Immunology, 1972, **22**, 1087.

Eosinophilia

I. EOSINOPHILIA IN GUINEA-PIGS MEDIATED BY PASSIVE ANAPHYLAXIS AND BY ANTIGEN–ANTIBODY COMPLEXES CONTAINING HOMOLOGOUS IgG1a AND IgG1b

W. E. PARISH

The Lister Institute of Preventive Medicine, Elstree, Herts.

(*Received 8th December* 1971)

Summary. There was an eight- to eleven-fold increase in the number of eosinophils in the peritoneal cavity of guinea-pigs repeatedly sensitized and subsequently challenged by that route. Peripheral blood eosinophilia was less and inconsistent. There were fewer eosinophils in the peritoneal cavity of guinea-pigs challenged intraperitoneally but sensitized by other routes, than in animals both sensitized and challenged peritoneally. Thus eosinophils were attracted to sites of antigenic challenge in anaphylactically sensitized tissue, particularly when sensitization occurred in the same tissue. In tissues repeatedly injected with antigen, there was an increase of mononuclear cells preceding and greater than the number of eosinophils.

The relation between eosinophilia and anaphylaxis was substantiated by evoking eosinophilia in normal guinea-pigs challenged after passive sensitization with homologous IgG1a (conferring PCA sensitivity for 3 to 4 days), and with IgG1b (conferring PCA sensitivity for 7 to 9 days) which mediate anaphylaxis, but not in animals treated with IgG2, which does not. Similarly complexes containing IgG1 antibodies evoke eosinophilia in normal animals, but complexes containing IgG2 do not.

Despite the association of eosinophilia with anaphylaxis, the number of peritoneal eosinophils following challenge is not greatly influenced by the susceptibility to fatal anaphylaxis, or by the serum PCA titre. Moreover it is doubtful whether passive sensitizing antibody mediates all the changes evoking eosinophilia on challenge, because guinea-pigs passively sensitized with IgG1 antibody for 16 hours had less eosinophilia on challenge than actively sensitized animals, though both passively and actively sensitized animals had the same titre of PCA antibody.

INTRODUCTION

Blood and local tissue eosinophilia is classically associated with anaphylaxis, anaphylactic disorders, and infestation with helminth parasites, probably reflecting a concomitant anaphylactic sensitization to helminth antigens. Experimentally, eosinophilia is induced in guinea-pigs by repeated injections of protein antigens (Biggart, 1932, Litt, 1960, 1964a); and by passive anaphylaxis (Redd and Vaughan, 1955; Litt, 1961). Litt (1961, 1964a) further demonstrated that antigen–antibody complexes induced eosinophilia, and he contended that the function of eosinophils was to ingest and destroy the complexes. All the

early studies were made with whole rabbit or guinea-pig sera, which are mixtures of antibodies with differing biological activities. However, when the globulins of guinea-pig sera are separated, the ability to prepare tissues for eosinophilia is found to be a property of IgG1 (γ1) anaphylactic sensitizing antibodies, and not of the IgG2 (γ2) non-anaphylactic sensitizing antibodies (Litt, 1967; Kay, 1970; Parish, 1970a). The IgG1 antibody mediates generalized and localized eosinophilia in passive anaphylaxis, and when injected as preformed antigen–antibody complexes, but complexes of antigen and IgG1 antibody are much less effective in attracting eosinophils *in vitro*, than those containing IgG2 antibody which are completely ineffective *in vivo* (Parish, 1970a). The investigations described in this paper show that eosinophilia in guinea-pigs is elicited during anaphylaxis mediated by two antibodies, IgG1a and IgG1b (Parish, 1970b), and by antigen–antibody complexes containing them. Nevertheless eosinophilia is greater after challenge of actively than of passively sensitized guinea-pigs, when both have the same serum titre of antibody conferring homologous passive cutaneous anaphylaxis (PCA). It is concluded therefore that the release of eosinophils from the bone marrow is not evoked by the anaphylactic antibody–antigen complexes, but by the synthesis or release of a selective eosinophilotropic substance.

MATERIALS AND METHODS

Sensitization of guinea-pigs

(a) *Active sensitization and priming for eosinophilia.* Guinea-pigs were sensitized to soluble diphtheria toxoid, or primed to respond readily to eosinophilotropic substances, by intraperitoneal injection of 1 ml diphtheria toxoid once weekly for 8–12 weeks; they showed mild or no anaphylaxis. Guinea-pigs receiving weekly injections of 1 ml of 0·1 per cent bovine plasma albumin (BPA) (Armour) (Tables 1 and 2) frequently had anaphylaxis. Before testing for eosinophilia they were rested 7 or 14 days, by which time they weighed about 650 g.

Guinea-pigs sensitized to diphtheria toxoid or to BPA in Freund's complete adjuvant (Table 2), received 200 μg intramuscularly, divided as 0·1 ml each hind leg. Those sensitized twice with antigen in adjuvant received the second injection about 1 cm higher than the first. Control animals were injected intraperitoneally with 1 ml saline, or intramuscularly with 0·2 ml, total, of saline in Freund's adjuvant.

Guinea-pig IgG2 (γ2) and IgG1 (γ1) antibodies to BPA were obtained by immunization according to the regimens of Benacerraf, Ovary, Bloch and Franklin (1963) and subsequently purified. The regimen to obtain mainly IgG1 was slightly modified, in that after the three consecutive daily intraperitoneal injections of 1 mg antigen, the animals were injected intradermally six times at 7-day intervals with 0·4 mg divided between four sites.

Guinea-pig IgG2 and IgG1a (analogous to the γ1 of Benacerraf *et al.*, 1963) anti-picryl-chloride antibodies, were prepared as above, using picryl-chloride guinea-pig albumin antigen. Guinea-pig IgG1b (Parish, 1970b) was obtained by injecting 50 μg unconjugated picryl-chloride in Freund's complete adjuvant divided between each hind footpad. On the 45th day 0·1 ml containing 5 μg picryl-chloride guinea-pig albumin was injected into a hind footpad, and repeated into the alternate footpad each week for 18 weeks.

(b) *Passive sensitization.* Guinea-pigs were sensitized intraperitoneally with fractions of homologous antisera by injecting 2 ml containing 50 μg Ab N and challenging intra-

peritoneally with 1 ml of 0·1 per cent BPA after 4 or 16 hours (Table 4). In other tests (Table 5) guinea-pigs receiving 2 ml containing 10 or 100 μg Ab N were challenged with 1 ml of 0·1 per cent picryl chloride-β-lactoglobulin after 4 hours, 4 and 7 days. Normal guinea-pigs had no eosinophilia after a single injection of all the antigens used.

To compare the eosinophilia induced in actively and passively sensitized animals, guinea-pigs weighing 200 g were sensitized passively with concentrated IgG1 anti-BPA by three or four intravenous injections at 3-hour intervals. Sixteen hours after the last injection, about 0·5 ml blood was taken from the ear for subsequent determination of the 4 hour PCA titre by the method of Ovary (1964), after which the animals were challenged intraperitoneally with 1 ml of 0·1 per cent BPA. The number of eosinophils in the peritoneal fluid and peripheral blood in passively sensitized animals with PCA titres of 8–16 were compared with those in actively sensitized animals with similar PCA titres.

Preparation of antigen–antibody complexes

In the preparation of BPA–anti-BPA complexes (Table 6) antigen–antibody equivalence was taken to be that amount of antigen that just neutralized the antibody as detected by antiglobulin-agglutination, using BPA coupled to sheep red cells by glutaraldehyde (Onkelinx, Meuldermanns, Joniau and Lontie, 1969) as the indicator system, and rabbit antiglobulin serum to the respective guinea-pig globulin. Complexes were formed by adding BPA in 8 times excess of equivalence to an equal volume of antibody at room temperature for 1 hour and at 4° for 24 hours. Complexes containing 100 or 250 μg Ab N were injected intraperitoneally.

Paracentesis and eosinophil counts

Peritoneal lavage was carried out by a modification of Litt's (1960) method. The needle used to drain the abdominal cavity was 5·5 cm long, 3 mm internal diameter and bored with ten holes 1·5 mm diameter in the distal 2·5 cm: it had a closely fitting stylet. The animal was anaesthetized with ether, laid on its back, a 1 cm incision made in the skin and the draining needle inserted into the abdominal cavity. The stylet was removed, and a syringe fitted with a disposable needle with a plastic connection was inserted into the draining needle and pushed so that the soft plastic made a tight seal. Ten millilitres of Hanks's salt solution with Hepe's buffer, or in some later tests physiological saline with 20 i.u. heparin ml, was injected, and the abdomen gently massaged for 15 seconds. Guinea-pigs weighing over 600 g were injected with 15 ml. The animal was then lifted, held with its abdomen dependent, the syringe with the plastic needle withdrawn, and the fluid from the draining needle flowed into ice cold plastic tubes with a non-wettable surface. When the flow ceased, the animal was laid back on the table, the needle removed and the skin incision closed with one suture. The operation took about 2 minutes.

All animals made a rapid recovery. The intestine was never punctured, and it was rare for blood to appear in the lavage fluid. If the omentum occluded the draining needle on the first or second lavage it could be dislodged by the stylet, but on repeated lavage the omentum more quickly and firmly blocked the needle. It was necessary to recover at least 4 ml and 7 ml from the 10 and 15 ml injected, respectively, to obtain a consistent eosinophil count, because the fluid recovered contains few cells initially, becoming more concentrated as the flow continues.

The volume of fluid recovered from 10 ml averaged 6·25, and from 15 ml, 11. Samples were diluted in Eosin Y solution (Discombe, 1946) for cell counts. In most tests

two samples were counted on four haemocytometer grids, but when many animals were examined daily (Tables 1, 2, 6) only one sample was counted on two grids. Peripheral blood was obtained by ear prick.

Since it was not possible to repeat Litt's (1960) recovery of 12–14 ml from 15 ml injected, probably because we used a smaller needle, our eosinophil counts were expressed as eosinophils/ml, instead of total eosinophils recovered. Nevertheless the numbers of eosinophils were very similar to those obtained by Litt when his figures are expressed in the same manner.

Preparation of antibody and normal IgG1 and IgG2 fractions

Globulin was precipitated twice from antisera with 35 per cent ammonium sulphate. The precipitate, dissolved in water and dialysed against 0·85 per cent NaCl pH 7·0, was applied to bromo-acetyl cellulose conjugated with BPA, or with picryl chloride–egg albumin, by the method of Robbins, Haimovich and Sela (1967), and the specific antibody eluted from the immuno-adsorbent with glycine-HCl buffer pH 2·5. The eluted antibody was concentrated with Carbowax, and then separated on DEAE cellulose into an IgG2 portion eluted with 0·005 M phosphate buffer pH 8·0 (Binaghi, 1966) followed by 0·01 M buffer, and an IgG1 portion (containing IgG1a or IgG1b antibody according to the mode of sensitization) eluted with a gradient of buffer from 0·01 to 0·15 M pH 8·0 (Oettgen, Binaghi and Benacerraf, 1965). The IgG1 was then passed down a Sephadex 200-gel column to remove any IgM. Finally, the preparations were freeze-dried. IgG2 and IgG1 from normal, unsensitized, animals were obtained in the same manner, omitting the elution from the immuno-adsorbent.

The antibody-containing fractions were shown to be pure IgG2 and IgG1 by antiglobulin agglutination, in which sheep red cells coupled with the relevant antigen by glutaraldehyde were treated with the antibody fraction and tested with specific rabbit anti-IgG2 or anti-IgG1. The IgG1 obtained from normal serum was slightly contaminated by IgG2, just detectable by precipitation in agar gel, and more easily by antiglobulin agglutination in which the fraction was coupled to the red cells for tests with the rabbit antisera.

RESULTS

EOSINOPHILIA FOLLOWING INTRAPERITONEAL CHALLENGE OF ACTIVELY SENSITIZED GUINEA-PIGS

The number of eosinophils in the peritoneal cavity of actively sensitized guinea-pigs 1 to 3 days after intraperitoneal challenge with antigen was eight to eleven times greater than that in sensitized animals challenged with saline, as in the first post-challenge counts of Groups 1, 2 and 3 (Table 1). These animals had been held for 14 days after their last sensitizing injection, during which the number of eosinophils in the peripheral blood and peritoneal fluid returns to normal after the increase induced by sensitization.

Most of the eosinophils enter the peritoneum within 24 hours of challenge (Group 1, Table 1): when the peritoneal lavage is delayed 48 hours after challenge, the eosinophilia is little greater than at 24 hours (Group 2, Table 1). Moreover, the depletion of eosinophils by lavage at 24 hours is not replaced at 48 and 72 hours (Group 1, Table 1). The eosinophilia persists about 3 days, decreasing on the third day (Group 3), and even more by the fifth day (Group 4, Table 1).

The number of eosinophils in the peripheral blood after intraperitoneal challenge tends

to be greatest at 12 to 16 hours, and decreases thereafter, but is still greater than normal at 24 hours (Table 1). However, eosinophilia of the blood appears less consistently than that of the peritoneal fluid; in some animals the increase in the peritoneal fluid was eightfold with no increase in the blood.

TABLE 1

NUMBER OF EOSINOPHILS PER CU MM PERIPHERAL BLOOD (B) AND PERITONEAL FLUID (P) AT INTERVALS FOLLOWING INTRAPERITONEAL INJECTION OF SALINE OR BPA IN GUINEA-PIGS SENSITIZED TO BPA

Group	Injection	No. of eosinophils in blood or peritoneum	Pre-challenge	Days post-challenge				
				1	2	3	4	5
1	Saline	Blood	25	19	7	6	9	6
		Peritoneum	260	83	25	19	11	8
	BPA	Blood	19	88	31	19	13	27
		Peritoneum	283	1105	360	126	63	21
2	Saline	B	31	38	38	25	28	21
		P	248		96			208
	BPA	B	28	69	41	30	23	25
		P	263		1606			280
3	Saline	B	28	21	19	17	18	23
		P	272			98		62
	BPA	B	31	82	21	16	19	26
		P	268			1548		139
4	Saline	B	21	24	22	26	26	23
		P	284					280
	BPA	B	24	73	38	19	16	18
		P	269					629

Each group contained thirty guinea-pigs; fifteen tested with antigen, fifteen with saline.
Eosinophils expressed as average number per cu mm of fifteen guinea-pigs in each group.
Animals sensitized by 8 weekly injections of 1 ml of 0·1 per cent BPA with an interval of 14 days before the pre-test count. They were challenged with the same antigen or 1 ml of saline.

Eosinophilia is evoked only by challenge with the antigen used for sensitization. Little was evoked in guinea-pigs sensitized to bovine plasma albumin by β-lactoglobulin, human IgG, egg albumin or diphtheria toxoid, though after these substances the number of eosinophils in the peritoneal fluid was slightly greater than in animals injected with saline.

Fatal anaphylaxis and eosinophilia

Some of the animals injected eight times with BPA to induce eosinophilia (Table 1) had severe anaphylaxis during sensitization when they had most eosinophils. Tests were therefore made to see which was the more potent in evoking eosinophilia, repeated exposure to antigen or the degree of anaphylactic sensitivity.

Guinea-pigs in groups of twenty were sensitized by different regimens to BPA, which is moderately anaphylactogenic, and to diphtheria toxoid, which is poorly so (see Materials and Methods and Table 2). In animals sensitized intramuscularly with adjuvant, the number of deaths from anaphylaxis in each ten animals of a group challenged intravenously was not consistently associated with the greatest eosinophilia (Table 2). In some groups in which all the animals challenged intravenously died of anaphylaxis, the number of eosinophils in those challenged intraperitoneally was lower after than before challenge; whereas, guinea-pigs sensitized by four or more weekly-intraperitoneal injections of soluble antigen were just as susceptible to anaphylaxis, and had many more eosinophils in

TABLE 2

RELATION BETWEEN FATAL ANAPHYLAXIS AND PERITONEAL EOSINOPHILIA IN GUINEA-PIGS SENSITIZED TO BPA OR TO DIPHTHERIA TOXOID AND CHALLENGED BY THE HOMOLOGOUS ANTIGEN

Sensitization antigenic:–			Sensitization[a] challenge interval	BPA challenge iv[b] ip eosinophilia[c]			Dip. toxoid challenge iv ip eosinophilia		
State	Site	Frequency		Deaths/10	Pre	Post	Deaths/10	Pre	Post
Adjuvant	I M	once	4 weeks	10	224	108	8	213	102
Adjuvant	I M	two, at 2 week interval	2 weeks	10	251	164	10	242	233
Adjuvant	I M	once	12 weeks	7	220	93	4	220	98
Saline only	I M	once	4 weeks	nd	226	61	nd	235	64
Soluble	I P	once	1 week	0	230	64	0	228	56
Soluble	I P	2, at 1 week interval	1 week	3	238	59	0	249	68
Soluble	I P	4, at weekly intervals	1 week	10	246	481	5	261	439
Soluble	I P	8, at weekly intervals	1 week	10	293	1551	10	323	1468
Soluble	I P	12, at weekly intervals	1 week	8	322	2490	9	389	2811
Saline only	I P	once	1 week	nd	214	48	nd	224	51

Guinea-pigs sensitized in groups of 20 (see Materials and Methods).
[a] Interval between last sensitizing injection and challenge.
[b] No. of guinea-pigs dying in 30 minutes out of each ten challenged intravenously.
[c] Average No. of eosinophils/cu mm/guinea-pig in each ten challenged intraperitoneally. Pre-challenge count and 24 hours post-challenge.
nd = not done.

the peritoneal fluid. Therefore eosinophilia was influenced more by the number of injections of soluble antigen than by susceptibility to fatal anaphylaxis.

INFLUENCE OF ROUTE OF ANTIGENIC SENSITIZATION ON POST-CHALLENGE PERITONEAL EOSINOPHILIA

In the tests summarized in Table 1, antigenic stimulation and challenge was made in the same tissue (peritoneum), subsequently examined for eosinophilia. In order to examine the role of local deposition of antigen in eliciting eosinophilia in a particular tissue, groups of guinea-pigs were sensitized to, and subsequently challenged with, soluble diphtheria toxoid by various routes other than the peritoneal (Table 3). The peritoneal eosinophilia

TABLE 3

INFLUENCE OF ROUTE OF SENSITIZATION ON THE EOSINOPHILIA FOLLOWING PERITONEAL CHALLENGE

Route of sensitization	1 mg. dip. toxoid in	Route of challenge	No. of g-pigs	Peritoneal pre-test	Eosinophilia post-test	Pre-challenge mean	PCA titre range
1P	1 ml	1P	38	246	1232	64	16–256 on eight pools
		SC	10	253	746		
SC	1 ml	1P	10	231	598	16	4–128 on five pools
		SC	10	237	202		
1M	0·25 ml	1P	8	224	392	64	16–128 on four pools
		IM	8	229	86		
1D	0·25 ml	1P	10	230	411	256	64–512 on five pools
		1D	10	218	89		
Saline control tests							
1P	Saline	1P	28	213	46	0 on three pools	
		SC	5	216	57		
SC	Saline	1P	5	231	63	0 on one pool	
		SC	5	240	61		

Animals sensitized by 8 weekly injections of diphtheria toxoid by volume and route stated. After an interval of 14 days they were challenged by the appropriate dilution of antigen for the route. One ml blood was taken from the ear 1 day before challenge to determine the PCA titre.

in these animals challenged intraperitoneally was compared with that in animals challenged by the same route by which they were sensitized. Before challenge, each animal was bled from the ear to measure the serum PCA titre, as an indication of anaphylactic sensitivity.

Eosinophilia was greatest when sensitization, challenge, and estimation of eosinophilia were all made in the same tissue, e.g. in the peritoneum (Table 3). Peritoneal eosinophilia was scanty in animals sensitized and challenged intramuscularly or intradermally, and though it was greater after intraperitoneal challenge of animals sensitized by the other routes, it was less than in animals both sensitized and challenged intraperitoneally. The peritoneal fluid of animals sensitized and challenged in tissues other than the peritoneum had only a few more eosinophils than that of animals prepared with saline and challenged once with antigen (Table 3), excepting after subcutaneous challenge of sensitized animals, probably because of the more rapid dispersion of 1 ml of antigen from the loose connective tissue than of 0·25 ml of antigen from muscle or skin.

The scanty peritoneal eosinophilia in animals sensitized and challenged by other routes is not due to lack of eosinophils because eosinophils were plentiful in impression and histological preparations of the challenge sites, though they were too numerous and too unevenly distributed for reliable counting. Thus eosinophils were attracted to these sites of antigen just as they were to the peritoneum. Such eosinophilia does not occur in nonsensitized animals injected for the first time with antigen.

The number of eosinophils infiltrating a tissue is determined more by the presence of antigen within the tissue than by anaphylactic sensitivity measured by the serum PCA titre. The titres after intraperitoneal, were similar to those after intramuscular sensitization, and less than those after intradermal sensitization (Table 3), yet intraperitoneal challenge evoked a much greater eosinophilia in animals sensitized by that route than in those sensitized by other routes.

It is concluded therefore that eosinophils are attracted to sites of antigenic challenge in anaphylactically sensitized tissue, and that local eosinophilia is greater when antigen is repeatedly deposited at the same site.

RELATION BETWEEN THE INCREASE IN NUMBER OF MONONUCLEAR CELLS AND OF EOSINOPHILS AT SITES OF ANTIGENIC SENSITIZATION

The peritoneal fluid of animals injected intraperitoneally once, twice or thrice with soluble antigen at weekly intervals contained increased numbers of mononuclear cells. The increase is evident before the eosinophilia which may first appear after three injections, and consistently after the 4th (Table 2). The increase in number of mononuclear cells between the 4th and 8th weekly injection is greater than the increase of eosinophils. Though macrophages and plasma cells are present, the majority of the mononuclear cells resemble large lymphocytes, but differ from them in having more cytoplasm and a less densely staining nucleus. In fixed and stained mesenteric spreads from these animals eosinophils tend to aggregate round groups of the mononuclear cells.

EOSINOPHILIA MEDIATED BY PASSIVE SENSITIZING ANTIBODIES

Since eosinophilia is associated with anaphylaxis, the antibodies mediating anaphylaxis were examined for their ability to prepare tissues for an eosinophilic response.

Challenge of guinea-pigs previously injected with homologous IgG1 antibody, which sensitizes the tissues anaphylactically, resulted in increased numbers of eosinophils in the peritoneum (Table 4). The eosinophilia was slightly greater in animals challenged 16 hours than in those challenged 4 hours after passive sensitization. Challenge of guinea-pigs given homologous IgG2 antibody, which does not sensitize their tissues anaphylactically, failed to evoke peritoneal eosinophilia (Table 4). In these tests it was necessary to use guinea-pigs primed to respond to eosinophil-evoking substances by previous injections of diphtheria toxoid, because little eosinophilia was evoked in unprimed animals, passively sensitized and anaphylactically challenged.

TABLE 4

EOSINOPHILIA FOLLOWING INTRAPERITONEAL CHALLENGE OF GUINEA-PIGS PREPARED WITH HOMOLOGOUS ANTISERUM TO BPA CONTAINING HIGH OR LOW TITRES OF PCA ACTIVITY

Antibody[a] mainly	TCT[b] aggl.	PCA[c] titre	Hours[d] of PS.	No. of g-pigs	Mean eosinophils[e]	
					Pre-challenge	Post-challenge
IgG1	256	1600	4	6	221	331
			16	4	246	408
IgG1	512	800	4	6	238	366
			16	4	209	382
IgG2	$>5\times10^5$	0	4	8	251	98
			16	3	233	63
IgG2	$>5\times10^5$	0	4	6	248	72
			16	5	226	68

[a] Antisera to BPA prepared to contain mainly IgG1 or IgG2 (Materials and Methods) and 2 ml injected intraperitoneally into normal recipients.
[b] Tanned cell test agglutination titre.
[c] PCA titre after 4 hours latent period.
[d] Period between injection of antibody and of antigen.
[e] Eosinophils/cu mm/mean pre- and 24 hours post-challenge.

TABLE 5

PERITONEAL EOSINOPHILIA 24 HOURS AFTER CHALLENGE OF RECIPIENTS OF IgG1a, IgG1b AND IgG2 GUINEA-PIG ANTI-PICRYL CHLORIDE

Antibody	Duration of PCA	μg Ab N. inj. ip.	Per cent change in eosinophils[a] 24 hours after challenge, following passive sensitization for:		
			4 hours	4 days	7 days
IgG1a	3–4 day	10	+56	+109	+12
IgG1b	7 day	10	+28	+143	+96
IgG2	Nil	10	−68	−79	−76
IgG1a	3–4 day	100	+188	+116	+43
IgG1b	7 day	100	+204	+269	+152
IgG2	Nil	100	−46	−74	−60

[a] Each percentage, the mean of four animals, represents the change in eosinophilia from 1 hour before, to 24 hours after challenge.

Guinea-pig IgG1 antibody has two forms, one (IgG1a) confers anaphylactic activity on guinea-pig skin for 3 to 4 days, the other (IgG1b) for 7 to 9 days (Parish, 1970b). Both IgG1a and IgG1b antibody passively sensitized guinea-pigs to eosinophilic responsiveness, but equal amounts of IgG2 antibody to the same antigen did not (Table 5).

With doses of 10 μg antibody N, the two forms of IgG1 antibody sensitized equally well 4 hours and 4 days after injection, but IgG1b prepared tissues for a greater eosinophilia at 7 days. However, a sensitizing dose of 100 μg Ab N IgG1b mediated a greater eosinophilia at all three time intervals than IgG1a.

These results establish that eosinophilia is mediated by two forms of anaphylactic sensitizing antibody, and the antibodies incapable of sensitizing tissues anaphylactically are ineffective.

EOSINOPHILIA INDUCED BY COMPLEXES OF ANTIGEN WITH ANAPHYLACTICALLY SENSITIZING ANTIBODY

Though IgG1a passive sensitizing antibody mediated a greater eosinophilia after a latent period of 16 hours than after 4 hours (Table 4), simultaneous injection of sensitizing antibody and antigen resulted in an eosinophilia detectable 24 hours later (Table 6). Complexes formed *in vitro* of antigen and IgG1a antibody, given intraperitoneally elicited such eosinophilia, whereas complexes of antigen and IgG2 antibody, and mixtures of antigen and normal (non-antibody) IgG1 and IgG2 globulins elicited no eosinophilia.

The results of this test on unprimed animals differed from those usually observed in unprimed animals in that they responded unusually well to the complexes containing anaphylactic antibody, with a substantial peritoneal eosinophilia.

However, in animals primed to an eosinophilic response by repeated injections of diphtheria toxoid, the response to complexes containing IgG1 anaphylactic antibody was even greater (Table 6), and moreover, eosinophilia occurred after injection of complexes containing IgG2 antibody, and even mixtures containing non-antibody globulin.

In a few animals receiving multiple injections for priming (Table 6) or for sensitization

TABLE 6

EOSINOPHILIA IN UNPRIMED (NORMAL) AND PRIMED GUINEA-PIGS FOLLOWING INTRAPERITONEAL INJECTION OF ANTIGEN–ANTIBODY COMPLEXES CONTAINING IgG1 AND IgG2

Type of Globulin	Concentration in Complex or mixture[a]		Eosinophilia in unprimed				Primed guinea-pigs[b]			
			Pre-test[c]		Post-test		Pre-test		Post-test	
			Blood	Perit.	Blood	Perit.	Blood	Perit.	Blood	Perit.
IgG1 antibody	μg Ab N	100	19	261	16	182	23	291	23	664
		250	18	273	24	298	21	287	29	931
IgG2 antibody	μg Ab N	100	18	268	13	82	25	311	21	217
		250	21	250	21	70	28	286	19	292
IgG1 normal	μg glob N	250	22	281	19	68	22	288	19	178
IgG2 normal	μg glob N	250	22	269	13	90	31	296	30	239

[a] Complexes in 8 × BPA antigen excess of equivalence. In the mixtures the higher concentration of antigen was added to the corresponding normal globulin.
[b] Primed by 8 weekly injections of diphtheria toxoid, and tested 1 week after last injection.
[c] Mean eosinophils in blood and peritoneal fluid of five g-pigs in each test.

(Table 1), abnormal neutrophils, or 'pseudo-eosinophils' appeared in the blood or peritoneum. These stain more brightly with eosin than normal neutrophils. They are not eosinophils because they had fewer and smaller granules, and are not stained by the modified peroxidase technique (Archer, 1963) which is selective for eosinophils.

COMPARISON OF EOSINOPHILIA INDUCED IN ACTIVELY AND PASSIVELY SENSITIZED GUINEA-PIGS

Though susceptibility to eosinophilia was passively transferable with homologous IgG1 antibody, the numbers of eosinophils in the peritoneal fluid 24 hours after challenge (Table 4) were much lower than after active sensitization to the same antigen (Tables 1 and 2). This could be due to the greater amount of antibody in the actively sensitized animals. To test this possibility, sufficient concentrated IgG1 anti-BPA was injected intravenously into normal guinea-pigs to achieve a serum PCA titre of 1/8 to 1/16 16 hours later. The eosinophilia after intraperitoneal challenge of the passively sensitized animals was less than that of animals actively sensitized by the subcutaneous or intramuscular route, and whose serum contained the same titre of PCA antibody (Table 3). It thus appears that the greater eosinophilia in actively sensitized animals is not due solely to the amount of anaphylactic sensitizing antibody in the serum.

DISCUSSION

Peritoneal lavage is a reliable technique for obtaining samples to count the number of infiltrating eosinophils. The counts so obtained probably reflect local tissue eosinophilia observed in lungs or muscle. The technique yielded results similar to that in another laboratory, for though we recovered less of the lavage fluid, only 6·25 or 11 from 10 or 15 ml respectively, than Litt (1960), who recovered 12 to 14 ml 'in most cases' from 15 ml, the eosinophil counts per cu mm of washout fluid were similar. Litt (1960) found 2·7 (± 1) million eosinophils in about 12 ml, i. e. 225 eosinophils per cu mm in a normal guinea-pig; we found 275 eosinophils per cu mm. The lesser yield of eosinophils on repeated washing out and the increase in numbers after antigenic challenge of actively sensitized animals (Table 1) were similar in both studies.

Eosinophilia is a manifestation of local or generalized anaphylaxis. It occurs in passive anaphylaxis, though seldom as strongly as occurs in active anaphylaxis, particularly in animals actively sensitized by repeated doses of antigen. It is consistent therefore that the antibodies separated from whole serum that prepare tissues for anaphylaxis also prepare them for an eosinophilic response (Litt, 1967; Kay, 1970; Parish, 1970a). In the guinea-pig there are two forms of IgG1 that mediate anaphylaxis, namely IgG1a conferring PCA sensitivity for 3 to 4 days, and IgG1b conferring PCA sensitivity for 7 to 9 days and differing slightly in electrophoretic mobility (Parish, 1970b). IgG1b prepared tissues for a greater eosinophilia for a longer time than IgG1a (Table 5) which reflected the more persistent passive sensitization by IgG1b. Nevertheless IgG1a prepared the peritoneum to a slight eosinophilia at 7 days, by which time it no longer conferred PCA sensitivity. Another anaphylactic antibody in guinea-pigs is heat-labile and confers a long-persisting passive sensitization, resembling human reagin (Catty, 1969; Parish, 1970b). This has not been tested for its ability to confer peritoneal eosinophilia, but sites of PCA reaction attract more than those of the IgG1 antibodies (Parish, 1972).

Antigen–antibody complexes prepared *in vitro* induce eosinophilia in the peritoneal cavity (Litt, 1961; 1964a), and in the peripheral blood of guinea-pigs (Parish and Coombs, 1968). The eosinophilotropic activity of these complexes depends upon the presence of passive anaphylactic IgG1 antibodies; complexes containing the non-anaphylactic IgG2 antibodies have little or no effect (Table 6). In these tests, normal guinea-pigs, not primed by previous injection of an unrelated antigen, responded unusually well to the first injec-

tion of complexes containing IgG1 antibody, because normal guinea-pigs seldom have much eosinophilia when injected with complexes. However, when complexes containing IgG1 antibodies are injected into primed guinea-pigs, the eosinophil response is very much greater than in unprimed animals. Moreover, the response to the non-anaphylaxis mediating, but complement-fixing IgG2 antibody-antigen complexes, and to non-antibody IgG1 and IgG2 is also enhanced (Table 6), though the eosinophilia is still much less than that induced by IgG1 antibody-antigen complexes. The enhanced response to non-anaphylactic complexes in primed animals is probably due not to the release of large numbers from the bone marrow, but to local attraction of the increased number of eosinophils already in the tissues, because, though complexes of IgG2 antibody with antigen do not elicit eosinophilia *in vivo*, after fixing complement they strongly attract eosinophils *in vitro* (Parish, 1970a), and complexes are ingested by these cells (Archer and Hirsch, 1963; Litt, 1964b; Parish, 1970a). Whatever the special evolutionary function acquired by eosinophils, they retain the phagocytic properties also present in neutrophils.

There appears to be two stages in an eosinophil infiltration. The first, which is selective for eosinophils, is the stimulus which releases eosinophils from the bone marrow. The second is the accumulation of eosinophils at the site of antigen. The substance responsible for the first stage probably participates in the second, but several other substances or cells influence the local accumulation, e.g. antigen–antibody complexes, mast cells and basophilic mononuclear cells (Parish, 1970a).

Eosinophils in sensitized animals are attracted to the site of antigen, especially when the tissue is subject to repeated antigenic stimulation. Fewer eosinophils infiltrate a new site of antigenic stimulus in animals primarily sensitized in other tissues, even though they may have as much or more PCA-mediating antibody than in animals sensitized and challenged in the same tissue (Table 3). It is evident that some substance other than antigen–antibody complexes evokes eosinophilia.

The greater attraction of eosinophils to sites of repeated exposure to antigen may be associated with the mononuclear cell infiltration at such sites, which precedes the eosinophilia and continues during the exposure to antigen. This may resemble the attraction between eosinophils and basophilic mononuclear cells in mesenteric milk spots after challenge of actively or passively sensitized guinea-pigs (Parish, 1970a). A diffusible substance from the lymphocytes of sensitized rats which induces eosinophilia has been described by Basten and Beeson (1970), but in experiments designed to test the relation between lymphocytes and eosinophils, Parish (1970a) found no association between lymphocytes conferring delayed-type sensitivity only and eosinophils, though anti-lymphocyte serum modified eosinophilia in animals with anaphylactic sensitivity. In further studies on guinea-pigs primed by repeated antigenic injection (Parish to be published) the cell-free peritoneal fluid was found to have an eosinophilotropic substance similar to that in extracts of anaphylactic guinea-pig lung (Parish and Coombs, 1968), which is not an antigen–antibody complex. The mononuclear cells in the peritoneal exudates also attract eosinophils *in vitro* and when injected into normal animals. Thus the evidence indicates that a selective eosinophilotropic substance is synthesized by, activated in, or released by anaphylactic tissues, possibly from cells of the lymphocyte-type.

ACKNOWLEDGMENTS

I thank Sir Ashley Miles F.R.S. for his advice and criticism of the manuscript.

I also thank Mr Kenneth Veal and Miss Susan Ramsden for their enthusiastic technical assistance.

REFERENCES

ARCHER, G. T. and HIRSCH J. G. (1963). 'Motion picture studies on degranulation of horse eosinophils during phagocytosis.' *J. exp. Med.*, **118**, 287.

ARCHER, R. K. (1963) *The Eosinophil Leucocytes*. p. 181, Oxford, Blackwell.

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

BENACERRAF, B., OVARY, Z., BLOCH, K. J. and FRANKLIN, E. C. (1963). 'Properties of guinea-pig 7S antibodies. I. Electrophoretic separation of two types of guinea-pig 7S antibodies.' *J. exp. Med.*, **117**, 937.

BIGGART, J. H. (1932). 'Some observations on the eosinophile cell.' *J. Path. Bact.*, **35**, 799.

BINAGHI, R. A. (1966). 'Production of 7S immunoglobulins in immunized guinea-pigs.' *J. Immunol.*, **97**, 159.

CATTY, D. (1969). *Immunology of Nematode infections. Trichinosis in Guinea Pigs as a Model*. Basel. S. Karger.

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

KAY, A. B. (1970).'Studies on eosinophil leucocyte migration. I. Eosinophil and neutrophil accumulation following antigen–antibody reactions in guinea-pig skin.' *Clin exp. Immunol.*, **6**, 75.

LITT, M. (1960). 'Studies in experimental eosinophilia. I. Repeated quantitation of peritoneal eosinophilia by a method of peritoneal lavage.' *Blood*, **16**, 1318.

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

LITT, M. (1964a). 'Eosinophils and antigen–antibody reactions.' *Ann. N.Y. Acad. Sci.*, **116**, 964.

LITT, M. (1964b). 'Studies in experimental eosinophilia. VI. Uptake of immune complexes by eosinophils.' *J. cell. Biol.*, **23**, 355.

LITT, M. (1967). 'Studies in experimental eosinophilia. VIII. Induction of eosinophilia by homologous 7Sγ1 antibody and by extremely minute doses of antigen. *Allergology*.' *Excerpta Medica Int. Congr. Series* No. **162**, p. 38.

OETTGEN, H. F., BINAGHI, R. A. and BENACERRAF, B. (1965). 'Hexose content of guinea-pig γ1 and γ2 immunoglobulins.' *Proc. Soc. exp. Biol. (N.Y.)*, **118**, 336.

ONKELINX, E., MEULDERMANS, W., JONIAU, M. and LONTIE, R. (1969). 'Glutaraldehyde as a coupling reagent in passive haemagglutination.' *Immunology*, **16**, 35.

OVARY, Z. (1964). 'Passive cutaneous anaphylaxis.' In *Immunological Methods*. (Ed. J. F. Ackroyd) p. 259. Oxford, Blackwell.

PARISH, W. E. (1970a). 'Investigations on eosinophilia. The influence of histamine, antigen–antibody complexes containing γ1 or γ2 globulins, foreign bodies (phagocytosis) and disrupted mast cells.' *Brit. J. Derm.* **82**, 42.

PARISH, W. E. (1970b). 'Homologous serum passive cutaneous anaphylaxis in guinea-pigs mediated by two γ1 or γ1-type heat-stable globulins and a non-γ1 heat-labile reagin.' *J. Immunol.* **105**, 1296

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

PARISH, W. E. and COOMBS, R. R. A. (1968). 'Peripheral blood eosinophilia in guinea-pigs following implantation of anaphylactic guinea-pig and human lung.' *Brit. J. Haemat.* **14**, 425.

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

ROBBINS, J. B., HAIMOVICH, J. and SELA, M. (1967). 'Purification of antibodies with immunoadsorbents prepared using bromoacetyl cellulose.' *Immunochemistry*. **4**, 11.