DISTRIBUTION OF LABELED LYMPH NODE CELLS IN MICE DURING THE LYMPHOCYTOSIS INDUCED BY *BORDETELLA PERTUSSIS**

BY ROBERT N. TAUB,‡ WALTER ROSETT, ANDY ADLER, AND
STEPHEN I. MORSE

(*From the Transplantation Immunology Laboratory, Departments of Surgery and
Medicine, Mount Sinai School of Medicine, New York 10029; and the
Department of Microbiology and Immunology, State University of
New York, Downstate Medical Center, Brooklyn, New York 11203*)

The mechanisms by which intravenously injected *Bordetella pertussis* organisms provoke striking lymphocytosis in mice are incompletely understood. The primary increase in blood lymphocytes is not due to newly formed cells, but to mature-appearing small lymphocytes that do not incorporate previously injected tritiated thymidine (1). Also, there is depletion of small lymphocytes from the lymph nodes, spleen, and thymus of mice during *B. pertussis*-induced lymphocytosis, suggesting that the cells in the blood may have been displaced from these organs (2). It has not been determined whether the principal locus of action of pertussis is on the blood lymphocytes or on the lymphoid organs through which they recirculate; nor has it been clarified how pertussis brings about the redistribution of lymphoid cells.

Recent studies from this laboratory have emphasized the relevance of changes in lymphocyte distribution in draining lymph nodes to the action of locally injected immunologic adjuvants, such as *B. pertussis* (3, 4). It was shown that *B. pertussis* organisms may indirectly influence lymphocyte traffic in the draining lymph node by affecting reticuloendothelial elements in the paracortical zone of the node (4, 5). The present studies deal with the direct effects of *B. pertussis* organisms and culture supernatant fluids on lymphocytes and their recirculatory properties.

Materials and Methods

Pertussis Organisms and Culture Supernatant Fluids.—Suspensions of killed *Bordetella pertussis* organisms in saline were obtained from Glaxo Ltd., London, England, washed twice with saline, and resuspended at a concentration of 5×10^9 organisms/ml. Supernatant fluids from *B. pertussis* cultures (BPCS)[1] were harvested after growth in liquid culture medium as described previously (6).

---

[1] *Abbreviations used in this paper:* BPCS, *B. pertussis* cultures; LPF, lymphocytosis-promoting factor.

Mice.—3-month old male CBA/J mice, obtained from the Jackson Laboratory, Bar Harbor, Maine, were used both as donors and recipients of radioactively labeled lymphoid cells.

Thoracic Duct Cannulation.—Performed by the method of Morse and Riester (7).

Preparation of Cell Suspensions and Radiolabeling.—Single-cell suspensions of mouse lymph node cells were obtained by pressing the lymph nodes of donor mice through nylon mesh and filtering through cotton wool as described previously (8). Thoracic duct cells were collected overnight in heparinized minimal Eagle's medium. Thrice washed cell suspensions were incubated with sodium chromate Cr-51 (Radiochemical Center, Amersham-Searle Corp., Des Plains, Ill., specific activity 5 mCi/mg) at a concentration of 10 μCi/10^8 cells for 30 min at 37°C. The suspensions were then washed three times and injected intravenously in dosages of 1.0–1.5 $\times$ 10^7 cells in a volume of 0.5 ml into each of three or more recipients per group. 18 hr later, the recipients were killed and samples of the "peripheral" (brachial, axillary, inguinal) nodes, mesenteric nodes, spleen, liver, and 0.25 ml samples of peripheral blood were assayed for radioactivity in a Picker Autowell gamma scintillation spectrometer (Picker X-Ray Corp., Cleveland, Ohio). The ability of the injected lymphoid cells to home to the lymph nodes of the recipients was assessed as a function of the proportion of the injected radioactivity lodging in the recipient lymphoid organs.

Blood Counts.—Peripheral leukocytes from treated mice were counted in a Neubauer hemacytometer and differential counts of 100 cells were made from Wright-stained blood smears.

RESULTS

Lymphocytosis.—Either whole *B. pertussis* organisms or the supernatants of *B. pertussis* cultures (BPCS) were capable of provoking a brisk leukocytosis, beginning several hours after intravenous injection, and reaching a peak between 3 and 6 days afterward (Fig. 1). If whole organisms were injected, there was a marked increase in both lymphocytes and mature granulocytes,
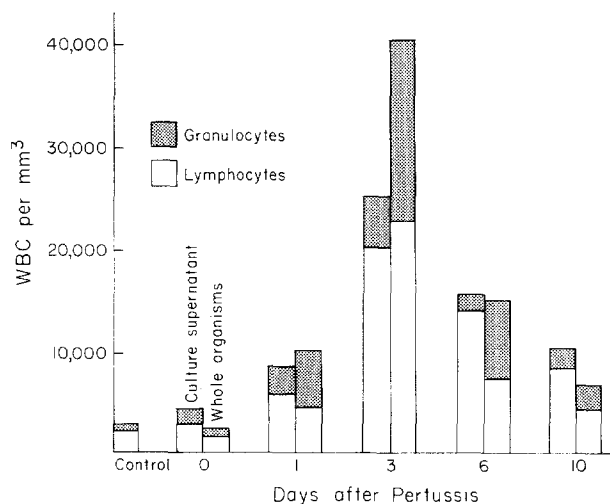

Fig. 1. Leukocytosis and lymphocytosis in mice given *B. pertussis* whole organisms or culture supernatant. Each bar graph represents the mean value obtained from four animals.

while after injection of BPCS lymphocytes predominated, with a lesser increment in granulocytes.

Effects of In Vitro Incubation of Cr-51-Labeled Lymph Node Cells with B. pertussis Culture Supernatants.—Cr-51-labeled lymph node cells were incubated for 15 min at 4°C with BPCS, washed thrice, and injected into syngeneic recipients in dosages of 10^7 cells each. Undiluted BPCS was toxic to lymphocytes and almost totally inhibited lymphocyte "homing" to lymph nodes or to spleen (Table I). BPCS diluted 1:10 showed no toxicity by the trypan blue exclusion

TABLE I

Effect of In Vitro Incubation with B. pertussis Culture Supernatant Fluids on the Migration of Cr-51-Labeled Lymph Node Cells and Thoracic Duct Cells

| Cells | Incubation with | Percentage of injected radioactivity distributing to: | | | | | Cell viability |
		Peripheral nodes	Mesenteric nodes	Spleen	Liver	Blood	
							%
Lymph node	Supernatant (undilute)	0.00* (0.02)	0.01 (0.02)	9.12 (0.88)	29.69 (3.00)	0.56 (0.04)	75
	Supernatant (1:10)	0.73 (0.07)	1.76 (0.31)	17.66 (4.08)	21.46 (2.22)	1.59 (0.14)	95
	Control	4.05 (0.46)	6.63 (0.96)	12.36 (0.95)	17.28 (1.50)	0.76 (0.06)	95
Thoracic duct	Supernatant (1:16)	0.26‡ 0.17‡	0.29 0.08	36.03 39.59	17.49 18.71	6.28 3.91	81
	Control	3.86 3.35	3.96 1.55	20.76 26.59	11.86 12.64	0.66 0.93	85

* Mean ± (SD) of four animals.

‡ Values in each of two animals.

test, yet it was almost as effective in inhibiting migration to lymph nodes. The proportion of cells migrating to the spleen was unaffected, and more of the injected radioactivity remained in the peripheral blood. As seen in Table I, when thoracic duct lymphocytes were employed in place of lymph node cells, incubation with BPCS also markedly inhibited lymphocyte migration.

Prevention of Lymphocyte Homing in Pertussis-Treated Animals.—Groups of mice were given a single intravenous injection of 0.2 ml of a saline suspension containing 1×10^9 pertussis organisms or 0.15 ml of undiluted culture supernatant. At various times afterward these mice were challenged intravenously with radioactively labeled lymphocytes. Significantly greater recovery of radioactively labeled cells in the blood was noted in pertussis-treated animals

(Table II). This effect was manifest as early as 1 hr after the injection of whole organisms and was present even when cells were given 6 days after pertussis injection. After injection of whole organisms, there was also usually a decrease in the percentage of label found in the mesenteric nodes, whereas there was a slight increase in the radioactivity in the spleen.

When labeled cells were injected into mice which had received BPCS 3 days previously, there was not only a higher level of radioactivity in the blood than that found in the controls but, in contrast to the findings in mice given whole organisms, there was a marked decrease in the radioactivity recovered in both

TABLE II

Distribution of Cr-51-Labeled, Normal, Syngeneic Donor Lymph Node Cells in Mice Treated with B. pertussis Organisms or Culture Supernatant

Treatment	Interval before injection of labeled cells	WBC at time of sacrifice	Percentage of injected radioactivity distributing to:				
			Blood	Peripheral nodes	Mesenteric nodes	Spleen	Liver
Intact orga-	1 hr	6,250	1.5*	3.1	4.1	20.7	15.5
nisms			(0.1)	(0.5)	(0.4)	(1.8)	(1.1)
	1 day	17,600	2.3	3.8	4.6	21.5	14.8
			(0.3)	(0.6)	(0.5)	(2.8)	(0.9)
	3 days	45,400	2.4	3.5	6.0	21.4	16.8
			(0.2)	(0.5)	(1.6)	(1.0)	(1.8)
	6 days	36,500	1.9	3.7	4.3	22.0	16.0
			(0.5)	(0.9)	(0.9)	(1.6)	(1.4)
Untreated	—	2,500	0.9	3.1	7.3	17.5	17.7
			(0.3)	(1.4)	(1.3)	(4.6)	(5.0)
Culture super-	3 days	25,300	3.4	3.9	5.8	6.6	18.6
natant			(0.7)	(0.7)	(0.9)	(1.6)	(1.2)
Untreated	—	4,100	0.7	6.0	11.9	12.7	15.4
			(0.2)	(0.5)	(1.9)	(1.3)	(1.0)

* Each value represents the mean ± (SD) obtained from four animals.

the peripheral and mesenteric nodes. Whereas there had been a slight increase in the quantity of label in the spleen in mice pretreated with whole organisms, a marked decrease was found in BPCS-treated animals.

Shift of Radioactively Labeled Cells from Lymphoid Tissues to Blood in Pertussis-Treated Recipients.—Groups of mice were injected intravenously with 1 × 10^8 Cr-51-labeled lymph node cells, and 24 hr later were given either a dose of BPCS or whole pertussis organisms, or were left untreated. At various times afterward three mice from each group were dissected and the proportion of injected radioactivity distributing to lymphoid organs was determined. The changes in distribution due to pertussis were assessed by determining the ratio between the treated animals and simultaneously sacrificed untreated controls. In those animals receiving BPCS there was a clear relative shift of radioactivity

from lymph nodes into the blood, approximating the kinetics of pertussis-induced lymphocytosis in these animals (Table III and Fig. 2). At the same time there was a relative loss of radioactivity in the mesenteric nodes, small intestine, pooled peripheral (brachial, axillary, inguinal) nodes, and the spleen.

In animals treated with whole pertussis organisms rather than BPCS, there was a similar change in the distribution of labeled cells in the blood and nodes as compared with control untreated animals. However, the spleens of treated mice contained paradoxically more radioactivity than did controls (Table IV).

TABLE III

Changes with Time in the Distribution of Cr-51-Labeled Lymphoid Cells in Normal Mice and in Mice Injected Intravenously with B. pertussis Culture Supernatant Fluid 24 hr after the Injection of Labeled Cells

Time after pertussis supernatant	Percentage of injected radioactivity distributing to:					
	Blood	Peripheral nodes	Mesenteric nodes	Spleen	Liver	Ileum
1 hr pertussis	1.7 (0.1)*	3.5 (0.4)	6.4 (0.3)	20.8 (0.6)	14.7 (1.3)	3.2 (0.4)
control	1.9 (0.1)	4.2 (0.3)	5.9 (0.5)	19.3 (2.4)	15.1 (1.4)	3.9 (0.1)
1 day pertussis	5.4 (0.7)	3.5 (0.2)	3.5 (0.3)	13.2 (1.7)	15.2 (1.5)	2.1 (0.1)
sis control	1.5 (0.1)	3.7 (0.1)	7.4 (0.6)	14.0 (0.2)	14.4 (1.6)	3.0 (0.2)
3 days pertussis	3.5 (0.1)	1.2 (0.1)	1.4 (0.1)	8.0 (0.8)	15.4 (1.4)	1.1 (0.1)
sis control	0.8 (0.1)	1.7 (0.4)	3.6 (0.3)	1.3 (0.4)	11.4 (2.8)	2.5 (0.7)
6 days pertussis	3.3 (0.1)	1.1 (0.3)	1.1 (0.3)	7.9 (2.6)	14.7 (1.2)	1.0 (0.1)
sis control	1.1 (0.1)	1.5 (0.1)	3.3 (0.4)	10.2 (1.7)	11.6 (0.6)	1.5 (0.1)
10 days pertussis control	2.0 (0.4)	1.0 (0.1)	1.3 (0.1)	6.9 (0.1)	14.1 (0.8)	0.9 (0.1)
	0.6 (0.1)	2.7 (0.4)	3.2 (0.8)	12.0 (2.0)	9.7 (1.0)	1.2 (0.2)

* Each value represents the mean ± (SD) obtained from four animals.

Secondary Transfer of Cr-51-Labeled Lymph Node Cells from Normal or Pertussis-Treated Primary Recipients.—Groups of mice were injected intravenously with 1 × 10⁸ Cr-51-labeled lymph node cells, and 24 hr later were given pertussis supernatant intravenously, or were left untreated. 3 days later, single-cell suspensions were made from the pooled lymph nodes of each group, and injected in doses of 3 × 10⁸ into groups of normal secondary CBA recipients. The pattern of distribution of cells obtained from pertussis-treated recipients showed some increase in the blood and a decrease in the nodes (Table V).

Distribution of Lymphocytes from Pertussis-Treated or Normal Donors in Normal or Pertussis-Treated Recipients.—In this experiment, an attempt was
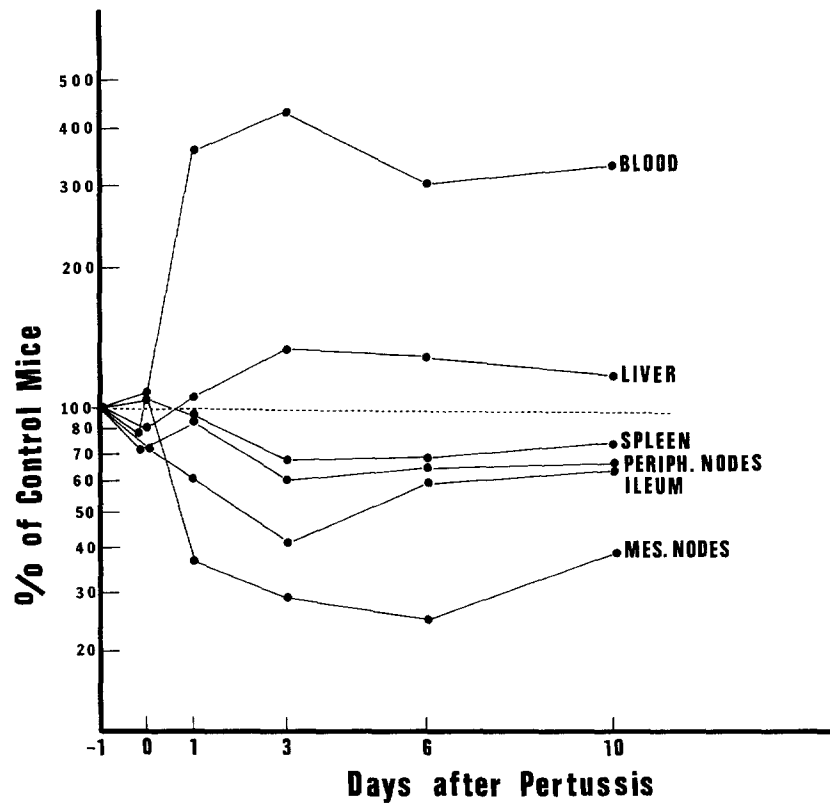
FIG. 2. Relative shifts of labeled cell activity after pertussis treatment. Each point represents: mean radioactivity of pertussis-treated group of three mice × 100; mean radioactivity of normal untreated group of three mice for each organ; the values from which this graph is derived are given in Table IV.

TABLE IV

Changes with Time in the Distribution of Cr-51-Labeled Lymph Node Cells in Normal Mice and in Mice Injected Intravenously with B. pertussis Organisms 24 hr after Labeled Cells*

	Percentage of injected radioactivity distributing to:				
	Blood	Peripheral nodes	Mesenteric nodes	Spleen	Liver
Injected with pertussis organisms	1.07 (0.14)‡	4.21 (0.17)	5.58 (0.70)	13.77 (0.86)	12.40 (0.70)
Controls	0.33 (0.04)	6.58 (0.98)	6.95 (1.07)	10.88 (0.73)	12.30 (1.51)
Ratio of pertussis to control	3.24	0.63	0.80	1.26	1.00

* The distributions shown were obtained 3 days after the injection of the bacterial cells.
‡ Each value is the mean ± (SD) of four animals.

made to determine if BPCS acted only on donor lymphocytes or whether homing could also be prevented at the lymph node sites of recirculation (e.g., the postcapillary endothelium in small venules) in recipients of Cr-51-labeled cells.

Table VI shows the distribution of lymph node cells taken from either

TABLE V

Secondary Transfer of Cr-51-Labeled Lymph Node Cells from Normal or Pertussis-Treated Primary Recipients

Cell source	Percentage of injected radioactivity distributing to:				
	Blood	Peripheral nodes	Mesenteric nodes	Spleen	Liver
Pertussis-treated primary recipients	1.08 (0.05)*	8.59 (0.37)	13.21 (1.10)	18.27 (1.61)	9.17 (0.50)
Normal primary recipients	0.44 (0.24)	6.68 (0.26)	17.27 (2.14)	18.28 (1 43)	8.68 (0.95)

* Each value represents the mean ± (SD) obtained from four animals.

TABLE VI

Effects of B. pertussis Culture Supernatant Given to Donors or Recipients of Cr-51-Labeled Lymph Node Cells

Treatment		Percentage of injected radioactivity distributing to:				
Donor	Recipient	Peripheral nodes*	Mesenteric nodes	Spleen	Liver	Blood
None	None	2.48 (0.60)‡	4.71 (0.14)	8.54 (1.01)	9.89 (0.69)	0.05 (0.01)
B. pertussis	None	1.32 (0.01)	3.05 (0.04)	9.42 (0.26)	18.39 (1.65)	0.12 (0.01)
None	B. pertussis	1.19 (0.20)	2.09 (0.45)	15.49 (2.01)	10.42 (1.51)	0.21 (0.08)
B. pertussis	B. pertussis	1.00 (0.20)	1.58 (0.04)	11.49 (0.80)	20.25 (1.03)	0.21 (0.08)

* Pooled brachial, axillary, inguinal.
‡ Each value is the mean ± (SD) of four animals.

normal or pertussis-treated donors injected into normal or pertussis-treated recipients. Pertussis-treated donors and recipients had received BPCS 4 days previously.

If pertussis had been given to either the donor or the recipient, the migration of donor cells to recipient lymph nodes was much reduced; more cells tended to stay in the blood. Cells from pertussis-treated donors, whether injected into normal or pertussis-treated recipients, showed greater localization in the liver than did cells from normal donors. Cells injected into pertussis-treated donors tended to show increased localization in the spleen.

Effect of In Vitro Incubation of Lymph Node Cells with Plasma from Pertussis-Treated Animals.—Plasma was harvested from animals injected 2 hr, 1 day, 3 days, or 6 days previously with 0.2 ml of *B. pertussis* supernatant fluid. Cr-51-labeled lymph node cells were incubated for 30 min in plasma from treated animals, thrice washed, and injected into syngeneic recipients. A slight but probably not significant inhibition of homing to mesenteric nodes was found when cells were incubated with plasma obtained either 2 or 24 hr after injection of BPCS.

Elution of Pertussis from Red Blood Cells and Lymphocytes.—The aim of this experiment was to allow the lymphocytosis-promoting factor of *B. pertussis* to attach to red blood cells of lymphocytes in vitro, wash the cells several times, and determine if activity detached from these cells and re-attached to a fresh inoculum of labeled lymphocytes. The scheme of this experiment is shown in Fig. 3. 0.5 ml of packed red blood cells or lymph node cells was incubated with
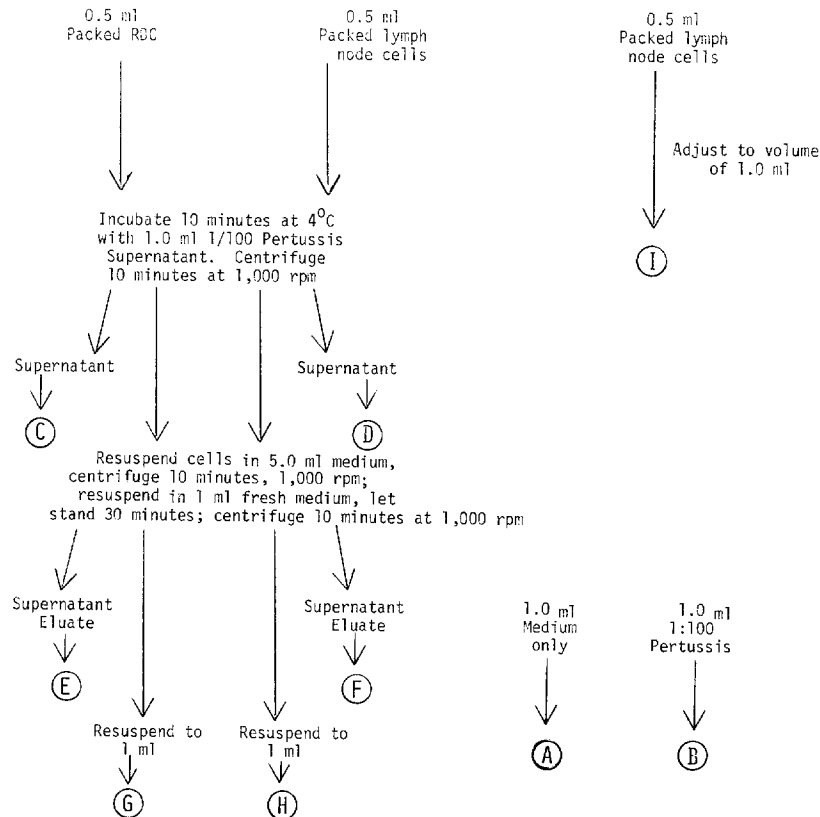

Fig. 3. Protocol of experiment on the elution of *B. pertussis* activity from red blood cells and lymph node cells. Add 1 million Cr-51-labeled cells in 0.5 ml to each lettered tube. Allow to incubate 30 min at 4°C, wash once, adjust volume to 3.0 ml, and inject three mice with aliquots of 0.5 cc, saving two aliquots for standard samples.

pertussis culture supernatant for 10 min and then washed once with medium. The cells were resuspended and allowed to stand in medium for 30 min and the eluate was removed and tested for its ability to interact with radioactivity tagged lymphocytes in vitro and prevent their later homing in syngeneic recipients (Table VII). Packed red cells bound a significant proportion of pertussis activity, which eluted readily back into fresh medium. Packed lymph node cells absorbed more of the pertussis activity and comparatively little could be eluted from the washed lymph node cells on incubation with

TABLE VII

Elution of Pertussis from Red Blood Cells or Lymph Node Cells

Cr-51-labeled lymph node cells incubated with	Percentage of injected radioactivity distributing to:			
	Blood*	Pooled lymph nodes‡	Spleen	Liver
Medium only	0.19§	10.36	17.02	9.39
	(0.01)	0.22	0.30	0.70
1/100 Pertussis	0.40	2.76	22.35	15.28
supernatant	(0.07)	0.00	1.28	2.14
Supernatant after absorption	0.52	5.74	22.02	14.53
by red blood cells	(0.10)	0.58	1.58	1.74
Supernatant after absorption	0.23	8.86	22.36	15.33
by lymph node cells	(0.12)	0.86	2.33	0.66
Eluate from pertussis-treated	0.46	1.88	21.61	13.77
red blood cells	(0.01)	0.81	1.11	2.57
Eluate from pertussis-treated	0.19	11.73	19.36	13.74
lymph node cells	(0.03)	0.55	1.55	1.25
Pertussis-treated red blood	0.51	4.30	23.65	13.70
cells	(0.05)	0.00	1.42	1.46
Pertussis-treated lymph	0.47	8.93	21.90	15.37
node cells	(0.05)	1.00	0.76	3.02
Normal unlabeled lymph	0.25	12.05	17.45	11.29
node cells	(0.08)	1.34	2.18	1.48

* 0.25 ml/mouse.

‡ Brachial, axillary, inguinal, mesenteric.

§ Mean ± (SD) of four animals.

fresh medium. Incubation of the treated, washed red blood cells or lymph node cells directly with radioactively labeled lymphocytes was sufficient to affect their ability to home to the lymph nodes of syngeneic recipients.

DISCUSSION

The studies of Gowans and his collaborators established that a certain subpopulation of lymphocytes recirculates from blood to lymph by traversing specialized endothelium in lymph nodes, spleen, and Peyer's patches of the ileum (9). Our present data indicate that the lymphocytosis-promoting factor (LPF) elaborated by B. pertussis acts to prevent lymphocyte recirculation, leading to an accumulation of affected cells in the bloodstream.

Incubation cf Cr-51-labeled thoracic duct cells or lymph node cells in vitro with BPCS abrogates their capacity to migrate to lymph nodes or normal recipients (Table I). Migration to recipient spleens is less affected, suggesting that LPF principally affects cells belonging to the lymph node-seeking recirculating cell pool.

Similarly, if pertussis is administered to prospective recipients of Cr-51-labeled mouse lymphocytes, the injected cells show a markedly diminished arrival in recipient lymph nodes, while more radioactivity remains in the peripheral blood (Table II). Analogous findings have been recorded in mice receiving uridine-labeled peripheral lymphocytes (10) and in LPF-treated rats given P-32-labeled thoracic duct cells or peripheral leukocytes (11). In mice, these effects are seen after treatment with either *B. pertussis* whole organisms or culture supernatant; however, there are differences in the distribution of cells after each agent. In animals treated with whole organisms, more injected lymphocytes reach periperal nodes and the spleen than in animals given BPCS, although in either case they remain unable to lodge in mesenteric nodes. It has been pointed out that whole pertussis organisms should be regarded as a mixture of antigens, endotoxins, and adjuvants (12, 13). It was previously noted that locally injected pertussis organisms affect reticuloendothelial cells in draining lymph nodes, causing increased trapping of lymphocytes in lymphoid tissue, an effect opposite to the lymphocytosis after intravenous administration (5). It may be that intravenously injected whole organisms simultaneously stimulate lymphocyte trapping as well as lymphocytosis, while the supernatant may lack the former activity.

The net effect of a decrease in homing of recirculating cells would be a gradual build-up of these cells in the peripheral blood and their depletion from specific regions of lymph nodes and spleen. This shift of cells was documented by our experiments in which animals were equipped with radioactivity labeled lymph node cells and injected 24 hr later with *B. pertussis* (Table III, Fig. 2). It has been previously shown that only recirculating lymphocytes lodge in recipient lymph nodes (14), and that the radioactivity declines gradually in these cells over a period of 2 wk or longer (8). If decay of the label was assumed to have proceeded equally in both treated and untreated animals, then cells belonging to the recirculating lymphocyte pool are displaced from lymph nodes, small intestine, and spleen into the peripheral blood of pertussis-treated mice. This shift of radioactivity is seen after injection of either *B. pertussis* whole organisms or supernatant extract, but is much less striking after the former. Also, after whole *B. pertussis* organisms the shift of labeled lymphocytes into the blood was accompanied by a paradoxical deposition of cells in the spleen, reflecting the simultaneous lymphocytosis and lymphocyte trapping effects (Table IV).

In order to determine whether the effect of pertussis was exerted on lymphocytes or on postcapillary endothelium of lymphoid tissues, a four-way transfer

experiment was performed in which cells from either normal or pertussis-treated donors were given to normal or pertussis-treated recipients. However, the net reduction in the ability of labeled cells to reach the lymph nodes of recipients was similar whether pertussis was given to the donor or to the recipient (Table VI). These results are in accord with those reported by Morse and Barron (10). This suggested that pertussis could act upon either lymphocytes or postcapillary endothelium to prevent lymphocyte recirculation, or alternatively that pertussis given to prospective recipients of normal lymph node cells is able to attach to the introduced lymphocytes.

Plasma taken from animals previously injected with pertussis was therefore assayed by in vitro incubation with labeled lymph node cells before their injection into normal recipients. Only miminal pertussis-like activity was found. However, our elution experiments established that pertussis attaches loosely and reversibly to erythrocytes and somewhat more firmly to lymphocyte surfaces. It is retained in pharmacologically active doses on red blood cells in vitro even after several washings, and may elute rapidly from these cells onto adjacent lymphocytes (Table VII).

Pertussis lymphocytosis thus appears to be a consequence of the attachment of the active principle of pertussis to surface sites on cells belonging to the mobilizable lymphocyte pool. Cells are likely to be affected while they are in the circulation rather than within the lymph node parenchyma, and for some time after the injection of pertussis cells are prevented from homing back to lymphoid tissue and progressively accumulate in the blood. Pertussis is bound loosely to red cells, and may persist as a reservoir on these cells until a lymphocyte is introduced.

These actions of B. pertussis organisms or culture supernatants on lymphocytes are sufficient to account for the magnitude and the kinetics of the observed lymphocytosis. It does not seem necessary to invoke a separate action of pertussis on postcapillary endothelium but this cannot be excluded, especially since pertussis organisms are known to have other effects on these or adjacent structures (5).

The thymus has been observed to decrease in cellularity during pertussis lymphocytosis (2). It cannot be told from our data whether there is a thymic contribution to the increase in cells in the peripheral blood, or whether the decrease in thymic cellularity reflects an endotoxic action of pertussis such as is observed after other endotoxins (12).

SUMMARY

The mechanism by which Bordetella pertussis organisms and their products induce lymphocytosis in mice was analyzed in terms of the localization of syngeneic Cr-51-labeled lymph node cells. Labeled lymphoid cells incubated in vitro with the supernatant of B. pertussis cultures and then injected intravenously into normal recipients, or labeled cells injected into pertussis-treated

recipients were unable to "home" to lymphoid organs but persisted for long periods in the blood. In animals "equipped" with a population of Cr-51-labeled lymphoid cells, administration of *B. pertussis* organisms or culture supernatant effected a shift of radioactivity from lymph nodes and spleen into the peripheral blood, coincident with the lymphocytosis.

In in vitro experiments it was found that the active principle could bind to both erythrocytes and lymphocytes and could spontaneously elute from these cells onto labeled lymphocytes which were then unable to home efficiently.

The data suggest that *Bordetella pertussis*-induced lymphocytosis involves a reversible attachment of the pertussis factor onto the surfaces of lymphocytes which prevents their recirculation to lymphoid organs. Recirculating lymphocytes are presumably affected as they emerge from lymphoid organs to enter the blood.

REFERENCES

1. Morse, S. I., and S. K. Riester. 1967. Studies on the leukocytosis and lymphocytosis induced by *Bordetella pertussis*. I. Radioautographic analysis of the circulating cells in mice undergoing pertussis-induced hyperleukocytosis. *J. Exp. Med.* **125**:401.

2. Morse, S. I. 1964. Studies on the lymphocytosis induced in mice by *Bordetella pertussis*. *J. Exp. Med.* **121**:49.

3. Dresser, D. W., R. N. Taub, and A. R. Krantz. 1970. The effect of localized injection of adjuvant material on the draining lymph node. II. Circulating lymphocytes. *Immunology.* **18**:663.

4. Taub, R. N., A. R. Krantz, and D. W. Dresser. 1970. The effect of localized injection of adjuvant material on the draining lymph node. I. Histology. *Immunology.* **18**:171.

5. Taub, R. N., and R. K. Gershon. 1972. The effect of localized injection of adjuvant material on the draining lymph node. III. Thymus dependence. *J. Immunol.* **108**:377.

6. Morse, S. I., and K. K. Bray. 1969. The occurrence and properties of leukocytosis and lymphocytosis stimulating material in the supernatant fluids of *Bordetella pertussis* cultures. *J. Exp. Med.* **129**:523.

7. Morse, S. I., and S. K. Riester. 1967. Studies on the leukocytosis and lymphocytosis induced by *Bordetella pertussis*. II. The effect of pertussis vaccine on the thoracic duct lymph and lymphocytes of mice. *J. Exp. Med.* **125**:619.

8. Taub, R. N., and E. M. Lance. 1968. Histopathologic effects in mice of heterologous antilymphocyte serum. *J. Exp. Med.* **128**:1281.

9. Gowans, J. L., and E. J. Knight. 1964. The route of re-circulation of lymphocytes in the rat. *Proc. R. Soc. Lond. B. Biol. Sci.* **159**:257.

10. Morse, S. I., and B. A. Barron. 1970. Studies on the leukocytosis and lymphocytosis induced by *Bordetella pertussis*. III. The distribution of transfused lymphocytes in pertussis-treated and normal mice. *J. Exp. Med.* **132**:663.

11. Iwasa, S., T. Yoshikawa, K. Fukumara, and M. Kurokawa. 1970. Effects of the

lymphocytosis-promoting factor from *Bordetella pertussis* on the function and potentiality of lymphocytes. I. Ability of lymphocytes to recirculate in the body. *Jap. J. Med. Sci. Biol.* **23:**47.

12. Farthing, J. R. 1961. The role of *Bordetella pertussis* as an adjuvant to antibody production. *Br. J. Exp. Pathol.* **42:**614.

13. Pieroni, R. E., and L. Levine. 1966. Adjuvant principle of pertussis vaccine in the mouse. *Nature (Lond.).* **211:**1419.

14. Lance, E. M., and R. N. Taub. 1969. Segregation of lymphocyte populations by differential migration. *Nature (Lond.).* **221:**841.

15. Rowlands, D. T., H. N. Claman, and P. D. Kind. 1965. The effect of endotoxin on the thymus of young mice. *Am. J. Pathol.* **46:**165.