INFECTION AND IMMUNITY, Sept. 1974, p. 489-498

Vol. 10, No. 3
Printed in U.S.A.

Inflammatory Lymphocyte in Cell-Mediated Antibacterial Immunity: Factors Governing the Accumulation of Mediator T Cells in Peritoneal Exudates

R. J. NORTH AND G. SPITALNY[1]
Trudeau Institute, Saranac Lake, New York 12983

Received for publication 8 April 1974

The lymphocytes which mediate immunity to infection with *Listeria monocytogenes* in the mouse accumulated in casein-induced peritoneal exudates. They were T cells, as evidenced by their susceptibility to anti-θ serum, but some were also destroyed by anti-immunoglobulin serum. For a given number of cells, exudate cells were at least six times more efficient than spleen cells in protecting normal recipients against lethal challenge. The extent to which mediator cells accumulated in exudates was found to be governed by the level of their production in responding lymphoid tissue and by the time available for them to migrate into an exudate. An intraperitoneal injection of casein at any stage of infection resulted in a progressive accumulation of mediator cells that continued for 3 days. The accumulation was not caused by continuous entry of cells during the whole of this period, but resulted from division of a limited number of cells that entered during the first 24 h. Accumulation of mediator cells in an exudate was associated with the conversion of a population of dividing cells into a population of nondividing T cells with a relatively short life-span.

Acquired immunity in mice to infection with the bacterial parasite *Listeria monocytogenes* is expressed by activated macrophages (12). The adaptive changes which enable macrophages to perform this function, however, are mediated by a population of specifically sensitized, short-lived, replicating, thymus-derived lymphocytes (15). Production of these T-cell mediators of antibacterial immunity begins in the spleen within 2 days of an intravenous infection; peak production is reached by day 6 and then rapidly declines. The kinetics of their production thus resembles the kinetics of production of the cells that mediate immunity to allografts which are also dividing T cells (21), and which decline rapidly in number after a quick rise to peak production in responding lymphoid tissues (2, 3).

Another property of the cellular mediators of anti-*Listeria* immunity, as revealed by experiments in the rat (10), is their propensity to enter sites of inflammation. Since the cellular mediators of allograft (9, 19) and other examples of cell-mediated immunity (1, 23, 25) also show this capacity to enter sites of tissue injury in the absence of a specific antigenic stimulus, it is apparent that all mediator lymphocytes share this property.

The purpose of this paper is to show that the cellular mediators of antibacterial immunity which enter sterile peritoneal exudates in the mouse are replicating T cells. It will further show that the extent to which they accumulate in peritoneal exudates is limited, among other things, by the level of their production in responding lymphoid tissue and by their capacity to replicate after they leave the circulation.

MATERIALS AND METHODS

Animals. Adult (8 to 14 weeks old) $AB6F_1$ (A $\times$ C57Bl/6) mice of both sexes were used. Mice of the same age and sex were confined to single experiments. AKR and C3H mice were used for producing anti-θ serum. All strains were purchased from Jackson Laboratories, Bar Harbor, Me.

Bacteria. *L. monocytogenes* (strain EGD) is passaged continuously in mice in this laboratory to maintain its virulence. The intravenous mean lethal dose (LD_{50}) of the organism was 5×10^3 at the time of the experiments. A log-phase culture (2×10^8 per ml) in Trypticase soy broth (Difco) was dispensed in small volume and frozen at -70 C. At the time of each experiment a tube was thawed in a 37 C water bath and diluted in a standard fashion in 0.85% sodium chloride solution for intravenous injection. The viability and virulence of the organism did not change in cold storage for at least 6 months. The standard

[1] Present address: Department of Preventive Medicine, School of Medicine, New York University, New York, N.Y. 10016.

immunizing dose was either 10^3 or 2×10^3, and the challenge dose 5×10^4.

Bacteria were enumerated in the spleen by plating 10-fold dilution of whole spleen homogenates on Trypticase soy agar (Difco).

Estimating mediator cells in spleens and peritoneal exudates. Changes in the relative number of mediator cells in the spleen and peritoneal cavity during infection were determined by measuring changes in the capacity of these cell populations to protect normal recipient mice against a standard lethal challenge infection. Protection was expressed as the $\log_{10}$ difference between the growth of the challenge infection in the spleens of recipients of "immune cells" and growth of the challenge infection in the spleens of recipients of normal cells at 48 h of the challenge infection.

Spleen cell suspensions were prepared as described previously (15).

Peritoneal exudates were induced by the intraperitoneal injection of 2 ml of sterile 5% casein solution. This was prepared by incubating 5 g of casein in 100 ml of 0.85% sodium chloride solution for 3 h at 55 C. The solution was neutralized with 10 N sodium hydroxide solution, filtered through surgical gauze, and stored at 4 C.

Exudate cells were harvested by folding back the abdominal skin and injecting 3 ml of Dulbecco phosphate-buffered saline (PBS) containing 10 U of heparin per ml intraperitoneally with a needle and syringe. After briefly massaging the abdomen, 2.75 ml of fluid was withdrawn. The procedure was repeated, and the cells were pooled. After one wash in PBS containing 5% fetal calf serum (PBS-FCS) the cells were diluted appropriately for counting in a hemocytometer and for intravenous infusion.

Antisera. ARK anti-C3Hθ serum was obtained by injecting AKR mice intravenously and intraperitoneally with 10^7 C3H thymocytes every 7 days for 4 weeks. Blood was collected by cardiac puncture 7 days after the last injection. The serum was diluted 1:5 in PBS, absorbed with 5×10^7 AKR thymocytes per ml for 30 min, and stored in small volumes at −20 C. The specificity of the antiserum for the θ antigen was tested by absorption with AKR and C3H brain tissue as described previously (16).

Rabbit anti-mouse (Ig) immunoglobulin serum (anti-Ig) was purchased from Miles Laboratories, Inc., Kankakee, Ill. It was supplied with immunoelectrophoretic data which showed that it was specific for mouse Ig.

The susceptibility of the cellular mediators of anti-*Listeria* immunity obtained from spleens and peritoneal exudates to anti-θ serum or anti-Ig serum was tested by incubating the cells at a concentration of 5×10^7 per ml in a 1:5 dilution of either antiserum at 37 C for 20 min. The 1:5 anti-Ig serum contained 260 μg of antibody protein per ml. The cells were then washed once in PBS-FCS and resuspended at the same concentration in 1:5 agarose-absorbed guinea pig serum (5) for 30 min at 37 C. They were then washed in PBS-FCS and resuspended for intravenous infusion. Control cells were treated in the same way except that they were incubated in normal AKR serum instead of anti-θ serum, and normal rabbit serum instead of anti-Ig serum. The anti-Ig serum was judged to be specific for Ig-bearing cells because when used as just described, it destroyed less than 0.2% thymocytes and about 40% spleen cells as determined by dye exclusion. Furthermore, it did not alter the phagocytic activity of macrophage monolayers (unpublished data).

Vinblastine sulfate. Vinblastine sulfate was obtained from Eli Lilly and Co., Indianapolis, Ind. It was dissolved in PBS and injected intravenously in a dose of 100 μg. This dose should have destroyed cells mitosing in vivo for a period of about 15 h (24).

RESULTS

Demonstration that mediator cells accumulate in 48-h casein-induced exudates. Evidence that the cellular mediators of anti-*Listeria* immunity accumulate in casein-induced peritoneal exudates is displayed in Fig. 1, which compares immunity transferred to normal recipients with peritoneal exudate cells from infected donors with immunity transferred with exudate cells from noninfected donors. Also shown is the protection transferred with cells obtained from the unstimulated peritoneal cavities of infected donors. All cells were harvested on day 7 of infection, and exudates were induced with casein on day 5. It can be seen that a high level of adoptive immunity was transferred with two equivalents of 48-h exudate cells (3.8×10^7) from infected donors, but not with two equivalents of 48-h exudate cells (3.6×10^7) from noninfected donors. It can be seen, in addition, that 2 equivalents of peritoneal cells (9×10^6) from unstimulated peritoneal cavities also possessed a substantial capacity to protect recipients against a challenge infection. It should be mentioned in this connection that these experiments were designed to determine the protective capacity of the total mediator cell population of the peritoneal cavity. Obviously, if a large enough number of cells were infused, the protection transferred with cells from the unstimulated peritoneal cavity would equal the protection transferred with exudate cells. Assuming equal efficiency per cell, the results simply mean that many more mediator cells were present in a 48-h peritoneal exudate than in an unstimulated peritoneal cavity.

Evidence that mediator cells in exudates are T cells. The effect of incubating peritoneal exudate cells, obtained as described above, with anti-θ serum and complement on their capacity to protect normal recipients against lethal challenge is shown in Fig. 2. Anti-θ serum completely abrogated their protective capacity. Since the activity of the anti-θ serum was

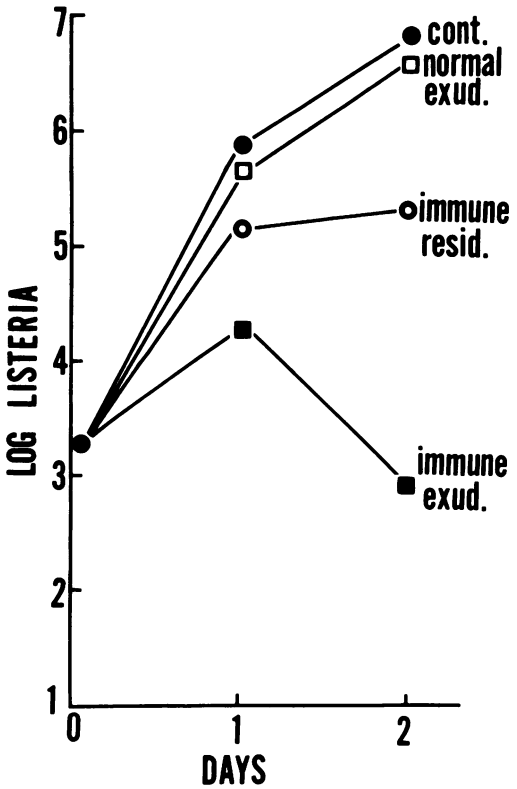

FIG. 1. *Growth curves of a Listeria challenge infection in the spleens of recipients of two equivalents of 48-h exudate cells from 7-day-infected donors (immune exud.) or noninfected donors (normal exud.), or two equivalents of cells from unstimulated peritoneal cavities of 7-day infected donors (immune resid.). A high level of adoptive immunity was transferred with immune exudate cells, but not with normal exudate cells. Resident peritoneal cells also transferred appreciable levels of immunity. Means of five recipients per time point.*

completely removed by absorption with C3H brain but not with AKR brain, the mediator cells that accumulate in exudates are θ-bearing T cells. In addition, however, anti-Ig serum and complement also reduced the protective capacity of exudate cells, although to a much lesser degree than anti-θ serum. It is apparent, therefore, that either a proportion of the mediator T cells contained Ig determinants on their surfaces, or that Ig-bearing cells other than T cells cooperated with T cells in mediating anti-*Listeria* immunity.

Exudates are a richer source of mediator cells than the spleens of intravenously infected donors. In comparing the levels of protection transferred with cells obtained from different tissue compartments, it is important to distinguish between the activity represented by all the cells in the compartment and the activity of a given number of them. The former allows a comparison to be made between the total number of mediator cells in the spleen and a peritoneal exudate, whereas the latter measures the relative efficiencies of the two populations. A comparison between the protection transferred with cells from 48-h peritoneal exudates and spleen cells harvested on day 7 of an immunizing infection is expressed in both ways in Fig. 3. One equivalent of peritoneal exudate cells transferred about the same level of protection as one equivalent of spleen cells. Assuming equal protective efficiency per cell, this means that each compartment contained about the same number of mediator cells. Also, when twofold dilutions of one exudate equivalent and one spleen equivalent of cells were infused, the levels of protection in recipients were correspondingly reduced by twofold steps. This means that the level of immunity conferred was directly proportional to the number of mediator lymphocytes infused.

When protection was plotted against the number of cells infused, however, peritoneal cells were much more efficient than spleen cells (Fig. 3, below). It was calculated that in this

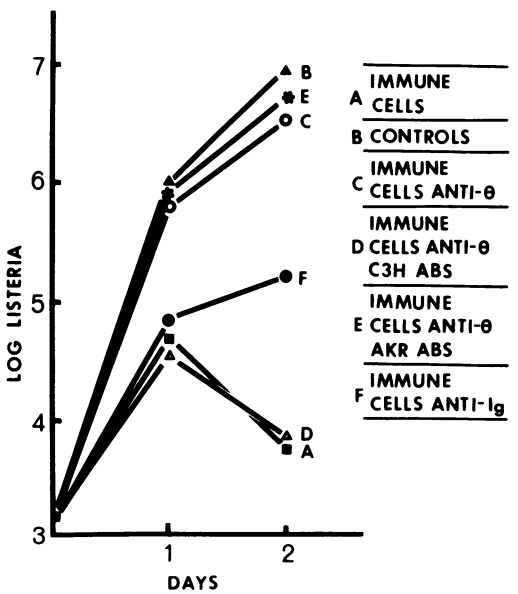

FIG. 2. *Incubation with anti-θ serum and complement completely eliminated the capacity of exudate cells to protect recipients. The cytoxic activity of the anti-θ serum was completely removed by absorption with C3H brain, but not with AKR brain. Anti-Ig serum also partly abolished the protective capacity of exudate cells. Means of five recipients per time point.*

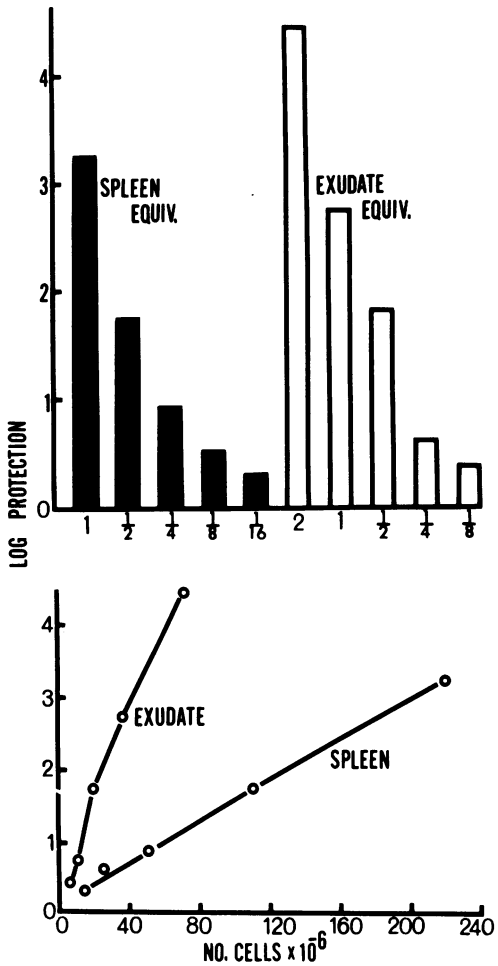

FIG. 3. *Comparison between protection transferred with 48-h exudate cells and spleen cells harvested on day 7 of infection. Histographs show protection transferred in terms of spleen and exudate equivalents and fractions of equivalents. The bottom graph compares protection on a cell for cell basis. The results show that although both compartments contained about the same number of mediator cells, exudate cells were about six times more efficient than spleen cells at protecting recipients against Listeria challenge. Means of five recipients.*

particular experiment, mediator cells were about six times more concentrated in 48-h peritoneal exudates than in spleens at the same stage of infection.

Number of mediator cells which can accumulate in exudates is determined by their level of production in the spleen. It would be expected that the number of mediator cells that can accumulate in a peritoneal exudate at any given time during infection would be a function of the numbers circulating in blood. This number would depend in turn on the numbers being released from responding lymphoid tissues. Since it had already been shown (15) that peak production of the cellular mediators of anti-*Listeria* immunity occurs in the spleen on day 6 of infection and declines thereafter, it was expected that mediator cells would accumulate in 48-h exudates in keeping with this pattern of production. The protective capacity of 48-h exudates induced at progressive stages of infection increased and decreased in parallel with the rise and fall in protective activity in the spleen, but with a delay of 48 h (Fig. 4). Thus, whereas the protective capacity of spleen cells increased from day 2 of infection to peak on day 6, that of 48-h exudate cells increased from day 4 to peak on day 8. The 48-h lag in reaching peak activity in exudates is explained below.

The protective activity of cells harvested from unstimulated peritoneal cavities at progressive stages of infection also increased and then decreased, but kept pace with the increase and decrease in the production of mediator cells in the spleen (Fig. 4).

The total numbers of nucleated cells harvested from spleen or peritoneal cavity at each time point are shown in Fig. 5. The rise and fall in the protective activity found in these cell populations were obviously related to the numbers of nucleated cells harvested. Presumably, therefore, the total number of cells present in

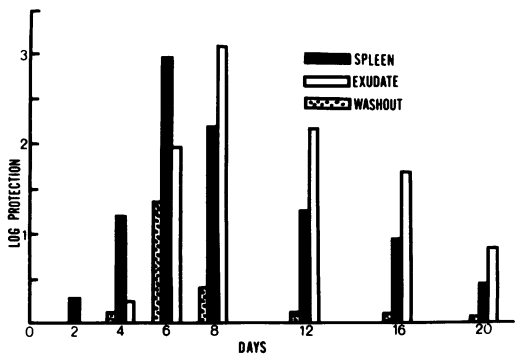

FIG. 4. *Evidence that the accumulation of mediator cells in exudates is dependent on the level of mediator cell production in the spleen. Shown are levels of protection transferred with two equivalents of 48-h exudate cells, one equivalent of spleen cells, and two equivalents of resident peritoneal cells (washouts) harvested at progressive stages of infection. The protective capacity of spleen cells and resident peritoneal cells peaked on day 6 and then declined. The protective capacity of 48-h exudate cells, as expected, peaked 48 h later and then declined. Means of five mice per time point.*

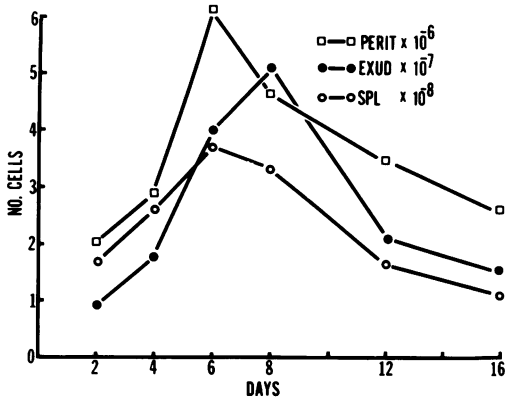

FIG. 5. *Number of nucleated cells harvested at progressive times of infection from two 48-h exudates, two unstimulated peritoneal cavities (perit.), and one spleen to give the levels of protection in recipients (Fig. 4).*

each compartment reflected their content of mediator cells at any given stage of infection.

Number of mediator cells in exudates is determined by the time allowed for accumulation. The preceding results showed that the number of mediator cells that accumulate in a 48-h exudate is determined by the prevailing level of production of these cells in the spleen. The following experiments show that the number of mediator cells which can be harvested from a peritoneal exudate is also influenced by the time allowed for them to accumulate.

In these experiments, a large number of mice were infected with *Listeria* and divided into groups according to whether they were given casein intraperitoneally on day 1, 4, 8, or 12 of infection. Exudates were harvested from each group at 24-h intervals after giving casein and the protective capacity of the recovered cells was measured by infusing two donor equivalents into groups of challenged recipients. Regardless of the stage of infection, mediator cells accumulated progressively for a period of 3 days (Fig. 6). In agreement with the preceding experiments, however, the largest 3-day accumulation of mediator cells occurred when exudates were induced during the time of peak production of mediator cells in the spleen (days 4 to 7). Maximum 3-day accumulation, in every case, was followed by a rapid fall in the number of mediator cells in the peritoneal cavity (Fig. 6). This decline is further illustrated in Fig. 7, which also shows changes in the number of nucleated cells harvested from exudates over a 5-day period. Stained smears of peritoneal cells showed that the large increase in the number of cells in 24-h exudates was caused mainly by the entry of polymorphonuclear leukocytes which progressively decreased in number after this time. If this peak is ignored, it can be concluded that the increase and decrease in protective capacity was associated with a rise and fall in the number of mononuclear cells. Additional information on this point will appear in a forthcoming publication.

Evidence that mediator cells enter the peritoneal cavity only during the first 24 h of inflammation. The preceding results showed that the mediators of anti-*Listeria* immunity accumulated progressively in peritoneal exudates of infected mice for a period of 3 days. The following experiment was designed to determine whether this accumulation was caused by continuous entry of mediator cells during this period.

A large group of noninfected, intermediate recipients was injected intraperitoneally with casein and divided into panels according to the time relative to the injection of casein that they were given a single intravenous infusion of 2×10^8 spleen cells from 6-day infected donors. Exudate cells were harvested at 24-h intervals from each panel of spleen cell recipients and

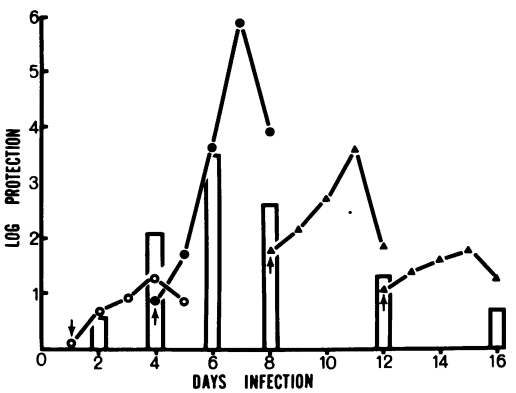

FIG. 6. *Evidence that mediator cells accumulate progressively in peritoneal exudates for 3 days and then decline in number. A large group of infected donors were divided into panels according to whether they were injected intraperitoneally with casein at day 1, 4, 8, or 12 of infection (arrows). Exudate cells were harvested every 24 h from each panel, and the protective capacity of two exudate equivalents of their cells was measured in challenged recipients. The histogram shows the time course of production of mediator cells in the spleen. An injection of casein at any time of infection resulted in a 3-day accumulation of mediator cells in the peritoneal cavity. The largest 3-day accumulation of mediator cells occurred during the period of maximum production in the spleen. Means of five recipients per time point.*

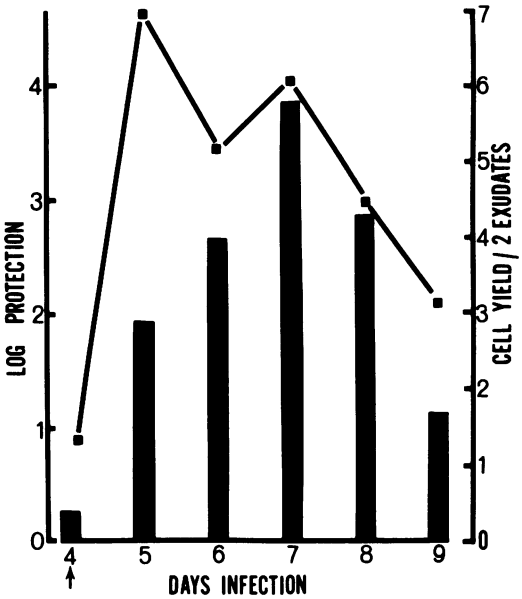

FIG. 7. *Additional evidence that a 3-day accumulation of mediator cells in exudates is followed by a progressive decline in their number (histogram). Casein was injected on day 4 of infection (arrow), and the protective capacity of two equivalents of exudate cells was harvested at 24-h intervals was tested in challenged recipients. There was a rapid decline in the protective capacity of exudate cells harvested after day 3. The total number of nucleated cells harvested from two peritoneal cavities at the times indicated are also shown. The large increase in the number of cells over the first 24 h was caused by the entry of polymorphonuclear leukocytes. Means of five mice per time point.*

assayed for protective activity in secondary recipients. In this way it was possible to detect the extent of entry of mediator cells into exudates of varying age. The experiment called for a fresh supply of spleen cells from 6-day-infected donors every 24 h.

Passively transferred mediator cells entered peritoneal exudates of normal recipients only during the first 24 h of inflammation, because protective activity was found only in the peritoneal cavity of recipients which were infused with immune spleen cells and injected with casein on the same day (Fig. 8). The cells from these exudates were capable of transferring about two logs of protection to challenged recipients. This represented about 60% of the known protective capacity of the infused donor spleen cells themselves. It will be noted, however, that the protective capacity of the cells quickly declines after 24 h, thus indicating that they either died or left the peritoneal cavity. This result indicated that the progressive accumulation of mediator cells over a 3-day period in the exudates of actively infected mice could not have resulted from continuous immigration. The only other way that the population of mediator cells could increase progressively for 3 days would be by replication of mediator cells that enter an exudate during the first 24 h of inflammation.

Evidence that mediator cells which enter exudates are actively dividing. The above results revealed an apparent contradiction. Although intravenously infused mediator cells were found to enter the peritoneal cavity of normal recipient mice only during the first 24 h of inflammation, mediator cell activity never-

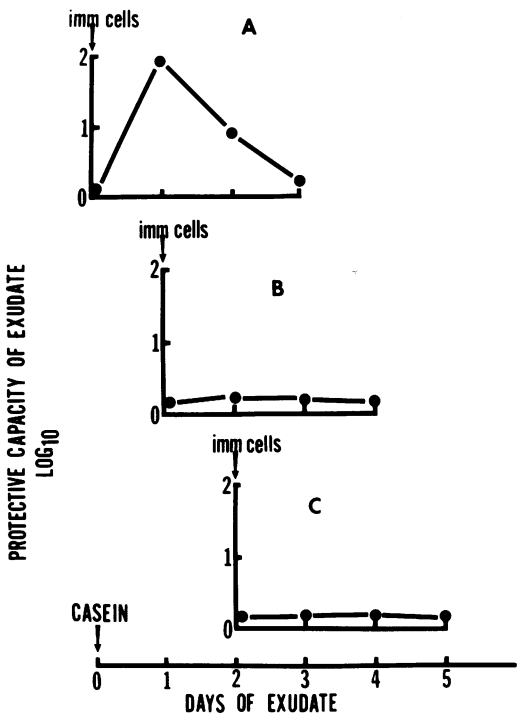

FIG. 8. *Evidence that mediator cells only enter exudates during the first 24 h of inflammation. Casein was injected intraperitoneally into a large group of noninfected intermediate recipients. These were divided into panels (A, B, C) according to the time after giving casein that they were infused intravenously with one equivalent of spleen cells from 6-day-infected donors. Exudate cells were harvested from each panel of recipients at 24-h intervals, and the protective capacity of two exudate equivalents of their cells was tested in challenged secondary recipients. Intravenously infused mediator spleen cells only entered the peritoneal cavity of intermediate recipients during the first 24 h of inflammation. Means of five recipients per time point.*

theless increased in the inflamed peritoneal cavities of actively infected mice for a full 3 days. This suggested that the 3-day accumulation of mediator cells was caused by replication of cells that entered during the first 24 h. To investigate this possibility, the antimitotic drug vinblastine was used to destroy dividing mediator cells in exudates.

A large group of mice was infected with *Listeria* and injected intraperitoneally with casein on day 4 of infection. They were given a single intravenous injection of vinblastine either at the time of, or at 24-h intervals after, injecting casein. Exudate cells were harvested at 24-h intervals after vinblastine, and their protective capacity was measured in challenged recipients.

When vinblastine was given at the time of injecting casein, the protective capacity of the 24-h exudate cells was almost completely eliminated. This means that most of the mediator cells that entered or would have entered the exudate the first 24 h were vinblastine sensitive. It can also be seen that the effect of a 15-h pulse of vinblastine on protective capacity decreased with time after inducing the exudates. Thus, whereas vinblastine destroyed the vast majority of protective cells during the first 24 h of inflammation, it destroyed only about 60% in 48-h exudates and 40% in 72-h exudates, and had no effect at all on the protective cells in 96-h exudates. It is almost certain, therefore, that although the mediator cells which entered exudates were rapidly dividing, their potential for division was progressively exhausted.

Evidence that mediator cells do not leave the peritoneal cavity. The decline in the protective capacity of exudate cells after day 3 of inflammation, as revealed in preceding experiments, means that mediator cells either lose their function or leave the peritoneal cavity. The possibility that mediator cells leave the peritoneal cavity was tested by measuring the capacity of 72-h exudate cells injected intraperitoneally to protect normal recipients against an intravenous challenge infection. It was reasoned that any appreciable migration of these cells from the peritoneal cavity into blood would be reflected in the expression of adoptive immunity in the recipients' spleens.

Normal recipient mice were injected intraperitoneally with either two donor equivalents of 72-h exudate cells (6×10^7) or one donor equivalent of spleen cells (2.5×10^8), both harvested from 7-day-infected donors. A group of recipients were also injected intraperitoneally with casein 3 days before receiving mediator

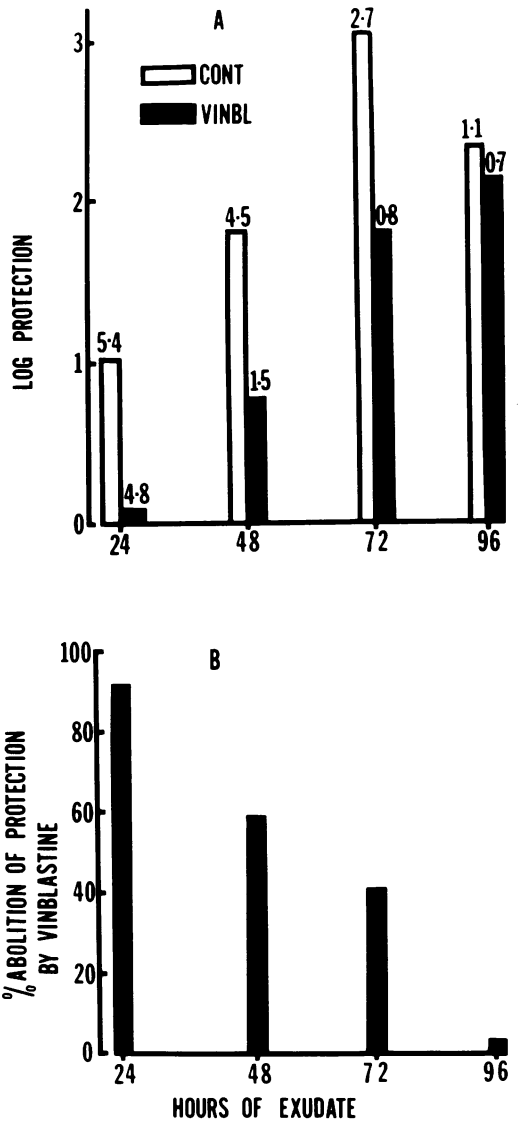

FIG. 9. *Evidence that the mediator cells that enter a peritoneal exudate during the first 24 h are dividing cells, but that there is a conversion from a dividing to a nondividing population over the next 72 h. A 15-h pulse of the antimitotic drug vinblastine eliminated the protective capacity of 24-h exudate cells, but the effect of the drug progressively diminished thereafter. It had no effect on the protective cells in 96-h exudates. The top histograms compare protection transferred with two equivalents of exudate cells harvested from control and vinblastine-treated donors. The figures on the histograph represent the number of nucleated cells harvested from two exudates* $\times 10^{-7}$. *The bottom histograph expresses the same results as percent abolition of protection by vinblastine. Means of five recipients per time point.*

cells intraperitoneally to simulate the conditions of inflammation associated with the normal loss of protective cells from this compartment. Other groups of recipients were given spleen and exudate cells intravenously. All mice were challenged intravenously with *Listeria* 1 h after cell transfer, and the growth of the challenge infection was followed in their spleens.

Neither exudate cells nor spleen cells injected intraperitoneally could protect normal recipients against an intravenous challenge infection (Fig. 10). In contrast, the same number of cells given intravenously caused the expression of high levels of adoptive immunity. It is almost certain, therefore, that functional mediator cells do not migrate from the peritoneal cavity into blood in significant numbers.

DISCUSSION

The results presented here confirm published findings in the rat (10) which show that the cellular mediators of anti-*Listeria* immunity accumulate in relatively large numbers in inflammatory exudates. This paper shows, in addition, that the mediator cells that accumulate in peritoneal exudates are T cells, and that the rate at which they accumulate at any time during infection is determined by the level of their production in responding lymphoid tissue. It also shows that mediator cells accumulate in a peritoneal exudate for 3 days after injection of casein, regardless of the stage of infection. Consequently, the largest accumulation of mediator cells occurs in 3-day exudates induced during the time of peak production of mediator cells in the spleen. Titration studies showed, moreover, that mediator cells were about six times more concentrated in an exudate than they were in the spleen, although the total number of these cells was about the same in each compartment.

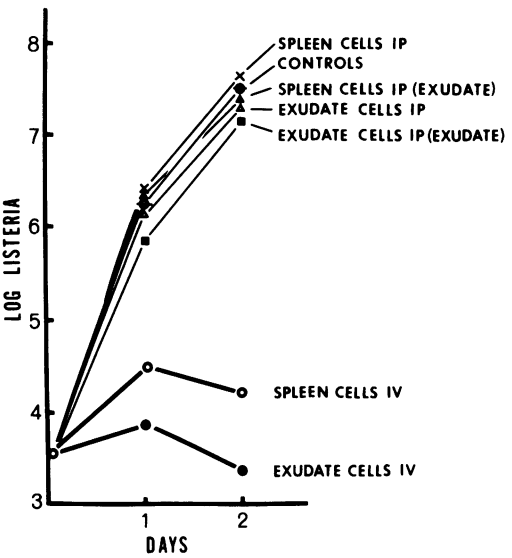

FIG. 10. *Evidence that mediator cells do not leave the peritoneal cavity. Neither two equivalents of exudate cells nor one equivalent of spleen cells from 7-day-infected donors could protect recipients against an intravenous challenge infection if the cells were infused intraperitoneally. In contrast, the same cells injected intravenously resulted in the transfer of high levels of immunity. Spleen and exudate mediator cells also failed to transfer protection if they were injected into the peritoneal cavities of recipients which had received casein intraperitoneally 72 h before (IP exudate) to simulate conditions associated with the normal loss of mediator cells from exudates. Means of five recipients per time point.*

The build-up of mediator cells in an exudate was not due to continuous entry from blood. On the contrary, cell transfer studies showed that passively administered mediator cells entered peritoneal exudates of normal recipients only during the first 24 h of inflammation. It can be postulated, therefore, that the protracted rise in the population of mediator cells found in exudates of actively infected mice is caused by the replication of mediator cells which enter during the first 24 h. This explanation is fully consistent with the finding that the mediator cells were destroyed by a 15-h pulse of vinblastine given while the exudate was forming, whereas those mediator cells found in the peritoneal cavity at a later stage were vinblastine resistant. This means that all mediator cells in 24-h exudates were dividing cells, whereas those present in 96-h exudates were not. Since it was shown, in addition, that the level of adoptive anti-*Listeria* immunity expressed by normal recipients was directly proportional to the number of donor mediator cells infused, it is easy to conceive how a threefold increase in protective capacity of exudates could occur over 48 h by division of mediator cells that entered during the first 24 h. It would take fewer than two complete divisions of the initial population of mediator cells to give the observed increase in protective capacity.

The stimulus for this division is not known. It was either the result of stimulation by systemically distributed soluble *Listeria* antigens (living *Listeria* were never found in exudates), or mediator cells may be obliged to complete mitotic cycles initiated while still in circulation or before release from a central lymphoid organ such as the spleen. Specific antigen almost certainly is required to sustain replication, because cell transfer studies showed that mediator cells which entered peritoneal exudates of non-

infected recipients did not increase in number. It should be remembered, however, that division decreased progressively to a very low rate by 4 days even in infected mice. It is interesting, furthermore, that the conversion from a dividing to a nondividing population coincided with the decline in protective activity in exudates. It is suggested, in view of this evidence, that mediator cells enter an exudate as rapidly dividing cells during the first 24 h of inflammation; that they enter in numbers determined by the level of their production in the spleen and their representation in blood; and that they continue to divide under the stimulus of soluble *Listeria* antigens, exhaust their mitotic capacity after one or two divisions, and then die or lose their protective function.

The finding that the mediators of anti-*Listeria* immunity are dividing when they enter an exudate agrees with findings in rats (13). It has been shown, moreover, that the lymphocytes which enter sites of allograft rejection (8, 9, 18) and autoimmune inflammation (25) are also predominantly newly formed cells. The conclusion that the mediators of anti-*Listeria* immunity which enter exudates are end cells is in keeping with the known properties of the majority of mediator cells produced in the spleen; namely, that they are rapidly dividing cells which disappear from the spleen and blood once they have ceased to divide (15). Again, mediator spleen cells only remain functional for 1 to 2 days after infusion into normal recipients (17). It is well to point out in this connection that the cellular mediators of allograft immunity also have a short half-life when infused into recipients (14) and a limited potential for division (4).

It is interesting that appreciable numbers of mediator cells could be harvested from the unstimulated peritoneal cavities of infected mice. The rise and fall in their number reflected a proportionate rise and fall of mediator cell production in the spleen. They could be produced locally, in concert with mediator cell production in the spleen, or reach the peritoneal cavity by constant "leakage" from blood. In either case, these cells contribute to the total protective capacity of exudate cells.

The cellular mediators of immunity in exudates were destroyed by incubation with anti-θ serum and complement. This is good evidence that they are θ-bearing T cells. A proportion of them, however, perhaps as many as 30%, were destroyed by anti-Ig serum and complement. It is apparent, therefore, that either a proportion of mediator T cells display Ig determinants on their plasma membranes, or that a class of Ig-bearing cells cooperates with T cells in the mediation of immunity. The first alternative seems possible in view of recent evidence that activated T cells can be stained with fluorescein-labeled anti-Ig (7) and may contain surface-bound specific antibody (6, 20). It is realized, nevertheless, that the possession of surface Ig by T cells remains a controversial issue. It is almost certain, on the other hand, that macrophages do not contribute to the adoptive immunity observed, because experiments still in progress have shown that when exudate cells which adhere firmly to plastic surfaces are removed, the remaining cells are six times more efficient on a cell for cell basis at adoptively immunizing normal recipients.

It is obviously important for the host to have a mechanism which enables it to focus sensitized lymphocytes at sites of tissue injury. Presumably, the mechanisms responsible for the entry of these cells into a sterile peritoneal exudate are the same as those responsible for focusing them at infective foci in the liver and spleen. It is not surprising, furthermore, that specific antigen need not be present to attract the mediators of anti-*Listeria* immunity and other examples of cellular immunity into inflammation. Since the entry of sensitized cells into inflammation must take place across the endothelium of small blood vessels, it is highly likely that sensitized lymphocytes are innately equipped to recognize, stick to, and migrate between capillary endothelial cells which have undergone physiological changes common to all types of inflammation. The presence of specific antigen in inflammation might be expected to be seen after entry of sensitized cells occurs. It is possible, for instance, that antigen could serve to cause division of sensitized cells and result in a local augmentation in their number. Unfortunately, those published studies which show that the mediators of allograft immunity either do (11, 22) or do not (9, 19) migrate preferentially to sites containing specific antigen were not designed to test this possibility. The events that occur when the T-cell mediators of anti-*Listeria* immunity enter a peritoneal exudate containing *Listeria* antigen will be the subject of a future publication.

It should be pointed out that it has been assumed, for the sake of discussion, that changes in the protective capacity of donor exudate cells as measured by log protection in the recipients' spleens were proportional to changes in the number of mediator cells present. While titration experiments showed that this was true for mediator cells harvested at any one time, it is nevertheless possible that the

protective efficiency, rather than the number of mediator cells, changes during the time course of infection and also during the development of an exudate. The same criticism can be leveled against all published studies that have quantified changes in the production of mediator cells during the time course of the immune response to allografts.

ACKNOWLEDGMENTS

This work was supported by Public Health Research grant AI-10351 from the National Institute of Allergy and Infectious Diseases.

The technical assistance of Jonathan Deissler is gratefully acknowledged.

LITERATURE CITED

1. Asherson, G. L., and G. G. Allwood. 1972. Inflammatory lymphoid cells: cells in immunized lymph nodes that move to sites of inflammation. Immunology **22**:493-502.
2. Biesecker, J. L. 1973. Cellular and humoral immunity as measured by ^{51}Cr cytotoxicity assay, after allogeneic tumor and renal transplantation. Transplantation **15**:298-307.
3. Canty, T. G., and J. R. Wunderlich. 1971. Quantitative assessment of cellular and humoral responses to skin and tumor allografts. Transplantation **11**:111-116.
4. Cheers, C., J. Sprent, and J. F. A. P. Miller. 1974. Interaction of thymus lymphocytes with histoincompatible cells. IV. Mixed lymphocyte reactions of activated thymus lymphocytes. Cell. Immunol. **10**:57-67.
5. Cohn, A., and M. Schlesinger. 1970. Absorption of guinea pig serum with agar: a method for eliminating its cytotoxicity for murine thymus cells. Transplantation **10**:130-132.
6. Cone, R. E., and J. J. Marchalonis. 1973. Antigen binding specificity of cell surface immunoglobulin isolated from T (helper) cells. Aust. J. Exp. Biol. Med. **51**:689-700.
7. Goldschneider, I., and R. B. Cohen. 1973. Immunoglobulin molecules on the surface of activated T lymphocytes in the rat. J. Exp. Med. **138**:163-175.
8. Gowans, J. L., D. D. McGregor, D. M. Cowen, and C. E. Ford. 1962. Initiation of immune responses by small lymphocytes. Nature (London) **196**:651-654.
9. Hall, J. G. 1967. Studies of the cells of afferent and efferent lymph nodes draining the site of skin homografts. J. Exp. Med. **125**:737-754.
10. Koster, F. T., D. D. McGregor, and G. B. Mackaness. 1971. The mediator of cellular immunity. II. Migration of immunologically committed lymphocytes into inflammatory exudates. J. Exp. Med. **133**:400-409.
11. Lance, E. M., and S. Cooper. 1972. Homing of specifically sensitized lymphocytes. Cell. Immunol. **5**:66-73.
12. Mackaness, G. B. 1962. Cellular resistance to infection. J. Exp. Med. **116**:381-406.
13. McGregor, D. D., and P. S. Logie. 1973. The mediator of cellular immunity. VI. Effect of the antimitotic drug vinblastine on the mediator of cellular resistance to infection. J. Exp. Med. **137**:660-674.
14. Mitchison, N. A. 1954. Passive transfer of transplantation immunity. Proc. Roy. Soc. London Ser. B **142**:72-87.
15. North, R. J. 1973. The cellular mediators of anti-*Listeria* immunity as enlarged population of short-lived, replicating T cells: kinetics of their production. J. Exp. Med. **138**:342-355.
16. North, R. J. 1973. Immunological control of macrophage proliferation in vivo. Infect. Immunity **8**:68-73.
17. North, R. J. 1974. T cell-dependent macrophage activation in cell-mediated anti-*Listeria* immunity, p. 166-179. *In* W. H. Wagner (ed.), Activation of macrophages. Excerpta Medica, Amsterdam.
18. Porter, K. A., and R. Y. Cane. 1960. The origin of the infiltrating cells in skin and kidney homografts. Transplant. Bull. **26**:458-469.
19. Prendergast, R. A. 1964. Cellular specificity of the homograft reaction. J. Exp. Med. **119**:377-387.
20. Roelants, G. E., A. Rydén, L-B. Hägg, and F. Loor. 1974. Active synthesis of immunoglobulin receptors for antigen by T lymphocytes. Nature (London) **247**:106-108.
21. Sprent, J., and J. F. A. P. Miller. 1972. Interaction of thymus lymphocytes with histoincompatible cells. III. Immunological characteristics of recirculating lymphocytes derived from activated thymus cells. Cell. Immunol. **3**:213-230.
22. Tilney, N. L., and W. L. Ford. 1974. The migration of rat lymphoid cells into grafts. Some sensitized cells localise preferentially in specific allografts. Transplantation **17**:12-21.
23. Turk, J. L., and J. Oort. 1963. A histological study of the early stages of the tuberculin reaction after passive transfer of cells labeled with (^{3}H) thymidine. Immunology **6**:140-148.
24. Valeriote, F. A., and W. R. Bruce. 1965. An *in vitro* assay for growth-inhibiting activity of vinblastine. Cancer Res. **35**:851-856.
25. Werdelin, O., and R. T. McCluskey. 1971. The nature and specificity of mononuclear cells in experimental autoimmune inflammations and the mechanisms leading to their accumulations. J. Exp. Med. **133**:1242-1263.