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VII. Localization of Sensitized Lymphocytes in Inflammatory Exudates*

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Earlier reports in this series (1, 2) focused upon the cytokinetics and properties of the specifically sensitized lymphocytes which collaborate with macrophages in host resistance to *Listeria monocytogenes*. Evidence was obtained that the sensitized lymphocytes are generated in animals primarily immunized with the living organism. As a population, they have a rapid turnover, short effective life-span, and fail to recirculate efficiently from the blood to lymph. These and other findings imply that immunoblasts or "large lymphocytes" are the principal, although possibly not the exclusive, specific effector cells responsible for transferring resistance to *L. monocytogenes*, at least in the rat.

Several lines of evidence indicate that specifically sensitized lymphocytes collaborate with macrophages in the expression of cellular resistance to infection (3, 4). Although the mechanism underlying their collaboration has not yet been determined, it is plausible to think that meaningful interactions occur locally in foci of infection. It may be significant therefore that after intravenous injection into rats, immunoblasts from the thoracic duct lymph of *Listeria*-infected donors localize in substantial numbers in inflammatory exudates induced in the peritoneal cavity (5). The results of the current investigation reaffirm and extend this observation by showing that immunoblasts move from the blood into bacteria-induced exudates in a functionally active condition and for reasons other than their immunological commitment. In addition, they indicate that the immigrant cells and small lymphocytes derived from them have protective properties.

Materials and Methods

Animals.—The subjects of this study were male and female (Lewis × DA) F_1 hybrid rats. Cell donors weighed 180–240 g. The recipients fell into two groups with respect to body weight: rats in whom antimicrobial resistance was measured weighed 70–100g, whereas those given radioactively labeled cells were approximately the same weight as the donors.

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Microorganisms.—*L. monocytogenes*, strain EGD, was passaged repeatedly in mice. A suspension of the organisms prepared from a 16 h trypticase soy broth culture of infected spleen was stored in liquid nitrogen from which it was recovered as needed. *Mycobacterium bovis*, strain BCG Montreal (Trudeau mycobacteria culture [TMC] no. 1066 B) was maintained by serial passage in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.). Cultures were used after 5–8 days of incubation at 37°C. *Francisella tularensis*, strain LVS[1] was passaged repeatedly in rats, recovered from infected spleen, and grown in "T" medium (6) to a density of approximately 5×10^8 organisms/ml. The cultures were maintained at 4°C for up to 2 wk without loss of viability or virulence.

Immunization of Rats and Measurement of Protective Immunity.—Rats were immunized with approximately 5×10^6 living organisms. The usual practice was to inject the organisms subcutaneously at multiple sites in the hind quarters; but in one experiment they were injected into the forequarters and nape of the neck. The immunity engendered by this procedure was measured indirectly, in terms of the specific resistance conferred on normal recipients by an injection of cells obtained from infected donors (1).

Cells.—Thoracic duct lymphocytes (TDL)[2] were obtained from freshly incannulated donor rats. The lymph was collected for 16–24 h into heparinized Ringer's solution without added antibiotics. Peritoneal exudate cells (PEC) were obtained from rats which had been simulated intraperitoneally with either 50 μg or 100 μg of alcohol-killed *L. monocytogenes*, killed BCG, or killed *Salmonella typhi*.[3] The technique for obtaining cells from the peritoneal cavity and the cellular composition of the inflammatory exudates have been described elsewhere (5).

Radioactive Labeling of TDL.—Immunoblasts were selectively labeled by incubating TDL in vitro for 1 h at 37°C in medium containing 0.5 μCi/ml of either [^{3}H]thymidine (3 Ci/mmol, New England Nuclear, Boston, Mass.) or [^{14}C]thymidine (40–60 mCi/mmol). This procedure labels 30–60% of immunoblasts in the thoracic duct lymph of infected rats; small lymphocytes do not become labeled, because the latter rarely if ever divide during the brief period that they are exposed to the radionucleoside (7, 8).

A labeled cell population made up almost entirely of small lymphocytes was obtained from the thoracic duct lymph of rats which had been given 14 daily injections of [^{3}H]thymidine (1 μCi/g). The last injection was given 15 days before incannulation. During the "rest" interval, two changes occur in the distribution of radioactivity among the cells in central lymph: radioactivity in immunoblasts is diluted as the cells divide, and the number of labeled small lymphocytes with a short-circulating life-span falls to a low value (9). The effect is to concentrate radioactivity in a subset of small lymphocytes that have a potentially long life-span.

An effort was made to minimize reutilization of radioactive residues. Prospective donors of labeled small lymphocytes were offered 10^{-4} M "cold" thymidine in their drinking water beginning immediately after the last injection of [^{3}H]thymidine. In addition, the recipients of either labeled small lymphocytes or labeled immunoblasts were injected subcutaneously with 1 ml of 0.85% sodium chloride containing 24 μg of cold thymidine. These animals also were offered 10^{-4} M thymidine in their drinking water beginning 48 h before cell transfer.

Measurement of Radioactivity.—10^8 TDL or PEC from individual rats were extracted with 15 ml of ice-cold 5% TCA. The insoluble pellet was then digested for 18 h at 50°C with 0.5 ml of NCS solubilized (Amersham/Searle Corp., Arlington Heights, Ill.), neutralized with 0.02 ml of glacial acetic acid and added to 10 ml of PCS scintillant (Amersham/Searle Corp.).

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[1] A gift from Dr. Quentin Myrvik, Bowman Gray School of Medicine, Winston-Salem, N. C.

[2] *Abbreviations used in this paper:* TDL, thoracic duct lymphocytes; PEC, peritoneal exudate cells; MGC, mean grain count.

[3] Kindly supplied by Dr. Philip Carter, Trudeau Institute, Saranac Lake, N. Y.

The mesenteric lymph node was homogenized in 5 ml of 5% TCA, washed twice in TCA, and digested in 1.0 ml of NCS solubilizer. In each case, radioactivity was measured in a Beckman LS-100 liquid scintillation system (Beckman Instruments, Inc., Fullerton, Calif.) and corrected for background radioactivity and quenching.

Autoradiography.—Methanol-fixed smears of TDL were extracted for 20 min with two changes of cold 5% TCA and then washed for at least 1 h in cold running water. They were subsequently coated with NTB-2 liquid photographic emulsion (Eastman Kodak Co., Rochester, N. Y.) and were exposed for 3 wk. The autoradiographs were stained after development with May-Grünwald-Giemsa stain.

Statistical Analysis.—Statistical significance between groups was determined by analysis of variance.

RESULTS

Transfer of Immunity by PEC.—

Peritoneal exudates induced in rats primarily immunized with *L. monocytogenes* contain many specifically sensitized lymphocytes. The protective cells emerge from the blood in response to inflammation; they do not arise from precursors normally present in the peritoneal cavity. This conclusion was drawn from experiments in which PEC and residential cells from the unstimulated peritoneal cavity of *Listeria*-infected rats were assayed for their protective capacity after transfer to normal recipients. In a typical experiment, a group of donor rats were each immunized with 5.2×10^6 *L. monocytogenes*. The organisms were injected subcutaneously into the forequarters and nape of the neck, the object being to concentrate the cellular response to infection in lymph nodes remote from the peritoneal cavity. 6 days later, some of the animals were stimulated intraperitoneally with 50 μg of killed *L. monocytogenes;* the remainder served as unstimulated controls. PEC obtained 24 h after stimulation, and residential cells from the peritoneal cavity of the control group, were transferred into normal recipients. The cells were injected intravenously1 h after the recipients had been challenged by the same route with 3.60×10^6 *L. monocytogenes*. With respect to the level of immunity conveyed by the donor cells, two comparisons were made: (*a*) PEC and the residential cells were compared on a cell-for-cell basis, i.e., equal numbers of cells were transferred, and (*b*) cells from 15 donors in each group were transferred into an equal number of recipients.

The results in Fig. 1 indicate that the residential cells had only a trivial influence upon the growth of the challenge organisms in the liver and spleen. By comparison, PEC conferred resistance in proportion to the number of cells transferred. Although the design of the experiment made no allowance for the different proportions of lymphocytes, macrophages, and other cell types in the two inocula, it is evident that protective cells were present in substantial numbers only in the cell population retrieved from exudates.

Delivery of Sensitized Lymphocytes to the Thoracic Duct and Induced Peritoneal Exudates.—

If the specifically sensitized lymphocytes which assemble in the inflamed peritoneal cavity of *Listeria*-infected rats arise in regionally stimulated lymphoid tissue, one might expect that their arrival in induced peritoneal exudates would coincide or closely overlap with their appearance in the blood. This proposition was tested in an experiment in which rats were infected in the hind quarters, a procedure that favors the delivery of sensitized lymphocytes to the blood by way of the thoracic duct. The adoptive transfer technique was then used to

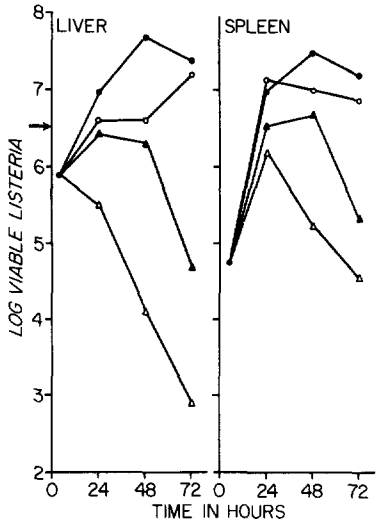

FIG. 1. Curves describing the growth of *L. monocytogenes* in the tissue of normal rats (●—●) and rats immunized adoptively with cells from *Listeria*-infected donors. ○—○: recipients of 19.4×10^6 cells from the unstimulated peritoneal cavity. ▲—▲: recipients of 19.4×10^6 PEC. △—△: recipients of 62.9×10^6 PEC (see text). Means of five animals per time point.

compare the level of immunity conveyed by a weight-adjusted dose of TDL or PEC obtained at predetermined intervals after infection. Since PEC out-perform TDL on a cell-for-cell basis (5), four times as many TDL were transferred into recipient rats so that similar levels of protection would be realized at the peak of the response. The cells were transferred intravenously 1 h after the recipients had been challenged by the same route with approximately 2×10^6 *L. monocytogenes*.

As there was a small but unavoidable difference in the number of organisms in the challenge inocula, the results are expressed in Fig. 2 as the mean difference in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized rats 48 h after challenge. They indicate that sensitized lymphocytes appeared in induced peritoneal exudates soon after they could be demonstrated in the thoracic duct, and that the protective capacity of PEC paralleled the performance of the lymph-borne cells. Since the latter have been identified as immunoblasts (2), it is plausible to think that cells of this type make an important contribution to the protective cell population in peritoneal exudates induced in *Listeria*-infected rats. Evidence to support this notion was obtained in the following experiments using the antimitotic drug, vinblastine.

Effect of Vinblastine on the Protective Capacity of PEC.—

Studies using autoradiographic techniques have shown that after intravenous injection, labeled immunoblasts from the thoracic duct lymph of donor rats accumulate in substantial numbers in peritoneal exudates induced by killed bacteria (2, 8). The exudate-seeking cells

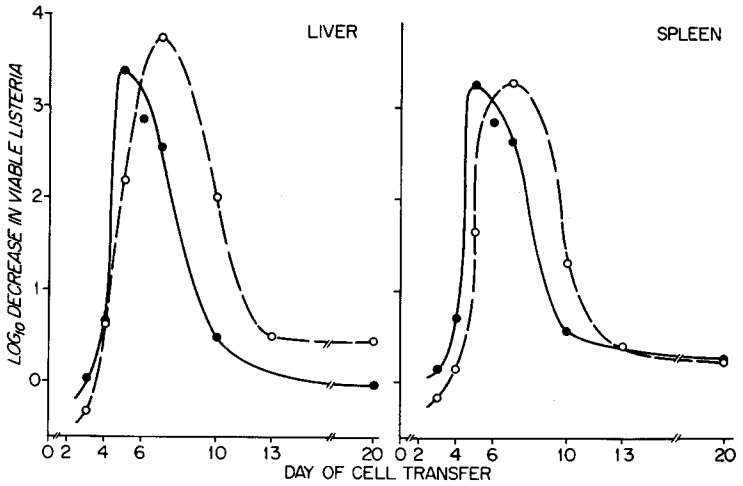

FIG. 2. Curves illustrating the rate of delivery of specifically sensitized lymphocytes to the thoracic duct and the arrival of such lymphocytes in the inflamed peritoneal cavity of *Listeria*-infected rats. Peritoneal exudates were harvested 24 h after stimulation with 50 μg of killed *L. monocytogenes*. Each point represents the mean difference in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls 48 h after an intravenous *Listeria* challenge. ●—●: immunity conveyed by 2×10^6 TDL/g. ○—○: Immunity conveyed by 5×10^5 PEC/g.

give rise by cell division and cell differentiation to a population of more lightly labeled small lymphocytes. Since immunoblasts have a rapid turnover while small lymphocytes do not (7), these two cell types differ in their susceptibility to inhibition by drugs such as vinblastine (2) that are selectively toxic for dividing cells. Vinblastine was therefore used in experiments designed to measure the contributions made by immunoblasts and small lymphocytes to protective cell populations obtained from the inflamed peritoneal cavity of *Listeria*-infected rats.

The general plan was as follows. A large panel of rats were primarily immunized with approximately 5×10^6 *L. monocytogenes*. 6 days later, exudates were induced by stimulating the animals intraperitoneally with killed *Listeria*. On the day of exudate induction, or at predetermined intervals thereafter, groups of five stimulated rats were given a single pulse of vinblastine (5 μg/g of body weight). The drug was injected intravenously 24 h before the exudates were harvested. PEC obtained from these animals, and exudate cells from similarly stimulated controls, were transferred intravenously into an equal number of recipient rats 1 h after the latter had been challenged with 5.7×10^5–1.4×10^6 *L. monocytogenes*.

The results of three separate experiments have been combined in Fig. 3 where the level of immunity conveyed by the individual inocula is expressed as the mean difference in log viable units found 48 h after challenge in the livers and spleens of adoptively immunized subjects and groups of nonimmunized controls. It is evident that maximum protection was transferred by PEC obtained from infected donors 24 h after stimulation, at a time when the cells were highly vulnerable to inhibition by vinblastine. Thereafter the level of immunity decreased while the protective cell population exhibited increasing resistance to the drug. The results accord with the view that protective cells arrive in exu-

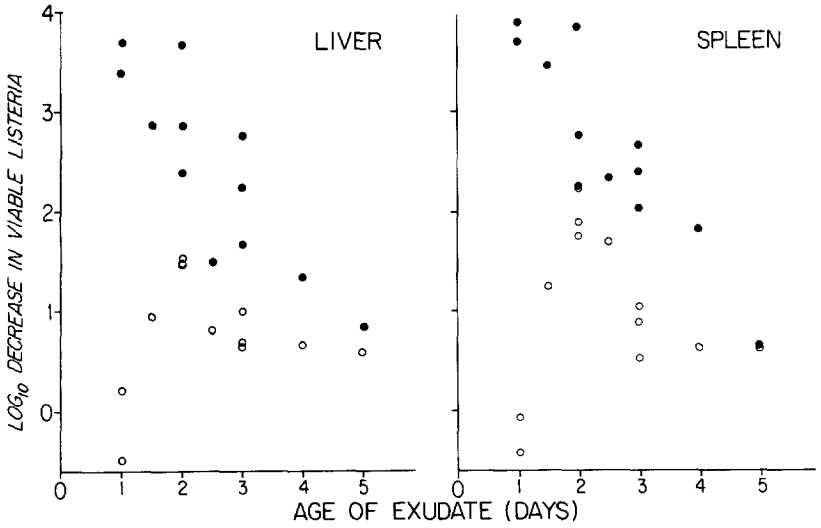

FIG. 3. Plots depicting the level of immunity conveyed by PEC obtained from donor rats which, on the 6th day of an immunizing *Listeria* infection, were stimulated intraperitoneally with 50 μg of killed *L. monocytogenes*. At predetermined intervals after stimulation, cells from five donors were transferred intravenously into an equal number of recipient rats. Each plot represents the mean difference in viable units found 48 h after challenge in the tissues of such adoptively immunized subjects and a group of five nonimmunized controls. The closed circles denote the level of resistance transferred by PEC from infected but otherwise untreated donors; the open circles denote the level of resistance conveyed by PEC from similarly infected donors that were given a single injection of vinblastine (see text). When given at the time of exudate induction, vinblastine abrogated the protective capacity of the transferred cells, thereafter the cells showed increasing resistance to the drug.

dates in a blast condition where they give rise locally to nondividing small lymphocytes that also have protective properties. It is to be emphasized, however, that exudates harvested up to 4 days after stimulation contained some vinblastine-sensitive lymphocytes. The question arises therefore as to whether these vinblastine-sensitive cells are recent immigrants from the blood or the dividing progeny of immunoblasts that localized in the inflamed peritoneal cavity during the early postinduction period. This problem was examined by measuring the assembly of labeled immunoblasts in peritoneal exudates over a 5-day period after stimulation.

Assembly of Labeled Immunoblasts in Exudates.—

2×10^8 TDL collected from donor rats on the 6th day of an immunizing *Listeria* infection were labeled in vitro with [^{14}C]thymidine, washed, and transferred intravenously into each of 30 unlabeled but similarly immunized recipients. The latter were stimulated intraperitoneally with 50 μg of killed *L. monocytogenes* either on the day of cell transfer or 1–4 days before transfer. A second panel of immunized recipients were given an equal number of labeled TDL which had been heated at 56°C for 30 min. The timing of the immunizing infection and stimulation of the recipients was such that all were transfused on the same day. 24 h after

cell transfer, the peritoneal exudates were harvested, the cells enumerated, and their radioactivity determined.

The results are shown in Fig. 4 where it can be seen that exudates induced in recipients of either living or heat-killed TDL contained approximately the same number of cells. However, only the former contained more than a trivial level of cell radioactivity. The second significant finding was that exudates induced on the day that living TDL were infused contained more labeled cells than exudates which had been induced up to 4 days before cell transfer. Since the radioactivity was concentrated in donor immunoblasts, the only cells to incorporate thymidine in vitro, it would seem that immunoblasts are recruited in substantial numbers from the blood only during the first 24 h after exudate induction.

Fate of Intravenously Injected Small Lymphocytes.—

It was concluded from an earlier study (8) that small lymphocytes rarely localize in acute inflammatory exudates. The investigations, however, were limited to an analysis of cell emigration during the early postinduction period. It was of interest therefore to determine

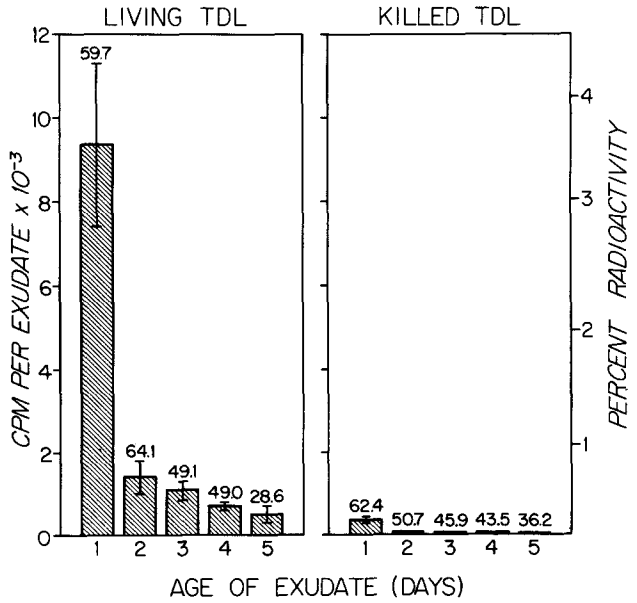

FIG. 4. Cell-associated radioactivity in peritoneal exudates of various ages 24 h after 2×10^8 [^{14}C]thymidine-labeled TDL were transfused into a panel of *Listeria*-infected rats. The labeled cells were obtained from similarly infected donors. They were transferred either on the day of exudate induction or 1–4 days after induction. Living cells alone localized in substantial number in the exudates, and then only when the cells were transferred on the day of exudate induction. The mean number of PEC obtained from the individual recipient groups is shown above each column. Means of 6 ± SD.

whether small lymphocytes extravasate later in the response. Their ability to do so could account, at least in part, for the observed presence in subacute exudates of sensitized lymphocytes which are resistant to inhibition by vinblastine. This possibility was tested by measuring the exudate-seeking capacity of radiolabeled small lymphocytes after their transfusion into *Listeria*-infected rats bearing peritoneal exudates of various ages.

The donor cells were obtained from the thoracic duct lymph of normal rats which had received the last of a series of [^{3}H]thymidine injections 15 days before incannulation. Autoradiographs prepared from a sample of the inoculum revealed that radioactivity was vested mainly, but not entirely, in small lymphocytes. 4×10^8 TDL were transfused into each of 25 recipients. The latter were sacrificed 24 h later at which time the exudates were harvested, the cells enumerated, and their radioactivity determined in the usual manner. In addition, the mesenteric lymph node from the same recipients was similarly processed for radiometric analysis.

The results in Fig. 5 indicate that exudates induced on the day of cell transfer contained a very low level of cell-associated radioactivity. Although the labeled cells were not identified morphologically, it is logical to postulate that they were lightly labeled immunoblasts, some of which had been transfused

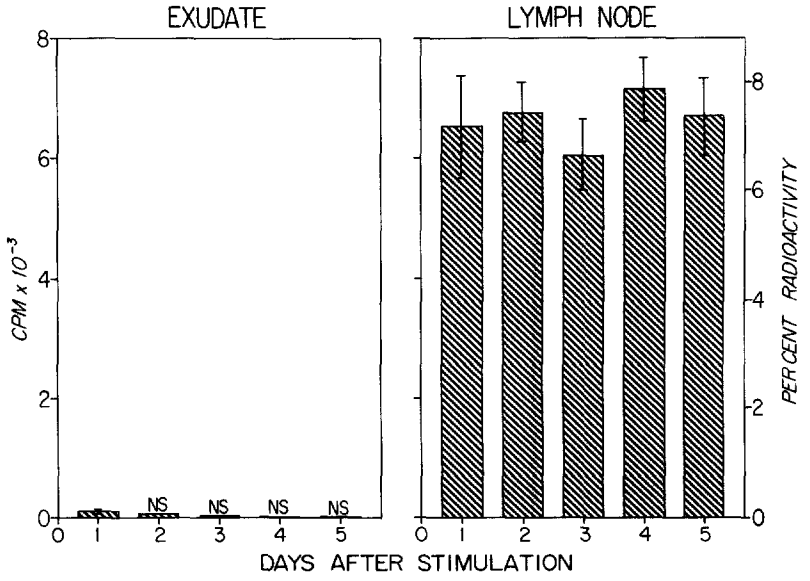

FIG. 5. Cell-associated radioactivity in peritoneal exudates of various ages and in the mesenteric lymph nodes of *Listeria*-infected rats 24 h after the animals had been transfused with 4×10^8 [^{3}H]thymidine-labeled TDL (see text). From an autoradiographic analysis, it was determined that the donor inoculum contained 52.9×10^6-labeled small lymphocytes (MGC, 10.1) and 0.4×10^6-labeled immunoblasts (MGC, 5.0). The donor cells were transferred either on the day of exudate induction or 1–4 days after induction. A few labeled cells localized in exudates induced on the day of cell transfer; thereafter the level of radioactivity in the exudates was not significantly elevated above the background. By comparison, a high and relatively uniform level of radioactivity was found in the mesenteric lymph nodes. Means of 5 ± SD. NS, not significant.

along with a much larger number of labeled small lymphocytes. Regardless of their identity, it is evident that even fewer labeled lymphocytes localized in exudates which had been induced 24 h or longer before cell transfer. In fact, the level of radioactivity in such exudates was not significantly elevated ($P > 0.05$) above the background radioactivity in exudates induced in a panel of untransfused controls. Failure of labeled small lymphocytes to extravasate in the inflamed peritoneal cavity cannot be ascribed to their poor condition or intensity of labeling, because labeled donor cells accumulated in substantial numbers in the mesenteric lymph node (Fig. 5), a tissue through which small lymphocytes normally pass as they recirculate from the blood to lymph (10).

Effect of Specific Antigen on the Localization of Immunoblasts in Exudates.—

The propensity of immunoblasts to localize in the inflamed peritoneal cavity during the immediate postinduction period raises the question as to whether their assembly in exudates is influenced by an immunological mechanism. To test this possibility, immunoblasts obtained from the thoracic duct lymph of rats infected with *L. monocytogenes* or BCG were radioactively labeled in vitro. Cells from the *Listeria*-infected donors were labeled with [^{14}C]thymidine while those from BCG-infected donors were labeled with [^{3}H]thymidine. After thorough washing, the two cell suspensions were mixed in equal numbers, and a total of 2×10^8 were transferred intravenously into normal recipients. The cells were transfused 4 h after the recipients had been stimulated intraperitoneally with 100 μg of killed *L. monocytogenes*, 100 μg of killed BCG, or 100 μg of killed *S. typhi*. Exudates induced in this manner were harvested 24 h after induction. PEC from individual animals were enumerated and their radioactivity determined by a dual channel counting procedure.

The results in Table I indicate that while there was no significant difference in total cell radioactivity in the various exudates, there was a highly significant difference ($P < 0.01$) in the proportion of ^{14}C-labeled cells and ^{3}H-labeled cells in exudates induced by *L. monocytogenes* or BCG. Paradoxically, more ^{14}C-labeled immunoblasts from donors immunized with *L. monocytogenes* accumulated in exudates induced by BCG than in exudates induced by the homologous organism. Similarly, exudates induced by *L. monocytogenes* were relatively rich

TABLE I

*Failure of Exudate-Seeking Immunoblasts from Infected Rats to Localize Preferentially in Peritoneal Exudates Induced by the Homologous Parasite**

Exudate stimulus	No. exudate cells $\times 10^{-6}$	cpm per exudate‡		
		Total	^{14}C	^{3}H
L. monocytogenes	36.3	3,390	1,742	1,648
BCG	32.2	3,673	2,388	1,285
S. typhi	49.9	3,305	1,945	1,360

* Rats stimulated intraperitoneally with 100 μg of killed bacteria. 4 h later, the same animals were injected intravenously with a mixture of 10^8 [^{14}C]thymidine-labeled TDL from *Listeria*-immune donors and 10^8 [^{3}H]thymidine-labeled TDL from BCG immune donors.

‡ Exudates harvested 24 h after cell transfer. Means of six.

in ^{3}H-labeled immunoblasts which had come from donors immunized with BCG. It would therefore seem that if immunologically specific forces are at work in directing the tissue disposition of immunoblasts, their effect is to deflect specifically sensitized cells from the inflamed peritoneal cavity.

Localization of Immunoblasts in Exudates Induced in Specifically Immunized Subjects.—

Although intravenously injected immunoblasts show no obvious preference for exudates induced by the homologous parasite, their entrapment in exudates is influenced by the immune status of the host, and presumably by the presence or absence of delayed-type hypersensitivity toward antigens of the eliciting stimulus. This conclusion was drawn from an experiment in which labeled immunoblasts were transfused into rats which had themselves been immunized with *L. monocytogenes* or BCG. The donor cells were obtained from rats infected with a third party organism, *F. tularensis*. The cells were labeled in vitro with [^{14}C]thymidine, and 2×10^{8} were transferred intravenously into individual recipients 4 h after the latter had been stimulated intraperitoneally with either killed *Listeria* or killed BCG. The number of cells and concentration of cell radioactivity in exudates induced in this manner were determined 24 h after cell transfer.

Fig. 6 indicates that labeled donor cells were found in all exudates. Considerable variation was observed from recipient to recipient; however, animals

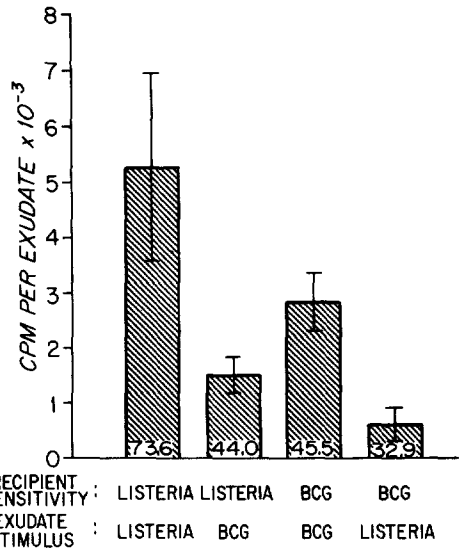

FIG. 6. Cell-associated radioactivity in peritoneal exudates 24 h after stimulation with 50 μg of killed *L. monocytogenes* or 50 μg of killed BCG. 4 h after exudate induction, the animals were transfused with 2×10^{8} [^{14}C]thymidine-labeled TDL from donors infected with *F. tularensis*. A higher level of radioactivity was found in exudates induced in rats stimulated by an organism to which they had been specifically sensitized. The mean number of PEC obtained from the individual recipient groups is shown at the base of each column. Means of $6 \pm$ SD.

stimulated by organisms to which they had been specifically sensitized developed exudates that were more cellular and contained higher levels of radioactivity. Since the labeled donor cells employed in this experiment came from rats immunized with *F. tularensis*, they would not be expected to show an obvious preference for exudates induced by *L. monocytogenes* or BCG. It would therefore seem that the assembly of immunoblasts in the inflamed peritoneal cavity was influenced by peculiarities of the inflammatory response. Apparently, the intense inflammation provoked in rats stimulated by an organism against which they had been previously immunized encouraged the local extravasation of immunoblasts regardless of their immunological leanings.

DISCUSSION

It has been previously shown that acquired resistance to *L. monocytogenes* is cell mediated in the rat as it is in the mouse (11, 12), and that the expression of resistance involves the collaboration of macrophages and specifically sensitized lymphocytes (13). The latter are generated in a response to an immunizing *Listeria* infection and can be demonstrated by their capacity to protect normal recipients against a challenge infection with this organism. Further, it has been demonstrated that peritoneal exudates induced in *Listeria*-infected rats are rich in specifically sensitized lymphocytes (5). The results of the current investigation not only substantiate this finding but go further in showing that the protective cells in exudates derive from the blood and are delivered to the peritoneal cavity in a blast condition.

Several lines of evidence indicate that the sensitized lymphocytes in induced exudates arise at sites remote from their final destination. In the current investigation, for example, a comparison was made of the protective capacity of PEC and residential cells obtained from the "unstimulated" peritoneal cavity of *Listeria*-infected rats. Whereas normal recipients could be adoptively immunized with PEC, the residential cells afforded little if any protection. This finding implies that sensitized lymphocytes have little tendency to leave the circulation except in the presence of inflammation.

Further evidence that the protective cells in exudates derive from the blood and are lineally related to those present in central lymph was provided by the results of a comparative analysis of the immunity transferred adoptively by TDL and PEC obtained from donor rats at various intervals after an immunzing *Listeria* infection. Protective cells appeared in the exudates soon after they could be demonstrated in the lymph. Thereafter a parallel was observed in the sequential increase and decay of immunity conveyed by the two inocula. The simplest, but not necessarily the only, interpretation of this finding is that the protective cells belong to a single population of sensitized lymphocytes.

Since the lymph-borne cells which transfer cellular resistance to *L. monocytogenes* are predominantly immunoblasts, it is plausible to think that cells of this type preferentially localize in inflammatory foci. That this in fact is the

case was demonstrated in experiments in which radiolabeled immunoblasts from the thoracic duct lymph of *Listeria*-infected rats were transfused into syngeneic recipients with induced peritoneal exudates. In these circumstances, a substantial portion of the donor cell radioactivity (up to 4%) was found later in the exudates. But, the timing of cell transfer with respect to challenge was crucial. Thus, labeled donor immunoblasts arrived in large numbers in the inflamed peritoneal cavity only when the cells were transferred on the day of exudate induction. The results imply that the extravasation of immunoblasts, including those which transfer cellular resistance to infection, is encouraged by evanescent changes in the microcirculation that follow the intraperitoneal injection of killed bacteria.

In contrast to immunoblasts, radiolabeled small lymphocytes rarely localize in induced peritoneal exudates. This is true regardless of whether the labeled cells are transferred on the day of exudate induction or up to 4 days after induction. Taken at face value, the results suggest that small lymphocytes lack surface features that enable them to engage complementary sites on inflamed vascular endothelium. It must be remembered, however, that the observations that support this contention were made in an experiment in which rats with induced peritoneal exudates were given TDL from donors that had received the last of a series of [^{3}H]thymidine injections more than 2 wk before incannulation. This procedure was used with the express purpose of labeling small lymphocytes at the expense of immunoblasts. While this objective was realized, the effect was to concentrate radioactivity in small lymphocytes that have a potentially long life-span (9, 14). Since small lymphocytes generated in the "rest" interval after the last injection of [^{3}H]thymidine would not be expected to be labeled, it could be argued that newly formed small lymphocytes or possibly a subset of "short-lived" cells have an exudate-seeking capacity similar to that demonstrated for immunoblasts. This possibility cannot be discounted; for when rats with induced peritoneal exudates are transfused with TDL obtained from donors given a course of [^{3}H]thymidine injections immediately before incannulation, labeled small lymphocytes, in addition to immunoblasts, are found later in the exudates (2). While some of these labeled small lymphocytes may have derived from exudate-seeking immunoblasts that escaped the inhibiting influence of vinblastine, others may have emigrated from the blood in a fully differentiated condition.

Although the exudate-seeking capacity of newly formed small lymphocytes has not yet been determined, it is clear that small lymphocytes with protective properties either fail to extravasate or do so only rarely during the early post-induction period. This conclusion was drawn from experiments in which vinblastine was used to evaluate the contributions made by immunoblasts and by nondividing (small) lymphocytes to the protective cell population in peritoneal exudates induced in *Listeria*-infected rats. A single injection of vinblastine given to such animals at the time of exudate induction virtually abolished

the protective capacity of cells obtained from the peritoneal cavity. Since small lymphocytes are unaffected by the drug (2) it would seem that the specifically sensitized cells which transfer resistance to *L. monocytogenes* are recruited from the circulation in a blast condition, at least during the first few hours after exudate induction.

An entirely different result was obtained when vinblastine was given to *Listeria*-infected rats 12 h or longer after the animals had been stimulated intraperitoneally. In these circumstances the drug failed to negate the protective immunity transferred by PEC. The latter finding is interesting for two reasons: (*a*) It implies that small lymphocytes as well as immunoblasts have protective properties, and (*b*) it accords with the results of the transfusion experiments and an earlier autoradiographic study (8) which indicate that many exudate small lymphocytes arise locally from immunoblasts that extravasate during the early postinduction period.

The impact of vinblastine on the sensitized lymphocytes which transfer cellular resistance to *L. monocytogenes* merits further comment. In the foregoing experiments, it was found that a measure of antimicrobial resistance was conveyed by PEC obtained from vinblastine-treated donors up to 5 days after stimulation. This observation is remarkable inasmuch as immunoblasts are recruited in substantial numbers from the blood only during the first 24 h after exudate induction. It would therefore seem that once exudate-seeking immunoblasts have emerged from the blood they generate more of their own kind before differentiating to small lymphocytes.

The results of our own earlier studies (8) and those of Moore and Hall (15) strongly suggest that immunoblasts are prone to leave the circulation at sites of bacterial implantation, and that their assembly in inflammatory exudates is determined by a general property of the cells rather than their immunological commitment. Further evidence to support this notion was obtained in the current investigation in which two radiolabeled preparations of thymidine were used to separately label immunoblasts from the thoracic duct lymph of rats infected with *L. monocytogenes* or BCG. When TDL labeled in this manner were mixed in equal numbers and transfused into syngeneic recipients they "homed" with approximately equal facility into exudates induced by *L. monocytogenes*, BCG, or the unrelated parasite *S. typhi*. Failure to demonstrate specific recruitment of immunoblasts into the inflamed peritoneal cavity does not exclude the possibility that the specificity of individual cells influences their localization and retention at sites of microbial invasion. Nevertheless, the results do indicate that the specificity of the cells is not a major factor influencing their recruitment into lesions.

It is to be emphasized that the foregoing conclusions were drawn solely from studies of the self-limiting inflammation induced by minute quantities of killed bacteria. Therefore quantitative or even qualitative differences in the influx of lymphocytes may occur during the protracted inflammation induced by liv-

ing mycobacteria and other organisms that are highly resistant to intracellular inactivation. Indeed, our own unpublished observations indicate that immunoblasts are recruited for at least 8 days into peritoneal exudates induced by living BCG. This finding accords with an earlier report by North et al. (16), which emphasized that pyroninophylic lymphocytes with the structural features of immunoblasts are a conspicuous element of the cell population in developing tubercles in the livers of intravenously infected mice.

Heterogeneity among the specifically sensitized lymphocytes involved in cell-mediated reactions has been described in a variety of experimental situations (17–20). The question arises therefore as to whether subpopulations of antigen-activated T cells, as defined by turnover, circulating life span, and tissue disposition, belong to the same cell line or to different cell lines. While this question cannot be answered categorically, the demonstrated relationship between labeled immunoblasts and small lymphocytes within inflammatory exudates provides inferential evidence that the expression of cellular resistance to infection involves the activity of a single line of specific effector cells.

It is possible of course that antigen-activated T cells contribute in different ways to the hosts' defense depending upon their stage of differentiation. For instance, the ability of T immunoblasts to divide locally in inflammatory foci could be the basis of an amplification device whereby the animal focusses his cellular defenses at sites of microbial invasion. The idea that nondividing (small) lymphocytes also have an effector function accords with the documented dissociation of the DNA synthetic response of lymphocytes to T mitogens and their cytotoxic properties (21–23), capacity to produce mediators (24–26) and ability to support the growth of certain RNA viruses (21).

Since circulating small lymphocytes either fail to localize in acute inflammatory foci or do so only rarely, they would seem to be at a strategic disadvantage in regard to their ability to influence events occurring at sites of microbial implantation. Nevertheless, circulating small lymphocytes could have an important role in protecting the host against reinfection. For instance, this would be true if the cells were to carry memory of microbial antigens. Small lymphocytes specifically committed to the antigens of a particular organism would be expected to settle out in the regionally stimulated lymphoid tissue of animals reinfected with the parasite. In this location, they would differentiate rapidly into the immunoblasts, thereby arming the host with an expanded cohort of specific effector cells with properties that enable them to assemble automatically in infected tissue.

SUMMARY

Peritoneal exudates induced in rats infected with *Listeria monocytogenes* contain sensitized lymphocytes which can protect normal recipients against a *Listeria* challenge. The protective cells arise in lymphoid tissue remote from the peritoneal cavity. Those formed in the caudal lymph nodes of subcutaneously

infected rats are delivered to the thoracic duct and hence to the blood from where they are drawn into exudates. Immunoblasts are the most immature members of this protective cell population and they alone among the cells in central lymph localize in exudates induced by killed bacteria. They do so in substantial numbers, but only during the early postinduction period.

The "homing" of immunoblasts to inflammatory foci seems to be determined by a general property of the cells rather than their immunological commitment; however, the intense inflammation induced by organisms to which an animal has been specifically sensitized is accompanied by an exuberant influx of immunoblasts into lesions. Sensitized lymphocytes that extravasate in the inflamed peritoneal may generate more of their own kind, but some give rise to small lymphocytes. The latter also have protective properties and, with time, comprise an increasing portion of the protective cell population.

The results imply that the tissue disposition of sensitized lymphocytes in the body is determined by a complementary relationship between blood-borne immunoblasts and vascular endothelium in inflamed tissue. The results also provide a plausible explanation for the concentration of sensitized lymphocytes at sites of microbial implantation where they alone would be expected to collaborate with monocyte-derived macrophages in the control of infection.

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REFERENCES

1. McGregor, D. D., F. T. Koster, and G. B. Mackaness. 1971. The mediator of cellular immunity. I. The life-span and circulation dynamics of the immunologically committed lymphocyte. *J. Exp. Med.* **133**:389.
2. 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.
3. Mackaness, G. B. 1971. Resistance to intracellular infection. *J. Infect. Dis.* **123**: 439.
4. McGregor, D. D., and P. S. Logie. 1974. Macrophage-lymphocyte interactions in infection immunity. *In* Mononuclear Phagocytes in Immunity, Infection and Pathology. R. Van Furth, editor. Blackwell Scientific Publications Ltd., Oxford. In press.
5. 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.
6. Tressett, H. B., and M. K. Ward. 1964. Blood-free medium for the rapid growth of Pasteurella tularensis. *Appl. Microbiol.* **12**:504.
7. Gowans, J. L. 1962. The fate of parental strain small lymphocytes in F_1 hybrid rats. *Ann. N. Y. Acad. Sci.* **99**:432.
8. Koster, F. T., and D. D. McGregor. 1971. The mediator of cellular immunity. III. Lymphocyte traffic from the blood into the inflamed peritoneal cavity. *J. Exp. Med.* **133**:864.

9. Caffrey, R. W., W. O. Rieke, and N. B. Everett. 1962. Radioautographic studies of small lymphocytes in the thoracic duct of the rat. *Acta Haematol. (Basel).* **28:**145.
10. Gowans, J. L., and E. J. Knight. 1964. The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **159:**257.
11. Mackaness, G. B. 1962. Cellular resistance to infection. *J. Exp. Med.* **116:**381.
12. Mackaness, G. B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. *J. Exp. Med.* **129:**973.
13. McGregor, D. D., and F. T. Koster. 1971. The mediator of cellular immunity. IV. Cooperation between lymphocytes and mononuclear phagocytes. *Cell. Immunol.* **2:**317.
14. Everett, N. B., R. W. Caffrey, and W. O. Rieke. 1964. Recirculation of lymphocytes. *Ann. N. Y. Acad. Sci.* **113:**887.
15. Moore, A. R., and J. G. Hall. 1973. Non-specific entry of thoracic duct immunoblasts into intradermal foci of antigens. *Cell. Immunol.* **8:**112.
16. North, R. J., G. B. Mackaness, and R. W. Elliott. 1972. The histogenesis of immunologically committed lymphocytes. *Cell. Immunol.* **3:**680.
17. Asofsky, R., H. Cantor, and R. E. Tigelaar. 1971. Cell interactions in the graft-versus-host response. *Prog. Immunol.* **1:**369.
18. Sprent, J., and J. F. A. P. Miller. 1971. Activation of thymus cells by histocompatibility antigens. *Nat. New Biol.* **234:**195.
19. Wagner, H. 1972. The correlation between the proliferative and the cytotoxic responses of mouse lymphocytes to allogeneic cells *in vitro*. *J. Immunol.* **109:** 630.
20. Stobo, J. D. 1972. Phytohemagglutinin and concanavalin A: prober for murine "T" cell activation and differentiation. *Transplant. Rev.* **11:**60.
21. Kano, S., B. R. Bloom, and M. L. Howe. 1973. Enumeration of activated thymus-derived lymphocytes by the virus plague assay. *Proc. Natl. Acad. Sci. U. S. A.* **70:**2299.
22. Bach, F. H., M. Segall, K. S. Zier, P. M. Sondel, B. J. Alter, and M. L. Bach. 1973. *Science Wash. D. C.* **180:**403.
23. Alter, B. J., J. Dolores, J. Schendel, M. L. Bach, F. H. Bach, J. Klein, and J. H. Stimpfting. 1973. Importance of serologically defined *H-2* regions. *J. Exp. Med.* **137:**1303.
24. Bloom, B. R., J. Gaffney, and L. Jimenez. 1972. Dissociation of MIF production and cell proliferation. *J. Immunol.* **109:**1395.
25. Wallen, W., D. Lucas, and J. Dean. 1972. Dissociation of interferon production from the DNA synthetic response to mitogens. *In* Proceedings of the Seventh Leukocyte Culture Conference. F. Daguillard, editor. Academic Press, Inc., New York. 375.
26. Rocklin, R. E. 1973. Production of migration inhibitory factor by non-dividing lymphocytes. *J. Immunol.* **110:**674.