (X—X).

reveal that the transplant of 100×10^6 anti-theta serum-treated cells was less active than the transplant of 100×10^6 control cells, and also less active than 25×10^6 untreated cells. Thus, the anti-theta serum experiments support the assumption that both early and late immune cells belong to the T-lymphocyte population.

DISCUSSION

Table V summarizes the results described in this paper. Where the difference in the size distribution of the lymphoid cells in the spleen and lymph nodes during the early and the late immune state to the LCM virus is concerned, our results correspond to those obtained by Shortman et al. (14) and Andersson (12) in allograft immunity experiments. Where the cytotoxicity is concerned, most workers seem to agree that it is the large blastlike cells which are the real killer cells, and this could correspond to the observation made by us and others that it is at the time at which these cells are most numerous that the cytotoxic activity of the cell population is at its peak. On the other hand, it is also well known that the blast cells can develop from small lymphocytes. Apparently few blast cells in a cell mixture used, therefore, do not imply that it is cytotoxically inactive. Accordingly, morphological studies cannot give any definite answer concerning the cytotoxic action of a cell mixture, but the presence of many

TABLE V

T Lymphocytes Immune to the LCM Virus

Cell criteria	Early immune cells	Late immune cells
Cell size	30–35% large	10–15% large
Cytotoxic activity	++++	(+)
Antiviral activity in acute inf.	++++	+
Protection against lethal inf.	++++	+
Adoptive immunization of virus carriers	+	++++
Resistance to X rays	++++	+
Susceptibility to anti-θ serum	+++	+++

large blastlike cells might indicate a strong cytotoxic activity. The relation of cell size to the other functions of the immune lymphoid cells described in this paper is completely unknown.

The rise and fall of cytotoxic activity of the cell populations in the spleen and lymph nodes during the different states of immunity to the LCM virus has been described in a previous paper from this laboratory (1). In the discussion of this presentation it was pointed out that it was very surprising that the cell activity vanished so quickly and that this was probably not merely a local organ phenomenon. Moreover, in the experiments described in this paper it has not been possible to demonstrate the presence of any blocking or inhibitory substances in the blood at the time when the cytotoxic activity was in a sharp decline, nor later when all the activity had disappeared. Nevertheless it is striking that the cytotoxic activity of the cells from the spleen and the lymph nodes vanishes at the time that viral antibodies begin to appear. It is therefore tempting to put forward the hypothesis that these antibodies are in some way involved in the phenomenon. However, in view of our results it seems unlikely that the antibodies themselves or the antigen-antibody complex produced by them have any direct influence on the active lymphoid cells or on the target cells. It therefore seems more probable that if antibodies do have a depressive effect on the cellular immunity it is an afferent or central effect on the development of cytotoxic cells. If this is the case, and if as assumed the disappearance of active cells is not only a local organ phenomenon, then our findings might indicate that the cytotoxic cells have a short life span and die out. We shall return to this point later in the discussion.

The anti-LCM viral activity of early immune cells in acutely infected mice described by Mims and Blanden (2) was readily confirmed by us. Moreover, our findings that the cells from the early state after transfer to infected mice could protect against a lethal infection correspond to and extend these observations. However, Gilden et al. (15) have clearly shown that the early immune cells can also kill the animal, but most probably the cell activity is lethal only when certain specific conditions are fulfilled. The data obtained by us and by others (1, 3, 15–17, footnotes 2, 3) indicate a hypothesis which suggests that

to kill the animal the cytotoxic cells must be present in sufficient numbers at a time when the virus has reached high titers and has been concentrated in a delicate organ such as the choriomeningeal plexus. If this happens, and that is the case after a lethal i.c. infection (or the situation can be induced by transplants of cytotoxic cells at the right time to immunodepressed infected mice), then the animal cannot survive the damage caused by the cytotoxic cells on the infected target cells. If, however, after i.c. inoculations of small doses of virus, a sufficient number of cytotoxic cells to check the virus develop before too many meningeal cells are infected, the damage done by the immune attack is not great enough to be lethal. With our strain of virus the same situation occurs after an i.p. infection. In this case the virus reaches the meninges late and will not be concentrated, nor reach high titers in this organ before the immune attack is mounted. The animal will, therefore, survive. On the other hand if, during an i.c. infection, the cytotoxic cells develop late, the virus, which is not in itself cytotoxic, will continue to grow uncontrolled for some time, the infection will spread to other organs and moreover the infection in the choriomeningeal plexus will diminish. This is the situation late after an immunodepressive treatment of acutely infected mice, and it is also the situation after infections of newborn mice. In these cases we know that the animal will survive the infection and we also know that it will survive transplants of cytotoxic cells. Probably as was the case in the i.p. infections the cell attack when it develops or is induced by transplants will be too dispersed and not sufficiently concentrated on vital organs to cause lethal damage.

Data obtained previously by Marker and Volkert (1), indicate that late immune cells can to some extent convert to cytotoxic cells. This seems contrary to the observations described in this paper which indicate that late immune cells, after transfer to infected mice, have little effect on the outcome of an acute infection. However, these last results are in agreement with the experiments carried out by Mims and Blanden (2) and by ourselves, which indicate that the late immune cells also have a very poor antiviral effect after transfer to acutely infected mice. Probably, therefore, in transplanted, acutely infected mice, the conversion of the late immune cells to active cells takes too long, or too few cells are converted, to give a sufficiently rapid retardation of the growth of the virus to save the lives of the animals.

In the adoptive immunity experiments on virus carriers it was also, and exclusively, the antiviral effect of the transplanted cells which was chosen as a parameter for cell activity. The humoral response, because of its dependence of B lymphocytes, was not taken into account. The results obtained demonstrated very clearly that the cells from the late immune state had an antiviral effect after transfer to the chronically infected virus carriers, whereas the early immune cells were much less effective. Even a very high dose of early immune cells had only a slight influence on the blood virus titers of the recipients during a period of 3 wk. These observations are contrary to those ob-

tained when early and late immune cells are transferred to acutely infected mice, and indicate a striking functional difference between the two kinds of cells. Moreover, in the acutely infected mouse it seemed quite reasonable to consider the cells which developed in the early immune state as precursors of those which developed later. If this were the case it seemed logical to assume that the early immune cells after transfer to virus carriers would by time convert to late immune cells and thereby achieve the same antiviral effect in the carrier mice as the ordinary late immune cells. This was not the case, and it therefore seems hard to believe that the cell mixture of early immune cells contains more than a few precursors of the antiviral cells in the late immune state. This means that the many cells which have an antiviral activity during the acute infection most probably do not convert to the cells which are antiviral in the chronic infection. Thus the data obtained give further support to the assumption mentioned above that the early immune cells with an antiviral activity are end cells.

It is well known that the X irradiation primarily causes damage to the cell mitoses. The fact that the function of early immune cells was difficult to destroy by X irradiation therefore indicates that the activity of these cells in our test system is not dependent on cell division but that it is a quality acquired before the cells were harvested from the donors. Furthermore, the relative ease with which X irradiation can damage the function of the late immune cells indicates that these are dependent on cell division before they acquire sufficient antiviral activity to influence the virus when transferred to virus carrier mice. Our results resemble the data obtained in allograft immunity experiments by Denham et al. (18) and by Stobo et al. (4) and we agree with these authors that the difference in X-ray sensitivity of T cells indicated that the cells tested belong to two different cell populations. As pointed out above, the results from all the other experiments described in this report support this assumption.

Concerning the classification of the active lymphocytes which develop during the different states of immunity to the LCM virus, Mims and Blanden (2) have presented a few data which indicate that the antiviral activity of early immune cells was T-cell dependent, and Cole et al. (19, 20) claim that they have been able to influence the cytotoxic activity by treatment with anti-theta serum. Our anti-theta experiments confirmed that this serum could abolish cytotoxic activity of early immune cells. However, the low cytotoxic index obtained by the control cells which had undergone the same physical manipulations as the anti-theta serum-treated cells indicates that the manipulation itself is rather harmful. Whether or not the anti-theta serum could abolish all or only some of the activity originally present in the fresh cell mixture is, therefore, not known. Anti-theta serum has, in our experiments, also been able to cause a considerable reduction in the ability of early immune cells to protect against a lethal infection. Moreover, when anti-theta serum was applied to late immune

cells their ability to cause an adoptive immunization of virus carriers was reduced by at least 80–85%. Both the early and the late immune cell populations accordingly contain a large percentage of anti-theta serum sensitive cells among the cells which are active in our assay systems. These cells are therefore probably T cells. However, further criteria for the T-cell classification of these cells are desirable, and attempts to obtain such are in progress in our laboratory. As the anti-theta serum treatment in our experiments did not completely abolish the cell activity, the experiments in progress are also aimed at elucidating the possible role played by lymphocytes that do not belong to the T-cell population.

The probable role of the early immune T cells has been discussed above, and it has been shown that these cells have little influence on the virus in chronically infected virus carriers. However, an antiviral effect in these mice is readily demonstrable when late immune T cells are transplanted to them. Probably, therefore, the late immune cells are specially suited to work in chronically infected animals. It seems tempting to postulate that these are the cells which eliminate remnants of virus which might be left over after the early immune cells have done their job during the early phase of the infection. Moreover, in agreement with the other data mentioned in the introduction of this paper, it seems reasonable to suggest that it is the late immune T cells which play the most important role for the surveillance of the occult virus which often persists after an acute LCM infection in mice. The mechanism by which they accomplish this is, however, not known.

SUMMARY

In this report seven different parameters were employed to investigate the spleen and lymph node cells from mice at the early and the late state of immunity to the lymphocytic choriomeningitis (LCM) virus. Distinct differences were observed. Morphological studies revealed a different size distribution of the cells in the preparations from the early and the late state of immunity. The cell mixtures of early immune cells contained many more large and blast-like lymphoid cells than the other. Where the cell function was concerned, the cytotoxic activity against LCM virus-infected target cells was almost entirely a function of the early immune cells, and our data strongly indicate that enhancement does not play any role for the disappearance by time of this cell activity. The antiviral effect after transfer to acutely infected animals was also predominantly a function of the early immune cells and the same was the case concerning the ability to protect against a lethal acute infection. However, the early immune cells were almost inactive after transfer to chronically infected virus carriers, whereas transplants of late immune cells to such mice had a very strong antiviral effect. The resistance to X irradiation also varied. Even high X-ray doses could not destroy the function of early immune cells, whereas the function of the late immune cells was readily impaired by X-ray treatment.

The early and the late immune cells have one thing in common—both are susceptible to treatment with anti-theta serum. Because of the differences observed between the early and the late immune cells, it is concluded that they belong to different cell populations. However, because of the common susceptibility to anti-theta serum, probably both populations are T-cell lymphocytes. The implications of the results and the role of the different cells in the combat of the viral infection are discussed.

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