Clin. exp. Immunol. (1977) 29, 75–83.

Eosinophilia

V. DELAYED HYPERSENSITIVITY, BLOOD AND BONE MARROW EOSINOPHILIA, INDUCED IN NORMAL GUINEA-PIGS BY ADOPTIVE TRANSFER OF LYMPHOCYTES FROM SYNGENEIC DONORS

W. E. PARISH, E. LUCKHURST & S. I. COWAN* *Lister Institute of Preventive Medicine, Elstree, Hertfordshire*

(*Received* 7 *February* 1977)

SUMMARY

Dinitrochlorobenzene (DNCB) induced delayed hypersensitivity but no eosinophilia in guinea-pigs from two colonies. Citraconic anhydride (CA) induced delayed hypersensitivity and eosinophilia of the blood and bone marrow, and sites of skin tests were also infiltrated by eosinophils. In adoptive transfer of lymphocytes separated from peritoneal exudate cells of strain XIII-sensitized donors, lymphocytes from DNCB-sensitized guinea-pigs transferred antigen-specific delayed hypersensitivity; lymphocytes from CA-sensitized guinea-pigs transferred delayed hypersensitivity, and induced eosinophilia of the blood and bone marrow of the recipients. Treatment of the lymphocytes before transfer with antilymphocyte (thymocyte) globulin or puromycin suppressed the manifestations in the recipients; normal globulin did not.

Active sensitization with DNCB induced formation of small amounts, and with CA larger amounts of anaphylactic antibody. Sera from the actively sensitized animals elicited no significant eosinophilia of blood or bone marrow in one group of recipients. Passive anaphylaxis elicited a transient eosinophilia of the blood, but not of the bone marrow.

It is postulated that T-helper cells interact with B-lymphocyte precursors, particularly IgE B cells, to stimulate eosinopoeisis. This results in a reserve of mature eosinophils that may be released in any subsequent anaphylactic event.

INTRODUCTION

Blood and tissue eosinophilia frequently occurs in generalized or local anaphylactic reactions, and is readily induced on challenge of guinea-pigs passively sensitized by antibody (Redd & Vaughan, 1955; Litt, 1961; Parish, 1972a,b). Eosinophilia also occurs in some lymphocyte-mediated responses not known to be associated with anaphylactic sensitivity, or considered to be independent of antibody formation. Passive transfer of eosinophilia by lymphocytes from sensitized to normal inbred rats or mice was used by Basten & Beeson (1970), by Spry (1972), and by Speirs (McGarry *et al.*, 1971; Ponzio & Speirs, 1975) to show conclusively that T or thymus-dependent lymphocytes mediate increased numbers of eosinophils in the blood. Active sensitization of rats results in increased rate of formation, and mobilization of eosinophils from the bone marrow into the blood (Spry, 1971a,b). Treatment of the recipients with antisera or immunosuppressive drugs before injection of the sensitized lymphocytes (Boyer *et al.*, 1970) or more critically, treatment of the lymphocytes about to be transferred with anti-theta serum (Ponzio & Speirs, 1975), which inhibits or destroys T-cell activity, also abolishes the stimulus to eosinophilia conferred by the transferred cells.

Some antigens that induce delayed hypersensitivity also induce eosinophilia (Parish & Luckhurst, 1977). These two concomitant lymphocyte-mediated phenomena were examined to determine if both occurred in recipients by adoptive transfer, and we present here the findings on eosinophilia in strain

* Present address: Hoechst Pharmaceuticals, Walton, Bedfordshire.

Correspondence: Dr W. E. Parish, Unilever Research, Colworth House, Sharnbrook, Bedfordshire.

XIII guinea-pigs, in which citraconic anhydride induces delayed hypersensitivity, eosinophilia of the blood and increased formation of eosinophils in the bone marrow. Transfer of purified suspensions of lymphocytes to normal recipients induces delayed hypersensitivity, blood and bone marrow eosinophilia. Treatment of the lymphocytes before challenge with antilymphocyte (thymocyte) globulin, or with puromycin, destroys their ability to transfer delayed hypersensitivity or eosinophilia.

MATERIALS AND METHODS

Guinea-pigs. The Lister colony inbred from Porton stock since 1956. Strain XIII guinea-pigs, some bought, others bred.

Eosinophil counts. These were made as before (Parish, 1972a) using the eosin Y of Discombe (1946). Bone marrow smears were prepared by the method of Cameron & Watson (1948), but a fine dental drill was used to open the marrow cavity and the needle contained 10% guinea-pig albumin in saline. Only mature, or band forms with numerous distinct eosinophilic granules were counted, so the numbers (percentages) tend to be lower than in other reports when early myelocyte forms were included.

Anti-guinea-pig lymphocyte globulin. This was prepared from thymocytes in Freund's complete adjuvant injected s.c. into rabbits, followed 1 month later by injection of a suspension of thymocytes into the same sites. Sera taken 8 days later were absorbed with homogenates of guinea-pig kidney. The IgG fraction was separated on DEAE cellulose, and repeatedly dialysed against buffered saline. The fraction was then absorbed twice with packed washed guinea-pig erythrocytes. The preparation was not toxic to monolayers of guinea-pig macrophages and lysed 50% guinea-pig lymphocytes at a dilution of 1/1000 with 1/10 dilution of guinea-pig complement.

Preparation of hapten–protein conjugates for skin and I..I.M. tests. A conjugate of 2,4-dinitrofluorobenzene was prepared with almost pure guinea-pig albumin in cold alkali to form DNP–albumin in the ratio 18 hapten : 1 protein. Citraconic anhydride was conjugated at room temperature and pH 8·0–8·5 to guinea-pig albumin previously oxidised by performic acid (Dixon & Perham, 1968) in the ratio of 16 hapten : 1 protein. A conjugate made by mixing citraconic anhydride with the albumin and precipitation with N HCl (Chase, 1947) was also effective in leucocyte-inhibition tests. To detect sensitivity in guinea-pigs sensitized passively by lymphocytes (Tables 3 and 4) 50 μg of the protein–hapten conjugates in 0·1 ml were injected i.d.

In the leucocyte-migration inhibition tests 500 μg of the conjugate was used. This was not toxic for normal leucocytes, though the DNCB conjugate tended to mildly stimulate migration, 112% compared to the non-antigen treated controls (Table 1).

Sensitization of guinea-pigs. Guinea-pigs sensitized to 2,4-dinitrochlorobenzene (DNCB) and to citraconic anhydride (CA) in the tests reported in Tables 1 and 2 were injected three times with 1% of the antigen in olive oil at 7-day intervals. On each occasion two injections of 0·05 ml were made i.d., one each side of the midline, the first over the base of neck, the second over the scapulae, the third just behind the scapulae. On the 14th day after the last injection the animals were challenged by topical application of 0·05% DNCB in acetone or CA in dioxane.

Guinea-pigs sensitized as donors of peritoneal exudate lymphocytes (Table 4) were treated as above, but 0·1 ml was injected at each site. One day after the last injection 0·1% antigen in solution, as above, was applied over the last injection sites, about 2 cm^2. Then on three occasions at 3-day intervals 0·1% antigen was applied each side, more posteriorly on each occasion. All animals showed strong reactions. They were injected i.p. with 10 ml paraffin oil at the time of the last antigenic stimulation, and killed 3 days later to harvest the peritoneal exudate cells.

Separation and treatment of lymphocytes for transfer. Actively sensitized animals (Tables 1 & 2) whose lymphocytes were transferred to normal recipients (Table 3) had lymph nodes removed from post-auricular, deep cervical, anterior and posterior scapular, tracheo- and interbronchial regions. Cells were teased from nodes by scalpel and sieving through fine stainless-steel mesh (30 gauge) into Eagle's MEM with 1% normal baby guinea-pig serum, and then passed through a double thickness of sterile well-washed cotton gauze which removed clumps, macrophages and most dead cells.

Peritoneal exudate cells (Table 4) were washed out with Eagle's MEM containing 5 u heparin/ml. Cells were sedimented and the aqueous/oil supernatant fluid removed; they were then washed thrice in Eagle's MEM with 1% guinea-pig serum, without heparin, and passed through a double thickness of cotton gauze.

Cells, $2–6\times 10^8$ in Eagle's MEM with 5% normal baby guinea-pig serum, were added to vertical columns of non-toxic sterile cotton wool equilibrated with the fluid medium (Table 3) or columns of nylon wool (Table 4), incubated at 37°C for 45 min and then eluted with 20 ml medium. The eluted cells were centrifuged in silicone-treated tubes and resuspended as required. They were about 96% viable in trypan blue exclusion tests when eluted.

Lymphocytes from peritoneal exudates were combined to form one pool from donors sensitized to DNCB and one pool sensitized to CA. Lymphocyte-transformation tests on other pools from strain XIII guinea-pigs showed the cells to be immunologically compatible, and a further control was a pool of cells from donors not treated with antigen (Table 4). Pools were divided into four samples, (1) untreated (Eagle's with 10% normal guinea-pig serum), (2) treated with medium containing 1/1000 normal globulin (NG), (3) with medium containing 1/1000 antilymphocyte globulin (ALG), and (4) with medium containing 5 μg puromycin/ml cell suspension. These were incubated for 2 hr at 37°C, gently centrifuged, washed briefly once with medium, and resuspended at $5–7\times 10^8$/ml. Recipients (Table 3) received about 3×10^6 cells i.v., donor: recipient ratio 1:1. Recipients (Table 4) received $5–7\times 10^7$ i.v., and the residuum, about 2×10^8, i.p., donor: recipient ratio

4·2:1. Untreated, and normal globulin/treated cells were about 90% viable, the puromycin-treated 81% and the anti-lymphocyte globulin-treated 73% without complement. Four recipients per group; total twenty-four.

RESULTS

Eosinophilia induced concomitantly with delayed hypersensitivity in strain XIII guinea-pigs

Strain XIII guinea-pigs, necessary for subsequent cell-transfer tests, were compared with those of the Lister colony for their susceptibility to delayed hypersensitivity, and to eosinophilia of the blood and bone marrow after sensitization to DNCB and to CA by three courses of injection.

Guinea-pigs from both sources had good delayed hypersensitivity responses to topical application of antigen as judged by the size and intensity of the local erythema. Reactions to CA were more erythematous than those to DNCB, and in some animals challenged with CA there was a diffuse erythema at the challenge site within 15 min, which did not occur in four unsensitized control animals used to examine the possible irritancy of the solution of test antigen.

The induction of delayed hypersensitivity was confirmed at the end of the test when the peripheral blood leucocytes taken 24 hr after challenge, incubated with the appropriate antigen, had their migration inhibited between 38 and 58% of that occurring in each individual cell sample without antigen (Table 1).

Sensitization by CA elicited increased numbers of eosinophils in the blood in animals from both sources, but more so in those of the Lister colony. The greatest numbers were seen 3 days after the last, i.e. third sensitizing injections, and the numbers decreased by the 13th day after sensitization and

TABLE 1. Number of eosinophils infiltrating delayed hypersensitivity challenge reactions in actively sensitized guinea-pigs and *in vitro* blood leucocyte-migration inhibition. Further data on these animals is reported in Table 3

	Sensitizing antigen	Eosinophilia at challenge site*		L.M.I.† (% migration)
		DNCB	CA	
Lister colony	DNCB	8	3	38
	CA	9	21	46
	—	—	—	112 (DNCB)
Strain 13	DNCB	4	1	58
	CA	5	14	50
	—	—	—	97 (CA)

* Challenge site 24 hr after topical application of dinitrochlorobenzene (DNCB) or citraconic anhydride (CA). Number of eosinophils per × 40 field, mean of twelve fields.

† Buffy coat leucocyte-migration inhibition on testing with the sensitizing antigen. Percentage difference between each individual sample without and with antigen. Nil Lister colony result of test with DNCB. Strain XIII result of test with CA.

did not increase significantly 24 hr after topical challenge made on the 14th day. A similar regimen of sensitization by DNCB did not elicit eosinophilia (Table 2).

Changes in the percentage of eosinophils in the bone marrow reflected the changes in the numbers appearing in the blood. 3 days after the last antigen sensitization to CA there was a mean of 12·2% in the bone marrow of the Lister animals, and 10·3% in the marrow of strain XIII animals, and the proportion in the marrow remained above that of the pre-sensitization observations, and controls. The animals sensitized to DNCB showed no such increase in bone marrow eosinophils (Table 2). The bone marrow eosinophilia recorded here is only the proportion of eosinophils in the total number of nucleated cells of the bone marrow. This may be misleading as there was an undoubted increase in the total number of mononuclear cells after sensitization. There was also an increase in the number of basophils in the marrow of animals sensitized to both antigens.

Eosinophils infiltrated the delayed hypersensitivity reaction sites of skin of animals sensitized to, and

TABLE 2. Eosinophilia in blood and bone marrow in guinea-pigs with delayed hypersensitivity to citraconic anhydride, but not in those sensitized to dinitrochlorobenzene (Table 1)

Guinea-pig colony	Sensitizing antigen	No./group	Mean changes in eosinophils: Blood (numbers)* Pre-S	Post-S	Pre-C	Post-C	Bone marrow (%)† Pre-S	Post-S	Pre-C	Post-C
Lister colony	DNCB	6	38	69	60	66	3·8	2·4	4·3	4·3
	CA	6	42	233	97	113	4·0	12·2	10·0	9·1
	Nil	28	29	34	36	31	3·7	3·5	3·8	3·3
Strain 13	DNCB	6	12	21	31	26	2·7	2·1	3·2	3·0
	CA	6	14	113	64	72	2·9	10·3	9·2	8·3
	nil	8	14	13	18	18	3·1	2·6	2·8	3·1

Pre-S 48 or 24 hr pre-sensitization; post-S 3 days after last sensitizing injection; pre-C 13 days after last sensitizing injection: 24 hr pre-challenge; post-C 24 hr post-challenge. Nil antigen controls were sampled at same intervals as the antigen-treated animals.

* Number of eosinophils/mm^3 blood.

† Mature or band form eosinophils as a percentage of total nucleated cells in the bone marrow.

challenged topically by, CA, but not to sites challenged by DNCB in animals sensitized to that antigen (Table 1). Furthermore, the site on each animal tested with the antigen to which the animals had not been sensitized elicited no inflammation and no local eosinophilia.

There was no correlation between the degree of delayed hypersensitivity, judged by the size of the skin test reaction and by the inhibition of blood leucocyte migration, and the presence of blood or bone marrow eosinophilia in individual animals sensitized to CA. Furthermore, DNCB, which did not elicit eosinophilia, was as potent or more potent as an allergen inducing delayed hypersensitivity as CA, which did elicit eosinophilia.

Transfer of stimulus to blood and bone marrow eosinophilia to normal animals by lymph-node lymphocytes

The possibility that the eosinophilia occurring concomitantly with the delayed hypersensitivity to citraconic anhydride was mediated by lymphocytes was examined by transfer of lymphocytes from sensitized to normal strain XIII guinea-pigs.

TABLE 3. Eosinophilia and delayed hypersensitivity on transfer of lymph node lymphocytes from sensitized (Tables 1 and 2) to normal strain XIII guinea-pigs

Donors: No.	Sensitizing antigen	Recipients: No. of eosinophils in blood* −1 day	+6 days	DH on day 7 to† DNCB	CA	Eosinophils/DH field‡ DNCB	CA
6	DNCB	22	38	11 (6/6)	0	1	0
6	CA	26	71	0	7 (4/6)	2	6
6	Nil	31	34	0	0	0	0

* Mean number of eosinophils/mm^3 blood of recipients 1 day before transfer and 6 days after transfer of donor lymphocytes.

† Delayed hypersensitivity response 24 hr after i.d. injection of antigen hapten conjugated to guinea-pig albumin. Mean size in mm, and (number responding/total).

‡ As in Table 2. Six of the DNCB recipients and four of the CA recipients examined.

Immediately after the tests on the sensitized strain XIII guinea-pigs described above (Tables 1 and 2), that is 15 days after the last sensitizing injection, and 24 hr after the skin test challenge, the lymphocytes were obtained from regional nodes and purified on nylon-wool columns. The lymphocytes from each donor were injected i.v. into one recipient of the same sex. The recipients had been selected to have approximately the same numbers of circulating eosinophils at the start of the test.

Lymphocytes from DNCB-sensitized donors transferred weak though definite delayed hypersensitivity to all six recipients but there was no blood spontaneous eosinophilia, or eosinophilia of the challenge site (Table 3). Lymphocytes from CA-sensitized donors transferred weak delayed hypersensitivity in only four of the six recipients, but a moderate increase in the number of eosinophils in the blood of all six, and a sparse infiltration of eosinophils into the delayed hypersensitivity reaction sites of the four responding to challenge.

The passive transfer of lymphocytes in this test did not induce antigen non-specific susceptibility to skin irritation in the recipients. Sites tested with the control antigen, not used to sensitize the donor, showed no reaction at 24 hr. Furthermore, two of the recipients showed no response to CA antigen though injected with lymphocytes from CA-sensitized donors. It is also evident that the skin test of the donors with the control antigen 24 hr before removal of the lymphocytes did not influence the antigen-specificity of the passively transferred lymphocytes.

Though few eosinophils infiltrated the CA challenge sites of the CA-sensitized recipients, the response appeared to be antigen-specific in that fewer eosinophils infiltrated the DNCB control antigen sites in the same animals (Table 3).

Transfer of stimulus to blood and bone marrow eosinophilia to normal animals by peritoneal exudate lymphocytes

In a more critical test to confirm the activity of the lymphocytes, donor animals were more exquisitely sensitized by repeated injections and topical applications (see the Materials and Methods section). During the last two topical applications some of the CA-sensitized guinea-pigs had a widespread generalized erythema and flare-reactions at previous sites. Peritoneal leucocytes were obtained 3 days

TABLE 4. Eosinophilia and delayed hypersensitivity on transferring peritoneal lymphocytes-untreated or after treatment with anti-lymphocyte globulin or puromycin, from sensitized to normal strain XIII guinea-pigs

Donor		Recipients					
		No. Eos blood†		Eos marrow (%)‡		DH (mm), +7d§	
Sensitization	Treatment* lymphocytes	−1d	+6d	−1d	+6d	DNCB	CA
DNCB	nil	32	19	2·8	3·1	14	4
CA	nil	35	146	3·4	7·1	3	11
	NG	31	127	3·1	6·2	2	10
	ALG	28	21	3·5	3·0	4	5
	PMYCN	33	42	2·6	3·1	2	2
Nil	nil	38	44	2·9	2·5	n.d.	n.d.

Four recipients per group, total twenty-four.

* Treatment of lymphocytes before transfer with NG, normal rabbit globulin; ALG, rabbit anti-lymphocyte globulin; PMYCN, puromycin.

† Mean number of eosinophils/mm^3 blood at 1 day before; and 6 days after injection of lymphocytes.

‡ Mean per cent eosinophils of nucleated cells in bone marrow at 1 day before and 6 days after injection of lymphocytes.

§ Mean size (mm) of erythematous induration of delayed hypersensitivity skin test response. All animals showed some response to the appropriate antigen.

after the last antigenic stimulation and passed through columns of nylon wool to prepare two pools, one from donors sensitized to DNCB and one from those sensitized to CA. Samples from each pool were untreated, or treated with normal rabbit globulin (control), rabbit anti-guinea-pig lymphocyte (thymocyte) globulin or with puromycin.

Recipients injected i.v. and i.p. with DNCB-sensitized lymphocytes which were untreated showed specific delayed hypersensitivity but no eosinophilia of the blood or bone marrow. Lymphocytes from CA-sensitized donors, untreated, or treated with normal globulin, passively transferred delayed hypersensitivity, and induced eosinophilia of the blood and of the bone marrow. Anti-lymphocyte globulin and puromycin destroyed these activities of the lymphocytes (Table 4).

The increase in the number of circulating eosinophils in recipients of CA-sensitized, untreated, lymphocytes started at day 3 and was most evident on days 4 and 5. The greatest individual variation was seen on day 5, being 128 ± 52 (Fig. 1).

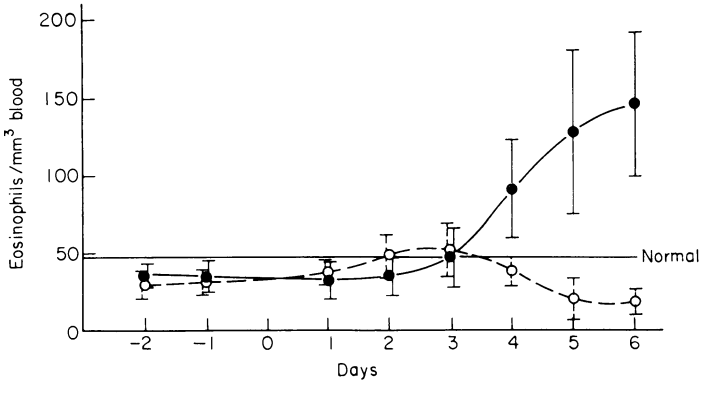

FIG. 1. Numbers of eosinophils in the blood of recipients following passive transfer of lymphocytes separated from peritoneal leucocytes of syngeneic donors sensitized to dinitrochlorobenzene (○) or to citraconic anhydride (●).

It is an important feature of the results that the percentage of eosinophils in the bone marrow was increased only in the two groups in which the lymphocytes induced blood eosinophilia (Table 4). The bone marrow was examined twice only.

Unlike the previous test (Table 3) the recipients in this test had some susceptibility to antigen non-specific irritation. The non-specific antigen elicited reactions of 2–5 mm size 24 hr after the skin test.

Results of treating the recipients with anti-lymphocyte globulin and puromycin before passive transfer of lymphocytes

Peritoneal lymphocytes from CA-sensitized donors injected into two untreated strain XIII guinea-pigs, and into two receiving two prior injections of rabbit normal globulin induced delayed hypersensitivity and eosinophilia of the blood and bone marrow. The responses, particularly of the bone marrow, were less than those reported in Table 4. Pre-treatment of the recipients by two injections of anti-lymphocyte serum or two of puromycin abolished the activity of the transferred cells.

Examination for blood eosinophilia following injections of sera from animals with delayed hypersensitivity

The following sera from strain XIII guinea-pigs injected i.v. into guinea-pigs from a commercial source did not induce eosinophilia of the blood at 6, 16, 24 hr, 2–6 days or any significant change in the percentage of eosinophils in the bone marrow on the 1st and 6th post-injection days: DNCB-sensitized donors, last antigen stimulus 15 and 3 days before obtaining serum samples, and CA-sensitized donors, 15 days after the last antigen stimulation.

The serum from donors sensitized to CA after multiple antigenic stimulations and obtained 3 days after

the last, elicited a mean increase in three recipients of eleven eosinophils per mm^3 blood at 6 hr, and 18 eosinophils at 16 hr which was not considered to be significant. There was no significant change in the percentage of eosinophils in the bone marrow.

Sera from donors actively sensitized to CA prepared normal guinea-pigs for passive cutaneous anaphylaxis at titres of 32–128 at 24 hr after sensitization. Sera of donors sensitized to DNCB had very weak anaphylactic activity. These results will be published in detail separately.

Changes in blood and bone marrow eosinophilia after passive generalized anaphylaxis

Guinea-pig sera containing anaphylactic IgGlb anti-bovine serum albumin or anti-β-lactoglobulin injected intravenously into normal guinea-pigs did not induce eosinophilia of the blood at 6, 16, 24 hr, 2, 6 and 12 days, and there was no change in the percentage eosinophilia of the bone marrow at 24 hr, 6 and 12 days.

When guinea-pigs passively sensitized by these sera were challenged i.p. after 6 hr or 3 days with enough antigen to elicit definite but non-fatal anaphylaxis, there was a moderate eosinophilia of the blood at 16 and 24 hr, which was much less at 2 days, and near the pre-test number in all animals at 6 days. The percentage of eosinophils in the bone marrow was decreased by a mean of 1·3%, with much individual variation, in animals challenged with bovine serum albumin, and 0·9% in the β-lactoglobulin-challenged animals. The proportion of eosinophils in the bone marrow returned to that pre-test by 6 days, and there was no increase at 12 days.

DISCUSSION

These findings show that blood eosinophilia can be induced in normal guinea-pigs by transfer of lymphocytes from compatible inbred sensitized donors, just as had been demonstrated in rats (Basten & Beeson, 1970) and mice (McGarry *et al.*, 1971). The findings provide further new evidence in that the transferred lymphocytes induce increased eosinopoeisis in the bone marrow of the normal recipients, not treated with antigen, similar to that seen in actively sensitized animals. Moreover, there is an association between the delayed hypersensitivity and stimulus to eosinophilia, in that both were induced in the actively sensitized animals by citraconic anhydride and both phenomena occurred in the recipients of the sensitized lymphocytes.

Some antigens are particularly potent in stimulating both delayed hypersensitivity and eosinophilia though much depends upon the regimen of sensitization (Parish & Luckhurst, 1977). Citraconic anhydride is very effective (Hunziker, 1965, 1969), whereas DNCB, though potent in stimulating delayed hypersensitivity in many tests, never induced eosinophilia of the blood and never or rarely in the infiltrate of patch tests (Hunziker, 1969). Citraconic anhydride also stimulates formation of anaphylactic antibodies (Chase, 1947; Hunziker, 1964), and Chase (1947) defined it as a compound of high reactivity, whereas DNCB had 'lesser chemical reactivity' being a weaker stimulant to formation of antibody. Our results are consistent with these findings. Antibodies probably contributed little to evoking eosinophilia of the actively sensitized animals, and almost certainly not at all in the recipients of the sensitized lymphocytes. Sera of actively sensitized animals when injected into normal recipients induced a slight increase in blood eosinophils, but less than that reported by Spry (1971b). Passive anaphylaxis also elicited eosinophilia of the blood and a transient decrease in the bone marrow, without any significant subsequent increase above normal, as will be described in detail separately. The stimulus to blood and bone marrow eosinophilia was in the T lymphocytes which when transferred to normal recipients spontaneously induced the eosinophilia without antigen challenge.

The transferred lymphocytes were probably T cells, because peritoneal exudate cells contain a high proportion of sensitized T lymphocytes in actively immunized donors (Rosenstreich *et al.*, 1971), one passage of cells over nylon wool or glass beads removes most of the lymphocytes with membrane-bound immunoglobulin (Rosenthal, 1972), and the rabbit antilymphocyte serum we used was prepared against guinea-pig thymocytes, though its T-cell specificity was not determined.

The conditions necessary to achieve a good transfer of lymphocyte activity are critical, among them

being the degree of sensitivity of the donor, the source of the lymphocytes transferred, e.g. lymph nodes or peritoneal cells, and time of harvest after the last antigenic sensitization. In preliminary tests we failed to achieve conclusive transfer of delayed hypersensitivity, though the recipients responded with eosinophilia if the lymphocytes were obtained from appropriate donors.

Separation of cells on cotton or nylon wool columns reduced the efficiency of the transferred activity. The transferred delayed hypersensitivity was more effectively detected by i.d. injection of the hapten conjugated to guinea-pig albumin than by topical application of the hapten. The conjugated hapten was non-irritant and used successfully in the leucocyte-migration inhibition tests. The skin responses were antigen-specific, but the skin of animals receiving large numbers of peritoneal lymphocytes become slightly susceptible to irritation (Table 4).

Inhibition of T-lymphocyte activity by removal of the thymus suppresses or reduces delayed hypersensitivity, and slightly or moderately reduces formation of IgG or IgM antibodies. It also abolishes the ability to respond with an eosinophilia on appropriate antigenic stimulation (Basten & Beeson, 1970; Walls *et al.*, 1971). Carrier-specific T cells are reported to be necessary for synthesis of IgE anti-hapten antibody (Tada & Okumura, 1971; Ishizaka & Okudaira, 1973; Hamaoka *et al.*, 1973). Thymectomy, however, enhances the ability of adult rats to synthesize IgE anaphylactic antibodies (Okumura & Tada, 1971), probably by removing a T-cell regulator or inhibitor. In active sensitization by hapten alone autologous proteins binding the hapten probably act as the hapten carrier.

In our study three phenomena, delayed hypersensitivity, eosinophilia and antibody formation, were observed which may be mediated by separate clones of T cells, or result from the interaction of enhancing or suppressing T cells. Delayed hypersensitivity and eosinophilia of blood and bone marrow were both mediated by T cells, and adoptive transfer was inhibitable by anti-thymocyte serum. They were probably mediated by separate clones of T cells, because DNCB induced delayed hypersensitivity without eosinophilia, and because in CA-sensitized animals there was no relation between the intensity of delayed hypersensitivity manifested by the skin response or inhibition of leucocyte migration, and the increase in blood eosinophils. Furthermore, in some preliminary tests before we achieved good adoptive transfer of delayed hypersensitivity, recipients that failed to respond to skin tests developed an eosinophilia.

The actively sensitized donors, particularly to CA, had anaphylactic antibodies, though their identity, IgG1b or IgE was not determined. Unfortunately we did not examine the recipients of the sensitized lymphocytes for antibody formation, but it is unlikely that significant amounts would be formed by T-cell activation within the 4–6 days during which there was an eosinophilia.

Eosinophilia frequently occurs in man concomitantly with increased amounts of IgE. Eosinophils also cluster round, or form rosettes with some large protein-rich mononuclear cells (Speirs, 1963; Parish, 1970, 1974). It is possible that there is an interacting stimulus to increased eosinopoeisis with eosinophilia of the blood, and B-cell precursors stimulated by T-helper cells. The relation between eosinophilia and anaphylactic IgE is a transient and mainly local phenomenon, partly mediated by mast-cell substances (Parish, 1972b, 1974). T-helper cells, or B-cell precursors activated by T cells may stimulate eosinopoeisis resulting in a reserve of eosinophils in the bone marrow. IgE B-cell precursors could be particularly potent. There is, therefore, a readily mobilizable reserve of eosinophils for any subsequent anaphylactic reaction of any antigenic specificity, not confined to the original sensitizing antigen. The eosinophils mobilized in the blood and tissues presumably are beneficial in limiting the effects of the anaphylactic pharmacological mediators and also limit further activation of B-lymphocyte precursors in lymphoid tissue or granulomata.

REFERENCES

BASTEN, A. & BEESON, P.B. (1970) Mechanism of eosinophilia. II. Role of the lymphocyte. *J. exp. Med.* **131**, 1288.

BOYER, M.H., BASTEN, A. & BEESON, P.B. (1970) Mechanism of eosinophilia. III. Suppression of eosinophilia by agents known to modify immune responses. *Blood*, **36**, 458.

CAMERON, D.G. & WATSON, G.M. (1948) Femoral bone marrow biopsy in the albino rat. *Blood*, **3**, 292.

CHASE, M.W. (1947) Studies on the sensitization of animals with simple chemical compounds. X. Antibodies inducing immediate-type skin reactions. *J. exp. Med.* **86**, 489.

DISCOMBE, G. (1946) Criteria of eosinophilia. *Lancet*, **i**, 195.

DIXON, H.B.F. & PERHAM, R.N. (1968) Reversible blocking of amino groups with citraconic anhydride. *Biochem. J.* **109**, 312.

HAMAOKA, T., KATZ, D.H. & BENACERRAF, B. (1973) Hapten-specific IgG antibody responses in mice. II. Cooperative interactions between adoptively transferred T and B lymphocytes in the development of IgE response. *J. exp. Med.* **138**, 538.

HUNZIKER, N. (1964) Experimental eczema. Passive transfer of guinea-pig's hypersensitivity to citraconic anhydride. *Dermatologica*, **129**, 475.

HUNZIKER, N. (1965) Experimental eczema. Eosinophilic leucocytes in the guinea-pig sensitized to citraconic anhydride. *Acta allergy*, XX, 164.

HUNZIKER, N. (1969) Experimental studies on guinea-pig's eczema. Springer-Verlag, Berlin, Heidelberg, New York.

ISHIZAKA, K. & OKUDAIRA, H. (1973) Reaginic antibody formation in the mouse. II. Enhancement and suppression of anti-hapten antibody formation by priming with carrier. *J. Immunol.* **110**, 1067.

LITT, M. (1961) Studies in experimental eosinophilia. III. The induction of peritoneal eosinophilia by the passive transfer of serum antibody. *J. Immunol.* **87**, 522.

MCGARRY, M.P., SPEIRS, R.S., JENKINS, V.K. & TRENTIN, J.J. (1971) Lymphoid cell dependence of eosinophil response to antigen. *J. exp. Med.* **134**, 801.

OKUMURA, K. & TADA, T. (1971) Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. *J. Immunol.* **106**, 1019.

PARISH, W.E. (1970) Investigations on eosinophilia. The influence of histamine, antigen–antibody complexes containing $\gamma 1$ or $\gamma 2$ globulins, foreign bodies (phagocytosis) and disrupted mast cells. *Brit. J. Derm.* **82**, 42.

PARISH, W.E. (1972a) Eosinophilia. I. Eosinophilia in guinea-pigs mediated by passive anaphylaxis and by antigen–antibody complexes containing homologous IgG1a and IgG1b. *Immunology*, **22**, 1087.

PARISH, W.E. (1972b) Eosinophilia. II. Cutaneous eosinophilia in guinea-pigs mediated by passive anaphylaxis with IgG1 or reagin, and antigen–antibody complexes; its relation to neutrophils and to mast cells. *Immunology* **23**, 19.

PARISH, W.E. (1974) Substances that attract eosinophils *in vitro* and *in vivo* and that elicit blood eosinophilia. *Antiobiotics and Chemotherapy*, volume 19, Chemotaxis: its biology and biochemistry, p. 233. Karger, Basel.

PARISH, W.E. & LUCKHURST, E. (1977) Eosinophilia. VI. Lymphocyte-mediated eosinophilia in guinea-pigs with delayed hypersensitivity to a chemical. *Perspectives in Inflammation: future trends and developments.* M.T.P. Press, London.

PONZIO, N.M. & SPEIRS, R.S. (1975) Lymphoid cell dependence of eosinophil response to antigen. VI. The effect of selective removal of T or B lymphocytes on the capacity of primed spleen cells to adoptively transferred-immunity to tetanus toxoid. *Immunology*, **28**, 243.

REDD, L. & VAUGHAN, J.H. (1955) Eosinophils in passive anaphylaxis. *Proc. Soc. exp. Biol. (N.Y.)*, **90**, 317.

ROSENSTREICH, D.L., BLAKE, J.T. & ROSENTHAL, A.S. (1971) The peritoneal exudate lymphocyte. I. Differences in antigen responsiveness between peritoneal exudate and lymph node lymphocytes from immunized guinea-pigs. *J. exp. Med.* **134**, 1170.

ROSENTHAL, A.S., DAVIE, J.M., ROSENSTREICH, D.L. & BLAKE, J.T. (1972) Depletion of antibody-forming cells and their precursors from complex lymphoid cell populations. *J. Immunol.* **108**, 279.

SPEIRS, R.S. (1963) Antigenic material: persistence in hypersensitive cells. *Science*, **140**, 71.

SPRY, C.J.F. (1971a) Mechanism of eosinophilia. V. Kinetics of normal and accelerated eosinopoiesis. *Cell Tissue Kinet.* **4**, 351.

SPRY, C.J.F. (1971b). Mechanism of eosinophilia. VI. Eosinophil mobilization. *Cell Tissue Kinet.* **4**, 365.

SPRY, C.J.F. (1972) The origin, kinetics and distribution of large lymphocytes from the thoracic duct of rats with trichinosis. *Immunology*, **22**, 663.

TADA, T. & OKUMURA, K. (1971) Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. *J Immunol.* **106**, 1019.

WALLS, R.S., BASTEN, A., LEUCHARS, E. & DAVIES, A.J.S. (1971) Mechanisms for eosinophilic and neutrophilic leucocytes. *Brit. med. J.* iii, 157.