MECHANISM OF EOSINOPHILIA

II. Role of the Lymphocyte*

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The preceding article describes findings which are compatible with the thesis that the eosinophil response to inoculation by a nematode parasite has characteristics of an immunologic phenomenon (1 *a*). Further evidence to this effect has been obtained in experiments employing various immunosuppressive agents (1 *b*). The present paper reports on tests for both humoral and cellular mediators of the reaction. Particular emphasis is placed on evidence that the lymphocyte plays a role, as it does in other expressions of immunity.

Materials and Methods

Unless stated, the experimental procedures in this work were those described in the preceding article (1 *a*).

Inbred Rats.—Two inbred rat strains were found to be suitable for this work. One, an SPF Wistar (WAfp), was obtained from the Medical Research Council Laboratory Animal Centre at Carshalton, Surrey; the other, a parasite-free strain (WAG), came from the Glaxo Research Farm, Harefield, Middlesex. The criterion for histocompatibility within each strain was survival of skin grafts for at least 100 days.

Antilymphocytic Sera.—Two types of rabbit, antirat, antilymphocyte serum were prepared. The first antiserum (ALS-1) was raised against thoracic duct lymphocytes using the method of Woodruff and Anderson (2). The second preparation (ALS-2) was obtained by immunizing another group of rabbits with lymphocytes derived from lymph nodes according to the method of Jeejeeboy (3). The rabbits were bled 10 days after the last injection of cells. Sera were inactivated by heating to 56°C for 30 min, absorbed with a quarter volume of packed erythrocytes, and stored in 1 ml portions at −20°C. Each serum was tested in a 200 g rat and was considered satisfactory for use if 4 hr after an intraperitoneal injection of 2 ml the blood mononuclear cell level had fallen to less than 15% of the preinjection figure.

Thoracic Duct Cannulation.—The thoracic duct was cannulated below the diaphragm by the method of Gowans and Knight (4). After surgery animals were placed in restraining cages and infused intravenously with isotonic saline containing 1 unit heparin/ml and 100 μg streptomycin/ml at a rate of approximately 2 ml/hr. Rats used as lymphocyte donors

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were usually killed after 36–48 hr but in some the fistula was kept patent for 5 days in order to deplete animals of the majority of their pool of circulating lymphocytes (5).

Lymph was collected in sterile 100 ml conical flasks containing 5 ml of Dulbecco balanced salt solution with 100 units heparin and 0.5 mg streptomycin added. Individual collections were made at room temperature for periods of not longer than 12–14 hr. To separate the cells lymph was centrifuged at 100 *g* for 10 min and the cell button resuspended in Dulbecco solution containing 1 unit heparin/ml and 100 μg streptomycin/ml (Dulbecco I). After a second centrifugation the lymphocytes were resuspended in Dulbecco I at a cell concentration not greater than 1×10^8/ml. Unless otherwise specified the cells were injected into syngeneic recipients by the intravenous route.

RESULTS

Attempts to Transmit the Stimulus to Eosinophil Production with Blood Plasma.—

Donor rats were inoculated with *Trichinella* larvae, by either the gastrointestinal or the intravenous route. In the case of natural infestation blood samples were collected on the 6th day, 13th day (at the height of the eosinophil response), and on the 35th day (at the time of peak antibody levels). When intravenous challenge was used collection was performed on the 1st, 2nd, and 5th days after inoculation. Groups of recipients were injected intraperitoneally and intravenously with doses of between 5 and 15 ml plasma/rat and eosinophil counts followed for 14 days.

The results with representative groups of rats are shown in Tables I and II. None of the test animals developed a significant eosinophil resonse.

In a second experiment 10 rats passively immunized with 15 ml of plasma of high antibody titer were rechallenged with parasites 24 hr later. In every case a normal but not an enhanced eosinophilia ensued. These studies provided no evidence that circulating antibody plays a significant role in either the primary or secondary eosinophil response to trichinosis.

Effect of Lymphocyte Depletion on the Eosinophil Response.—

Neonatal Thymectomy: The technique described by Jankovic et al. (6) was employed using 1–2½ day-old rats born to SPF Wistar strain mothers. Litter mates underwent sham operation but the thymus was not disturbed. Approximately 1 in 10 of the thymectomized animals developed a wasting syndrome and was discarded. The remainder which appeared comparable to their sham operated litter mates in weight and behavior were challenged at 8 wk of age with larvae by the gastrointestinal route. At the time of inoculation the mean blood lymphocyte count in the thymectomized animals was 4300 cells/mm^3 compared with a level of 9225/mm^3 in the sham operated controls.

The effect of this producedure on the eosinophil response was found to depend on the size of parasitic inoculum used. Thus when a small oral dose, i.e. 5–10 larvae/g body weight, was given, a significant reduction in eosinophila was observed (Fig. 1). If the strength of stimulus was increased to 15 larvae/g body weight or more the eosinophil peak of test animals became similar to that of the controls.

Antilymphocyte serum: Test animals were inoculated with 10,000 larvae by the intravenous route. Fig. 2 illustrates the effect of ALS-1 on the ensuing eosinophil response in three groups of recipients. Members of the first group received a 6 day course of ALS-1 beginning 24 hr before challenge, which resulted in marked inhibition of the expected rise in eosinophil level. When normal

TABLE I

Passive Transfer of Plasma at Varying Intervals after Inoculation of Trichinella Larvae by the Gastrointestinal Route

Time of collection of donor blood following infestation	Number of recipients	Day 0	Peak response (days 4–8)
Days		*Mean eosinophil counts/mm³* ± SE	
0	6	71 ± 9.5	127 ± 19
3–6	5	29 ± 3.5	85 ± 8
12–15	9	68 ± 11	81 ± 11.5
35	5	86 ± 10.5	96 ± 20
56	4	85 ± 14.5	105 ± 25

Donors were inoculated with 25 larvae/g body weight. Each recipient was given 15 ml plasma intraperitoneally.

TABLE II

Passive Transfer of Plasma at Varying Intervals after Inoculation of Trichinella Larvae by the Intravenous Route

Time of collection of donor blood following inocultion	Donors inoculated with 50,000 larvae i.v.	Number of recipients	Day 0	Peak response (days 4–8)
Days			*Mean eosinophil counts/mm³* ± SE	
–	–	6	52 ± 4.5	103 ± 21
1	+	10	107 ± 23.5	143 ± 28
2	+	6	56 ± 9.5	97 ± 17.5
5	+	4	40 ± 4	136 ± 21

Each recipient was given 5 ml plasma, half intravenously and half intraperitoneally.

rabbit serum was substituted or administration of ALS-1 was postponed until 72 hr after challenge a normal eosinophil response ensued. Thus the antiserum did not exert its effect by nonspecific inhibition of eosinopoiesis.

When a course of ALS-2 was given a slight reduction in eosinophilia appeared to result, but the response did not differ significantly from that of controls when subjected to statistical analysis (Table III).

Prolonged thoracic duct drainage: A group of rats was subjected to thoracic duct drainage for a period of 5 days. 2 days later they were given 10,000 larvae intravenously. The effect of this, shown in Table III, indicates that thoracic

duct drainage by itself did not prevent the eosinophil response to this form of challenge. A trial was therefore undertaken combining two procedures, neither of which alone had been capable of preventing eosinophilia, i.e., prolonged thoracic duct drainage and ALS-2 administration. The mean eosinophil response of nine animals treated in this way is presented in Table III. At no stage did their counts rise significantly above the initial level. It will be noted that the mean cell count on day 0 of 213 ± 62/mm³ exceeded the upper limit of the

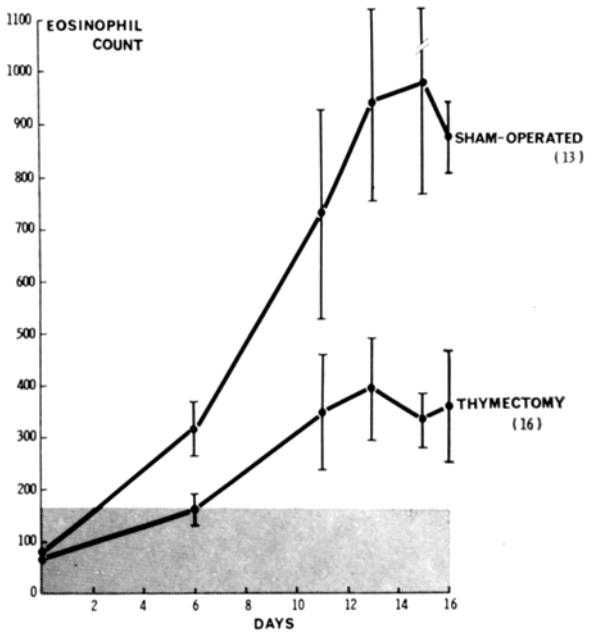

FIG. 1. Effect of neonatal thymectomy on the eosinophil response. Animals were inoculated with 5–10 larvae/g body weight via the gastrointestinal route. The difference between the two responses was significant ($P<0.05$).

normal range. This high level was thought to represent a reaction to cessation of the stress of prolonged restraint.

Adoptive Transfer of the Stimulus to Eosinophilia.—

Lymphocytes from thymus, spleen, and lymph nodes: Initial attempts at adoptive transfer were made with lymphoid cells obtained from thymus, spleen, and lymph nodes.

Donor animals were killed by exsanguination 6 days after infestation by the gastrointestinal route, and relevant organs transferred to Medium 199 containing 1 unit heparin/ml. They were then teased carefully with forceps, fibrinous debris being removed by filtration through

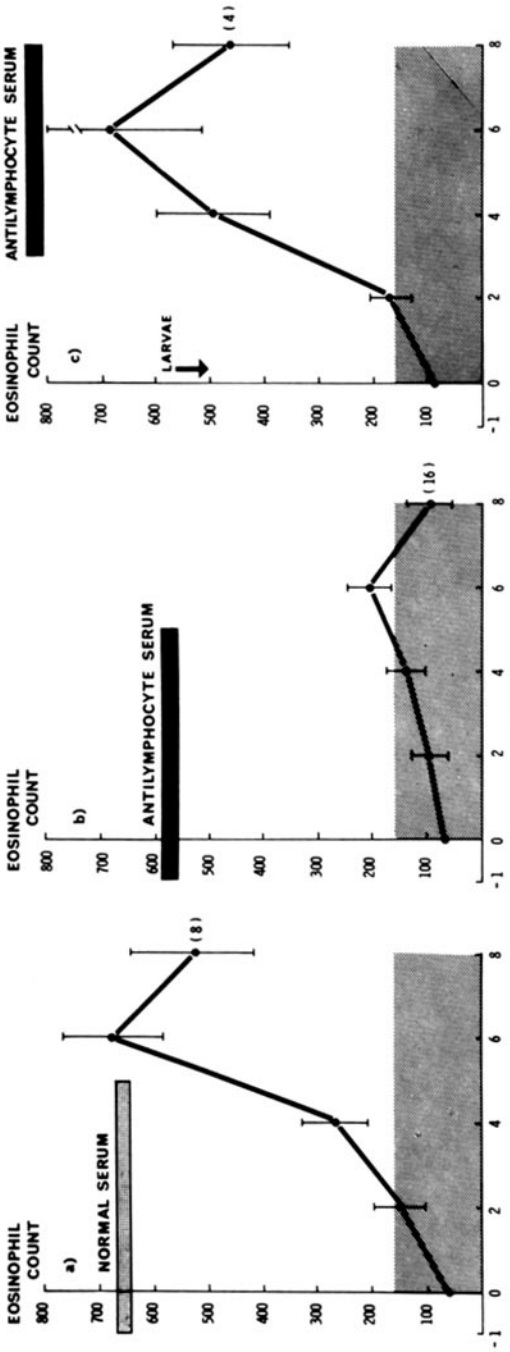

FIG. 2. Effect of antilymphocyte serum (ALS-1) on the eosinophil response. Sera were given in a dose of 1 ml/rat per day. The difference between the response in (*b*) was significant when compared with that in (*a*) or (*c*) ($P<0.05$).

fine sterile gauze. Before intravenous injection the cells were centrifuged at 100 *g* for 10 min and redispersed in fresh heparin 199 solution to form a single cell suspension.

The results, shown in Table IV, indicate that none of the three groups of recipients developed eosinophilia. Failure to demonstrate an effect with cells from these sources will be discussed later.

TABLE III

Effect of Lymphocyte Depletion on the Eosinophil Response to Trichinella Larvae

Method of depletion	Number	Day 0	Peak response (days 4–8)	Significance levels (t test)
		Mean eosinophil count/mm³ ± SE		
Normal rabbit serum	8	56 ± 17	719 ± 81	—
ALS-1	16	66 ± 10	247 ± 42	$P < 0.05$
ALS-2	6	72 ± 14	406 ± 57	$0.10 < P < 0.15$
Thoracic duct fistula	6	187 ± 60	565 ± 24	$0.05 < P < 0.10$
Thoracic duct fistula + ALS-2	9	213 ± 62	256 ± 63	$P < 0.01$

Method of challenge: 10,000 larvae intravenously on day 0. Antilymphocyte sera were administered in a dose of 1 ml/rat per day from day −1 to day +5. Thoracic duct fistulae were maintained for 5 days from day −8 to day −3.

TABLE IV

Attempted Adoptive Transfer of Eosinophilia with Thymocytes, Lymph Node, and Spleen Cells

Cells transferred	Number of cells × 10^8	Number of recipients	Day 0	Peak response (days 4–8)
			Mean eosinophil counts/mm³ ± SE	
Lymph node	6–10	5	84 ± 14	147 ± 53
Thymus	3–4.5	5	70 ± 7	73 ± 11
Spleen	6–10	6	33 ± 6	85 ± 17

Thoracic duct lymphocytes: Zaiman and Villaverde (7), in their studies with parabiotic rats, demonstrated that the stimulus to eosinophil production in trichinosis arises during the first few days of infestation. At this stage the parasites are localized within the intestinal wall where they form an antigenic focus drained by the thoracic duct. It was therefore reasoned that thoracic duct lymphocytes collected at that time might be capable of mediating the stimulus to eosinopoiesis.

Inbred rats of the WAfp strain were inoculated by the gastrointestinal route with 15 larvae/g body weight. The animals were then subjected to thoracic duct cannulation at intervals after inoculation and the lymphocytes injected intravenously into groups of normal syngeneic recipients.

Transfer of the stimulus to eosinophilia was achieved with cells collected between the 3rd and 7th days after infestation, if at least 6–8 × 10^8 lymphocytes were used. Subsequently it was found that collections obtained as late as 6–7 days contained migrating second generation larvae. Thereafter only cells collected between the 3rd and 5th days of infestation were employed for

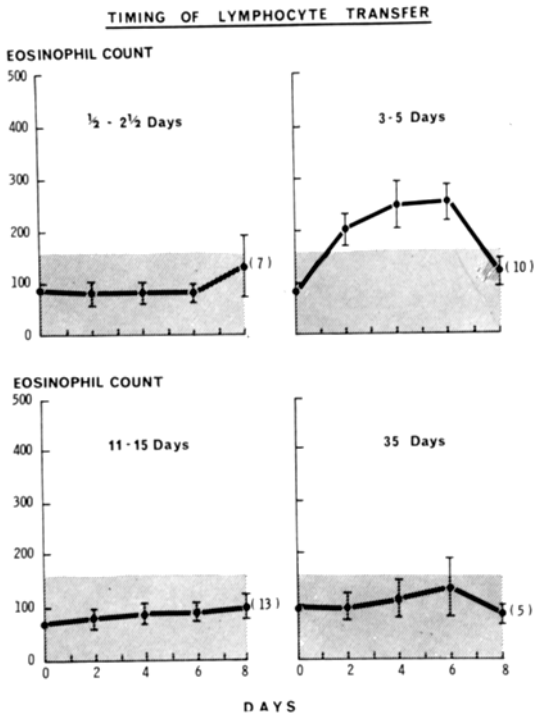

FIG. 3. The eosinophil response to "sensitized" thoracic duct lymphocytes obtained from donors at various intervals following larval inoculation by the gastrointestinal route. Only 3–5-day collections were capable of transferring the response.

experimental purposes. In Fig. 3 are shown the results with cells collected before, during, and after this period. As can be seen, lymphocytes obtained up to 2½ days proved inactive as did those collected either at the height of the eosinophil response in the donor (days 11–15) or at the time of maximal antibody level (day 35).

To ensure that 3–5-day cells were parasite-free the carcasses of all recipients were subjected to peptic digestion at the end of each experiment. No larval forms were found in any animal which had been given 3–5-day lymphocytes although the carcasses of 6–7-day recipients contained up to 50,000 larvae each. These findings indicated that a significant number of second generation

larvae did not begin to migrate via lymphatic channels until about the 6th day after infestation, and are in agreement with the work of Zaiman and Villaverde (7).

To confirm these observations a larger group of rats (WAG strain) was inoculated with 6–8 × 10^8 3–5-day (sensitized) lymphocytes. Their eosinophil counts were compared with those of a group of animals given a similar number of normal thoracic duct lymphocytes (Fig. 4 *a*). There is a clear and statistically significant difference between the two responses ($P < 0.005$). Of the 21 recipients 19 did not develop a detectable hemagglutinin response; the remaining 2 had titers of 1:2 and 1:4.

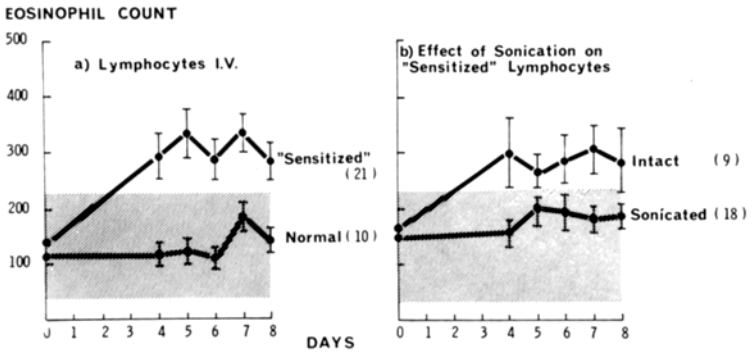

FIG. 4 *a*. Adoptive transfer of the eosinophil response with thoracic duct lymphocytes. Each recipient was given 6–8 × 10^8 lymphocytes. The difference between the responses of test and control animals was significant ($P<0.005$).

FIG. 4 *b*. Thoracic duct lymphocytes lost capability of stimulating eosinopoiesis when disrupted by ultrasound

Inactivity of thoracic duct lymph: Cell-free lymph obtained from the same donors which had provided an active population of lymphocytes was given to other recipients, either by repeated intraperitoneal injection or in the form of a continuous intravenous infusion from a reservoir kept at 4°C. No eosinophil response was observed in the recipients even when as much as 100 ml of lymph was administered to a single animal.

Proportion of large lymphocytes in thoracic duct lymph:

Smears of thoracic duct cells collected at different times after infestation were stained with methyl green pyronine. The proportion of large lymphocytes[1] at any given time was determined by counting a minimum of 500 cells/smear.

The results are shown in Fig. 5. Normal lymph was found to contain 4–5%

[1] The criterion for classification as a large lymphocyte was that of Gowans and Uhr (8), i.e., a thoracic duct cell with diameter exceeding 8 μ.

large lymphocytes. After inoculation there was an increase beginning on the 3rd day, reaching a peak of 16% by the 6th day and declining thereafter, so that by the time peak blood eosinophil levels would have been expected (11th–15th days) the percentage had returned to within the normal range. The eosinophil response generated by transfer of thoracic duct cells thus appeared to be correlated with the content of large lymphocytes.

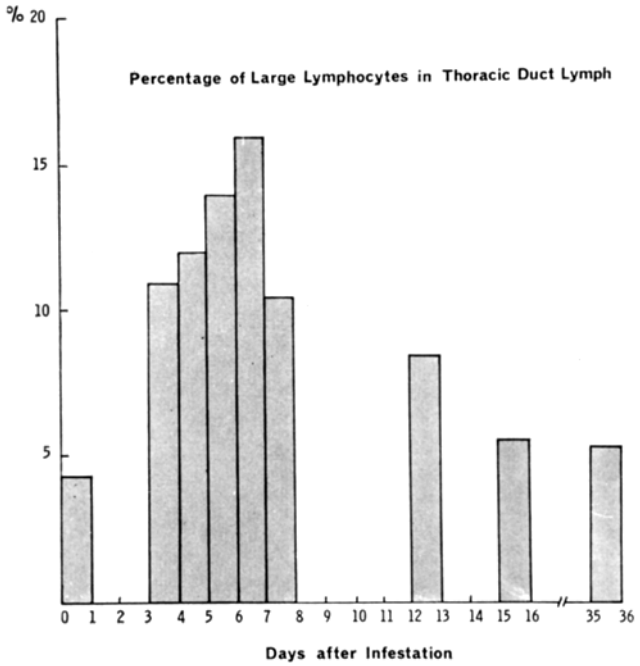

FIG. 5. Histogram of the percentage of large lymphocytes in thoracic duct lymph at intervals following inoculation by the gastrointestinal route.

The proliferation of pyroninophilic cells noted here appeared comparable in magnitude and timing to that observed by Hall et al. (9) in the efferent lymphatics of lymph nodes which had been the site of recent antigenic stimulation.

Modification of Lymphocytes before Adoptive Transfer.—

Ultrasonic disruption: One of the possible ways in which lymphocytes could transfer the stimulus to eosinopoiesis is by passive carriage of parasitic products adsorbed on their surfaces. To test this possibility thoracic duct cells were exposed to ultrasonic disruption before transfer.

Groups of three to six animals were infested with 15 larvae per g of body weight and thoracic duct cells obtained from them between the 3rd and 5th days after inoculation. 12-hourly

collections were divided into two portions, one of which was transferred unaltered and the other after ultrasonic treatment. For disruption lymphocytes were suspended in Dulbecco I as described previously and exposed for a period of 15 sec at 4°C to an ultrasonic disintegrator. Each inoculum was examined microscopically for adequate fragmentation, then injected into recipients in the usual manner.

The results of this experiment, illustrated in Fig. 4 *b*, show that the injured cells were incapable of transferring eosinophilia whereas the controls did so as in other tests.

Selective depletion of large lymphocytes: The differential counts of smears of thoracic duct cells had suggested that the ability of lymphocytes to transfer the stimulus to eosinophilia might be correlated with the large lymphocyte component. To test this by another means a population of thoracic duct cells deficient in large lymphocytes was prepared by the procedure of Gowans and Uhr (9) which takes advantage of the relative fragility of large lymphocytes when cultured under unfavorable conditions in vitro.

Groups of three to six rats were inoculated with 15 larvae per g of body weight and then cannulated 72 hr later. 12-hourly collections of lymphocytes were divided into two portions to provide final inocula of 8×10^8 cells/rat. One sample was injected into test animals untreated; the other fraction was suspended in Dulbecco I solution at a concentration of 5–10 $\times 10^7$ cells/mm^3 in Medium 199 to which had been added 1% inactivated rat serum and 20% v/v phosphate-buffered saline, pH 7.3 (Dulbecco A). The mixture was incubated for 24 hr at 37°C in 25 ml stoppered conical flasks in a shaking water bath. After this the cells were separated by centrifugation for 10 min at 100 *g* and resuspended for injection in the usual way. Smears of "incubated" cells were stained with methyl green pyronine and the proportion of surviving large lymphocytes determined. The procedure was found to have produced a reduction in this type of cell from 15% to less than 5%.

The eosinophil responses to inocula of fresh and "incubated" lymphocytes have been compared in Fig. 6 *a*. "Incubated" cells proved incapable of transmitting the stimulus while the controls did so as usual. These results seem consistent with the assumption that eosinophil inducing activity is a function of large lymphocytes.

Enclosure of lymphocytes in diffusion chambers: Despite the negative results which had followed tests of blood and lymph plasma it still seemed conceivable that an eosinophilia might be transferred by lymphocytes enclosed in cell-tight diffusion chambers in a manner analogous to that observed in other immunologic phenomena such as homograft rejection (10) and experimental autoallergic encephalomyelitis (11).

Chambers were made from small lucite rings 2 mm in depth with external diameter 14 mm and internal 10 mm. They were stored under saline containing penicillin (10,000 units/ml) and streptomycin (10 mg/ml). Using sterile precautions a Millipore filter 13 mm in diameter and 0.1 μ pore size (Millipore Filter Corp., Bedford, Mass.) was glued with MF1 Millipore cement over one end of the ring. After partial immersion in medium 199 containing 1 unit heparin/ml, the chamber was filled with a suspension of lymphocytes. Finally it was sealed with a second filter and inserted into the peritoneal cavity of a syngeneic recipient. Usually

three such chambers were placed in each test animal, in order to provide the desired number of cells, i.e., 8×10^8. Two groups of recipients were used, one of which was given normal thoracic duct lymphocytes and the other cells obtained 3–5 days after infestation.

As shown in Fig. 6 *b* the active population of 3–5-day lymphocytes retained their capacity to transfer an eosinophilia even when confined in a cell-tight chamber.

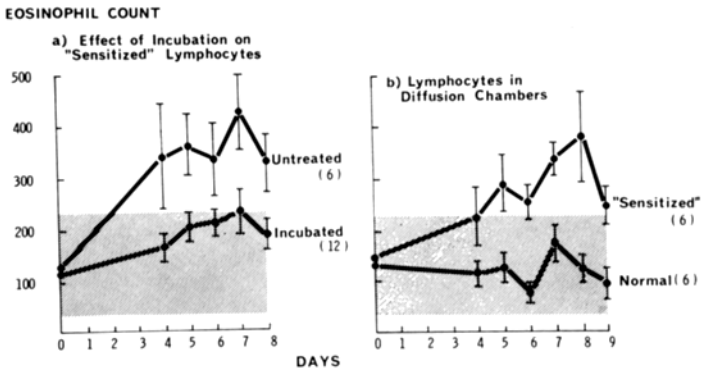

FIG. 6 *a*. Thoracic duct lymphocytes lost capability of stimulating eosinpoiesis after incubation at 37°C for 24 hr.

FIG. 6 *b*. Thoracic duct lymphocytes confined in peritoneal diffusion chambers were still capable of stimulating eosinpoiesis.

Adoptive Transfer of Eosinophilia with Peripheral Blood Leucocytes.—

When the intravenous rather than gastrointestinal route of larval inoculation was employed thoracic duct cells proved incapable of adoptive transfer of eosinophilia. In this situation where the stimulus was localized in the lungs it was considered that the bloodstream became the most readily available source of lymphocytes which had passed through the site of antigenic challenge. Therefore an attempt was made to induce an eosinophil response in naive recipients by means of peripheral blood leukocytes.

For this purpose donor animals received 10,000 larvae intravenously, and cells were obtained either 1 day or 5 days later. Buffy coat cells were separated from the erythrocyte component by the method of Hullinger and Blazkovec (12). Heparinized blood was layered on a column of high density material composed of methyl cellulose (Methocel 25 centripoises, Dow Chemical Co., Midland, Mich.) and sodium metrizoate (Triosil 45, Glaxo Laboratories, Ltd., Greenford, Eng.). At room temperature this mixture caused rapid agglutination and sedimentation of erythrocytes within 40 min, leaving a supernatant plasma layer containing leukocytes. After removal this layer was centrifuged at 100 *g* for 10–15 min. Before use the cell button was resuspended in Dulbecco I solution at a cell concentration of not greater than 2×10^7/ml. This suspension was injected into syngeneic recipients intravenously.

Table V presents the mean peak eosinophil responses of various groups of recipients including those given cells subjected to the same modifications as were used for thoracic duct lymphocytes. The results show that leukocytes (containing approximately 75% mononuclear cells) obtained 24 hr following challenge were capable of inducing a significant eosinophilia when compared with normal controls provided that at least 4–5 $\times$ 10^7 cells were transferred. This eosinophilic activity was markedly diminished by incubation at 37°C or by ultrasonic treatment. Transfer was likewise unsuccessful with cells collected 5 days after inoculation, at a time when the eosinophil response in the donors would have been reaching its maximum. The ability of buffy coat leukocytes

TABLE V

Adoptive Transfer of Eosinophilia with Peripheral Blood Leukocytes

Donors inoculated with saline	10,000 larvae	Treatment of donor leukocytes	Time of transfer	Recipients		
				Number	Day 0	Peak response (days 4–8)
					Mean eosinophil counts/mm³ ± SE	
+	−	Nil	24 hr	5	72 ± 12	149 ± 26
−	+	Nil	24 hr	9	109 ± 14	416 ± 98*
−	+	Ultrasonication	24 hr	4	77 ± 10	128 ± 41
−	+	Incubation	24 hr	4	66 ± 22	262 ± 25
−	+	Nil	5 day	4	106 ± 23	205 ± 32

* Significantly different from saline controls ($P < 0.05$).

to transfer eosinophilia again appeared to be a function of the large lymphocyte component. In the active 24 hr population of leukocytes the proportion of large lymphocytes among the mononuclear fraction was approximately 8–9% compared with a figure of only 2–3% in normal rats and in those tested 5 days after larval injection.

Restoration of the Eosinophil Response after Irradiation.—

Irradiation: Rats were exposed to the gamma beam from a cobalt 60 source (Department of Biochemistry, Oxford University). Each animal received a total dose of 600 rad at a rate of approximately 150 rad/min.

Bone marrow cell suspension: To obtain bone marrow adult rats were given an intravenous injection of 100–150 units heparin and killed 5 min later. After thorough external cleaning, the femurs were split and the contents transferred to Medium 199 containing 1 unit heparin/ml. Cell masses were broken up by agitation with a Pasteur pipette. Before intravenous injection the cells were separated by centrifugation at 100 *g* for 10 min and resuspended in fresh heparin 199 solution to form a single cell suspension. Each recipient of these cells was given the contents of two femurs intravenously in a volume of 2–3 ml.

Lymphocytes: Lymphocytes were obtained from the thoracic duct as described previously. Each recipient was injected intravenously with 8 $\times$ 10^8 cells.

5 days after exposure to 600 rad gamma irradiation, each animal was injected intravenously with 10,000 larvae. Between the time of irradiation and parasitic challenge, reconstitution was carried out with various combinations of thoracic duct lymphocytes and bone marrow cells from normal syngeneic donors. The eosinophil counts of each group of recipients were measured for a period of 12 days after injection of parasites.

The responses are shown in Fig. 7 and for comparison the eosinophil response of a group of nonirradiated rats to the same number of *Trichinella* larvae has been included. Inocula of normal lymphocytes alone, normal bone marrow cells alone, or sensitized marrow[2] failed to restore the eosinophil response of irradiated recipients. On the other hand reconstitution with both normal lymphocytes and normal bone marrow did restore the capacity to respond to parasitic challenge, the magnitude of eosinophilia resembling that of nonirradiated animals. In the final portion of this experiment reconstitution was again carried out with lymphocytes and bone marrow cells, but on this occasion the lymphocytes were collected from recipients infested with trichinosis 5 wk previously. As shown in Fig. 7 *d*, the resultant mean eosinophil response of this group of rats reached a peak of about 1150 cells/mm^3 compared with between 600 and 650 cells/mm^3 in control groups. It will be recalled that this population of cells contained a normal proportion of large lymphocytes and was incapable of adoptive transfer of eosinophilia. This would appear to resemble a "secondary" type of response, and suggested that "memory" resided with lymphocytes rather than a bone marrow element.

DISCUSSION

The work described here demonstrates that immunologically competent lymphocytes participate in events leading to increased eosinophil production. Evidence of this relationship was first obtained from procedures which depleted or inactivated the pool of recirculating lymphocytes. Antilymphocyte serum raised against thoracic duct cells caused marked diminution of the eosinophil response to *Trichinella* larvae. Neonatal thymectomy likewise diminished the reaction although its ability to do so could be overcome by giving a large inoculum of parasites. A similar dose-dependent effect has been reported by Taylor and Wortis (13) in their studies on antibody production to sheep erythrocytes in thymectomized mice. Prolonged thoracic duct drainage was not capable of suppressing eosinophilia unless it was combined with an antilymphocyte serum (raised against lymph node cells) which by itself was also ineffective. This result may be comparable with the observation of a synergistic action by this combination in prolonging survival of skin homografts (14).

[2] Obtained from donors exposed 5 wk previously to gastrointestinal inoculation of *Trichinella* larvae.

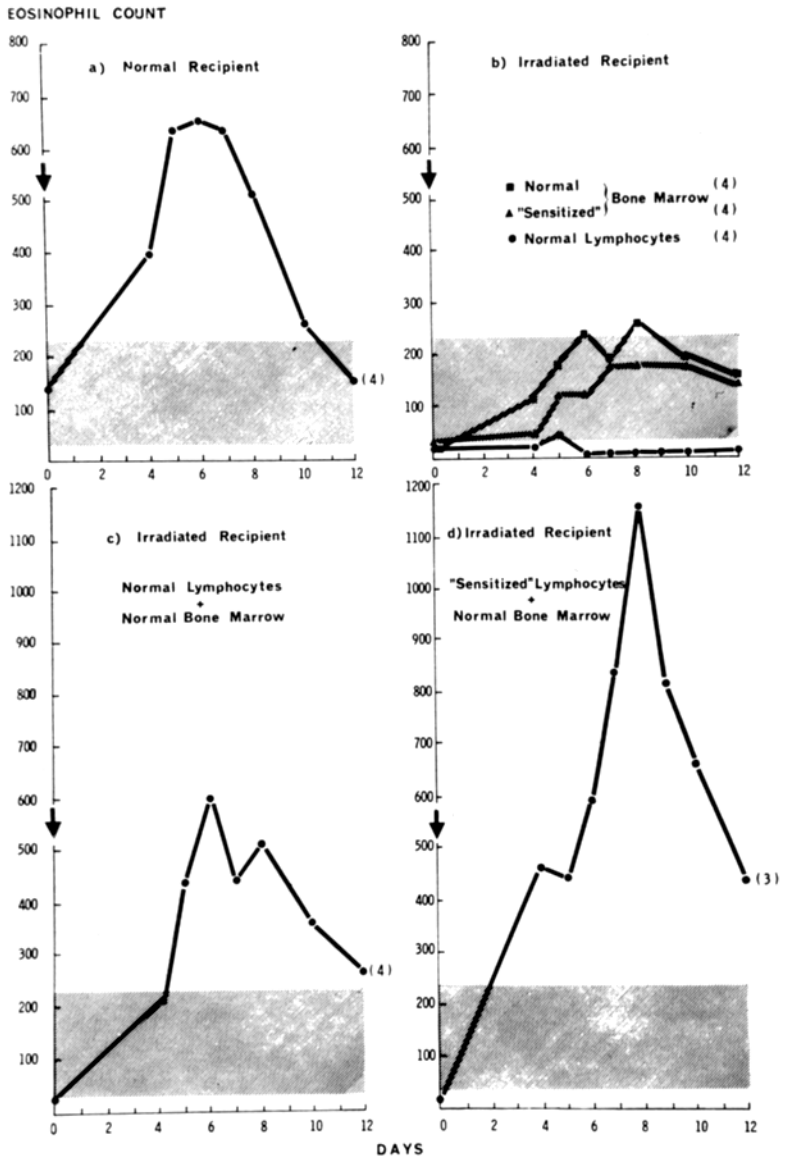

FIG. 7 The eosinophil response of irradiated hosts to *Trichinella* larvae after reconstitution with various combinations of bone marrow and thoracic duct cells. Test animals were irradiated on day 0, reconstituted with cells from day +1 to day +3, and challenged intravenously with 10,000 larvae on day +5.

An essential role for the lymphocyte was demonstrated by the finding that irradiated animals exposed to parasitic challenge did not develop eosinophilia unless reconstituted with both lymphocytes and bone marrow cells. Furthermore, when reconstitution was carried out with lymphocytes collected from donors infested 5 wk previously, a "secondary" type of reaction developed. No such effect was observed if only bone marrow cells from such donors were employed. That there is "memory" for eosinophil proliferation was suggested by the experiments employing immunosuppressive agents. The findings reported here confirm this and indicate that "memory" is a property of circulating lymphocytes rather than a bone marrow element.

Transfer of the eosinophil response was achieved adoptively with "sensitized" cells from both thoracic duct lymph and peripheral blood. The inability of lymphocytes from thymus, spleen, and lymph nodes to transfer the reaction was thought to indicate that the active population of cells does not remain in or circulate between these organs for a sufficient period of time. The association of eosinophil-stimulating activity with large lymphocytes, which probably have a short life span, is compatible with this viewpoint. Failure of disrupted cells to stimulate eosinophil production suggests that a population of metabolically active lymphocytes is required, and argues against a possible role for these cells as passive vectors of adsorbed antigen.

When cells capable of adoptive transfer were enclosed in diffusion chambers they retained ability to participate in the reaction. Thus lymphocytes apparently exerted an effect on bone marrow by release of a diffusible factor. They did not act as direct precursors of eosinophils, as shown in this and in the irradiation experiments. The results of the latter studies are in line with other work showing that thoracic duct cells (in contrast to peripheral blood leukocytes) are incapable of reconstituting either the erythroid or granulocyte components of irradiated hosts (15). The existence of a diffusible factor raises the question why the response could not be transferred by blood plasma or cell-free lymph which might have contained the eosinopoietic material. Possibly this simply reflects an insufficient quantity of active substance in the circulation at a given time. Similar findings have been noted in two other systems where successful transfer could only be achieved with a population of living cells, namely, transplantation immunity (10) and experimental allergic encephalomyelitis (11). The precise nature of the diffusible factor remains to be determined. It could of course be a form of antibody, for example homocytotropic antibody, not detectable by the hemagglutination technique employed in our work. However, Sadun et al. (16) have shown that passive cutaneous anaphylaxis is not detectable in mice given trichinosis until the 4th wk of infestation long after the eosinophil response has passed its maximum.

Categorization of increased eosinophil production as a form of immunologic reaction raises the question why it does not occur regularly in response to anti-

genic stimulation. In clinical medicine eosinophilia usually is observed when repeated exposure to antigen has taken place, as seen in allergic phenomena, and when the host is subjected to prolonged stimulation as in a course of drug treatment or in homograft rejection (17). A single exposure to conventional antigen, while leading to antibody formation, does not provoke a detectable increase in eosinopoiesis. Development of eosinophilia after one injection of *Trichinella* larvae must therefore result from cellular events which differ from those culminating in antibody synthesis. Lack of correlation between eosinophil and humoral responses was described in a preceding article (1 *a*), and clinical evidence of this is found in the report of massive eosinophilia in an agammaglobulinemic human subject suffering from visceral larva migrans (18). The difference between the two expressions of immunity may be related to the way in which the antigenic stimulus is presented to host cells during the induction phase. Thus, we were able to elicit a sharp eosinophil response when intact parasites were deposited in lung or muscle (1 *a*), but if the same material was homogenized before intravenous inoculation, presumably with rapid delivery to reticulo-endothelial tissue, its handling became similar to that of most other antigens, in that eosinophilia failed to develop whereas the antibody response was brisk.

The findings reported in the present series of studies indicate that eosinophils share with lymphocytes, plasma cells, and macrophages the property of proliferation in response to antigenic challenge. Although the induction of this proliferative process, which seems to be lymphocyte mediated, may be immunologically specific, eosinophils themselves probably lack such specificity in their function—whatever that may be. Their paucity of endoplasmic reticulum (19) seems inconsistent with antibody formation and in their phagocytic activities they exhibit little or no preference for particular antigenic materials (20, 21). On present evidence eosinophilia appears to have features in common with the altered macrophage capability known to characterize various forms of acquired cellular resistance (22).

SUMMARY

A possible role for the lymphocyte in the mechanism of eosinopoiesis has been examined.

Procedures known to deplete or inactivate the pool of recirculating lymphocytes such as neonatal thymectomy, administration of antilymphocyte serum, and prolonged thoracic duct drainage, either singly or in combination, resulted in a highly significant reduction in the eosinophil response to trichinosis.

Irradiated animals exposed to parasitic challenge did not develop eosinophilia unless reconstituted with lymphocytes as well as bone marrow cells. When "memory" cells were used instead of normal lymphocytes, a "secondary" type of eosinophil response was observed.

Transfer of a primary eosinophilia was achieved adoptively with a population of living large lymphocytes from thoracic duct lymph and peripheral blood, but not with blood plasma or cell-free lymph. The potency of the active lymphocytes was not impaired by enclosing them in cell-tight diffusion chambers, indicating that they exerted an effect on bone marrow by agency of a diffusible factor.

The demonstration of a role for lymphocytes in induction of the eosinophil response to this kind of stimulus supports the conclusion that eosinophilia belongs in the category of immunologic phenomena.

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