ANTIBODY-DEPENDENT EOSINOPHIL-MEDIATED DAMAGE TO ^{51}CR-LABELED SCHISTOSOMULA OF *SCHISTOSOMA MANSONI*: DAMAGE BY PURIEID EOSINOPHILS*

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Although acquired immunity to *Schistosoma mansoni* infection has been described in a number of experimental models (1–4), the mechanism of such immunity has proved difficult to study in vivo. For many years, attempts to transfer immunity with either serum or lymphoid cells were unsuccessful (5, 6). Recently, however, Sher et al. (7) succeeded in transferring immunity in mice with serum. Subsequent experiments in rats revealed a brief period, 2–4 wk after a primary infection, when immunity could be transferred with lymphoid cells (8), but at the peak of immunity, 6–8 wk after a primary infection, it was again serum that was effective in transferring resistance (8, 9). Successful transfer depended on the presence in the recipient of one or more types of radiosensitive cell (9).

These findings suggested that some form of antibody-dependent, cell-mediated effector mechanism might be involved. In an attempt to analyze this mechanism in vitro, we developed (10, 11) a ^{51}chromium release technique for estimating damage to schistosomula, the larval stage of the parasite which is found immediately after the infective cercariae penetrate the skin of a mammalian host, and against which protective immune responses are most probably directed (12). With this technique, we identified an antibody-dependent, complement-independent release of ^{51}Cr from labeled schistosomula induced by human peripheral blood leukocytes. In this system, effector cell activity was associated with a leukocyte fraction rich in granulocytes, in contrast to a mononuclear-rich fraction which was almost inactive (13). Effector cell activity could be ablated by pretreatment of a mixed leukocyte preparation with an anti-eosinophil serum and complement, whereas an antineutrophil serum had relatively little effect.

These results implied that the eosinophil was the major cell responsible for antibody-dependent damage to schistosomula, and led Mahmoud et al. (14) to test the effect of anti-eosinophil serum on immunity in mice. Ablation of circulating eosinophils was associated with loss of immunity, strongly suggesting that the eosinophil mediated mechanism observed in vitro reflected events that occur in vivo.

An alternative approach to investigating the ability of eosinophils to mediate antibody-dependent damage to schistosomula has now been developed. In this paper, we report that populations of cells highly enriched in eosinophils and

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devoid of neutrophils mediate such damage. In contrast, populations enriched in mononuclear cells or neutrophils, but devoid of eosinophils, are not able to do so. Furthermore, the addition of homologous lymphocytes, monocytes, or neutrophils from normal individuals to purified eosinophils does not enhance the cytotoxic effect of these eosinophils.

Also of interest is the finding that eosinophils from patients with *S. mansoni* were often less active cell per cell than eosinophils from normal subjects. Whether this is partly due to a blockade of an Fc receptor on the eosinophils by antigen-antibody complexes, or to a functional heterogeneity of eosinophils associated either with the influx of many immature cells into the blood of these patients or with damage to such cells in the circulation, remains to be determined.

Materials and Methods

Parasite Cycle and Preparation of Schistosomula. A local strain of *S. mansoni*, recovered from naturally infected *Biomphalaria pfeifferi*, was maintained by passage in laboratory-bred snails and CBA mice, as described elsewhere (15). Schistosomula were prepared by allowing cercariae to penetrate an isolated preparation of mouse skin in vitro (16). The organisms recovered, which were contaminated with not more than 5–10% cercariae, were stored overnight at 4°C in Hank's balanced salt solution, pH 7.4, containing 0.5% lactalbumin hydrolysate, 100 U/ml penicillin, and 100 μg/ml streptomycin, together with 10% heat-inactivated fetal calf serum (FCS).

After overnight storage, schistosomula were labeled with ^{51}Cr sodium chromate (The Radiochemical Centre, Amersham, England), 1,000 $\mu Ci/10^4$ organisms, for 3–4 h at 37°C. Labeled organisms were washed four times in Eagle's minimal essential medium (MEM) containing 20 mM Hepes, 100 U/ml penicillin, and 100 μg/ml streptomycin, and twice in MEM containing 10% heat-inactivated fetal calf serum (MEM/FCS).[1] After checking that the final wash contained <0.5% of the total counts present in the schistosomula, the organisms were resuspended in MEM/FCS at 500/ml. Such preparations routinely showed a count rate of about 100 cps in an aliquot containing 50 schistosomula.

Antisera. Serum samples were obtained from patients with *S. mansoni* infection, and were inactivated at 56°C for 1 h before testing for their ability to induce release of chromium from labeled schistosomula in the presence of unpurified normal peripheral blood leukocytes. Dilutions in MEM/FCS were chosen which gave high levels of cell-dependent cytotoxicity. These gave no direct toxicity to schistosomula. Pooled samples of human sera were used in some experiments.

Preparation and Purification of Effector Cells. Peripheral blood leukocytes were prepared from normal individuals, from patients with eosinophilia associated with *S. mansoni*, with or without other helminth infections, or from a patient with eosinophilia of unknown origin, without detectable helminth infection. 5 vol of freshly-drawn, heparinized blood was allowed to sediment with 1 vol of 4.5% dextran in phosphate-buffered saline (Dextran 150,000; Sigma Chemical Co., St. Louis, Mo.) for 30 min at 37°C. The leukocyte-rich supernate was withdrawn and washed twice in MEM by centrifugation at 1,000 rpm to remove platelets.

Such unpurified preparations either were tested directly for cytotoxicity after appropriate dilution in MEM/FCS, or were subjected to further purification. Eosinophil-rich and eosinophil-depleted preparations were obtained by taking the pellet and interface, respectively, after centrifugation for 40 min at 400 *g* and 4°C over diatrizoate (Hypaque 50, Winthrop Laboratories, New York) diluted 1:1.7 with distilled water according to Mahmoud et al. (17).

It should be noted that the number of neutrophils present in the various Hypaque gradient fractions depends on the way that the cells are treated before they are centrifuged on the Hypaque cushion. When leukocytes are sedimented in dextran, washed, and then placed directly on Hypaque at high cell concentrations, the cells obtained, either from the interface or from the pellet, are essentially devoid of neutrophils. A small visible clump forms by the interface and it is removed by suction with a water vacuum apparatus. Presumably, the neutrophils are in this

[1] *Abbreviations used in this paper:* FCS, fetal calf serum; LSD, least significant difference.

clump. On the other hand, if the cells are first centrifuged on Ficoll (Pharmacia Fine Chemicals Inc., Piscataway, N. J.)-metrizoate (Nyegaard) and the granulocyte-rich cells are recentrifuged on Hypaque at lower cell concentrations, neutrophils are found both in the interface and in the pellet while the eosinophils are present almost exclusively in the pellet. Thus, granulocyte-enriched preparations were obtained by taking the pellet after centrifugation of unpurified cells for 20–40 min at 400 *g* over a Ficoll-metrizoate mixture (Ficoll, 6.35 g/100 ml, and metrizoate, 10 g/100 ml in distilled water) according to Böyum (18). After washing, granulocyte-enriched preparations were then enriched in or depleted of eosinophils by centrifugation over Hypaque, as above, followed by recovery of the pellet and interface, respectively.

Purified lymphocyte preparations were obtained by taking the mononuclear-rich interface after centrifugation on Ficoll-metrizoate, as above, and removing the phagocytic and adherent cells with carbonyl iron and a magnet. In some experiments, the cells from the interface after Hypaque centrifugation of buffy coat cells were used without carbonyl iron extraction as preparations enriched in both lymphocytes and monocytes.

Partially purified cells prepared in this way were washed twice in MEM. Some preparations were then tested directly for cytotoxicity, after appropriate dilution in MEM/FCS. Others were cultured overnight in MEM/FCS at 1–2 × 10^6/ml in Falcon tissue culture flasks (Biocult Laboratories). Others were treated with trypsin (trypsin-TPCK, Worthington Biochemical Corp., Freehold, N. J.), 10 or 1 mg/ml, in Hank's balanced salt solution for 1 h at 37°C with frequent mixing. They were then washed twice in MEM before overnight culture in MEM/FCS. After culture, cells were removed from the flask by gentle, repeated aspiration into a Pasteur pipette; they were then washed once in MEM, and resuspended in MEM/FCS.

At various stages during and at the end of preparation, total leukocyte counts were carried out with the aid of a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.). Absolute eosinophil counts were done, with Discombe's fluid, and differential counts were carried out on Jenner-Giemsa-stained smears on 1,000 cells.

Cytotoxicity Assay. The cytotoxicity assay has been described in detail elsewhere (10, 11). Briefly, aliquots of 0.1 ml of schistosomula (500/ml) were dispensed into 38 × 7-mm round-bottomed plastic tubes (LP/2, Luckham, Ltd.). These tubes were then randomized to eliminate systematic variation during dispensing. Dilutions of antiserum (0.1 ml) and effector cells (0.1 ml) were added to each of four replicate tubes, and the tubes were incubated in humidified airtight plastic boxes at 37°C. Control tubes received an appropriate volume of medium alone, cells and medium, or antiserum and medium. Maximum release of isotope was induced by freezing and thawing of mixtures of 0.1 ml schistosomula and 0.9 ml distilled water in 3-ml polystyrene tubes. In experiments involving more than one preparation of effector cell, 0.1-ml aliquots of each cell type were mixed in cytotoxicity tubes. Antibody (0.1 ml) and schistosomula (0.1 ml) were then added, giving a final volume of 0.4 ml. Alternatively, in other experiments, the schistosomula and antibody were previously mixed, and 0.1 ml of each effector cell was then added with a 10–20-min delay between each addition.

At the end of the incubation period (7 or 15 h, in different experiments), the contents of each tube were resuspended and centrifugated at 200 *g* for 5 min. One-half of the supernate was then withdrawn into a second tube and both tubes were counted for ^{51}Cr in a Packard well-type gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The percentage of isotope release was calculated from the corrected count rates in both tubes, thereby avoiding the errors attributable to variation in total count rate which occur if calculations are based on the count rate in the supernate alone.

Statistical Analysis. Results from individual experiments were tested by multifactorial analysis of variance on the logarithmically transformed percent release data (19). Individual means were then compared by Duncan's multiple range tests (20), with error estimates derived from the residual mean square in the analysis of variance. In each figure or table, the geometric mean of four replicate tubes is given, together with an indication of the significance of their difference from the appropriate controls, as judged by multiple range tests. In addition, where feasible, a summary is given in the legend to each table, indicating the significance of effects or interactions in the analysis of variance. This is followed by a least significant difference ("LSD") figure, representing the difference required by the multiple range tests between two $\log_e$ figures for a probability of $P < 0.05$. Since this figure is logarithmic, for the sake of clarity, an indication of the arithmetic difference required is given in parentheses for one or two arbitrary but appropriate levels of isotope release.

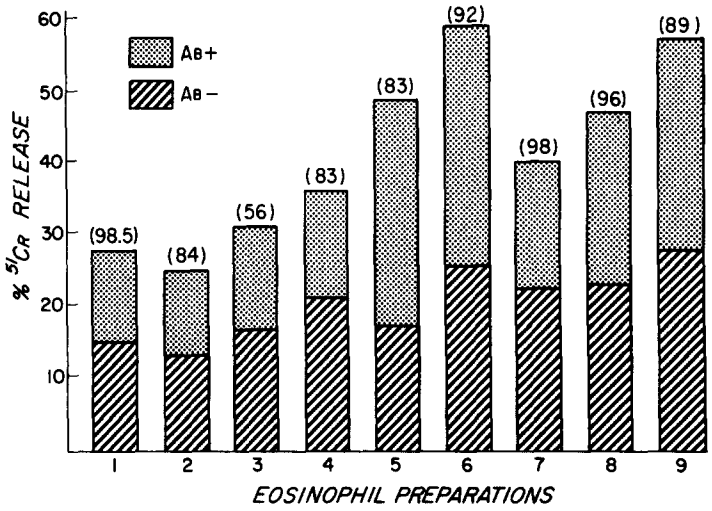

FIG. 1. Antibody-dependent cell-mediated damage to schistosomula by purified eosinophils. Release of ^{51}chromium from labeled schistosomula induced by purified or partially purified eosinophils (percentages for each preparation shown in parentheses). Preparations 1–7 were cultured overnight before assay, whereas preparations 8 and 9 were tested immediately after purification. Preparations 1–6 were from patients with *S. mansoni*, with or without additional helminth infestation. Preparations 7–9 were from a patient with eosinophilia of unknown cause, with no detectable parasitic infection. Cells in preparation 1 were trypsinized for 1 h, washed, and then cultured overnight before testing (the untrypsinized cells from this patient showed no activity). All cell preparations were tested for cytotoxicity to schistosomula, with or without antibody, at various effector to target ratios: only the highest ratios tested, ranging from 3,200:1 to 5,000:1, are shown in this figure. In all cases, ^{51}Cr release in the presence of antibody differed from release with no antibody at $P < 0.05$ or less. Dose-response curves for some preparations are shown in Fig. 2.

Results

Cytotoxic Activity of Eosinophil-Enriched Cell Preparations. Human blood leukocyte preparations highly enriched in eosinophils were consistently cytotoxic to schistosomula in the presence of antibody (see Fig. 1). For reasons discussed later, some of these preparations were cultured overnight before testing. It can be seen that all but one of the cell preparations studied contained 83–98.5% eosinophils; the remaining cells were mainly lymphocytes and monocytes which have been shown to be inactive (see next section and Ref. 13). It is of note that four of the populations studied contained no detectable neutrophils, and three contained only 1% neutrophils. The eosinophil-enriched preparations in the presence of antibody caused a significantly greater release of ^{51}Cr from labeled schistosomula than that induced by cells in the absence of antibody. In evaluating the data, it should be recalled that the maximum release of isotope after freeze-thawing the labeled schistosomula is not 100% but 55–68%, and that the schistosomulum, in contrast to the targets usually tested in assays of this type, is a large multicellular organism.

The first six preparations shown in Fig. 1 were taken from patients who had eosinophilia secondary to *S. mansoni* infection; some of these patients also had additional heminthic infections. The last three preparations were from a patient with eosinophilia of unknown etiology.

Dose-response curves, shown in Fig. 2, demonstrate isotope release by differ-

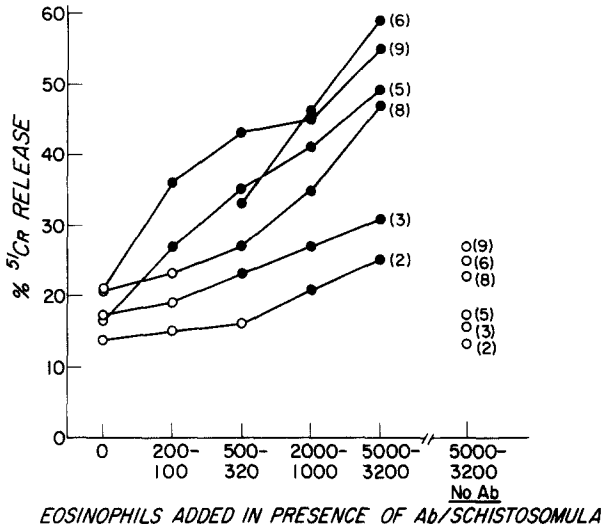

FIG. 2. Dose-response curves for six of the eosinophil-rich preparations shown in Fig. 1. All solid circles differ at $P < 0.05$ or less from the controls (i.e., release induced by cells from the same preparation in the absence of antibody). Least significant differences for $P < 0.05$: preparation 2, 0.151 (+ 3.0% at 15%); preparation 3, 0.166 (+ 3.6% at 20%); preparation 5, 0.148 (+ 3.2% at 20%); preparation 6, 0.174 (+ 4.8% at 25%); preparation 8, 0.176 (+ 3.8% at 20%); preparation 9, 0.120 (+ 5.1% at 40%).

ent ratios of cells to schistosomula for six of the eosinophil-enriched preparations. It can be seen that with an increasing ratio of cells to schistosomula, there was a progressive increase in ^{51}Cr release in the presence of antibody. In some cases, significant isotope release was found with the lowest ratio tested. Antibody in the absence of cells had no effect when compared with medium alone nor did the highest ratio of cells without antibody.

Lack of Cytotoxicity by Preparations Depleted of Eosinophils. The studies described above clearly showed that cell preparations enriched in eosinophils and depleted of neutrophils were capable of mediating antibody-dependent cell damage to schistosomula. It was therefore of interest to test whether or not cell preparations depleted of eosinophils were active. In two experiments, the cells from patients with *S. mansoni*-induced eosinophilia which sedimented at the interface after Hypaque centrifugation were assayed for cytotoxicity after overnight culture, at the same time as the eosinophil-enriched pellet. The interface preparations contained less than 0.1% eosinophils, and more than 99% of the cells were lymphocytes or monocytes. As is shown in Table I, these cells were not cytotoxic for schistosomula in the presence of antibody. In contrast, the eosinophil-rich pellet cells were active, as described in the previous section. In separate experiments (Table III, discussed later), it was also observed that fresh lymphocytes from normal individuals had little or no capacity to damage schistosomula in the presence of antibody.

An additional experiment was carried out to test whether neutrophil-rich populations, depleted of eosinophils, were cytotoxic in this sytem. The granulocyte fraction obtained by Ficoll-metrizoate centrifugation from a normal individual was further centrifuged on Hypaque, as described in Methods, before testing

TABLE I
Lack of Damage to Schistosomula by Cell Preparations Depleted of Eosinophils

Exp.	Eosinophils	Ab	^{51}Cr release				
			5,000–3,200:1	2,000–1,000:1	500–320:1	200–100:1	No cells
	%				%		
1	< 0.1	+	19	17	17	19	17
		–	16	18	16	15	17
2	< 0.1	+	19	17	16	14	15
		–	16	18	19	15	19

Preparations depleted of eosinophils were obtained by centrifugation of the leukocyte-rich supernate of dextran-sedimented blood from patients with *S. mansoni* infection on Hypaque. Cells recovered from the interface and subsequently cultured overnight contained not more than 0.1% eosinophils; the remaining cells were lymphocytes and monocytes. These cells failed to induce antibody-dependent release of ^{51}Cr under conditions in which the eosinophil-enriched pellets showed significant effects at cell to schistosomulum ratios of 400:1 and 320:1, for experiments 1 and 2, respectively. LSD = 0.166 (+ 3.6% at 20%) for experiment 1 and 0.178 (+ 3.9% at 20%) for experiment 2. No significant differences were observed between cells with antibody and cells without antibody at any ratio.

immediately against schistosomula. The cell pellet, containing 21% eosinophils and 75% neutrophils, showed significant antibody-dependent cytotoxicity at cell:schistosomula ratios from 3,200:1 to 100:1. In contrast, the cells from the interface which contained 0.5% eosinophils and 45% neutrophils were not cytotoxic at the highest ratio tests, namely, 1,000:1. These experiments indicate that populations rich in lymphocytes, monocytes, or neutrophils, but depleted of eosinophils, do not mediate antibody-dependent, cell-mediated damage to schistosomula.

Comparison of Cytotoxicity Mediated by Cells from Normal and Eosinophilic Subjects. The experiments described so far strongly suggest that eosinophils mediate antibody-dependent damage to schistosomula, and that populations of lymphocytes, monocytes, and neutrophils depleted of eosinophils do not. During the course of these studies, however, we had noted that eosinophils from patients with eosinophilia caused by *S. mansoni* appeared to be less cytotoxic, cell for cell, than those from normal persons. Three experiments that illustrate this observation are presented in Table II. In these experiments, fresh preparations of unpurified leukocytes, collected by dextran sedimentation, were tested directly with schistosomula. At given cell to schistosomula ratios, the ^{51}Cr release by cells from the normal subjects was similar to that of the cells from the eosinophilic subjects. If eosinophils were the only effector cell, one would have predicted that the populations from the subjects with eosinophilia should have been 10 times more effective because they contain approximately 10 times more eosinophils. In other words, cells from eosinophilic individuals at a ratio of 100:1 should have induced the same isotope release as cells from normal individuals at a ratio of 1,000:1. This was not the case. Furthermore, unpurified leukocytes from two patients with marked eosinophilia of 59 and 45% were not cytotoxic. One possible reason for this was that the Fc receptors of these eosinophils were blocked by immune complexes taken up in the circulation (21). We reasoned that

TABLE II

Antibody-Dependent, Cell-Mediated Damage to Schistosomula. Comparison of Unpurified Cells from Normal and Eosinophilic Subjects

Exp.	Source of cells	Eosinophils	% ^{51}Cr release					
			Ratio cell/somula in presence of Ab					No cells
			1,000:1	320:1	100:1	32:1	10:1	
		%						
1	*Normal*	3	55 (32)	45	36	33	29	28
	S. mansoni	47	47* (33)	39*	36	31	30	28
	Normal	2	40 (21)	28	24	22	21	18
	S. mansoni	16	34 (21)	30	28	22	20	18
3	Normal	3	62 (36)	53	46	38	37	33
	S. mansoni	44	55 (35)	47	41	39	35	33

Freshly prepared, unpurified peripheral blood leukocytes from normal subjects and from patients with eosinophilia induced by *S. mansoni* infection were tested for cytotoxicity to schistosomula at various effector to target ratios with or without antibody over 15-h incubation periods. Numbers in parentheses represent ^{51}Cr release of cells at 1,000:1 with no antibody. Values that are underlined differ from these at $P < 0.05$. (*) Refers to paired values that differ from each other at $P < 0.05$ or less in patient 1.

Statistical summary: Experiment 1. Ratio effect $P < 0.001$ ($F = 51.1$; 5 and 36 degrees of freedom). Effect of cell source $P < 0.05$ ($F = 5.1$; 1 and 36 df). LSD = 0.118 (+ 3.8% at 30%, + 6.3% at 50%). Experiment 2. Ratio effect $P < 0.001$ ($F = 44.5$; 6 and 37 df). LSD = 0.147 (+ 3.2% at 20%, + 6.3% at 40%). Neither cell effect nor cell with ratio interaction significant. Experiment 3. Ratio effect $P < 0.05$ ($F = 2.49$; 6 and 40 df). Ratio effect $P < 0.001$ ($F = 39.9$; 6 and 40 df). LSD = 0.139 (+ 6.0% at 40%, + 8.9% at 60%). Freezing and thawing of cells in experiments 1–3 released in ^{51}Cr: 59, 59, and 66.

if this were so, eosinophils might be more active if they were trypsinized before testing. In addition, to allow recovery from the enzyme treatment, we also planned to incubate the cells overnight in culture medium with FCS, a procedure which itself would encourage shedding of complexes.

The eosinophil-enriched population of one patient was incubated in 1% trypsin for 1 h, washed, and then cultured overnight. The results (patient 1, Table I) show that these cells mediated antibody-dependent cytotoxicity. An aliquot of the same cells cultured overnight, but not trypsinized, was not active. However, in similar experiments with cells from the next two patients, the cells that were simply cultured overnight were cytotoxic, whereas those that were trypsinized were not. We cannot explain the dramatic effect of trypsin in the first case, although it could have been attributable to a higher affinity of the Fc receptor for the particular complexes circulating in that patient. The occasional finding that eosinophil-enriched cells from patients with eosinophilia do not mediate antibody-dependent damage at the highest cell:schistosomula ratio (5,000:1), whereas others do at 32:1, suggests that the function of eosinophils may be altered by the patient's condition, or that eosinophils are a heterogenous population.

A problem in these experiments was that we could not assess the function of eosinophils from the same patient immediately after collection and also after overnight culture because of technical difficulties associated with availability of parasites and accessibility of patients. Therefore, in all experiments, the purified cell populations were cultured overnight, except for preparations 8 and 9, from a patient with eosinophilia without *S. mansoni* infection, which were tested on the same day they were collected and purified.

Eosinophil-Mediated Damage to Schistosomula not Enhanced by the Addition of Lymphocytes, Neutrophils, or Monocytes. These studies were carried

out to investigate further the finding that eosinophils from patients with *S. mansoni* were less active, cell for cell, than normal eosinophils. This might be partly explained if the patients lacked cells which themselves did not mediate cytotoxicity but which could act synergistically with eosinophils and enhance their cytotoxicity. Fresh purified preparations containing 97–99.5% lymphocytes, or neutrophil-enriched, eosinophil-depleted preparations, were obtained from normal subjects (see Methods). These were added to eosinophil-enriched preparations obtained from patients with *S. mansoni* and cultured overnight. Data from three experiments are summarized in Table III. In experiments 1 and 2, it can be seen from the last column, in which no eosinophils were present, that fresh lymphocytes alone had little or no effect, even at the high lymphocyte to schistosomulum ratio of 3,200:1. In contrast, eosinophils alone (top line of each set) showed a marked effect at ratios down to 320:1. Addition of lymphocytes at 3,200:1 to varying ratios of eosinophils (bottom line of each set) did not enhance the effect of eosinophils alone. For the sake of simplification, data obtained from the addition of lymphocytes at lower ratios (1,000:1 down to 32:1) are not shown, as no enhancement was detected at these ratios either. Similarly, in experiment 3, a fresh preparation of normal cells, containing 49% neutrophils, 50% lymphocytes and monocytes, and 1% eosinophils had no direct effect on schistosomula at ratios of 3,200:1, nor did it enhance the effect of a preparation containing 92% eosinophils. Thus, neither direct effects nor synergy could be detected with fresh preparations either of lymphocytes or of mixed neutrophils and mononuclear cells.

Discussion

In previous studies, it was shown that antibody-dependent cell-mediated damage to schistosomula was ablated when the unpurified human blood leukocytes used as the effector cells were first treated with anti-eosinophil serum and complement, whereas treatment with antineutrophil serum had relatively little effect (13). This, together with the findings that mononuclear cells were not active, strongly suggested that the eosinophil was the cell primarily responsible for mediating damage in this reaction. In this paper, we report results in which a more direct approach, namely, cell purification, was used to define the effector cell. It was found that cell populations containing up to 98% eosinophils, and devoid of neutrophils, were effective in damaging antibody-coated schistosomula. Cells depleted of eosinophils, but rich in lymphocytes, monocytes, or neutrophils were ineffective. Additional studies also essentially rule out the basophil as the effector cell. Basophils sediment with the lymphocytes and monocytes after centrifugation on Ficoll-metrizoate; it was previously shown that this mononuclear-rich interface fraction, with or without subsequent removal of phagocytic cells with carbonyl iron, failed to damage antibody-coated schistosomula. Moreover, Butterworth and Mahmoud (unpublished observations) have found that pretreatment of unpurified buffy coat preparations with a monospecific antibasophil serum (22) and complement did not affect subsequent cytotoxicity under circumstances in which pretreatment with anti-eosinophil serum caused a 90% reduction in cytotoxicity.

Experiments were carried out to test for the possible cooperative effect of other cell types with eosinophils. Eosinophil-enriched preparations were mixed with

TABLE III

Damage by Eosinophils Not Enhanced by Addition of Lymphocytes or Polymorphonuclear Leukocytes (PMNL) and Monocytes

Exp.	Other cells added‡	% ^{51}Cr release			
		Eosinophils* (ratio/"somula")			
		3,200:1	1,000:1	320:1	0
1	None	49	42	35	17
	Lymphocytes§	49	40	33	24
2	None	59	46	33	25
	Lymphocytes§	53	45	35	27
3	None	59	46	33	25
	PMNL + Mono‖	54	43	36	26

* Eosinophil-rich leukocyte preparations were obtained from a patient with *S. mansoni* infection by centrifugation of unpurified dextran-sedimented leukocytes over Hypaque. The preparations were cultured overnight before assay. Final composition: experiment 1, 83% eosinophils, 17% mononuclear cells, no detectable neutrophils; experiments 2 and 3, 92% eosinophils, 7% mononuclear cells, 1% neutrophils.

‡ Other cells added were at ratios 3,200:1 down to 32:1. Since lesser ratios gave similar results, only those of the highest ratio, 3,200:1, are given.

§ Freshly prepared lymphocytes from a normal individual were obtained by centrifugation of a dextran-sedimented leukocyte preparation over Ficoll-metrizoate, followed by extraction of interface cells with carbonyl iron. Final composition: experiment 1, 97% lymphocytes, 2.7% neutrophils, 0.3% eosinophils; experiment 2, 99.5% lymphocytes, 0.5% neutrophils. In experiment 1, eosinophils and lymphocytes were mixed in the ratios shown, and schistosomula preincubated with antibody were then added. In experiment 2, eosinophils were added to schistosomula with antibody, and lymphocytes were then added after a 10–20-min delay. Isotope release was determined after 7 h.

‖ Eosinophil-depleted, neutrophil-enriched cells were obtained by centrifuging the pellet from a Ficoll-metrizoate preparation of mixed normal cells over Hypaque, and recovering the interface (49% neutrophils, 1% eosinophils, 50% mononuclear cells). Eosinophils were added to schistosomula 10–20 min before the neutrophil-rich preparation; isotope release was determined after 7 h of incubation.

Statistical summary: Experiment 1. Interaction of eosinophil ratio with lymphocyte ratio $P < 0.01$ ($F = 2.64$; 25 and 107 df); LSD = 0.152 (+ 3.3% at 20%, + 7.4% at 45%). Experiment 2. Interaction of eosinophil ratio with lymphocyte ratio $P < 0.05$ ($F = 2.39$; 9 and 47 df); LSD = 0.128 (+ 3.4% at 25%, + 6.8% at 50%). Experiment 3. Eosinophil ratio effect $P < 0.001$ ($F = 135$; 3 and 9 df); neutrophil ratio effect and interaction of neutrophil ratio with eosinophil ratio not significant; LSD = 0.174 (+ 4.8% at 25%, + 9.5% at 40%).

All values that are underlined differ from controls containing no eosinophils (last column) at $P < 0.05$. There was no significant increase in ^{51}Cr release by a given ratio of eosinophils to schistosomula attributable to the addition of either the lymphocytes or the neutrophil-mononuclear cell preparations.

eosinophil-depleted preparations of lymphocytes, neutrophils and monocytes. The addition of these cells did not enhance the cytotoxicity of the eosinophils. Indeed, in one experiment, the addition of lymphocytes in high concentrations partially inhibited the effect of eosinophils, possibly by competing for bound antibody on the schistosomula. Thus, no evidence was found that other cells are involved in an ancillary fashion during the early stages of damage to schistosomula (the period during which the ^{51}Cr is being released).

These studies also raise an important yet unresolved question: why is it that eosinophils from patients with eosinophilia induced by *S. mansoni* infection are less cytotoxic, cell per cell, than eosinophils from normal individuals? Several possible reasons for this have been considered. First, such eosinophils may be less active because their Fc receptors are partially blocked by immune complexes which have been taken up from the circulation. This explanation is supported by the observations that immune complexes can elicit both local (23) and systemic (24, 25) eosinophilia when injected into animals; circulating immune complexes involving several antigens have been demonstrated during schistosome infections (26–30). Moreover, we have found that antigen-antibody complexes could completely block the antibody-dependent cell-mediated damage to schistosomula induced either by unpurified buffy coat leukocytes from normal subjects or by purified eosinophils from patients with eosinophilia.[2]

Another possible explanation is that eosinophils may be heterogeneous, or that the different stages of maturation may have different capabilities: cells responding to a stimulus which induces eosinophilia may be less mature, or may be altered or injured as a result of the stimulus. This possibility cannot be excluded, especially in light of several observations (31–33) and in particular those of Tai and Spry (34, 35) that eosinophils from patients with eosinophilia induced by a variety of conditions are markedly heterogeneous, with many cells showing vacuolation or degranulation. Such cells may be depleted in vivo of some effector agent and this could be partly regenerated during the period of culture in vitro.

Whereas the data reported here clearly implicates the human eosinophil as the effector cell in antibody-dependent cell-mediated damage to schistosomula, observations by others in different species have implicated other cells. For example, Dean et al. have reported that antibody and complement-dependent damage to schistosomula may be effected by rat neutrophils (36), while in the guinea pig, eosinophils adhere but do not damage schistosomula treated with antibody and complement (37). In an antibody-dependent, complement independent system, analogous to the human system reported here, Sher has found that purified rat eosinophils are inactive against schistosomula and postulates that the neutrophil is the major effector cell of the rat ^{51}Cr release reaction (personal communication), while both Perez (9) and Capron et al. (38) have reported antibody-dependent, macrophage-mediated damage to schistosomula. In contrast to the present system, the macrophage-mediated damage described by Perez involved IgG cytophilic antibody, while that described by Capron et al. was particularly interesting in that it involved IgE.

These reports describe a plethora of mechanisms by which the combination of various cells with different antibody, with and without complement, can damage schistosomula in vitro. It is quite possible that any or all of these mechanisms could contribute to immunity to reinfection in a given species, and that several mechansims may be involved at different times in the same species. An example of a possible interaction between two mechanisms is the question of

[2] Butterworth, A. E., H. G. Remold, V. Houba, J. R. David, D. Franks, P. H. David, and R. F. Sturrock. Antibody-dependent eosinophil-mediated damage to ^{51}Cr-labeled schistosomula of *Schistosoma mansoni:* mediation by IgG, and inhibition by antigen-antibody complexes. Manuscript submitted for publication.

how the eosinophils reach the site of the schistosomula in the first place. It would be quite possible that a reaction involving mast cells and IgE would generate chemotactic factors of anaphylaxis (39, 40) which would in turn call forth the eosinophils to the site. Thus, the finding that drugs that deplete animals of mast cells also abrogate immunity to reinfection is not evidence against the hypothesis that it is the eosinophils which are actually doing the damage (41). In this context, Sher has recently shown that mast cells will adhere to schistosomula through complement components generated by the alternative pathway (42). This could lead to the generation of chemotactic factors of anaphylaxis which could attract eosinophils to the site.

The eosinophil-mediated damage described here is likely to be of particular importance in the mouse, since Mahmoud et al. have shown that the reduction of circulating eosinophils by administration of anti-eosinophil serum abolishes immunity, whereas other anticell sera do not (14). Moreover, von Lichtenberg et al. have found an eight- to tenfold increase in eosinophils at the sites of cercarial challenge in the skin of immune mice in comparison to similar sites in normal mice (43). Eosinophils have also been described around schistosomula of *S. japonicum* in monkeys (44, 45). It is of interest that eosinophils do not gather at the site of cercarial challenge in immune mice depleted of mast cells by administration of reserpine (A. Sher, personal communication). Although it is not possible to determine the mechanisms of immunity in man, nor even to state conclusively that immunity exists, the data presented here with human eosinophils and antibody from patients with *S. mansoni,* indicate that eosinophil-mediated damage should be considered as a strong candidate.

If eosinophil-mediated damage to schistosomula is an important mechanism of immunity in vivo, it is possible that the limiting factor in immunity is not simply the level of antibody, but rather a highly complex interaction between free antibody, immune complexes involving different schistosomal antigens, eosinophil levels, and eosinophil activity. This possibility is emphasized by the finding, described here, that not all eosinophils are equally active, and that cells from patients with schistosomiasis may be relatively inactive. Immunity may, therefore, depend at least partially on the availability of a sufficient number of active eosinophils at the site of cercarial penetration. The limitation of immunity by lack of sufficient numbers of eosinophils, by lack of eosinophil activity, or by interaction of eosinophils with immune complexes or with aggregated immunoglobulins, may explain some curious features about immunity to schistosomiasis, including the failure of some serum pools to transfer immunity in spite of containing high antibody levels (7), the failure to immunize with antigenic extracts in spite of the development of antibodies (46), and the possible loss of immunity after treatment or self cure. All of these procedures could entail a relative reduction in active eosinophils. Conversely, immunization with heterologous organisms may be successful because the organisms survive at least temporarily within the host (4), and may therefore elicit an eosinophilia. Equally, attempts to increase eosinophil levels nonspecifically in the absence of circulating immune complexes might enhance the immunity conferred by natural infection or by the passive transfer of serum. This possibility is currently being tested.

Summary

After earlier observations that antibody-dependent, cell-mediated damage to ^{51}Cr-labeled schistosomula can be ablated by pretreatment of a mixed preparation of human peripheral blood leukocytes with an anti-eosinophil serum and complement, we investigated the cytotoxic effects of eosinophil-enriched cell preparations.

Preparations containing up to 98.5% eosinophils and devoid of neutrophils were effective in mediating antibody-dependent damage to schistosomula. Preparations enriched in mononuclear cells or in neutrophils, and devoid of eosinophils, were inactive.

Eosinophils from some patients with eosinophilia induced by schistosomiasis were less active on a cell-to-cell basis than cells from normal individuals. The possibility that such cells were initially blocked by immune complexes was considered, and it was found that reasonable cytotoxicity by purified eosinophils from patients with eosinophilia could be generated by overnight cultures.

A possible requirement for cooperation between eosinophils and other cell types was also studied. Lymphocytes, neutrophils and monocytes failed to enhance eosinophil-mediated cytotoxicity.

These results provide further evidence that the eosinophil is the only cell in man responsible for antibody-dependent, complement-independent damage to schistosomula in vitro. Eosinophils from individuals, however, differ in their cytotoxic potential by a mechanism yet to be elucidated. The possible relationship of these findings to immunity in vivo is discussed.

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