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Transfer of Immunity Against *Listeria monocytogenes* by T Cells Purified by a Positive Selection Technique

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Affinity columns prepared with rabbit antibody to the $F(ab')_2$ fragment of rat immunoglobulin were used to separate rat thoracic duct lymphocytes into subpopulations that differ with respect to the density of their surface membrane immunoglobulin. Using this technique, it was shown that lymphocytes in the DNA synthetic (S) phase of the mitotic cycle are added in increased number to the lymph of rats infected with *Listeria monocytogenes*. The great majority of these S-phase cells lacked a high density of surface immunoglobulin as indicated by their failure to bind to the immunoabsorbent. Cells which can protect recipient rats against a challenge infection with *L. monocytogenes* also segregated with nonadherent thoracic duct lymphocytes obtained from *Listeria*-immune donors. These protective cells realized their full immunological potential only in recipients that shared histocompatibility-gene-coded structures with the immune lymphocyte donors. The above findings accord with the view that immunity to *L. monocytogenes* is mediated in rats by activated T cells which are formed as part of the animal's cell-mediated response to infection. Although *Listeria*-protective lymphocytes concentrate in the nonadherent, T-cell-enriched fraction, it was consistently observed that the adherent, B-cell-enriched fractions of immune donor thoracic duct lymphocytes also could transfer a low level of antimicrobial resistance. This immunity was restricted in allogeneic recipients, a finding which implies that the protection afforded by the adherent population is related to its content of T cells. Nonadherent S-phase lymphoblasts moved in substantial numbers from the blood into peritoneal inflammatory exudates induced by *L. monocytogenes*. The above finding encourages the belief that recently activated T cells realize their protective function locally in centers of infection where they have secondary effects on macrophages.

Affinity techniques using anti-immunoglobulin gels have been widely used to "purify" the two major classes of lymphocytes, namely, T and B cells (2, 6, 7, 11, 28). The technique described by Schlossman and Hudson (28) makes use of antibody to the $F(ab')_2$ fragment of immunoglobulin. By linking such antibodies to Sephadex G-200, immunoabsorbents are prepared which can selectively bind B cells. The procedure was used by Crum and McGregor (7) to fractionate rat thoracic duct lymphocytes (TDL) into populations that were enriched in either T or B cells.

In the current investigation, affinity columns prepared with rabbit antibody to rat $F(ab')_2$ were used to identify cells in the thoracic duct of specifically immunized rats which can transfer resistance to *Listeria monocytogenes*. The results indicate that the great majority of *Listeria*-protective cells have a low density of surface membrane immunoglobulin, as evidenced by their failure to adhere to the immunoabsorbent gel. The above finding, in conjunction with the fact that the cells concerned realize their full protective power only in recipients that share certain histocompatibility-gene-coded structures (13), implies that resistance to *L. monocytogenes* is mediated in rats by lymphocytes which belong to a T-lymphocyte line.

Listeria-protective cells are delivered to the thoracic duct and hence to the blood while still in active cycle (21). It will be shown that these newly formed T cells (T lymphoblasts) have a penchant to localize in peritoneal inflammatory exudates induced by *L. monocytogenes*. The above finding gives credence to the view that *Listeria*-immune T cells realize their protective function in centers of infection where they promote the focal accumulation and activation of

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blood monocytes and monocyte-derived macrophages (20, 23).

MATERIALS AND METHODS

Animals. Specific-pathogen-free Lewis rats, Wistar-Furth rats, and F_1 hybrids between the Lewis and Brown Norway strains were purchased from Microbiological Associates, Walkersville, Md. Specific-pathogen-free (Lewis × DA) F_1 hybrid rats were obtained from the Trudeau Institute, Saranac Lake, N.Y. Donors of TDL weighed 160 to 280 g, whereas the recipients weighed 70 to 100 g.

L. monocytogenes. *L. monocytogenes* strain EDG was used for both immunization and challenge. A suspension of organisms, prepared from a Trypticase soy broth (Baltimore Biological Laboratory, Cockeysville, Md.) culture of infected spleen, was stored up to 3 months in liquid nitrogen.

Antimicrobial immunity. Rats were immunized with approximately 5×10^6 living *L. monocytogenes.* The organisms, in a volume of 0.6 ml, were injected at multiple sites in the hind quarters: both footpads, the base of the tail, and subcutaneously over the lower abdomen. Protective immunity engendered by this procedure was measured indirectly, in terms of the resistance conferred on normal rats by an intravenous (i.v.) injection of immune TDL (19).

Cells. TDL were collected for 16 to 24 h into heparinized Ringer solution without added antibiotics. Peritoneal exudate cells (PEC) were obtained from rats at predetermined intervals after the animals had been stimulated intraperitoneally with 50 μg of alcohol-killed *L. monocytogenes.* The procedures for inducing exudates and harvesting the cells and the cellular composition of the exudates have been described elsewhere (15).

Radioactive labeling of TDL. TDL were labeled in the DNA synthetic (S) phase of the mitotic cycle by incubating the cells in vitro for 1 h in medium 199 containing 5% fetal calf serum, 1 U of heparin per ml, and [^{14}C]thymidine (40 to 60 mCi/mmol; New England Nuclear, Boston, Mass.) at a final concentration of 0.5 μCi/ml. This procedure labels 30 to 60% of the lymphoblasts; small lymphocytes are not labeled.

In cell transfer experiments, the localization of labeled S-phase lymphoblasts in peritoneal inflammatory exudates was measured radiometrically (22). Results are expressed as percent counts per minute originally present in the TDL inoculum.

Fractionation of cells on affinity columns. TDL and PEC were fractionated on immunoabsorbent columns which selectively bound cells that had a high density of membrane immunoglobulin. The columns were prepared by coupling rabbit anti-rat $F(ab')_2$ antibody to cyanogen bromide-activated Sephadex G-200 (Pharmacia, Upsala, Sweden) (7).

In one experiment, an immunoabsorbent was prepared with $F(ab')_2$ antibody fragments. To this end 120 mg of immunoabsorbent purified anti-rat $F(ab')_2$ in 60 ml of 0.7 M acetate–0.05 M NaCl, pH 4.0, was incubated with 4 mg of pepsin (Worthington Biochemicals Corp., Freehold, N.J.) for 18 h at 37°C. Thereafter, the pH of the reaction mixture was increased to 8.0 with 1 M NaOH and dialyzed for 48 h against borate-buffered saline, pH 8.3. Destruction of the Fc portion of the molecule was confirmed by gel diffusion, using an anti-rabbit immunoglobulin serum: a line of partial identity was demonstrated between the pepsin-treated antibody and untreated rabbit immunoglobulin. $F(ab')_2$ antibody fragments prepared in this way were incubated with 9 g of cyanogen bromide-activated Sephadex. Each gram of gel bound approximately 8 mg of protein.

One hundred milliliters of immunoabsorbent was packed into 50-mm columns. One hundred milliliters of TDL at a concentration of 1×10^7 to 2×10^7/ml in Hanks balanced salt solution containing 5% fetal calf serum was added to each column. The columns were washed with 450 ml of Hanks balanced salt solution containing 5% fetal calf serum, and the nonadherent cells (lymphocytes lacking detectable surface immunoglobulin) were recovered from the effluent. Thereafter, the adherent (immunoglobulin-bearing) cells were eluted from the gel with 400 ml of Hanks balanced salt solution containing 20% rat serum. Cells recovered in the eluate were washed twice with Hanks balanced salt solution containing 5% fetal calf serum before their use in experiments. In one experiment, column-adherent cells were recovered by digesting the immunoabsorbent with dextranase (28).

PEC were fractionated in the same way; however, the cell population was first depleted of macrophages by adding carbonyl iron to the cells and extracting iron-laden macrophages with a magnet (10). The depletion procedure removed 35 to 40% of the cells from the exudate. This intermediate step did not obviously affect the protective function of immune PEC and greatly expedited passage of the cells through the gel.

Immunofluorescence. Immunoglobulin-bearing cells were identified by indirect immunofluorescence, using a rabbit anti-rat $F(ab')_2$ antiserum diluted 1:8 and a fluorescent goat anti-rabbit immunoglobulin G (Cappel Laboratories, Downington, Pa.) diluted 1:20 (7).

Statistical analysis. Differences between means were tested by analysis of variance and the Q test (29).

RESULTS

Protective capacity of column-fractionated TDL. TDL obtained from donor rats on day 6 of an immunizing *Listeria* infection were fractionated on anti-rat $F(ab')_2$ columns. Nonadherent lymphocytes and adherent lymphocytes eluted from the columns were enumerated, and the cells were examined by indirect immunofluorescence for the presence of membrane immunoglobulin. Samples of the two cell fractions were then infused in equal numbers into recipient rats 1 h after the recipients had been challenged i.v. with approximately 3×10^6 *L. monocytogenes.*

The results of three consecutive experiments are summarized in Table 1. In each, the nonadherent cell fraction was severely depleted of immunoglobulin-bearing lymphocytes. Substantial variation was observed from experiment to

TABLE 1. *Distribution of Listeria-immune TDL on immunoabsorbent columns prepared with rabbit antibody to rat F(ab')$_2$*

Expt	Cell fraction	Lymphocytes transferred × 10^{-4}/g[a]			Log_{10} protection[b]	
		Total	Immunoglobulin positive	Immunoglobulin negative	Liver	Spleen
1	Unfractionated	200	60	140	2.78	3.74
	Nonadherent	200	6	194	3.35	3.55
	Nonadherent	25	<1	24	0.79	1.36
	Adherent	200	186	14	0.52	0.73
2	Unfractionated	200	56	144	2.00	2.77
	Nonadherent	200	4	196	2.27	3.15
	Nonadherent[c]	200	1	199	2.07	3.00
	Nonadherent	10	<1	10	0.44	0.54
	Adherent	200	184	16	1.03	1.61
3	Unfractionated	200	60	140	2.13	3.27
	Nonadherent	200	3	197	2.74	3.46
	Adherent	200	182	18	1.39	1.43

[a] Obtained from donor rats on day 6 of an immunizing *Listeria* infection. The cells were infused into normal recipients 1 h after the recipients had been challenged i.v. with approximately 3 × 10^6 *L. monocytogenes*.

[b] Mean difference 48 h after challenge in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls.

[c] Passaged serially through two immunoabsorbent columns.

experiment in the protective power of both nonadherent and adherent lymphocytes. But on a cell-for-cell basis, nonadherent lymphocytes outperformed adherent cells in their capacity to immunize the recipients against the challenge infection. Segregation of protective lymphocytes in the nonadherent cell fraction accords with the results of experiments in mice which indicate that T cells are specific mediators of resistance to *L. monocytogenes* (3, 17, 26).

Although the above findings imply that T cells have a similar mediator function in rats, it is evident from Table 1 that lymphocytes in the column-adherent, B-cell-enriched fraction also transferred a low but still highly significant ($P < 0.01$) level of resistance. Therefore, the question arises whether B cells have a limited capacity to mediate immunity to *Listeria* challenge or whether the protective power of the adherent cell population is vested in "contaminating" T cells. In this connection, it should be noted that up to 9% of lymphocytes in the adherent cell fractions lacked detectable surface immunoglobulin. At least a portion of these might be T cells that were trapped at random in the gel. Alternatively, *Listeria*-immune T cells, the great majority of which are S-phase lymphoblasts (16, 21), might have a greater tendency to adhere to anti-rat F(ab')$_2$ columns than do T cells at large. Evidence to support this proposition was secured in the following experiments in which S-phase TDL were radioactively labeled before the cells were applied to the immunoabsorbent gel.

Fractionation of S-phase lymphoblasts. TDL from either normal or 6-day *Listeria*-immune donors were incubated with [^{14}C]thymidine to label a cohort of lymphoblasts. The TDL were then applied to Sephadex anti-rat F(ab')$_2$ columns.

Table 2 indicates that approximately two-thirds of the lymphocytes segregated in the nonadherent fraction; the remainder were found in the adherent fraction. It is evident that the lymph of immune donors was enriched in labeled lymphoblasts. Since the latter were concentrated in the nonadherent fraction, it can be concluded that they had a low density of surface immunoglobulin.

Having shown that the lymph of *Listeria*-immune rats is enriched in nonadherent S-phase lymphoblasts, the possibility was tested that these newly formed cells have a greater tendency to bind to the immunoabsorbent than do T cells at large. TDL from 6-day *Listeria*-immune donors were labeled in vitro with [^{14}C]thymidine and applied to Sephadex anti-rat F(ab')$_2$ columns. Lymphocytes in the nonadherent, T-cell-enriched fraction were then fractionated on a second set of immunoabsorbent columns.

As regards nonadherent lymphocytes, Table 3 shows that 96% of the applied sample segregated again in the nonadherent fraction. Although only 4% of the cells were retained on the columns, this subpopulation had a higher specific activity than the nonadherent cell population that was applied to the gel. The results could be interpreted in several ways; however, they accord with the notion that S-phase lymphoblasts have a tendency to become trapped

TABLE 2. *Distribution of labeled S-phase TDL on immunoabsorbent columns*[a]

Expt	TDL donors	Applied sample		Nonadherent lymphocytes			Adherent lymphocytes		
		% Immunoglobulin positive	Sp act (cpm/10^5 TDL)	% TDL recovered	% Immunoglobulin positive	% cpm recovered	% TDL recovered	% Immunoglobulin positive	% cpm recovered
1	Immune	28	100	68	1	71	32	95	29
2	Immune	26	122	69	3	73	31	90	27
3	Immune	26	97	74	1	77	26	95	23
4	Immune	23	102	69	1	63	31	96	37
5	Immune	27	127	65	3	63	35	95	37
6	Normal	30	42	68	2	48	32	94	52
7	Normal	26	56	66	3	28	34	95	72
8	Normal	20	44	67	1	28	33	ND[b]	ND

[a] TDL from either normal or 6-day *Listeria*-immune donors were labeled in vitro with [^{14}C]thymidine and then fractionated on Sephadex anti-rat F(ab')$_2$ columns.
[b] ND, Not done.

TABLE 3. *Preferential trapping of labeled S-phase TDL on immunoabsorbent columns*

Applied sample[a]	Cell fraction	Lymphocytes recovered (%)	Immunoglobulin-positive lymphocytes (%)	Radioactivity recovered (%)	Sp act (cpm × 10^3/10^8 TDL)
Unfractionated TDL	Nonadherent	69	2	71	26
	Adherent[b]	31	93	29	24
Nonadherent TDL	Nonadherent	96	<1	89	25
	Adherent[c]	4	ND[d]	11	86

[a] TDL from 6-day *Listeria*-immune donors were labeled in vitro with [^{14}C]thymidine and then fractionated on Sephadex anti-rat F(ab')$_2$ columns. Thereafter, samples of the unfractionated cell population and nonadherent subpopulation were applied to similar immunoabsorbent columns.
[b] Recovered by elution of the columns with 20% rat serum.
[c] Recovered by digestion of the gel with dextranase
[d] ND, Not done.

on immunoabsorbent columns for reasons other than the density of their membrane immunoglobulin.

Failure of *Listeria*-immune lymphocytes to bind to affinity column via Fc receptors. Several investigators (1, 5, 30–32, 34) have reported that receptors for the Fc region of immunoglobulin are expressed on at least some antigen-activated T cells. In light of this finding, it was important to determine whether *Listeria*-protective lymphocytes can bind to Sephadex anti-rat F(ab')$_2$ columns via Fc receptors. This proposition was tested by determining the distribution of *Listeria*-immune TDL on conventional immunoabsorbent columns and columns containing an immunoabsorbent prepared with pepsin-treated F(ab')$_2$ antibody fragments (see Materials and Methods). The two absorbents contained approximately the same amount (weight/weight) of antibody protein. Samples of the applied TDL samples, and the nonadherent and adherent cell fractions, were examined for their content of immunoglobulin-bearing lymphocytes; the remainder were assayed for their protective function in recipient rats.

Table 4 indicates that the two immunoabsorbents selected immunoglobulin-bearing lymphocytes with approximately the same efficiency. With each, lymphocytes segregating in the nonadherent cell fractions were at least as effective as unfractionated TDL in their capacity to protect recipient rats against an i.v. *Listeria* challenge. But an additional finding of interest was the presence of protective cells in the adherent cell fractions. The cells were not identified with respect to class, but their retention on columns prepared with F(ab')$_2$ antibody fragments indicates that they did not bind to the gel via Fc receptors.

Allogeneic restriction of *Listeria*-immune lymphocytes. Recent studies in mice (39, 40) and rats (13) have shown that the efficient transfer immunity to *L. monocytogenes* depends upon the sharing between donor and recipient of specificities which are coded by major histocompatibility complex-associated genes. This restriction applies to activated T cells but not to B cells, at least in respect to their response

TABLE 4. *Failure of Listeria-immune TDL to bind to immunoabsorbent columns via Fc receptors*

Immunoabsorbent column	Cell fraction	Lymphocytes transferred × 10^{-4}/g[a]			Log_{10} protection[b]	
		Total	Immunoglobulin positive	Immunoglobulin negative	Liver	Spleen
Unfractionated		200	60	140	2.13	3.27
Rabbit anti-rat $F(ab')_2$[c]	Nonadherent	200	3	197	2.74	3.56
	Adherent	200	182	18	1.39	1.43
Rabbit $F(ab')_2$ Anti-rat $F(ab')_2$[d]	Nonadherent	200	5	195	2.55	3.20
	Adherent	200	182	18	1.03	1.27

[a] Obtained from 6-day *Listeria*-infected donors. The cells were infused into normal recipients 1 h after the recipients had been challenged i.v. with 3.32×10^6 *L. monocytogenes*.

[b] Mean difference 48 h after challenge in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls.

[c] Immunoabsorbent prepared in the usual manner by coupling rabbit immunoglobulin to Sephadex G-200.

[d] Immunoabsorbent prepared by coupling pepsin-treated rabbit immunoglobulin to Sephadex G-200.

to certain thymus-independent antigens (34, 35). Restriction was therefore used to identify *Listeria*-protective lymphocytes in the lymph of specifically immunized rats.

In the first experiment, 24 (Lewis × Brown Norway)F_1 hybrid rats (AgB_1, AgB_3) were incannulated on day 5 of an immunizing *Listeria* infection. TDL obtained during the first 24 h of lymph drainage were fractionated in the usual manner on anti-$F(ab')_2$ columns. Cells from the nonadherent and adherent cell fractions, and a sample of the unfractionated cell population, were then assayed for their protective power in recipients belonging to the Lewis (AgB_1) and Wistar-Furth (AgB_2) strains. In each case, the immunity transferred by an inoculum of 2×10^6 lymphocytes/g of body weight was determined by measuring the growth of *L. monocytogenes* in the liver and spleen of i.v. challenged cell recipients and a group of similarly infected but otherwise untreated controls belonging to the same inbred strain.

The results in Fig. 1 indicate that the unfractionated TDL transferred resistance that was more efficiently expressed in Lewis recipients that shared a haplotype with the immune lymphocyte donors. The immunity conveyed by nonadherent ("T") cells was similarly restricted. On the other hand, the level of resistance in recipients of adherent ("B") cells was too low and the variation from animal to animal was too great to determine whether it too was restricted.

In view of the indeterminant results obtained with the cells from the adherent fraction, a second experiment was undertaken in which nonadherent and adherent lymphocyte populations were prepared from the lymph of *Listeria*-immune (Lewis × DA)F_1 hybrid rats (AgB_1, AgB_4). The cells were assayed in syngeneic and allogeneic (Wistar-Furth) recipients. Figure 2 shows the log linear relationship between the level of immunity expressed in the spleen and the number of nonadherent lymphocytes transferred. A similar dose-response relationship was observed in syngeneic and allogeneic recipients, although on a cell-for-cell basis the immunity in syngeneic subjects was approximately 3 log_{10} units greater than that observed in allogeneic rats. There were insufficient cells to perform a similar dose response analysis for lymphocytes in the adherent fraction. Nevertheless, it is evident that the immunity conveyed by cells from the column-adherent fraction was restricted in the manner demonstrated for nonadherent lymphocytes. The above finding implies that the immunity conveyed by the adherent fraction is related to its content of contaminating T cells.

Localization of lymphoblasts in *Listeria*-induced exudates. Lymphoblasts from the thoracic duct have a penchant to localize in inflammatory foci (12, 14, 22). But an important and still unanswered question is whether these exudate-seeking cells belong to a particular class or whether lymphoblasts at large have an affinity for inflamed vascular endothelium. The problem was studied by labeling *Listeria*-immune TDL with [^{14}C]thymidine before fractionating the cells on immunoabsorbent columns. Both the nonadherent and adherent cell fractions contained labeled S-phase lymphoblasts. Inocula containing these labeled cells were infused in equal numbers (5×10^7) into similarly immunized recipients 1 h after the recipients had been stimulated intraperitoneally with 50 μg of killed *L. monocytogenes*. All were sacrificed 24 h after stimulation, at which time the exudates were harvested and analyzed radiometrically.

The nonadherent TDL fraction contained

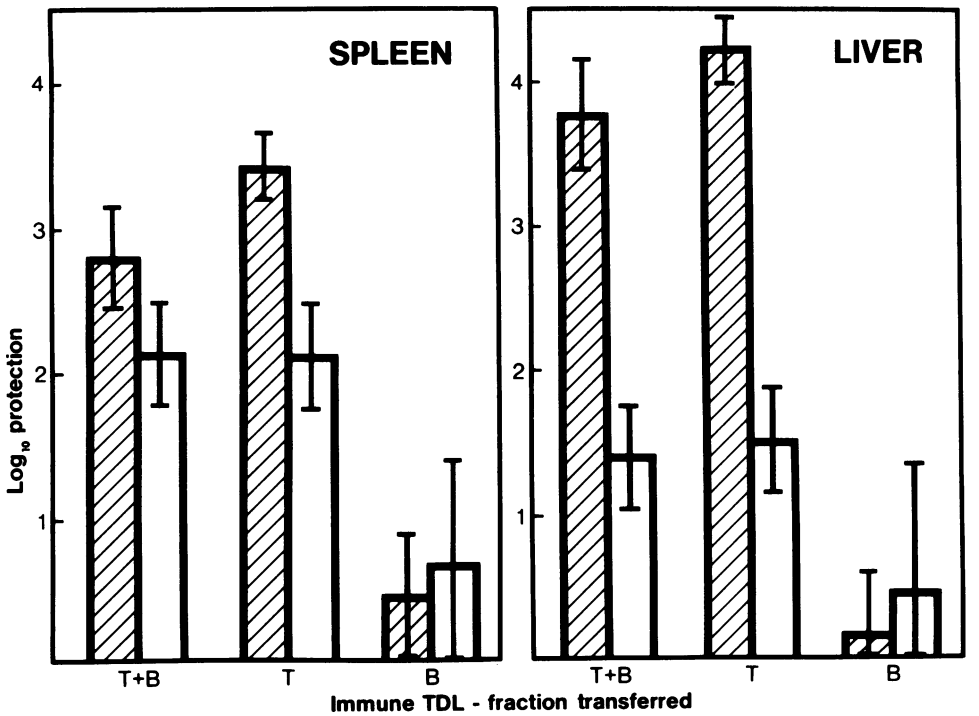

FIG. 1. *Immunity to L. monocytogenes conferred on semisyngeneic (▨) and allogeneic (□) rats by an i.v. inoculum of TDL obtained from 6-day Listeria-immune donors. Unfractionated TDL and cell populations enriched in either T or B cells were compared on a cell-for-cell basis, 2 × 10^8 lymphocytes per g of body weight. The immunity conveyed by the unfractionated cell population (T + B) and by T cells was restricted in allogeneic recipients. The level of immunity was too low and the variation too great to determine whether the B-cell population was similarly restricted. Means of five ± standard deviation.*

more labeled S-phase lymphoblasts than did the adherent fraction. Therefore, the localization in the exudates of labeled donor cells has been expressed in Table 5 as percent counts per minute originally present in the i.v. inoculum. All exudates contained labeled donor cells. However, the exudate-seeking capacity of lymphoblasts from the nonadherent fraction exceeded that of the adherent cells.

In the foregoing experiments, the localization in exudates of labeled lymphoblasts was compared at one time point only, namely, 23 h after transfer. In reality, however, nonadherent S-phase cells move in substantial numbers into exudates soon after their infusion into recipient rats. This was shown in another experiment in which rats injected with labeled lymphoblasts were sacrificed at predetermined intervals during the first 30 h after cell transfer. Figure 3 shows that at all time points, the exudate-seeking capacity of labeled nonadherent lymphoblasts exceeded that of similarly labeled adherent cells.

Protective capacity of column-fractionated PEC. Peritoneal exudates induced in *Listeria*-infected rats are an especially potent source of protective lymphocytes (15, 22). Since the cells concerned originate elsewhere and are, in fact, lineally related to S-phase TDL (16, 22), it is logical to postulate that they would segregate in a similar manner on affinity columns. To test this proposition, a large panel of specifically immunized rats was stimulated intraperitoneally with killed *L. monocytogenes*. Exudates induced in this manner were harvested 24 h after stimulation and depleted of macrophages (10). The macrophage-depleted population was then applied to Sephadex anti-rat $F(ab')_2$ columns.

Table 6 shows the results of two experiments with column-purified PEC. It is evident that protective cells were found in both the nonadherent and adherent fractions. On a cell-for-cell basis, however, nonadherent PEC were more effective than adherent cells in their capacity to transfer immunity against *L. monocytogenes*. The above finding accords with earlier reports that T cells accumulate in substantial numbers in allografts undergoing rejection (2) and implies

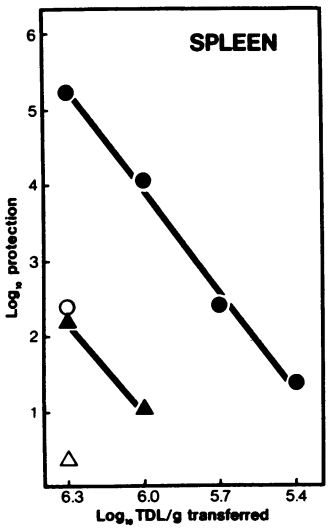

FIG. 2. *Dose response relationships between the level of antimicrobial immunity expressed in the spleen and the number of Listeria-immune TDL transferred into recipient rats. Nonadherent lymphocytes conveyed immunity in proportion to the number of cells transferred. However, the resistance expressed in syngeneic rats (●) was higher than that in allogeneic recipients (▲). The level of immunity conveyed by adherent lymphocytes (open symbols) was far less than that transferred by nonadherent cells, but it too was restricted. Means of five rats per group.*

that cellular resistance depends for its full expression upon the local deployment of T cells in centers of infection.

DISCUSSION

The results reported here indicate that immunity to *L. monocytogenes* is mediated in rats by lymphocytes that lack a high density of membrane immunoglobulin. Antisera of the appropriate specificity and potency were unavailable to identify the cells concerned; nevertheless, there are reasons for thinking that they belong to a T-lymphocyte line as they do in mice (3, 17, 26). Aside from lacking a high density of surface immunoglobulin, the protective rat cells realize their full immunological potential only in recipients that share certain histocompatibility-gene-coded structures (13; Fig. 1 and 2). Allogeneic restriction has been observed in respect to a variety of activated T-cell functions (13, 25, 33, 39). This restriction has been taken as evidence that two specificity requirements must be satisfied for secondary stimulation of T cells. One set of specificities is dictated by antigen; the other is determined by surface membrane structures that are coded by genes associated with the major histocompatibility complex (25).

An interesting and unexpected observation was the ability of column-adherent lymphocytes to transfer a low but still highly significant level of resistance against *L. monocytogenes*. In retrospect, this might have been anticipated, because the fractionation procedure was less efficient in preparing purified subpopulations of immunoglobulin-bearing lymphocytes than it was in removing these cells from the lymph. Thus, up to 9% of column-adherent TDL lacked detectable surface immunoglobulin. Since the great majority (perhaps all) of lymphocytes in rat thoracic duct lymph display either T- or B-cell markers (9), and the majority of B-TDL express membrane immunoglobulin (27), it is logical to postulate that some T cells become trapped in the gel for reasons other than the density of immunoglobulin they display at the cell surface.

The above finding implies that the column-adherent fraction of immune TDL contained protective lymphocytes of the kind found in higher concentration in the nonadherent fraction. Several lines of evidence support this notion. First, inocula prepared from the lymph of *Listeria*-immune donors convey immunity in proportion to their content of lymphocytes that

TABLE 5. *Localization of labeled S-phase TDL in peritoneal inflammatory exudates*[a]

Expt	Donor TDL	Immunoglobulin-positive lymphocytes (%)	Radioactivity in exudate (%)[b]
1	Unfractionated	26	3.75 ± 0.90
	Nonadherent	1	3.88 ± 0.27
	Adherent	95	2.40 ± 0.37
2	Unfractionated	27	3.25 ± 0.21
	Nonadherent	3	4.56 ± 0.49
	Adherent	95	0.85 ± 0.23
3	Unfractionated	26	4.28 ± 0.65
	Nonadherent	2	5.53 ± 1.08
	Adherent	90	3.34 ± 0.49
4	Unfractionated	28	6.41 ± 0.42
	Nonadherent	1	5.75 ± 0.84
	Adherent	95	3.81 ± 0.52

[a] TDL from 6-day *Listeria*-immune donors were labeled in vitro with [^{14}C]thymidine before fractionation of the cells on Sephadex anti-rat F(ab')$_2$ columns. A total of 5×10^7 lymphocytes from the individual fractions were injected i.v. into similarly infected recipients 1 h after the recipients had been stimulated intraperitoneally with 50 μg of killed *L. monocytogenes*.

[b] Exudates harvested 24 h after stimulation. Radioactivity expressed as percent counts per minute originally present in donor TDL inoculum. Means of five ± standard deviation.

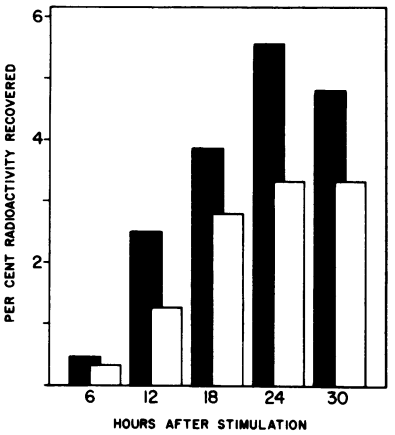

FIG. 3. *Histogram describing the localization of radioactively labeled lymphoblasts in peritoneal inflammatory exudates. Cells from the thoracic duct lymph of Listeria-immune donors were labeled in vitro with [^{14}C]thymidine and then fractionated on anti-F(ab')$_2$ columns. Lymphocytes from the nonadherent and adherent cell fractions were infused in equal numbers into syngeneic recipients 1 h after the recipients had been stimulated intraperitoneally with killed L. monocytogenes. Labeled lymphoblasts from the nonadherent fraction (closed bars) localized in larger numbers in the exudates than did lymphoblasts from the adherent fraction (open bars). Means of five rats per group.*

TABLE 6. *Distribution of Listeria-immune PEC on immunoabsorbent columns prepared with rabbit antibody to rat F(ab')$_2$*

Expt	Cell fraction[a]	No. of PEC transferred ($\times 10^{-4}$/g)	Log_{10} protection[b]	
			Liver	Spleen
1	Unfractionated	50	1.82	2.24
	Nonadherent	50	2.25	3.18
	Adherent	50	1.25	1.45
2	Unfractionated	20	1.51	1.44
	Nonadherent	20	1.52	1.67
	Adherent	20	0.80	0.83

[a] Obtained from 7-day *Listeria*-immune rats 24 h after the animals had been stimulated intraperitoneally with 50 μg of killed *L. monocytogenes*. PEC from 15 donors were pooled and depleted of macrophages (13) before application of the cells to the column.

[b] Mean difference 48 h after challenge in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls.

lack detectable surface immunoglobulin; the level of immunity is unrelated to the number of immunoglobulin-bearing lymphocytes transferred. Second, lymphocytes from the adherent cell fraction are restricted in the manner observed for T cells; i.e., the immunity they convey is dependent upon the sharing of histocompatibility-gene-derived products between the immune lymphocyte donor and the animals into which they are infused (Fig. 2). Finally, it was shown that nonadherent TDL that have been labeled in the S phase of the mitotic cycle have a greater tendency to become "trapped" on immunoabsorbent columns than do nonadherent lymphocytes at large (Table 3). The latter observation becomes significant when it is remembered that S-phase lymphoblasts are the principal mediators of resistance to *L. monocytogenes*, at least in rats (16, 21).

Whereas recently activated T cells have a tendency to adhere to anti-F(ab')$_2$ columns, it is not known whether they do so because they have a relatively high density of membrane immunoglobulin. However that may be, the presence of many *Listeria*-immune lymphocytes in the nonadherent cell fraction implies that these lymphocytes either lack receptors that are recognized by anti-rat F(ab')$_2$ or possess such receptors in insufficient number to cause attachment of the cells to the immunoabsorbent gel.

It is unlikely that *Listeria*-immune lymphocytes adhere to the gel via naked Fc receptors. Parish and Hayward (27)and Mason (24) used rosetting techniques to quantify membrane receptors on rat TDL. Although receptors for Fc and C3 were demonstrated on a portion of small lymphocytes, these were either absent or expressed in very low density on the surfaces of lymphocytes that were labeled in the S phase of the mitotic cycle. Moreover, in the present investigation *Listeria*-protective lymphocytes were retained in approximately equal numbers by immunoabsorbents that were prepared with either whole antibody or pepsin-treated antibody to rat F(ab')$_2$. Since pepsin treatment destroys the Fc portion of the molecules, it can be concluded that *Listeria*-protective cells are retained on the immunoabsorbent columns for reasons other than their capacity to bind via Fc receptors.

The present findings merit further comment insofar as they revealed several limitations of affinity chromatography as a procedure for preparing cell populations enriched in T or B cells. Aside from the inability of the immunoabsorbent to bind a few immunoglobulin-bearing lymphocytes and the possibility that some T cells are entrapped because of size or other reasons, it was observed that PEC could not be fractionated as efficiently as TDL. Indeed, PEC could be fractionated in large numbers only after the cell population had been deleted of macrophages by treatment with carbonyl iron.

The results reported here, in conjunction with earlier studies in mice and rats (20, 23), point to T cells as the specific mediators of immunity to *L. monocytogenes*. However, a role for B cells

cannot be discounted categorically, despite compelling evidence that serum antibodies do not protect (18) and that the immunity conveyed by B-cell-enriched TDL fractions is related, at least in part, to their content of contaminating T cells. B cells might operate as mediators of resistance if they, like T cells, were to release factors that influence the local deployment and/or functional activation of macrophages in centers of infection. Wall et al. (37, 38) have shown that chemotactic activity is generated in vitro by polyclonal stimulation of B cells, provided a small number of "helper" T cells are present in the incubation mixture. Likewise, Bloom and Shevach (4) reported that the production of macrophage migration inhibitory factor by B cells is dependent upon the presence of activated T cells in the reaction mixture. It is not known whether B cells can be stimulated in this way to release mediators that have a purposeful role in the expression of resistance to *L. monocytogenes*. In our experience (unpublished observations) we observed an additive effect, but not synergism, between adherent and nonadherent cells prepared by affinity chromatography from the thoracic duct lymph of *Listeria*-immune rats. The addition of a small number of nonadherent cells to a much larger number of adherent lymphocytes increased the protective power of the mixture in proportion to the number of nonadherent T cells in the mixture.

Whereas a role for B cells cannot be excluded, their capacity to operate as mediators of resistance to *L. monocytogenes* is clearly subordinate to that of T cells. The reasons are not far to seek when it is remembered that *L. monocytogenes* provokes a vigorous T-cell response and a conspicuous increase in the delivery of nonadherent S-phase lymphoblasts to the thoracic duct. This consideration, together with the fact that on a cell-for-cell basis nonadherent S-phase lymphoblasts exceed adherent cells in both their exudate-seeking capacity and protective function, indicates that recently activated T cells are ideally suited to operate as mediators of cellular resistance to infection.

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LITERATURE CITED

1. **Anderson, C. L., and H. M. Grey.** 1974. Receptors for aggregated IgG on mouse lymphocytes. Their presence on the thymocytes, thymus-derived and bone-marrow-derived lymphocytes. J. Exp. Med. **139**:1175–1188.
2. **Bhan, A. K., C. L. Reinisch, R. H. Levey, R. T. McCluskey, and S. F. Schlossman.** 1975. T-cell migration into allografts. J. Exp. Med. **141**:1210–1215.
3. **Blanden, R. V., and R. E. Langeman.** 1972. Cell-mediated immunity to bacterial infection in the mouse. Thymus-derived cells as effectors of acquired resistance to *Listeria monocytogenes*. Scand. J. Immunol. **1**:379–388.
4. **Bloom, B. R., and E. Shevach.** 1975. Requirement for T cells in the production of migration inhibitory factor. J. Exp. Med. **142**:1306–1311.
5. **Brown, G., and M. F. Greaves.** 1974. Cell surface markers for human T and B lymphocytes. Eur. J. Immunol. **4**:302–310.
6. **Chess, L., R. T. MacDermott, and S. F. Schlossman.** 1974. Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. J. Immunol. **113**:1113–1121.
7. **Crum, E. D., and D. D. McGregor.** 1976. Functional properties of T and B cells isolated by affinity chromatography from rat thoracic duct lymph. Cell. Immunol. **23**:211–222.
8. **Erb, P., M. Feldman, and N. M. Hogg.** 1976. Role of macrophages in the generation of T helper cells. IV. Nature of genetically related factor derived from macrophages inoculated with soluble antigen. Eur. J. Immunol. **6**:365–372.
9. **Goldschneider, I., and D. D. McGregor.** 1973. Anatomical distribution of T and B lymphocytes in the rat. Development of lymphocyte-specific antisera. J. Exp. Med. **138**:1443–1465.
10. **Goldstein, P., and H. Blomgren.** 1973. Further evidence for autonomy of T cells mediating specific *in vitro* cytotoxicity: efficiency of very small amounts of highly purified T cells. Cell. Immunol. **9**:127–141.
11. **Jaffer, A. M., G. Jones, E. J. Kasdon, and S. F. Schlossman.** 1973. Local transfer of delayed hypersensitivity by T lymphocytes. J. Immunol. **111**:1268–1269.
12. **Jungi, T. W., and D. D. McGregor.** 1978. Activated lymphocytes trigger lymphoblast extravasation. Cell. Immunol. **38**:76–83.
13. **Jungi, T. W., and D. D. McGregor.** 1978. Allogeneic restriction of acquired antimicrobial resistance in the rat. J. Immunol. **121**:449–455.
14. **Jungi, T. W., and D. D. McGregor.** 1978. Allogeneic restriction of the delayed inflammatory reaction in the rat. J. Immunol. **121**:456–463.
15. **Koster, F. T., D. D. McGregor, and G. B. Mackaness.** The migration of immunologically committed lymphocytes into inflammatory exudates. J. Exp. Med. **133**:400–409.
16. **Kostiala, A. A. I., D. D. McGregor, and M. J. Lefford.** 1976. The mediator of cellular immunity. XI. Origin and developments of MIF producing lymphocytes. Cell. Immunol. **24**:318–327.
17. **Lane, F. C., and E. R. Unanue.** 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. J. Exp. Med. **135**:1104–1112.
18. **McGregor, D. D., H. H. Hahn, and G. B. Mackaness.** 1973. The mediator of cellular immunity. V. Development of cellular resistance to infection in thymectomized irradiated rats. Cell. Immunol. **6**:186–199.
19. **McGregor, D. D., F. T. Koster, and G. B. Mackaness.** 1971. The mediator of cellular immunity. I. The lifespan and circulation dynamics of the immunologically committed lymphocyte. J. Exp. Med. **133**:389–399.
20. **McGregor, D. D., and A. A. I. Kostiala.** 1976. Role of lymphocytes in cellular resistance to infection, p. 237–266. *In* W. O. Weigle (ed.), Contemporary topics in immunology. Plenum Press, New York.
21. **McGregor, D. D., and P. S. Logie.** 1973. The mediator

of cellular immunity. VI. Effect of the antimitotic drug vinblastine on the mediator of cellular resistance to infection. J. Exp. Med. **137**:660–674.

22. **McGregor, D. D., and P. S. Logie.** 1974. The mediator of cellular immunity. VII. Localization of sensitized lymphocytes in inflammatory exudates. J. Exp. Med. **139**:1415–1430.
23. **Mackaness, G. B.** 1972. Lymphocyte-macrophage interaction, p. 163–176. *In* I. H. Lepow and P. A. Ward (ed.), Academic Press Inc., New York.
24. **Mason, D. W.** 1976. The requirement of C_3 receptors on the precursors of 19S and 7S antibody-forming cells. J. Exp. Med. **143**:1111–1121.
25. **Miller, J. F. A. P., and M. A. Vadas.** 1976. Antigen activation of T lymphocytes: influence of major histocompatibility complex. Cold Spring Harbor Symp. Quant. Biol. **41-2**:579–588.
26. **North, R. J., and G. Spitalny.** 1974. Inflammatory lymphocytes in cell-mediated antibacterial immunity: factors governing the accumulation of mediator T cells in peritoneal exudates. Infect. Immun. **10**:489–502.
27. **Parish, C. R., and J. A. Hayward.** 1974. The lymphocyte surface. II. Separation of Fc receptor, C'_3 receptor and surface immunoglobulin-bearing lymphocytes. Proc. R. Soc. London Ser. B **187**:65–81.
28. **Schlossman, S. F., and L. J. Hudson.** 1973. Specific purification of lymphocyte populations on populations on a digestible immunoabsorbent. J. Immunol. **110**: 313–319.
29. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods. Iowa State University Press, Ames.
30. **Soteriades-Vlachos, C., M. I. C. Gyöngyössy, and J. H. L. Playfair.** 1974. Rosette formation by mouse lymphocytes. III. Receptors for immunoglobulin on normal and activated T cells. Clin. Exp. Immunol. **18**: 187–192.
31. **Stout, R. D., and L. A. Herzenberg.** 1975. The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. J. Exp. Med. **142**:611.
32. **Stout, R. D., and L. A. Herzenberg.** 1975. The Fc receptor on thymus-derived lymphocytes. II. Mitogen responsiveness of T lymphocytes bearing the Fc receptor. J. Exp. Med. **142**:1041.
33. **Thomas, D. W., U. Yamashita, and E. M. Shevach.** 1977. The role of Ia antigens in T cell activation. Immunol. Rev. **35**:97–120.
34. **Toivanen, A., and P. Toivanen.** 1975. Histocompatibility requirements for cooperation between donor and host cells in transplantation of bursal stem cells in immunodeficient chicks. Transplant. Proc. **7**:165–171.
35. **Toivanen, P., A. Toivanen, and T. Sorvari.** 1974. Incomplete restoration of the bursa-dependent immune system after transplantation of allogeneic stem cells into immunodeficient chicks. Proc. Natl. Acad. Sci. U.S.A. **71**:957–961.
36. **Van Boxel, J. A., and D. L. Rosenstreich.** 1974. Binding of aggregated α-globulin to activated T lymphocytes in the guinea pig. J. Exp. Med. **139**:1002–1012.
37. **Wall, S. M., G. M. Iverson, and J. J. Oppenheim.** 1974. Induction of guinea pig B-cell lymphokine synthesis by mitogeneic and non-mitogeneic signals to Fc, Ig and C_3 receptors. J. Exp. Med. **140**:1631–1645.
38. **Wall, S. M., and D. L. Rosenstreich.** 1976. Role of B lymphocytes in cell-mediated immunity. I. Requirement for T cells or T-cell products for antigen induced B-cell activation. J. Exp. Med. **144**:1175–1187.
39. **Zinkernagel, R. M.** 1974. Restriction by the H-2 gene complex of the transfer of cell-mediated immunity to *Listeria monocytogenes*. Nature (London) **251**: 230–232.
40. **Zinkernagel, R. M., A. Althage, B. Adler, R. V. Blanden, W. F. Davidson, U. Kees, M. B. C. Dunlop, and D. C. Shreffler.** 1977. H-2 restriction of cell-mediated immunity to an intracellular bacterium. Effector T cells are specific for *Listeria* antigen in association with H-2 I region-coded self markers. J. Exp. Med. **145**: 1353–1367.