

## THE MEDIATOR OF CELLULAR IMMUNITY

### VI. EFFECT OF THE ANTIMITOTIC DRUG VINBLASTINE ON THE MEDIATOR OF CELLULAR RESISTANCE TO INFECTION\*

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(Received for publication 16 October 1972)

Bacterial parasites that can survive and multiply intracellularly are notable for their ability to induce delayed-type hypersensitivity and a concomitant state of resistance that is expressed in the enhanced capacity of macrophages to kill ingested organisms (1). The mechanism by which macrophages become activated in this respect has not been determined, but the process clearly involves an activity of specifically sensitized lymphocytes. These are formed in the infected animal and can be detected by their ability to protect recipients against the homologous parasite.

Cells that can protect normal rats against a challenge infection with *Listeria monocytogenes* (2) or *Mycobacterium tuberculosis* (Lefford, McGregor, and Mackaness, unpublished data) are delivered to the thoracic duct of donors infected with these organisms. The appearance of protective cells in the lymph of *Listeria*-infected subjects coincides with the influx of many newly formed lymphocytes: large, medium, and small (2). If the protective cells belong to a rapidly proliferating cell population, as the foregoing observation suggests, they should be vulnerable to agents that inhibit cell replication. Support for this notion was obtained in the current investigation in which the plant alkaloid vinblastine sulfate (Vbl)<sup>1</sup> was used to analyze the part played by dividing and nondividing lymphocytes in the transfer of cellular resistance to infection.

It will be shown that a single injection of Vbl given to rats at the peak of their response to a primary infection with *L. monocytogenes* deletes specifically sensitized effector cells from the thoracic duct and from an inflammatory exudate induced in the peritoneal cavity. The results point to dividing (large) lymphocytes as the specific mediators of host resistance to *L. monocytogenes* and provide a plausible explanation for their rapid turnover and short circulating life-span (2).

\* Supported by the Jacob C. Korb Memorial grant for Cancer Research from the American Cancer Society and grant AI-08642 from the National Institute of Allergy and Infectious Diseases.

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<sup>1</sup> Abbreviations used in this paper: BSS, Hanks' balanced salt solution containing 1% fetal calf serum; Cy, cyclophosphamide; PEC, peritoneal exudate cells; T cells, thymus-derived lymphocytes; TDL, thoracic duct lymphocytes; Vbl, vinblastine.

### Materials and Methods

*Rats.*—Male and female (Lewis × DA)F<sub>1</sub> hybrid rats were used. Cell donors weighed 160–250 g; the recipients in which the microbicidal assays were performed weighed 60–90 g. In one experiment, thoracic duct lymphocytes from Lewis donors were transferred to female recipients belonging to the (Lewis × BN)F<sub>1</sub> hybrid cross. The latter weighed 60–80 g at the beginning of the experiment.

*Microorganisms.*—*L. monocytogenes*, strain EGD, was used for immunization and challenge. Suspensions of the organisms usually were prepared from trypticase soy broth cultures of infected mouse spleen. In some experiments, however, the organism was reconstituted from a standard culture maintained in liquid nitrogen (3).

*Immunization of Rats and Assay of Antimicrobial Resistance.*—Rats were immunized against *L. monocytogenes* by injecting the organisms subcutaneously into both hind feet, the base of the tail, and over the lower abdomen. The total injection volume of 0.6 ml contained approximately 10<sup>7</sup> viable units. Protective immunity engendered by this procedure, or by an injection of lymphoid cells from specifically immunized donors, was measured in terms of the number of viable bacteria present in the liver and spleen at intervals after intravenous challenge with 8.5 × 10<sup>5</sup>–2.5 × 10<sup>6</sup> living *Listeria* (2). In some experiments, rats were given a single subcutaneous injection of cyclophosphamide (Cy<sup>2</sup>) (200 mg/kg) 24 h before adoptive immunization and challenge. This large dose not only inhibited the recipients' immune response but also amplified the immunity afforded by cells from actively immunized donors.

*Vinblastine.*<sup>3</sup>—Vbl was dissolved in 0.85% sodium chloride and injected intravenously in a single dose equivalent to 5 μg/g of body weight.

*Preparation of Cell Suspensions.*—Thoracic duct lymphocytes (TDL) were obtained from freshly incannulated rats during the first 12 h of lymph drainage. The cells were pooled with those from donors belonging to the same group. They were then washed once in Hanks' balanced salt solution containing 1% fetal calf serum (BSS) and resuspended in BSS or tissue culture medium 199 (Microbiological Associates, Inc., Bethesda, Md.) at a concentration suitable for labeling or injection.

Peritoneal exudates were harvested 24 h after a local injection of 50 μg of alcohol-killed *L. monocytogenes*. The technique for obtaining the cells and the cellular composition of the exudates have been described elsewhere (4).

*Radioactive Labeling of Lymphocytes.*—TDL were labeled *in vivo* by injecting prospective donors repeatedly with [<sup>3</sup>H]thymidine (3 Ci/mmol, New England Nuclear Corp., Boston, Mass.). The nucleoside was injected subcutaneously approximately every 8 h in a dose equivalent to 0.34 μCi/g of body weight. An inoculum in which large lymphocytes and a few medium lymphocytes alone were labeled was prepared by incubating TDL *in vitro* in medium 199 containing 5% fetal calf serum, 1 U of heparin/ml, and [<sup>3</sup>H]thymidine at a final concentration of 0.2 μCi/ml. The mixture was incubated for 1 h at 37°C in a water bath oscillating at 120 cpm.

*Transfer of Labeled Cells.*—Labeled TDL were washed twice in BSS and resuspended in 1–2 ml of fresh medium for intravenous injection into unlabeled recipients. In order to minimize reutilization of radioactive residues, individual recipients were housed in a restraining cage (5), where they received a continuous intravenous infusion of Ringer's solution containing nonradioactive thymidine at the rate of 1.2 mg daily. The infusion began several hours before cell transfer and was continued to the end of the experiment.

*Autoradiography.*—Methanol-fixed smears of TDL or peritoneal exudate cells (PEC) were extracted for 20 min at 4°C with two changes of 5% TCA, then washed for at least 1 h in cold

<sup>2</sup> Cyclophosphamide (Cytoxan) was generously supplied by Mead Johnson & Co., Evansville, Ind.

<sup>3</sup> Vinblastine (Velban) was a gift from Eli Lilly & Co., Indianapolis, Ind.

running water. They were subsequently coated with a 2:1 dilution of K-5 nuclear emulsion (Ilford, Ltd., Ilford, England) and exposed for 5-7 wk. Autoradiographs prepared in this manner were stained after development with May-Grünwald-Giemsa stain.

#### RESULTS

*Effect of Vbl on Specifically Sensitized Lymphocytes.*—Cells that can protect recipient rats against a challenge infection with *L. monocytogenes* appear within 72 h in the thoracic duct of rats infected with this organism. The delivery of protective cells to the lymph coincides with the influx of many newly formed cells (2), and it is among these that we should look for the mediators of cellular resistance to infection.

The possibility that the protective cells belong to a dividing cell population was tested by injecting 5  $\mu\text{g/g}$  of body weight of Vbl into 6-day *Listeria*-infected rats. Immediately thereafter the thoracic duct was incannulated, and cells issuing from the fistula during the first 12 h of lymph drainage were transferred intravenously into Cy-treated recipients. The cells, in a dose equivalent to  $2 \times 10^6/\text{g}$  of body weight, were injected 1 h after the recipients had been challenged intravenously with  $9.4 \times 10^5$  *L. monocytogenes*.

Fig. 1 shows that lymphocytes from Vbl-treated animals were less effective than those from similarly infected but untreated donors in their ability to contain the challenge infection in the livers and spleens of recipient rats. The results imply that large lymphocytes are the principal effector cells responsible for mediating host resistance to *L. monocytogenes*, because they alone incorporate [ $^3\text{H}$ ]-thymidine into nuclear DNA and are thus subject to the inhibitory effects of the drug.

Although Vbl clearly inhibited the great majority of sensitized lymphocytes in the thoracic duct of *Listeria*-infected donors, it failed to erase their protective

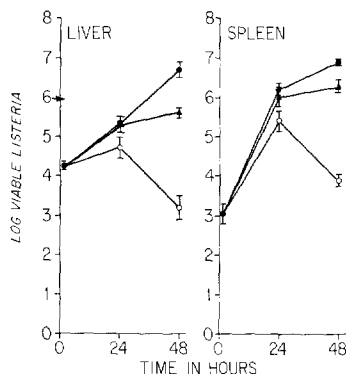


FIG. 1. Curves describing the growth of *L. monocytogenes* in the livers and spleens of Cy-treated rats infected with  $9.7 \times 10^5$  living organisms. The animals were nonimmunized controls (●—●) or rats immunized adoptively with  $2 \times 10^6$  TDL/g of body weight. These were obtained from either 6-day *Listeria*-infected donors (○—○) or similarly infected donors given Vbl (▲—▲). Means of  $5 \pm \text{SD}$ .

function entirely. Fig. 1 indicates that recipients of lymphocytes from Vbl-treated donors enjoyed a low but significant level of immunity 48 h after challenge. Apparently, the lymph of *Listeria*-infected rats contains some Vbl-resistant cells, or else those that are vulnerable to the drug continue to function for a time in their adopted hosts. The latter possibility was tested by allowing lymphocytes from infected donors to reside in recipient rats for 24 h before challenging the animals with *L. monocytogenes*. In the interval between cell transfer and challenge, virtually all donor large lymphocytes would be expected to reach metaphase, the point at which they are inhibited by Vbl (6, 7). Normal rats, rather than Cy-treated animals, were employed as recipients. These were injected intravenously with TDL from 6-day *Listeria*-infected donors to some of which Vbl had been given immediately before incannulation. In each case,  $2 \times 10^6$  cells/g of body weight were transferred either 24 h before or 1 h after the recipients were challenged with approximately  $10^6$  *L. monocytogenes*.

Table I shows that Vbl had a severe impact upon the immunity conveyed by the donor cells. It is evident, nonetheless, that lymphocytes from Vbl-treated donors afforded a significant ( $P < 0.05$ ) level of protection that remained in force for at least 24 h. The cells that convey this protection have not been identified morphologically; however, their resistance to Vbl and their ability to function for at least 24 h after transfer suggest that they are small lymphocytes. Presumably, they are mature members of an immunologically committed cell line that was established in the donor in response to the immunizing infection. Support for this notion was obtained in the following experiments, in which a single injection of Vbl was given to prospective lymphocyte donors at various intervals after infection.

*Effect of Vbl on the Protective Capacity of Lymphocytes Obtained at Various Intervals after Infection.*—Nine separate experiments were undertaken in which

TABLE I  
*Protective Immunity in Recipients of TDL from Listeria-Infected Rats to Some of Which a Single Injection of Vbl Had Been Given at the Time of Incannulation*

Donor treatment	<i>Listeria</i> challenge (relative to cell transfer)*	Log <sub>10</sub> protection†	
		Liver	Spleen
	<i>h</i>		
None	-1	2.38	2.25
Vbl§		0.40	0.48
None	+24	2.53	1.95
Vbl§		0.94	0.69

\* Cells obtained from 6-day *Listeria*-infected donors during the first 12 h of lymph drainage were transferred intravenously in a dose equal to  $2 \times 10^6$  cells/g of body weight.

† Difference in viable *Listeria* in tissues of adoptively immunized recipients and non-immunized controls 48 h after challenge. Mean of 5.

§ 5 µg/g of body weight injected intravenously.

Vbl was administered to donor rats 5–15 days after an immunizing *Listeria* infection. In each case the drug was injected intravenously immediately before incannulation of the thoracic duct. Lymphocytes obtained from these animals, or from a panel of similarly infected but untreated donors, were transferred intravenously into normal recipients 1 h after the recipients had been challenged with approximately  $10^6$  *L. monocytogenes*. As there was a small but unavoidable variation in the number of organisms used for immunization and challenge, the results have been expressed in Fig. 2 as the difference in log viable units found in the livers and spleens of adoptively immunized rats and nonimmunized controls 48 h after challenge.

It is evident that the lymph of 5-day *Listeria*-infected donors contained protective lymphocytes and that these were completely inhibited by Vbl. Vbl-resistant cells were delivered to the thoracic duct between the 5th and 11th days of the infection, and with advancing time accounted for an increasing portion of the protective cell population. Beyond the 11th day, however, protective cells could no longer be detected in the lymph. The results give credence to the notion that the Vbl-resistant lymphocytes are mature members of an immunologically committed cell line.

The large dose of Vbl ( $5 \mu\text{g/g}$ ) employed in these experiments not only inhibited the great majority of lymphocytes specifically committed to *Listeria* antigens

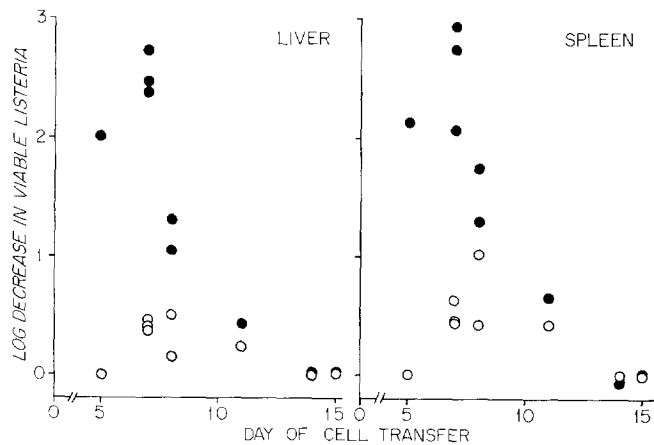


FIG. 2. Plots depicting the development of a population of specifically sensitized lymphocytes in the thoracic duct of *Listeria*-infected rats to some of which a single injection of Vbl had been given immediately before incannulation.  $2 \times 10^6$  lymphocytes/g of body weight were transferred intravenously into recipient rats 1 h after the recipients had been challenged with approximately  $10^6$  living organisms. Each plot represents the mean difference in log viable units in the livers and spleens of five adoptively immunized subjects and five nonimmunized controls 48 h after challenge. The closed circles denote the protective immunity conveyed by lymphocytes from infected donors; the open circles denote the immunity transferred by cells from similarly infected donors given Vbl.

but also caused a reduction in output of cells from the thoracic duct. During the first 12 h of lymph drainage, the mean output from 15 Vbl-treated donors and 9 untreated subjects was  $386 \pm 74 \times 10^6$  and  $713 \pm 93 \times 10^6$  cells, respectively. The low output from Vbl-treated animals and the fact that the great majority of cells in central lymph are small lymphocytes impelled us to ask whether these nondividing cells were affected by the drug. The problem was examined in the following experiment, in which the aggressive behavior of TDL was measured in a graft-vs.-host assay.

*Effect of Vbl on the Ability of Lymphocytes to Initiate a Graft-vs.-Host Reaction.*

—Lymphocytes from the thoracic duct of normal adult rats can cause a fatal wasting illness in  $F_1$  hybrid recipients that have a parent belonging to the donors' inbred strain. The aggressor cells have been identified as small lymphocytes (8, 9). Since their capacity to induce a graft-vs.-host reaction is potentiated by irradiation of the host (10), this procedure was exploited to measure the effect of Vbl on the activity of potentially reactive cells. A single injection of the drug was given to each of several normal Lewis rats. Immediately thereafter their thoracic ducts were incannulated. Cells obtained from these animals and from a panel of untreated controls were pooled with cells from the same group, and a weight-adjusted dose was injected intravenously into irradiated recipients belonging to the (Lewis  $\times$  BN) $F_1$  hybrid cross.

The injected cells were highly aggressive in their adopted hosts. Table II indicates that approximately 10 million donor lymphocytes were lethal for most recipients and that their ability to cause a graft-vs.-host reaction was unimpaired by treatment of the donors with Vbl. The results buttress the view that dividing (large) lymphocytes are the targets of Vbl and virtually exclude the possibility that lymphocytes are inhibited by the drug regardless of their position in the division cycle.

TABLE II  
*Aggressive Behavior of TDL from Normal Lewis Rats to Some of Which a Single Injection of Vbl Had Been Given at the Time of Incannulation*

Donor treatment	Lewis TDL	Fate of (Lewis $\times$ BN) $F_1$ hybrid recipients*		
	Lymphocytes transferred $\times 10^{-4}$ per g body wt	Rats inoculated	Rats ill	Rats killed
None	15	11	11	11
	10	13	13	12
	5	25	23	17
Vbl‡	15	10	10	10
	10	10	10	9

\* Female rats 60–80 g in body weight were exposed to 350 rad of whole body X and gamma irradiation 24 h before lymphocyte transfer.

‡ 5  $\mu$ g/g of body weight injected intravenously.

*Effect of Vbl on the Protective Capacity of PFC.*—In an earlier study (4) it was shown that specifically sensitized lymphocytes of the kind that mediate cellular resistance to infection with *L. monocytogenes* can be recovered in a fully functional condition from inflammatory exudates induced in the peritoneal cavity of rats infected with this organism. If the protective cells are mainly large lymphocytes, they should be vulnerable to Vbl. This proposition was tested by administering a single dose of Vbl to each of several rats on the 6th day of an immunizing *Listeria* infection. Immediately thereafter the animals were stimulated intraperitoneally with 50  $\mu$ g of killed *Listeria*. Peritoneal exudates harvested 24 h after stimulation were transferred to Cy-treated recipients in a dose equivalent to  $5 \times 10^5$  cells/g of body weight. The cells were injected intravenously 1 h after the recipients had been challenged by the same route with  $1.15 \times 10^6$  *L. monocytogenes*.

Fig. 3 indicates that a measure of protective immunity was conveyed by PEC obtained from either Vbl-treated donors or similarly infected but untreated controls. However, the recipients of cells from Vbl-treated donors enjoyed substantially less protection in both the liver and spleen. The results suggest that the effector cells belong to a proliferating lymphocyte population similar to that present in the lymph of infected rats.

Since the specifically sensitized lymphocytes that transfer resistance to *L. monocytogenes* are either absent or present in only small numbers in the uninfamed peritoneal cavity (4), they must be members of a cell population that emerges from the blood in response to inflammation. Autoradiographic studies (11) of the tissue disposition of newly formed lymphocytes obtained from *Listeria*-infected donors support this contention, and point again to a large

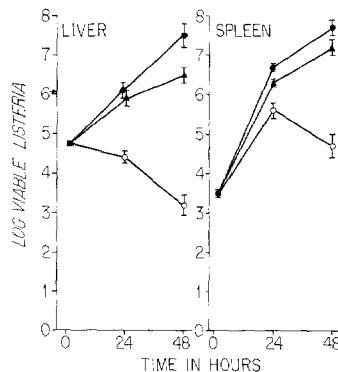


FIG. 3. Curves describing the growth of *L. monocytogenes* in the livers and spleens of Cy-treated rats infected with  $8.5 \times 10^5$  living organisms. The animals were nonimmunized controls (●—●) or rats immunized adoptively with  $5 \times 10^5$  PEC. The latter were obtained from either 7-day *Listeria*-infected donors (○—○) or similarly infected donors given Vbl (▲—▲) at the time of exudate induction. Means of  $5 \pm$  SD.

lymphocyte as the principal cell type responsible for transferring resistance to this infectious agent.

*Effect of Vbl on the Exudate-Seeking Capacity of Newly Formed Lymphocytes.*—Prospective donors were injected repeatedly with [<sup>3</sup>H]thymidine during the first 6 days of an immunizing *Listeria* infection. A number of these animals were then given a single injection of Vbl immediately before incannulation of the thoracic duct. Cells issuing from the fistula during the first 12 h of lymph drainage were pooled with cells from animals in the same group, and  $3.6 \times 10^8$  were transferred intravenously into each of several recipients. The recipients were given a continuous infusion of nonradioactive thymidine at the rate of 1.2 mg daily and, immediately after transfer, were stimulated intraperitoneally with killed *L. monocytogenes*. Smears prepared from samples of the donor inocula and from cells obtained from the peritoneal cavity of individual recipients 24 h after transfer were examined autoradiographically.

Table III indicates that the inoculum prepared from Vbl-treated donors contained fewer large and medium lymphocytes, but approximately the same number of labeled small lymphocytes, as the "control" inoculum, i.e., TDL obtained from labeled *Listeria*-infected rats that had not been given Vbl. However, there was a remarkable difference in the exudate-seeking capacity of the labeled cells. In recipients of cells from Vbl-treated donors, only one labeled medium lymphocyte and a few labeled small lymphocytes were found among the more than 5,000 cells examined from each exudate. By comparison, a sub-

TABLE III  
*Effect of Vbl on the Exudate-Seeking Capacity of TDL Labeled with [<sup>3</sup>H]Thymidine in Vivo\**

Donor treatment	Labeled lymphocytes transferred ( $\times 10^{-4}$ )		Lymphocytes in recipient exudate† ( $\times 10^{-4}$ )		
	Large and medium	Small	Total	Labeled large and medium	Labeled small
None	1,974	3,484	758	42	50
			901	39	52
			806	45	55
			794	38	57
			964	53	87
Vbl‡	896	3,554	287	1	5
			558	<1	6
			519	<1	10

\* Donor rats given 18 injections of [<sup>3</sup>H]thymidine during the first 6 days of a primary *Listeria* infection.  $3.6 \times 10^8$  cells collected from the thoracic duct during the first 12 h after the last injection were transferred intravenously into each of several recipients. Immediately thereafter the recipients were stimulated intraperitoneally with 50  $\mu$ g of killed *L. monocytogenes*.

† Harvested 24 h after exudate induction. Autoradiographs exposed for 7 wk.

‡ 5  $\mu$ g/g of body weight injected intravenously immediately before incannulation.



stantial number of labeled lymphocytes originally present in the control inoculum were found in exudates induced in similar recipients. The large number of labeled small lymphocytes found in this group suggests that many derived from replicating precursors of the kind inhibited by Vbl. Evidence to support this notion was obtained in the following experiment, in which the fate of donor large lymphocytes was determined by labeling the cells with [ $^3\text{H}$ ]thymidine *in vitro*.

*Effect of Vbl on the Exudate-Seeking Property of Large Lymphocytes.*—Inocula in which large and medium lymphocytes were the only radioactively labeled cells were prepared by incubating TDL *in vitro* for 1 h in medium containing [ $^3\text{H}$ ]thymidine. The donors in this experiment were 6-day *Listeria*-infected rats to some of which a single injection of Vbl had been given at the time of incannulation. Cells issuing from the fistulae were transferred intravenously into syngeneic recipients, the usual precautions being taken to minimize reutilization of radioactive residues released from labeled donor cells. Each recipient was given  $4 \times 10^8$  cells. Immediately thereafter all were stimulated intraperitoneally with killed *L. monocytogenes*. PEC obtained from these animals 24 h after stimulation were examined autoradiographically.

Table IV shows that the lymph of both Vbl-treated donors and their respective controls contained labeled large and medium lymphocytes; labeled small lymphocytes were not found among the more than 5,000 donor cells examined. Only two labeled lymphocytes (both small) were found in recipients of cells from Vbl-treated rats. In contrast, a moderate number of labeled lymphocytes were

TABLE IV  
*Effect of Vbl on the Exudate-Seeking Capacity of TDL Labeled with [ $^3\text{H}$ ]Thymidine in Vitro\**

Donor treatment	Labeled lymphocytes transferred ( $\times 10^{-4}$ )		Lymphocytes in recipient exudate† ( $\times 10^{-4}$ )		
	Large and medium	Small	Total	Labeled large and medium	Labeled small
None	433	<1	527	1	8
			849	5	14
			496	7	18
			762	14	28
Vbl‡	90	<1	407	<1	<1
			602	<1	<1
			532	<1	1
			668	<1	1

\* Cells obtained from 6-day *Listeria*-infected donors during the first 12 h of lymph drainage. After *in vitro* labeling with [ $^3\text{H}$ ]thymidine (see Methods),  $4 \times 10^8$  cells were transferred intravenously into each of several recipients. Immediately thereafter the recipients were stimulated intraperitoneally with 50  $\mu\text{g}$  of killed *L. monocytogenes*.

† Harvested 24 h after exudate induction. Autoradiographs exposed for 5 wk.

‡ 5  $\mu\text{g}/\text{g}$  of body weight injected intravenously immediately before incannulation.

found in exudates induced in the four recipients of "control" cells. The labeled exudate cells varied in size: some were large lymphocytes, similar to those that had incorporated [<sup>3</sup>H]thymidine in vitro; others were typical small lymphocytes. It is evident, therefore, that some donor large lymphocytes had migrated from the blood into the inflamed peritoneal cavity of the recipients, where they gave rise to smaller lymphocytes.

#### DISCUSSION

Acquired resistance to *L. monocytogenes* is mediated by lymphocytes that are represented among the free-floating cells in the central lymph of animals infected with this organism. Protective cells appear in the thoracic duct within 72 h of an immunizing infection. Their sudden influx at this time suggests that they belong to a rapidly dividing cell population. The results of the current investigation give credence to this notion, for they indicate that the cells that can protect normal rats against a *Listeria* challenge are highly vulnerable to Vbl.

Vbl is an appropriate drug for studies of this type, because it has a short half-life in vivo (12, 13) and acts selectively upon dividing cells, causing metaphase arrest and cell death (6, 7). In the current investigation, small lymphocytes, which divide infrequently or not at all, were shown to be highly resistant to Vbl. Their resistance was revealed in the failure of Vbl to inhibit the aggressive activity of Lewis TDL in a graft-vs.-host assay (Table II). The ability of Vbl-treated cells to transfer immunological memory of the bacteriophage  $\Phi$ X-174 (McGregor and Mackaness, unpublished data) provides further evidence to this effect and implies that dividing lymphocytes are selectively destroyed by the drug.

It is to be emphasized that the specifically sensitized lymphocytes that mediate cellular resistance to *L. monocytogenes* do not belong to a single, homogeneous class. The present studies using Vbl substantiate this contention and suggest that the makeup of the protective cell population changes as the response evolves. Vbl has a major impact on the protective function of lymphocytes obtained from the thoracic duct of *Listeria*-infected donors, but the timing of the drug injection is crucial. Thus, Vbl deletes sensitized lymphocytes from the duct on the 5th day of a primary immunizing infection (Fig. 2). Thereafter, Vbl-resistant cells are added to the lymph and comprise an increasing proportion of the protective cell population.

The foregoing observations suggest that large lymphocytes are immature members of a specifically committed cell line and are the first to be released into the circulation of *Listeria*-infected rats. Vbl-resistant lymphocytes are delivered to the blood later in the infection. The latter have not yet been identified morphologically, although it seems likely that they are small lymphocytes. Two lines of evidence support this conclusion: (a) an injection of Vbl has no effect on the proportion of newly formed small lymphocytes delivered to the thoracic duct of 6-day *Listeria*-infected rats (Table III), and (b) the drug

has only a trivial effect on the protective capacity of lymph-borne cells obtained later in the response, when the proportion of large lymphocytes in the lymph has declined to the "normal" value.

Taken at face value, the results of the current investigation imply that large lymphocytes are the principal, although probably not the exclusive, effector cells involved in transmitting immunity to *L. monocytogenes*, at least in the rat. It cannot be concluded, however, that large lymphocytes have a predominant role in host resistance to other infectious agents. Lefford, McGregor, and Mackaness (unpublished data) found that TDL from rats infected with *M. bovis* (strain BCG) can protect normal recipients against a challenge infection with either attenuated (R<sub>1</sub>R<sub>v</sub>) or virulent (H<sub>37</sub>R<sub>v</sub>) strains of *M. tuberculosis*. Since lymphocytes obtained from Vbl-treated donors 4 wk after infection also afford protection, it would seem that small lymphocytes have an important role in host resistance to this organism.

Factors peculiar to the host-parasite relationship could explain the rate at which committed lymphocytes are generated, the makeup of the protective cell population, and the cell susceptibility to Vbl. *L. monocytogenes* causes an acute infection in rats, as it does in mice (14). The organisms grow vigorously for several days in the livers and spleens of intravenously infected subjects. Thereafter the bacteria are rapidly destroyed by macrophages that acquire an enhanced listericidal capability (14, 15). Since *Mycobacteria* are more resistant to intracellular inactivation (16), it is plausible to think that they provide a more enduring immunogenic stimulus. The committed cell population generated in response to a BCG infection would therefore be expected to include a large proportion of "mature" (nondividing) small lymphocytes.

In an earlier study (2) it was shown that the cells that transfer resistance to *L. monocytogenes* have a short circulating life-span. After intravenous transfer into uninfected recipients, protective donor lymphocytes fail to recirculate to the thoracic duct in sufficient numbers to confer protective immunity upon a second panel of normal recipients. Failure of the cells to recirculate readily from blood to lymph implies that they arise in stimulated lymphoid tissue in the regional lymphatic bed. Their precise origin in parent lymph nodes has not been determined. It may be significant, however, that the delivery of protective cells to the thoracic duct is preceded by the appearance of blast cells in the paracortex of nodes draining sites of bacterial implantation (1). These blast cells are morphologically indistinguishable from the large pyroninophilic cells that develop from small lymphocytes during graft-vs.-host reactions (8). Since they arise in areas of lymphoid tissue which are normally occupied by thymus-derived lymphocytes (T cells) (17, 18) and through which T cells continuously recirculate (19, 20), it is likely that they, too, are thymus dependent. This notion accords with the fact that host resistance of *L. monocytogenes* is cell mediated and that the protective function of lymphoid cells obtained from *Listeria*-infected mice is abrogated by anti- $\theta$  serum (21).

Regardless of their lineal relationship to T cells, the short circulating life-

span of lymphocytes specifically committed to *Listeria* antigens sets them in a class apart from the majority of lymph-borne cells. The inability of protective cells to recirculate from blood to lymph could reflect a readiness to leave the blood in areas of inflammation. It is to be noted in this connection that protective cells can be recovered in a fully functional condition from inflammatory exudates induced in the peritoneal cavity of *Listeria*-infected rats (4). Since protective cells are either absent or present in only small numbers in the unstimulated peritoneal cavity, they must belong to a mobile population that emerges from the blood in response to inflammation. The results of the present investigation substantiate this contention and show, in addition, that a dividing (large) lymphocyte is the principal effector cell type present in exudates. There can be little doubt that the effect of the drug is on large lymphocytes, for when these alone were radioactively labeled before intravenous transfer, labeled cells from Vbl-treated donors were rarely found in peritoneal exudates induced in recipient rats.

The remarkable capacity of large lymphocytes to leave the blood in areas of inflammation has important implications in infections caused by facultative intracellular parasites in which host resistance rests ultimately with macrophages (1). Several lines of evidence support the view that host resistance to *L. monocytogenes* depends on the collaborative participation of specifically sensitized lymphocytes and mononuclear phagocytes (1). The requirement for macrophages is illustrated in the failure of *Listeria*-infected animals to benefit from an injection of committed lymphocytes when they are unable to assemble monocyte-derived macrophages in an inflammatory exudate. This is the case, for example, in mice exposed to a large dose of whole body X irradiation (22, 23) and rats given a potentially lethal dose of Cy (24).

Since lymphocytes from animals infected with *L. monocytogenes* can confer specific antimicrobial resistance upon uninfected recipients, it seems likely that an important function of these cells is to provide the stimulus required to enhance macrophage microbicidal function. The events underlying this activation process have not been determined, but they could involve the activity of a lymphokine. In fact, it has been reported recently that a product of antigen-stimulated lymphocytes can influence the microbicidal capacity of macrophages in vitro (25, 26).

The propensity of dividing (large) lymphocytes to localize in areas of non-specific inflammation could explain how specific effector cells assemble in infective foci. It is conceivable that inflammation initiated by the parasite or by an immunologically specific event involving either cells or antibody creates circumstances in which dividing lymphocytes, regardless of the immunological commitment, assemble in lesions. Here specifically sensitized cells might be stimulated by microbial antigens to release products that attract macrophages and give them an enhanced microbicidal capability.

Since small lymphocytes either fail to localize in inflammatory foci (11) or

do so only rarely, they are clearly at a strategic disadvantage with respect to their ability to modify macrophage microbicidal function. It is plausible, however, that they serve as vehicles for the carriage of immunological memory, a function they are known to discharge with respect to conventional antigens (27, 29). According to this notion, small lymphocytes specifically committed to the appropriate microbial antigens would rapidly transform into large lymphocytes upon reinfection with the homologous parasite, thereby arming the host with effector cells that localize preferentially in infected tissue.

#### SUMMARY

The antimitotic drug vinblastine (Vbl) has a profound impact upon the specifically sensitized lymphocytes that transfer cellular resistance to *Listeria monocytogenes*. A 12-h pulse of the drug given to prospective donors during the first week of an immunizing *Listeria* infection inhibits the delivery of protective lymphocytes to the thoracic duct and their subsequent movement into an inflammatory exudate induced in the peritoneal cavity. The effect of Vbl is clearly related to its antimitotic activity, not to an effect on lymphocytes regardless of their position in the division cycle. This conclusion was drawn from an autoradiographic analysis of cells in the lymph of Vbl-treated rats and from failure of the drug to abrogate a known function of small lymphocytes, namely, their ability to initiate a graft-vs.-host reaction. The results imply that large lymphocytes, the rapidly proliferating cells in central lymph, are the principal effector cells responsible for transmitting resistance to *L. monocytogenes* and provide a plausible explanation for their rapid turnover and short circulating life-span.

We thank Mrs. Patricia Scheefer and Mrs. Judith Smith for their valuable technical assistance.

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