

THE MEDIATOR OF CELLULAR IMMUNITY
III. LYMPHOCYTE TRAFFIC FROM THE BLOOD INTO THE INFLAMED
PERITONEAL CAVITY*

BY F. T. KOSTER,‡ AND D. D. MCGREGOR,§ M.D.
(From the Trudeau Institute, Saranac Lake, New York 12983)

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Mononuclear cells with the structural features of lymphocytes are especially numerous in delayed-type hypersensitivity (DTH)¹ reaction sites, in allografts undergoing rejection, and in the lesions of certain autoimmune diseases (1-4). The origin of these "small round cells" and their possible role in cell-mediated immunity have been the subject of an unduly prolonged debate, based mainly upon morphological evidence. But the issue cannot be resolved by morphological analysis, because the small round cells in exudates ultimately derive from a heterogeneous population of blood-borne lymphocytes. The latter differ with respect to turnover (5), circulating life-span (5, 6), and possibly with respect to origin and function.

In an earlier study, using normal nonimmunized rats (7), it was found that a portion of the small mononuclear cells in peritoneal exudates induced by casein derive from newly formed lymphocytes which enter the blood by way of the thoracic duct. Small lymphocytes of the kind which have a slow turnover rate and a potentially long circulating life-span were excluded, although cells of this type comprise approximately 90% of those normally present in central lymph (8). Results of the present investigation reaffirm and extend these observations by showing that newly formed lymphocytes from rats infected with *Listeria monocytogenes* can move in substantial numbers into exudates induced by casein bacteria. The disposition of these lymphocytes assumes importance when one considers that they include the specific mediators of cellular resistance to infection (6). The ability of specifically committed lymphocytes to localize in inflammatory foci provides a plausible explanation for the protective property of

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‡ Present address: Case Western Reserve University School of Medicine, Cleveland, Ohio.

§ Research associate, American Cancer Society.

¹ Abbreviations used in this paper: DTH, delayed-type hypersensitivity; MGC, mean grain count.

peritoneal exudate cells obtained from *Listeria*-immune donors (9), and points to a mechanism by which the infected animal marshals its cellular defenses at sites of bacterial implantation.

Materials and Methods

Animals.—The subjects of the experiments were male and female Lewis rats, 175–250 g body weight.

Microorganisms.—*Listeria monocytogenes*, strain EGD, was used for immunization. Suspensions of the organism were prepared from 16 hr trypticase soy broth cultures of infected mouse spleen. *Mycobacterium tuberculosis*, strain R₁R_v, was obtained from the mycobacterial culture collection of the Trudeau Institute (TMC No. 205). The organism was grown on Proskauer and Beck medium for 3 wk before the pellicle was removed, heated to 100°C, and lyophilized. *Mycobacterium bovis*, strain Calmette-Guerin bacillus (BCG) (TMC No. 1012), was grown for 10 days in Tween-albumin liquid medium (Difco Laboratories Inc., Detroit, Mich.). The organisms were then killed in 70% ethanol, washed twice in 0.85% sodium chloride, and lyophilized.

Immunization.—Prospective donors of thoracic duct cells were specifically immunized against *L. monocytogenes* with living organisms injected subcutaneously into both hind footpads and over the lower abdomen. The total injection volume was 0.5 ml; it contained 8.2×10^5 – 2.0×10^6 viable units. In some experiments, the recipients of thoracic duct cells were also immunized by a footpad injection of living *L. monocytogenes* or of 300 µg of heat-killed *M. tuberculosis* in 0.1 ml of mineral oil.

Preparation of Cell Suspensions.—Thoracic duct cells were obtained on the 7th day of the immunizing *Listeria* infection. The lymph from freshly cannulated donors was collected at 4°C for 18–24 hr into sterile flasks containing 5 ml of Ringer's solution, 100 units of heparin, and 60 µg of penicillin. Collections from several animals were pooled and filtered through surgical gauze to remove fibrin clots. The cells were then centrifuged for 10 min at 500 g, washed once in Hanks' balanced salt solution containing 1% fetal calf serum, and resuspended in fresh medium at a suitable concentration for labeling or injection. Cells were expressed from the mesenteric lymph node by teasing the node in tissue culture medium 199 (10) containing 1% fetal calf serum and 1 unit of heparin/ml. The technique for obtaining cells from the peritoneal cavity, the cellular composition of the inflammatory exudates, and the method for measuring cell radioactivity have been described elsewhere (9). Blood leukocyte concentrates were prepared by adding cardiac blood to 2 volumes of 3% dextran having an average molecular weight of 204,000 (Mann Research Laboratories Inc., New York). In order to facilitate erythrocyte sedimentation, the blood-dextran mixture was centrifuged for 5 min at 50 g. Leukocytes were then sedimented from the supernatant by centrifugation for 10 min at 1000 g.

Radioactive Labeling of Cells.—Thoracic duct lymphocytes were labeled in vivo by injecting prospective donors every 8 hr with 0.34 µCi/g body weight of tritiated thymidine (³H-thymidine, 3 Ci/mole, New England Nuclear Corp., Boston, Mass.). Inocula containing labeled large and medium lymphocytes and labeled short-lived small lymphocytes were prepared from lymph collected within 24 hr after the last of 18 ³H-thymidine injections. Cell populations, in which long-lived small lymphocytes were the predominantly labeled cell-type, were obtained by cannulating the thoracic duct 24–30 days after the last of a longer series of ³H-thymidine injections. In one experiment, large lymphocytes alone were labeled by incubating thoracic duct cells in vitro in medium 199 containing 5% fetal calf serum, 1 unit of heparin/ml, and ³H-thymidine at a final concentration of 0.2 µCi/ml. The mixture was incubated for 1 hr at 37°C in a water bath oscillating at 120 cpm.

Injection of Thoracic Duct Cells.—Thoracic duct cells labeled with ³H-thymidine were

centrifuged for 10 min at 500 g, washed once in Hanks' balanced salt solution containing 1% fetal calf serum, and resuspended in fresh medium at a concentration of 10^8 cells/ml. More than 98% of the cells prepared in this manner were viable as judged by their appearance in counting chambers and by their ability to exclude trypan blue. The cells were injected intravenously in a dose equivalent to 10^6 /g of body weight. Individual recipients were housed in a restraining cage (11) and given a continuous infusion of Ringer's solution containing non-radioactive thymidine at the rate of 1.2 mg/day. The infusion began several hours before the cell injection and continued to the end of the experiment.

Radioautography.—Air-dried smears of cells were fixed in methanol, extracted for 20 min at 4°C with two changes of 5% trichloroacetic acid, then washed for at least 1 hr in cold running water. The smears were coated with a 2:1 dilution of K-5 nuclear research emulsion (Ilford Ltd., Ilford, Essex, England), and exposed for 5 or 6 wk. The radioautographs were stained after development with May Grünwald-Giemsa stain.

RESULTS

Migration of Newly Formed Lymphocytes into the Inflamed Peritoneal Cavity.—Peritoneal exudate cells from rats which have survived an infection with *L. monocytogenes* can confer a substantial level of antimicrobial resistance upon normal recipients (9). The protective cells have not been identified morphologically, but there are reasons for thinking that they are lymphocytes rather than macrophages (12). This notion would be strengthened, however, if it could be shown that the thoracic duct lymph of infected animals contains newly formed lymphocytes which can find their way from the blood into an inflammatory exudate. Normal rats and rats infected with *L. monocytogenes* were therefore injected every 8 hr for 6 days with ^3H -thymidine. Thoracic duct cells collected within 24 hr after the last injection were introduced intravenously into normal recipients. Immediately after transfer, the animals were stimulated intraperitoneally with casein, killed *L. monocytogenes*, or both.

Table I indicates that a substantial number of labeled lymphocytes were found in the lymph nodes, blood, and inflamed peritoneal cavities of all recipients. The labeled exudate cells were all scored as lymphocytes; labeled macrophages were not found, although macrophages accounted for up to 94.4% of the mononuclear cells examined. Labeled lymphocytes were especially numerous in the inflamed peritoneal cavities of animals given thoracic duct cells from *Listeria*-infected donors. In one animal stimulated with killed *L. monocytogenes*, for instance, approximately 6.2% of all mononuclear cells in the exudate were radioactively labeled. A smaller proportion of labeled cells were found in exudates induced by casein and *Listeria*. This can be explained by the much larger number of cells (mostly unlabeled macrophages) that were recovered when casein was used.

It is apparent from Table I that the mean grain count (MGC) per labeled small lymphocyte in exudates was greater than that of small lymphocytes in the donor inoculum, or in the blood and lymph nodes of recipients. The heavy labeling among the exudate cells implies that they belong to a special subpopu-

lation of small lymphocytes or that a significant portion of exudate-containing small lymphocytes derive from still more heavily labeled large and medium lymphocytes originally present in the donor inoculum. The latter possibility was tested in the following experiment.

TABLE I
Distribution of ^3H -Thymidine-Labeled Cells in Rats Injected Intravenously with Thoracic Duct Cells Labeled Predominantly in the Short-Lived Component*

Donor status	Exudate stimulus	Mesenteric lymph node		Blood		Peritoneal exudate†		
		Small lymphocytes	Large and medium lymphocytes	Small lymphocytes	Large and medium lymphocytes	Small lymphocytes	Large and medium lymphocytes	Macrophages
Normal§	Casein	48/3000 (18.2)	1/105 (67)	34/3000 (18.1)	3/79 (44.0)	6/500 (27.0)	1/26 (39)	0/8850 —
	None	34/3000 (14.9)	0/49 —	24/3000 (10.9)	0/130 —	1/3000 (28.0)	0/0 —	0/10,637 —
Immune	Casein	47/3000 (20.6)	1/132 (33)	40/4000 (18.8)	2/42 (63.0)	16/500 (36.8)	6/53 (48.6)	0/8000 —
	<i>Listeria</i>	68/3000 (26.6)	1/98 (76)	61/3000 (30.3)	5/33 (54.8)	49/500 (40.2)	23/95 (42.1)	0/568 —
	Casein + <i>Listeria</i>	56/3000 (23.6)	1/145 (36)	53/3000 (26.2)	5/41 (49.8)	21/500 (30.2)	7/54 (48.0)	0/9330 —

* Donor rats were given 18 injections of ^3H -thymidine over a 6 day period before cannulation. Cells obtained during the first 24 hr after the last injection were given intravenously in a dose equivalent to 10^6 cells/g of body weight. Results recorded as No. labeled cells per No. examined. MGC per labeled cell enclosed by parentheses. Radioautographs exposed for 7 wk.

† Harvested 24 hr after intraperitoneal injection of casein, killed *L. monocytogenes*, or both.

§ Thoracic duct cell inoculum contained 97.3% small lymphocytes (MGC, 24.9) and 2.7% large and medium lymphocytes (MGC, 44.7).

|| Infected subcutaneously with 8.2×10^5 *L. monocytogenes*. First injection of ^3H -thymidine given on the day of infection. Thoracic duct cell inoculum contained 95.3% small lymphocytes (MGC, 23.4) and 4.7% large and medium lymphocytes (MGC, 42.7).

The Distribution of Thoracic Duct Cells Labeled In Vitro with ^3H -Thymidine.—Large and medium lymphocytes in the thoracic duct lymph of 7-day *Listeria*-infected rats were radioactively labeled by incubating the cells in vitro for 1 hr with ^3H -thymidine. This procedure labels few (if any) small lymphocytes (13). Radioautographs prepared from the cells after incubation revealed that 62.9% of the large lymphocytes and 16.9% of the medium lymphocytes were radioactively labeled. The MGC per labeled medium lymphocyte was 69.8, whereas that of large lymphocytes was too high to be counted accurately. Thoracic duct

cells prepared in this manner were injected intravenously into normal recipients in a dose equivalent to $10^6/g$ body weight. The recipients were given the usual infusion containing nonradioactive thymidine and all were stimulated intraperitoneally with 50 μg of killed *L. monocytogenes*.

Table II shows the disposition of the labeled cells and their progeny in the mesenteric lymph nodes and peritoneal exudates of recipient rats 24–72 hr after transfer. Only two labeled lymphocytes (both small) were found among the more than 40,000 lymph node cells examined. In striking contrast, labeled lymphocytes were frequent among the cells found in the inflamed peritoneal

TABLE II
Distribution of Labeled Cells in Rats Injected Intravenously with Thoracic Duct Cells Labeled with 3H -Thymidine In Vitro*

Time of sacrifice (hr after cell transfer)	Mesenteric lymph node		Peritoneal exudate‡		
	Small lymphocytes	Medium and large lymphocytes	Small lymphocytes	Medium and large lymphocytes	Macrophages
24	0/10,000	0/517	18/1000	7/53	0/1016
	—	—	(52.9)	(72.2)	—
	0/10,000	0/252	23/1000	6/70	0/3120
48	—	—	(43.0)	(63.0)	—
	2/10,000	0/437	11/1000	0/24	0/1711
72	(29.0)	—	(15.7)	—	—
	0/10,000	0/239	13/1000	0/19	0/3486
	—	—	(18.2)	—	—

* Cells obtained on the 7th day of an immunizing *Listeria* infection were labeled in vitro with 3H -thymidine. The inoculum contained 95.5% small lymphocytes (< 0.05% labeled), 1.7% large lymphocytes (62.9% labeled), and 2.8% medium lymphocytes (16.9% labeled). Results recorded as No. labeled cells per No. examined. MGC enclosed by parentheses. Radioautographs exposed for 5 wk.

‡ Harvested 24 hr after intraperitoneal injection of killed *L. monocytogenes*.

cavity. At 24 hr, the radioactivity was present in large, medium, and small lymphocytes, but at 48 and 72 hr, it was found only in small lymphocytes. The results indicate that large and medium lymphocytes, the only cells which had incorporated 3H -thymidine in vitro, had migrated from the blood into a peritoneal exudate where they differentiated into small lymphocytes. The decrease in mean grain count with advancing time implies that cell division intercedes in this process.

Disposition of Newly Formed Lymphocytes in Normal and Infected Rats.—In the foregoing experiments, it was found that the traffic of newly formed lymphocytes from the blood into the inflamed peritoneal cavity was especially heavy in animals given thoracic duct cells from *Listeria*-infected donors. The results suggested that lymphocytes, of the kind which have a short-circulating

life-span and are produced in increased numbers in infected rats (6), have a special affinity for the endothelium of inflamed blood vessels. This, and the possibility that the emigration of lymph-borne cells into exudates is influenced by the immune status of the recipient, were tested by infusing normal and *Listeria*-infected donors with radioactively labeled thoracic duct cells.

The donors employed in this experiment were given 18 injections of ^3H -thymidine over a 6 day period immediately before cannulation. Lymphocytes issuing

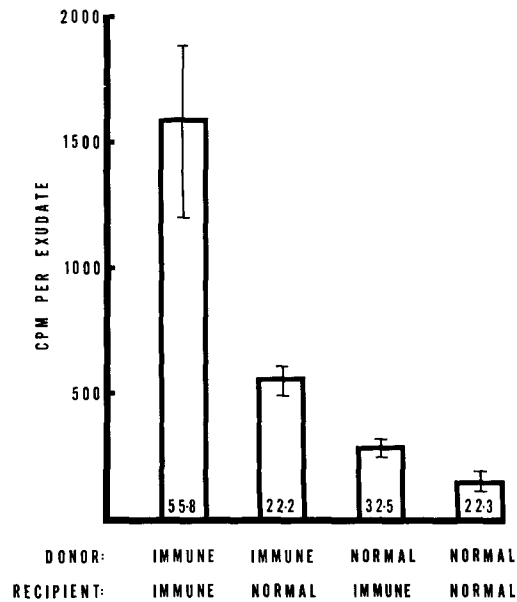


FIG. 1. Radioactivity in cells from the peritoneal cavities of rats 24 hr after an intra peritoneal injection of killed *L. monocytogenes*. The animals were injected intravenously with labeled thoracic duct cells at the time of exudate induction (see text). Means and ranges of 4. The mean number of exudate cells $\times 10^6$ is indicated at the base of each column.

from the fistulae during the first 16 hr of lymph drainage were injected intravenously into normal recipients and *Listeria*-infected rats in a dose equivalent to 10^6 cells/g of body weight. Immediately after transfer, the recipients were stimulated intraperitoneally with 50 μg of killed *L. monocytogenes*.

Fig. 1 shows that the exudates raised in animals given thoracic duct cells from infected donors contained a relatively high concentration of cell radioactivity and hence more labeled donor lymphocytes. But the immune status of the recipient also affected the disposition of the labeled cells. It is apparent from Fig. 1 that recipients which had themselves been infected with *L. monocytogenes* mounted a particularly violent inflammatory response. Peritoneal exu-

dates induced in these animals contained more labeled donor lymphocytes than exudates induced in normal nonimmunized recipients. The results suggest that the flow of newly formed lymphocytes into an inflammatory focus is related not only to the number of lymphocytes present in the blood, but also to the intensity and duration of the inflammatory response, the assumption being that the infected animals alone displayed DTH toward *Listeria* antigens.

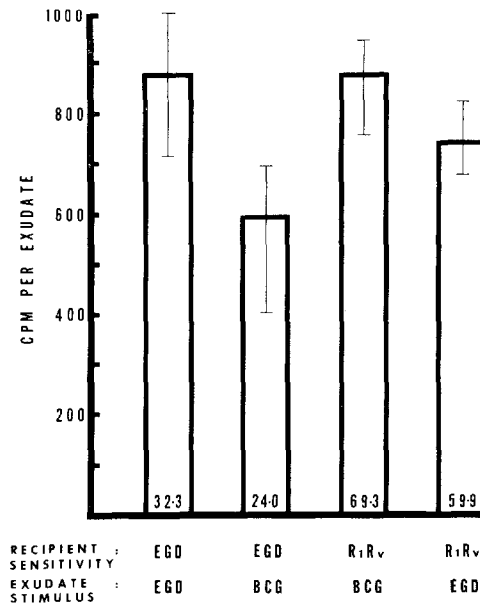


FIG. 2. Radioactivity in cells from the peritoneal cavities of rats 24 hr after an intraperitoneal injection of killed *L. monocytogenes* or killed BCG. The animals were injected intravenously with labeled thoracic duct cells at the time of exudate induction. The lymphocyte donors and the recipients were immunized with either *L. monocytogenes*, strain EGD, or *M. tuberculosis*, strain R₁R_v (see text). Means and ranges of 4. The mean number of exudate cells is indicated at the base of each column.

In the foregoing experiment, the peritoneal cavity was inflamed by the local injection of killed *L. monocytogenes*. It could be argued therefore that the lymphocytes of donors infected with this organism migrated into the exudates under the chemotactic influence of *Listeria* antigens. A selective process was therefore sought by measuring the flow of labeled thoracic duct cells into peritoneal exudates induced by either *L. monocytogenes* or the antigenically unrelated organism, BCG. The influence of DTH on the disposition of the labeled cells was studied simultaneously by sensitizing the recipients to either *L. monocytogenes* or *M. tuberculosis*.

Accumulation of Newly Formed Lymphocytes in Inflammatory Exudates In-

duced by *Specific and Nonspecific Stimuli*.—Newly formed lymphocytes, including those formed in response to *Listeria* antigens, were labeled in vivo by a series of 18 ^3H -thymidine injections given during the first 6 days of a primary *Listeria* infection. Thoracic duct cells obtained on the 7th day were injected intravenously into recipients which had been sensitized 7 days earlier with living *L. monocytogenes*, or 8 days earlier with heat-killed *M. tuberculosis*, strain R₁R_v, in oil. Immediately after transfer, the animals were stimulated with *L. monocytogenes* or BCG.

Fig. 2 indicates that a substantial number of labeled donor lymphocytes accumulated in all exudates. Considerable variation was observed among in-

TABLE III
Distribution of Labeled Cells in Rats Injected Intravenously with Thoracic Duct Cells Having the ^3H -Thymidine Label only in the Long-Lived Component*

Exudate stimulus	Mesenteric lymph node		Blood		Peritoneal exudate†	
	Small lymphocytes	Large and medium lymphocytes	Small lymphocytes	Large and medium lymphocytes	Small lymphocytes	Large mononuclear cells (lymphocytes and macrophages)
Casein	19/3000	0/186	21/3000	0/88	0/1000	0/10,350
<i>Listeria</i>	6/3000	0/143	5/3000	0/82	0/3000	0/9,832
	9/3000	0/73	—	—	0/3000	0/14,540

* Donor rats rested for 24–30 days before cannulation. Inocula contained > 96% small lymphocytes of which 5.6–7.5% were labeled. Radioautographs exposed for 5 wk. Results recorded as No. labeled cells per No. examined.

† Harvested 24 hr after intraperitoneal injection of casein or killed *L. monocytogenes*.

dividual recipients in each group; however, animals stimulated by the organism to which they had been specifically sensitized usually developed a more cellular exudate, and one which contained a larger number of labeled donor lymphocytes. The fact that *L. monocytogenes* and BCG were equally effective in promoting the emigration of labeled cells implies a lack of immunological specificity, at least with respect to the process which calls newly formed lymphocytes into the inflamed peritoneal cavity.

Failure of Long-Lived Small Lymphocytes to Enter Exudates.—It was concluded from an earlier study (7) that long-lived small lymphocytes are excluded from peritoneal exudates induced by casein. Since killed *L. monocytogenes* seem more effective than casein in recruiting lymph-borne cells from the circulation (Table I), it was of interest to determine whether long-lived small lymphocytes are more numerous in exudates induced by this organism. Normal, non-sensitized rats were therefore injected intravenously with thoracic duct cells from donors which had been rested for as long as 30 days after the last of a

series of ^3H -thymidine injections. During the interval between the last injection of ^3H -thymidine and cannulation of the thoracic duct, two changes occur in the distribution of cell radioactivity in lymph: radioactivity in large and medium lymphocytes is diluted by cell division, and the number of labeled small lymphocytes with a short circulating life-span falls to a low value (14). The effect is to concentrate radioactivity in a population of lightly labeled small lymphocytes that have a potentially long circulating life-span. Radioautographs prepared from the two inocula used in this experiment revealed that they contained 5.6% and 7.5% of labeled small lymphocytes. Only one labeled medium lymphocyte, but no labeled large lymphocytes, were found among more than 2000 cells examined in each specimen.

Table III shows the disposition of these labeled lymphocytes 24 hr after intravenous injection into rats stimulated with casein or killed *L. monocytogenes*. Lightly labeled small lymphocytes were found in the mesenteric lymph node and the blood, but close examination of more than 10,000 cells from each exudate failed to reveal a single labeled cell. This adds strongly to the view that large and medium lymphocytes, and possibly short-lived small lymphocytes, are specialized with respect to their ability to move from the blood into an inflammatory focus.

DISCUSSION

The results of the current investigation indicate that a portion of the lymphocytes in an induced peritoneal exudate derive from cells which enter the blood by way of the thoracic duct. Their identity was revealed in radioautographs prepared from exudates harvested 24–72 hr after radioactively labeled thoracic duct cells were injected intravenously. Labeled cells originally present in the donor inoculum were found later in the exudates. All were scored as lymphocytes: labeled macrophages were not found, although macrophages were the dominant cell type in the exudates. This does not say that macrophage antecedents are absent from lymph, but only that they are uncommon or fail to differentiate into macrophages under the conditions prevailing in the present experiments.

The lymph-borne cells which participate in inflammation have a rapid turnover and are delivered in increased numbers to the blood of *Listeria*-infected rats. At least some are large and medium lymphocytes. This conclusion was drawn from an experiment in which the thoracic duct cells of prospective donors were labeled *in vitro* with ^3H -thymidine. Large and medium lymphocytes were the only cells which became radioactively labeled. After intravenous injection, however, labeled lymphocytes (large, medium, and small) were found in induced peritoneal exudates. The results indicate that a portion of the small mononuclear cells in exudates can derive from replicating precursors (large and medium lymphocytes) which enter the blood by way of the thoracic duct. But

they also imply that the movement of labeled lymphocytes into the inflamed peritoneal cavity reflects a physiological event and is not due to the selective emigration of cells damaged by incorporated tritium. Although ^3H -thymidine can inhibit the proliferative capacity of mammalian cells (15), the concentrations employed in the current investigation failed to prevent large and medium lymphocytes from generating small lymphocytes by a process involving both cell division and cell differentiation.

The remarkable capacity of newly formed lymphocytes to localize in inflammatory foci merits further comment. 24 hr after intravenous injection, large and medium lymphocytes which had been labeled *in vitro* with ^3H -thymidine were identified in induced peritoneal exudates, but not a single labeled cell was found among a much larger number of lymphocytes expressed from mesenteric lymph nodes. This difference in the tissue distribution takes on added significance when it is considered that large lymphocytes from rat thoracic duct lymph "home" preferentially upon lymphoid tissue of the intestinal lymphatic bed (16).

Recent evidence obtained by Werdelin and McCluskey² strongly support the view that newly formed lymphocytes are especially prone to leave the circulation in areas of inflammation. These investigators studied the development of experimental allergic encephalomyelitis and adrenalitis in rats injected intravenously with lymph node cells from donors sensitized to spinal cord and adrenal tissue antigens, respectively. When cells labeled *in vitro* with ^3H -thymidine or ^3H -adenosine were transferred, a substantial number of labeled cells (the great majority of which were lymphocytes) accumulated at the margin of heat-induced lesions in the corresponding target tissue. In each case, however, ^3H -thymidine-labeled cells showed a greater tendency to enter reaction sites. In experiments in which ^3H -thymidine labeled cells from one donor source were given together with unlabeled cells from the second source, labeled lymphocytes were found in approximately equal proportions in both brain and adrenal lesions. The latter results are especially interesting for they imply that newly formed lymphocytes, irrespective of their immunological commitment, have an affinity for the endothelium of inflamed blood vessels.

It is not yet known whether large and medium lymphocytes are the only lymph-borne cells which participate in inflammation. However, long-lived small lymphocytes seem to be excluded, although they are the predominant cells in rat thoracic duct lymph. This conclusion, drawn from an earlier study (7), was substantiated in the present investigation when rats were injected intravenously with inocula consisting predominantly of long-lived small lymphocytes. In this experiment, labeled donor small lymphocytes were found later in the recipients'

² Werdelin, O., and R. T. McCluskey. 1970. The nature and the specificity of mononuclear cells in experimental autoimmune inflammations and mechanisms leading to their accumulation. Submitted for publication.

lymph nodes and blood, but not at all in a sample of more than 40,000 exudate cells. This implies that if small lymphocytes do cross the endothelium of inflamed blood vessels in the manner of large and medium lymphocytes, they too have a rapid turnover and a short circulating life-span.

Experiments using thoracic duct cells from rats given a course of ^3H -thymidine injections immediately before cannulation add force to this argument and point to a possible difference in the origin of short- and long-lived small lymphocytes. The experimental conditions were such that the great majority of short-lived lymphocytes and a proportion of the donor's long-lived small lymphocytes were radioactively labeled. After intravenous injection into rats with induced peritoneal exudates, labeled cells were found not only in lymph nodes and the blood, but also in the exudates. An analysis of the grain count distribution among labeled small lymphocytes revealed that those in the exudates were more heavily labeled. Since many (perhaps all) of these cells were generated by large and medium lymphocytes, it is possible that the more lightly labeled small lymphocytes in lymph nodes and blood have a different life history.

Comment should be made on the finding that lymphocyte traffic into the inflamed peritoneal cavity is influenced not only by the number of short-lived lymphocytes delivered to the blood, but also by the intensity and duration of the inflammatory stimulus. This was shown by a counting procedure which enabled the disposition of labeled lymphocytes to be compared in a larger number of recipient rats. Exudates induced in animals specifically sensitized to the inflammatory stimulant, *L. monocytogenes* or BCG, contained a higher concentration of cell radioactivity, and hence more labeled donor lymphocytes, than did exudates raised in either normal recipients or recipients sensitized to the heterologous organism. But it cannot be concluded that the labeled lymphocytes were recruited from the blood, because they alone were attracted by the antigens of a particular organism. A more plausible explanation is that the flow of lymphocytes into an inflammatory focus is governed by the intensity and duration of the inflammatory process, and this in turn depends upon the presence or absence of hypersensitivity to the irritant used to provoke a response.

There are reasons for thinking that the lymph-borne cells which participate in inflammation include the mediators of cellular resistance to infectious disease (17). Both are released in increased numbers into the thoracic duct lymph of *Listeria*-infected rats (6); they have a short circulating life-span and are capable of localizing in inflammatory exudates (9). The property of lymphocytes which enables them to leave the blood has not yet been determined. It is conceivable, however, that short-lived lymphocytes as a class have an affinity for the endothelium of inflamed blood vessels. According to this theory, lymphocytes arrive at random in inflammatory foci where those which can react specifically with antigen could trigger secondary events that would involve blood monocytes and macrophages in a process known as acquired cellular resistance (18).

SUMMARY

A substantial portion of the lymphocyte-like cells in induced peritoneal exudates derive from cells which enter the blood by way of the thoracic duct. The migrant cells have been identified as large and medium lymphocytes, but they may also include short-lived small lymphocytes derived from them. Small lymphocytes which have a potentially long circulating life-span are excluded from exudates, although cells of this type predominate in thoracic duct lymph.

The results imply that many (perhaps all) of the small round cells in inflamed tissue are members of a line of rapidly proliferating lymphocytes. Specifically committed lymphocytes with precisely these properties are added to the blood of rats infected with *Listeria monocytogenes*. The localization of committed lymphocytes in inflammatory foci could be the crucial event which enables the host to focus his cellular defenses at sites of bacterial implantation.

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