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Modulation of Eosinophil Cytotoxicity by Blood Mononuclear Cells from Healthy Subjects and Patients with Chronic *Schistosomiasis mansoni*[1]

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Human blood mononuclear cells in culture release a factor(s) that markedly enhances eosinophil cytotoxicity. This factor(s) stimulates eosinophils to kill *Schistosoma mansoni* larvae at low antibody concentrations and cell/target ratios. A study of the mononuclear cells of 78 subjects with chronic schistosomiasis mansoni and 33 controls suggests that the production of eosinophil cytotoxicity enhancing activity (ECEA) is suppressed in most patients with *S. mansoni* infections. Suppression of ECEA production was not observed, however, with cells from many patients with heavy infections, including patients with hepatosplenomegaly. The possible role of ECEA in the development of pathology is discussed.

INTRODUCTION

Helminth infections are associated with elevated blood and tissue eosinophilia, and several studies (reviewed in (1, 2)) have suggested that eosinophils play an important protective role in these infections. On the other hand, heavy infiltration of eosinophils may also cause tissue damage (3–6) because eosinophils release several substances, such as eosinophil peroxidase and cationic or basic proteins, that are toxic for mammalian cells (7, 8). Secretion of these toxic mediators is apparently enhanced in certain pathological conditions associated with chronic eosinophilia (5, 9).

There is good evidence that eosinophil cytotoxicity is regulated *in vivo* (10, 11), and the mechanisms of this control have been studied in several laboratories. James and Colley (12) have shown that stimulated T lymphocytes from the egg granuloma of *Schistosoma mansoni* infected mice release eosinophil cytotoxicity enhancing activity. Anwar *et al.* (13) and Capron *et al.* (14, 15) have reported that mediators released by mast cells potentiate the helminthotoxicity of human and rat eosinophils. This laboratory has shown (10) that eosinophils from individuals with high eosinophilia

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demonstrate enhanced cytotoxicity, an effect which could be caused by a substance like the eosinophil colony stimulating factor(s) (16).

This paper shows that human blood mononuclear cells release, in resting cultures, factors that enhance eosinophil cytotoxicity. Adherent cells, probably monocytes, are the source of this activity whereas T lymphocytes do not release a similar activity in these resting cultures (17).

The effect of schistosome infections on the production of this factor(s) is shown and discussed in relation to the severity of the disease.

MATERIALS AND METHODS

Life cycle of S. mansoni. A Puerto Rican strain of *S. mansoni* was maintained by passage through CBA/J mice and *Biomphalaria glabrata* snails. Schistosomula were prepared by allowing cercariae to penetrate an isolated preparation of rat skin *in vitro* (18, 19).

Antisera. Sera from patients with *S. mansoni* infection were used as a source of antischistosomular antibodies. All sera were heat inactivated at 56°C for 1 hr and had previously been tested for their ability to mediate eosinophil-dependent damage to schistosomula *in vitro* (20).

Effector cells. Neutrophils and eosinophils were recovered from the blood of healthy individuals (unless specified) by fractionation on Metrizamide gradients, as previously described (20). Cytocentrifuge smears of different cell fractions were stained with Wright's Giemsa for immediate examination, and appropriate fractions were pooled. Purity of eosinophil preparations was >88 to 99% and of neutrophil preparations > 95%. In the case of the eosinophils, the contaminating cells were neutrophils; in the case of the neutrophils, the contaminating cells were eosinophils, with occasional mononuclear cells and basophils. Cells and schistosomula were washed and resuspended in minimal essential Eagle's medium supplemented with 25 m*M* Hepes, 100 U/ml penicillin G, 100 μg/ml streptomycin, 1% glutamine, 10% fetal calf serum (FCS), and 30 mg/liter deoxyribonuclease, as previously described (20). Cell concentrations were adjusted to 2×10^6 cells/ml, yielding effector cell-to-target schistosomulum ratios of 1000/1 unless specified elsewhere.

Killing assay. Eosinophils (10^5; 50 μl) were incubated for 30 to 60 min with culture supernatant (50 μl). Then 100 schistosomula (50 μl) and an appropriate dilution of antischistosomular antiserum (50 μl) were added, and the tubes were incubated for 18 to 24 hr in humidified airtight boxes at 37°C. At the end of the incubation period, schistosomula and cells that had pelleted at the bottom of the tubes were gently resuspended in 50 μl of assay medium and placed on a slide previously coated with 2 drops of 0.1% toluidine blue in methanol. The number of dead organisms was then counted at a magnification of 100. Larvae were scored as dead if they were immotile and had taken up toluidine blue in an intense and granular fashion (20). Separate experiments have shown that schistosomula considered dead by these criteria are unable to mature into adult worms when reinjected into mice (21).

Preparation of enhancing culture supernatants. Human blood mononuclear cells, prepared by centrifugation on Ficoll–Hypaque density gradients, were cultured (2 to 5×10^6 cells/ml) in serum-free minimal essential Eagle's medium supplemented with 25 m*M* Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 m*M* glutamine.

This medium is referred to as culture medium. Cells were placed in tissue culture plates (1 ml/well, Linbro 76-033-05, Flow Laboratories) or in 20-ml flasks (2 to 5 ml per Costar flask). After 24 hr of culture, supernatants were collected by centrifugation (200*g*, 15 min) and stored at −20°C. Any modifications of this protocol are specified in the text.

Blood mononuclear cell fractionation. Cell fractionation by adherence to plastic was done according to Fisher *et al.* (22). Ficoll–Hypaque purified blood mononuclear cells were washed three times in culture medium; then cells were resuspended ($3–4 \times 10^6$/ml) in culture medium supplemented with 10% autologous serum (or FCS) and incubated (37°C, 3 hr) in plastic petri dishes (5 ml/dish, 60 × 15 mm Falcon) previously coated with autologous serum. At the end of the incubation period, nonadherent cells were washed out with warm culture medium, and the adherent cells were incubated for a further 15 min with 5 ml of a 0.02% EDTA solution in buffered saline (Versene, Gibco). Then, 1 ml of serum was added to the plates, and adherent cells were scraped off with a rubber policeman. Adherent and nonadherent cells were washed five times in culture medium before being placed ($1.5–5 \times 10^6$ cells/ml) in culture.

T-Lymphocyte rosetting with sheep erythrocytes was done according to Engleman *et al.* (23). E-Rosetting T cells were recovered after lysis of the SRBC with Tris-buffered NH_4Cl.

The purity of cell fractions was determined on cytocentrifuge smears stained with Wright's Giemsa and stains for peroxidase activity (24). T cells were enumerated by direct immunofluorescence with anti-Leu-1 monoclonal antibody (Ab) (Becton–Dickenson). Viability was assessed by the acridine orange ethidium bromide method.

Patient clinical status. Individuals infected with *S. mansoni* were selected from the population of Caatinga do Moura, a village in a northeast state of Brazil (Bahia). This study was carried out in January and February 1981 and 1982, during the season when exposure to cercariae is maximum. Caatinga do Moura is a hyperendemic area for schistosomiasis; almost all of the inhabitants are infected. Some had been treated with schistosomicidal drugs, but most had been reinfected within a year after treatment. A small number of individuals stayed away from infected water after treatment and did not excrete *S. mansoni* eggs (five stools examined). These individuals are referred to as "not reinfected." Malaria and leishmaniasis, two major causes of hepatosplenomegaly in the tropics, have never been observed in Caatinga (20-year survey).

Infected subjects were randomly selected from individuals living in Caatinga. Egg counts were determined on three stools by the Kato–Katz procedure (25). Patients with different clinical forms of schistosomiasis were selected from a pool of 200 individuals who volunteered for this study.

Clinical status was determined by physical examination (all patients were examined by the same physician) as previously defined by Prata and Bina (26). Examination of the liver was done with the patient in a supine position, and the size (impalpable, palpable at deep breath, palpable at or below the costal margin), the consistency (soft or hard), and the characteristics (smooth or nodular) were recorded. The size of the spleen was assessed as for the liver; the spleen was considered not palpable only after examination with the patient lying on his or her right side. Enlargement of the spleen and hardening and enlargement of the liver indicate an evolution to hepatosplenomegaly. According to these criteria (26), three different clinical stages have been

defined. These are the intestinal form in which the spleen and liver are impalpable, but eggs are present in the feces; the hepatointestinal form, in which the liver is palpable, but soft and smooth and usually not nodular; and the hepatosplenomegalic form, with the liver palpable at or below the costal margin and often hard and nodular, with the spleen palpable. In this latter group, 15 individuals had a spleen only palpable at deep respiration, and 9 patients showed severe hepatosplenomegaly.

Patients and control individuals. All Caatinga's inhabitants are or have previously been infected by *S. mansoni.* Therefore, controls were selected from Jacobina (30 miles from Caatinga) or Salvador (150 miles from Caatinga). These individuals had no history of schistosomiasis and did not excrete *S. mansoni* eggs (three stool examinations).

Nineteen control subjects (11 females and 8 males, aged 18 to 65, mean 40) and 26 *S. mansoni* infected individuals (12 females and 14 males, aged 15 to 57, mean 39) were included in the 1981 study. Parasitological examination of these control (C) and infected (Inf) subjects showed *Ascaris lumbricoides* in 33% C and 10% Inf, *Ancylostoma duodenale* in 6% C and 5% Inf, *Trichuris trichiura* in 13% C and 0% Inf, and *Entamoeba histolytica* in 20% C (10 tested) and in 50% Inf. Subjects included in the 1982 study are described in Table 1.

TABLE 1

Patients (1982 Study)[a]

	Never infected	Not reinfected	Intestinal/ hepatointestinal		Hepatosplenomegaly
			<400 eggs	>500 eggs	
Origin	Salvador	Caatinga	Caatinga	Caatinga	Caatinga
n (%)	13 (54)	9 (44)	17 (70)	12 (75)	22 (55)
Age					
Mean	32	26.6	16.7	16.2	18.3
Range	17–65	12–52	11–23	11–30	10–34
Treated	0	9	6	0	5
Eggs/g					
Mean	0	0	220	870	312
Range			10–390	500–1350	50–800
Parasitology[c]					
E. coli	14%	44%	65%	50%	52%
E. histolytica	14	0	59	42	29
G. lambia	0	33	29	33	30
A. lumbricoides	40	11	12	50	8
T. trichiura	40	0	6	8	5

[a] Groups were defined as described in the text: never infected; not reinfected, previously infected individuals who have been treated and subsequently do not eliminate eggs in their stools; infected individuals with the intestinal or hepatointestinal clinical form and <400 eggs/g or >500 eggs/g; and infected individuals with hepatosplenomegaly.

[b] Eggs per gram of stool in three different samples (Stoll thick-smear method).

[c] The most common intestinal parasites (in addition to *S. mansoni*) in these individuals were *Entamoeba coli, Entamoeba histolytica, Giardia lamblia, Ascaris lumbricoides,* and *Trichuris trichiura.*

RESULTS

Cultured Human Blood Mononuclear Cells Release Factor(s) That Enhance Ab-Dependent Eosinophil-Mediated Killing of Schistosomula

Mononuclear cells were purified from the blood of healthy volunteers and cultured in serum-free medium. Culture supernatants were collected after 24 hr and added to the *in vitro* killing assay containing purified human blood eosinophils, *S. mansoni* larvae, and human antischistosomular antisera. Culture supernatants enhanced severalfold the killing of schistosomula by eosinophils (Fig. 1); no increase in larval mortality was observed in the control tubes containing schistosomula and supernatants incubated with or without eosinophils in the absence of antibody. The increase of schistosomula death was caused by eosinophils and not by neutrophils contaminating the eosinophil preparations, because purified neutrophils incubated with supernatants did not kill Ab-coated schistosomula. Hereafter, the eosinophil cytotoxicity enhancing activity will be referred to as ECEA.

Enhancement by ECEA of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) by Eosinophils: Effect of Varying Ab Concentration, Eosinophil-to-Schistosomulum Ratio, and Culture Supernatant Dilution

The effect of blood mononuclear cell culture supernatants on the killing of schistosomula by eosinophils was studied at various antibody concentrations (Fig. 2), eosinophil/target ratios (Fig. 3), and supernatant dilutions (Fig. 4). Culture supernatants containing less than 10- to 100-fold the antiserum concentration and 10-fold the eosinophil/target ratio were required for the killing reaction. Most culture supernatants could be diluted more than 20 times and still demonstrate detectable enhancing activity.

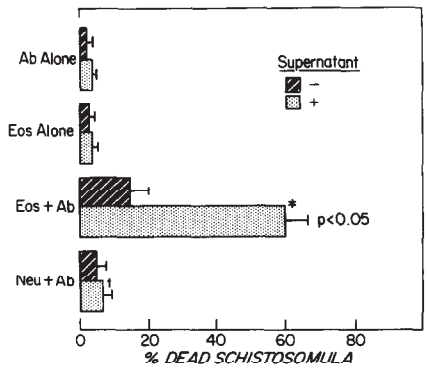

FIG. 1. Enhancement of the Ab-dependent eosinophil-mediated killing of schistosomula by factor(s) released by human blood mononuclear cells *in vitro*. Peripheral blood mononuclear cells from 19 healthy individuals were cultured in serum-free medium. Culture supernatants were collected after 24 hr and their effects on ADCC by purified human blood eosinophils were tested as described under Materials and Methods. Results are represented as the mean of these determinations ± SEM in three separate experiments performed with eosinophils from three different donors.

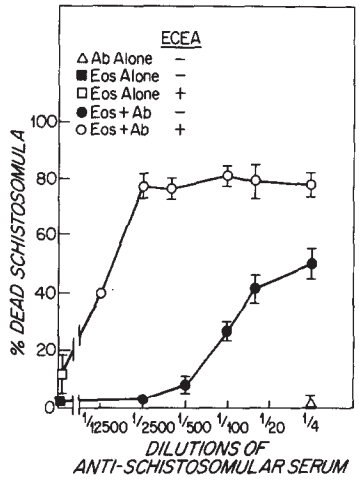

FIG. 2. Effect of ECEA on the killing of schistosomula by eosinophils at various antischistosomular antisera dilutions. Purified eosinophils were incubated (for 60 min at 37°C) in ECEA containing culture supernatants (1/5 dilution), then, human antischistosomula antisera and schistosomula (1 per 1000 eosinophils) were added. Dead schistosomula were scored 18 hr later. Each point represents the mean ± SD of determinations obtained in three separate experiments, with eosinophils of three different donors.

Effect of ECEA on Eosinophils from Individuals with Atopy, Helminth Infections, and Tropical or Idiopathic Eosinophilia

Since eosinophils from eosinophilic subjects are capable of enhanced cytoxicity (10), we wished to determine whether they differ from eosinophils from noneosinophilic individuals in their response to ECEA. The effect of ECEA on eosinophils purified from the blood of individuals with atopy, helminth infections, and tropical or idiopathic eosinophilia are shown in Table 2. ECEA enhanced the cytotoxicity of almost all eosinophil preparations, although some responded better than others to the addition

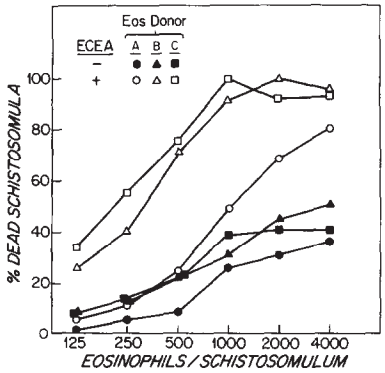

FIG. 3. Effect of ECEA on the Ab-dependent eosinophil-mediated killing of schistosomula at various eosinophil/schistosomulum ratios. Eosinophils were purified from the blood of one *S. mansoni* infected (A) and two healthy (B, C) donors and added in graded numbers to ECEA containing culture supernatants (1/5 dilution), then Ab and schistosomula were added as in Fig. 2. Schistosomula death was scored 18 hr later. Each point is the mean of duplicate determinations obtained in two separate experiments.

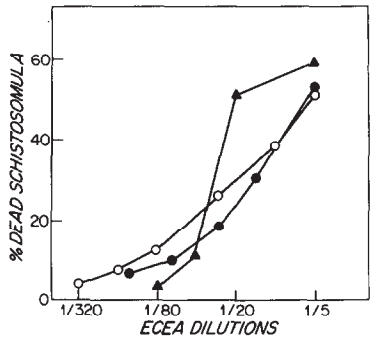

FIG. 4. Titration of ECEA in three different cultures of blood mononuclear cells. Blood mononuclear cells from three different healthy individuals were cultured in serum-free medium and supernatants were collected after 24 hr as described under Materials and Methods. The killing assay was performed at a 1/500 antischistosomular antisera dilution and 1000/1 cell/target ratio. Each point represents the mean of duplicate determinations obtained in two separate experiments with eosinophils from two donors.

of ECEA, eosinophils from atopic individuals responding best. However, differences in responsiveness between clinical groups were not dramatic.

In view of these results, all subsequent experiments were carried out with eosinophils purified from healthy subjects, with an eosinophilia between 2 and 8% of the total white blood cell count.

Optimal Culture Conditions for ECEA Production

The optimal cell density for the production of ECEA in serum-free culture is between 1.5×10^6 and 5×10^6 cells per milliliter in 2-ml wells of Linbro plates. ECEA levels in culture supernatants decrease markedly at cell concentrations greater than 10^7 cells/ml, probably because of rapid acidification of the cultures.

At a cell density of 2×10^6/ml, ECEA is detectable in supernatants as soon as 3 hr after the initiation of the culture. Maximal ECEA levels are reached between 10 and 18 hr of culture and plateau thereafter.

Addition of certain batches of fetal calf serum to the culture medium (10% FCS) resulted in an increase of ECEA levels. Both endotoxin-contaminated and endotoxin-"free" (<0.06 ng/ml) FCS enhanced ECEA production. These sera did not by themselves enhance eosinophil cytotoxicity when added directly to the killing assay.

On the basis of these results, the culture supernatants used in all subsequent experiments were obtained after 18 to 24 hr culture in serum-free media (unless specified) at a cell density of 2 to 5×10^6 cells per milliliter.

Origin of ECEA in Culture of Human Blood Mononuclear Cells

Human blood mononuclear cells from healthy subjects were fractionated by adherence to plastic, and the ability of adherent and nonadherent cells to release ECEA *in vitro* was tested (Table 3). Both adherent cells (containing 91 ± 6% peroxidase-positive cells) and nonadherent cells released ECEA in culture. When nonadherent cells were fractionated further by rosetting with sheep erythrocytes, ECEA producing cells could only be demonstrated in the T-lymphocyte-depleted nonadherent cell

TABLE 2

Effect of ECEA on Eosinophils from Different Individuals

Eosinophil donor	% Dead schistosomula ± SD	
	EOS + Ab	EOS + Ab + supernatants[a]
Normal		
H (0.9%)	11 ± 4	29 ± 5[b]
Ma (4%)	32 ± 5	84 ± 5
Ta (1.1%)	4 ± 1	68 ± 11
A (0.5%)[c]	25 ± 11	27 ± 8
6 additional individuals[d]	14 ± 5	55.8 ± 12
Atopy		
Ro (1%)	7 ± 2	87 ± 12
Lo (7%)	5 ± 1	66 ± 5
Da (14%)	14 ± 1	50 ± 9
Dn (8%)	4 ± 5	75 ± 6
8 additional individuals	11 ± 3	68.2 ± 10
Helminth infection		
Ja (9%)	43 ± 4	56 ± 2
E (10%)	8 ± 2	22 ± 9
Cn (14%)	21 ± 3	35 ± 4
V (20%)	57 ± 2	88 ± 4
6 additional individuals	27.5 ± 7	56.5 ± 11
Tropical eosinophilia		
Te (26%)	34 ± 3	86 ± 4
Idiopathic hypereosinophilia		
Ih (80%)	74 ± 4	96 ± 5

[a] Eosinophils were incubated with supernatants for 1/2 hr at 37°C then Ab and schistosomula were added.

[b] Differences between groups with and without supernatants were significant ($P < 0.05$) except with cells from Donor A.

[c] % blood eosinophilia.

[d] Mean of separate experiments performed with supernatants of cells from additional individuals.

fractions (containing 8 ± 4% peroxidase-positive cells and less than 6% T lymphocytes). Purified T lymphocytes failed to release ECEA in these culture conditions, even with the addition of 5% monocytes or 5×10^{-5} *M* 2-mercaptoethanol.

Effect of Chronic Schistosomiasis on the Production of ECEA by Blood Mononuclear Cells

Cells from individuals with chronic schistosomiasis were tested for their capacity to produce ECEA *in vitro*. Few cultures with cells from infected individuals showed detectable ECEA. Of 26 individuals 7 (27%) (1981 study) and of 51 individuals 12 (23%) (1982 study) were ECEA positive (ECEA (+)) *in vitro*. Supernatants that enhanced ADCC by two standard deviations were considered to be positive. In contrast, cultures performed at the same time and under the same conditions with cells from

TABLE 3

Cell Origin of ECEA in Resting Cultures of Human Blood Mononuclear Cells

Supernatant of cell culture	% Dead schistosomula		
	Expt 1	Expt 2	Expt 3
—	8 ± 0[a]	4 ± 3	5 ± 2
Total PBMC[b]	32 ± 0	44 ± 7	75 ± 1
Adherent	25 ± 5	75 ± 8	94 ± 1
Nonadherent			
Total	12 ± 0	70 ± 3	ND
E (+)	4 ± 1	7 ± 4	10 ± 3
E (−)	28 ± 19	69 ± 7	88 ± 0

Note. Cells were purified and cultured, and supernatants were tested as described under Materials and Methods. Peroxidase-positive cells represented 91 ± 6% of the plastic adherent cells and 8 ± 4% of the nonadherent cells. T lymphocytes (Leu-1-positive cells) represented 6 ± 1% of the adherent cells, 87 ± 5% of the E (+) nonadherent cells, and 19 ± 10% of the E (−) plastic nonadherent cells.

[a] Mean ± SD.

[b] Peripheral blood mononuclear cells.

matched controls were ECEA (+) in 16 out of 19 (84%) (1981) and 11 out of 13 (84%) (1982) cases. Of the cultures of cells from 10 healthy subjects from our laboratory in Boston 80% were also found to be ECEA (+). ECEA levels in cultures of these control and infected individuals are shown in Fig. 5.

Since mixing ECEA (−) supernatants with ECEA (+) supernatants does not lower the enhancing activity of active supernatants (Table 4), there is probably no inhibitor or inactivator of ECEA in the negative cultures. Furthermore, eosinophils preincubated

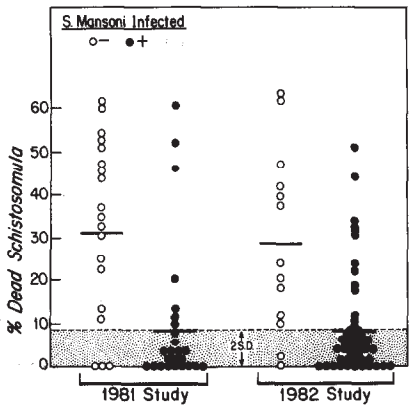

FIG. 5. The selection and description of the control and *S. mansoni* infected individuals are indicated under Materials and Methods. Blood mononuclear cells from these individuals were cultured (5×10^6 cells/ml) in serum-free medium for 24 hr. Then supernatants were collected and stored at −20°C. All supernatants were tested at least twice on two different preparations of eosinophils. Results are represented as ADCC with culture supernatants minus ADCC without culture supernatants. Killing in absence of culture supernatants was 5 to 15% ± 4 (SD). Each point represents the mean of duplicate determinations in two separate experiments.

TABLE 4

No Inhibitor of ECEA in ECEA (−) Culture Supernatants

	Culture supernatants	% Dead schistosomula
Expt 1[a]	None	20 ± 6
	A	12 ± 7
	B	17 ± 11
	C	60 ± 10
	D	71 ± 4
	A + B + C	80 ± 7
	A + B + D	70 ± 9
Expt 2[b]	None	19 ± 6
	E	12 ± 11
	F	16 ± 13
	G	57 ± 10
	E + G	42 ± 3
	F + G	71 ± 7
Expt 3[c]	None	26 ± 2
	H	24 ± 5
	K	75 ± 2, 51 ± 10, 43 ± 9, 30 ± 20
	H + K	73 ± 4, 56 ± 7, 43 ± 8, 38 ± 8

Note. ECEA (−) supernatants (A, B, E, F, H) were prepared with cells from infected patients and ECEA (+) supernatants (C, D, G, K) with cells of control individuals. Supernatants were combined as indicated below and residual ECEA tested as indicated under Materials and Methods.

[a] A + B + C for 1 hr at 37°C, then +EOS for a 1/2 hr at 37°C, then Ab + schistosomula.

[b] E or F + EOS for 1 hr at 37°C, then +G for a 1/2 hr at 37°C, then Ab + schistosomula.

[c] H + K, K at dilution of (1/7, 1/14, 1/28, 1/56), +EOS for a 1/2 hr at 37°C, then Ab + schistosomula.

in ECEA (−) supernatants respond to active supernatants as well as fresh eosinophils (Table 4; Experiments 2 and 3), showing that no factor in the inactive supernatants prevents eosinophil stimulation by ECEA.

Influence of the Clinical Status of S. mansoni Infected Patients on the Production of ECEA by Peripheral Blood Mononuclear Cells

In order to evaluate whether ECEA production correlates with clinical status, we selected 24 patients with hepatosplenomegaly, 30 patients with hepatointestinal schistosomiasis (12 with heavy infections (>500 eggs excretion) and 18 with low to moderate infection (<400 eggs)), and the ability of their blood mononuclear cells to release ECEA *in vitro* was tested. To optimize ECEA production, cultures with the cells of these individuals were carried out in 10% FCS. In order to control for variations in culture conditions, cells from one author (A.J.D.) were cultured in parallel with each set of cultures. Twelve of these control cultures (A.J.D.) were performed over a 2-month interval and exhibited an enhancing activity (average increase killing 160 ± 70%).

Infected patients differed markedly in their ability to release ECEA *in vitro*. Blood mononuclear cells from most patients with hepatointestinal schistosomiasis and low to moderate infections did not release detectable levels of ECEA *in vitro*, even when

the cells were cultured in 10% FCS. Only 3 out of 18 (17%) of the cultures with cells of these individuals were ECEA (+) and the average increase in killing for the 18 cultures was 30%.

However, cultures of cells from individuals with hepatointestinal disease and severe infections differed markedly from the previous cultures. Of 12 cultures 8 (67%) were ECEA (+), and the average increase in killing for the 12 cultures was 170%. Finally, 15 out of 24 (62%) individuals with hepatosplenomegaly were also ECEA (+), with an average increase in killing for the 24 cultures of 140%.

ECEA levels in cultures of cells from these individuals and the controls are shown in Fig. 6 (average increase in killing was 250% with control supernatants).

DISCUSSION

The data presented in this paper show that mononuclear cells purified from the blood of healthy subjects release, in culture, factor(s) that enhance the antibody-dependent killing of schistosomula by purified human blood eosinophils. In the presence of this factor(s), fewer eosinophils per schistosomulum and 10 to 100 times less antibody are required for the killing. This factor(s) enhances the cytotoxicity of eosinophils from most individuals tested, including patients with atopy, helminth infections, and tropical or idiopathic eosinophilia.

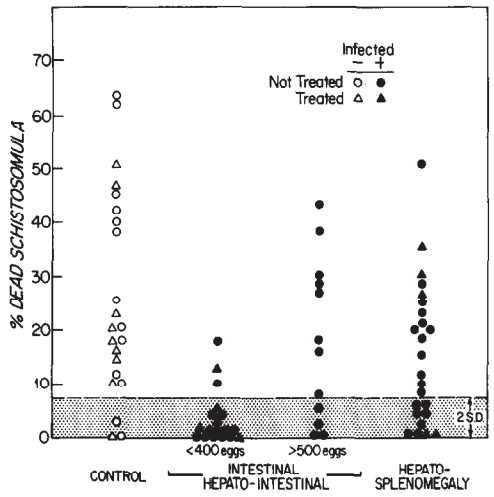

FIG. 6. Production of ECEA by blood mononuclear cells of patients with different clinical status. Clinical status was determined as indicated under Materials and Methods. Cells were cultured at 5×10^6 cells/ml in 10% FCS minimal essential medium. Supernatants were collected after 24 hr of culture, and their ability to enhance eosinophil cytotoxicity was tested at least on two different preparations of eosinophils. Each point represents the mean of these two or more determinations. Results are represented as in Fig. 5. Eighteen infected individuals were bled twice at 1-month intervals and results for these patients are represented as the mean ECEA titer in these two cultures, each culture being tested at least twice. The hepatointestinal group (<400 eggs) differs significantly ($P < 0.01$) from control, hepatointestinal (<500 eggs) and hepatosplenomegaly groups. The hepatosplenomegaly group differs significantly ($P = 0.02$) from the hepatointestinal group. Other differences are not significant ($P > 0.05$). Lymphocytes, monocytes, neutrophils, eosinophils, basophils (% ± SD; 15 cultures in each group): ECEA (+) cultures—64 ± 16, 31 ± 15, 1.3 ± 1.5, 0.2 ± 0.5, 2 ± 0.2; ECEA (−) cultures—52 ± 15, 42 ± 14, 3.4 ± 0.9, 0.2 ± 0.2, 1.8 ± 1.5.

This increase of schistosomula death in the tubes containing ECEA + eosinophils + Ab is not due to a lethal or sublethal effect of the supernatants on the larvae because (1) schistosomula viability or motility was not decreased when the larvae were cultured for 48 hr in ECEA and (2) ECEA-incubated eosinophils could be washed prior to their addition to schistosomula and still demonstrate enhanced cytotoxicity. Moreover, we have shown that ECEA directly enhances eosinophil degranulation on schistosomula (J. C. Caulfield, H. L. Lenzi, and A. D. Dessein, unpublished data).

ECEA is spontaneously released in culture in the absence of mitogenic or antigenic restimulation. This feature has been reported for certain monocyte products (27). Indeed, the data presented here show that adherent blood mononuclear cells that contain >90% monocytes spontaneously release ECEA in culture whereas purified T lymphocytes do not (even in the presence of 5% monocytes). We show elsewhere that treatment of the adherent cells with antilymphocyte sera and complement does not affect ECEA production, confirming that monocytes are likely to be the source of ECEA in resting cultures. Finally, Veith *et al.* (28) also reported that monocytes secrete an eosinophil cytotoxicity enhancing activity.

The origin of ECEA in resting cultures of E (−) plastic nonadherent cells is unclear. The monocyte content of these cultures is probably too low to explain the high ECEA levels; we are presently analyzing whether the large granular lymphocytes could be the source of this activity.

ECEA secreted by mononuclear cells has been characterized: it is relatively heat stable (15 min, 90°C), is associated with a MW between 14,000 and 60,000 (17, 28), and has an isoelectric point of 3.1 to 4.8 (17). These properties suggest that ECEA is probably not carried by the molecules responsible for the activities of Interleukins 1 and 2.

Secretion of ECEA by mononuclear cells is altered by schistosome infections whereas it is apparently not affected by a variety of intestinal parasites. Inhibition of the human immune response by schistosomes has been documented in several studies (29–37). Chronic schistosomiasis reduces the peripheral blood mononuclear cell proliferative response (29–31) and, to some extent, the delayed skin reaction (32, 33) to *S. mansoni* antigens. Adherent suppressor cells and suppressor factors have been demonstrated in the blood of certain infected individuals (34–37). This and another report (38) show that production of certain monokines and lymphokines are also depressed in chronic human schistosomiasis.

The delayed-type hypersensitivity reactions that develop around *S. mansoni* eggs is most affected by this suppression (27, 28, 32). The egg granuloma is infiltrated by numerous eosinophils that are thought to take a part in the destruction of the eggs and in the development of the inflammation. It is, therefore, possible that ECEA production is suppressed by the same mechanisms that modulate the egg granulomatous reaction, as it has been shown previously in the mouse for MIF and ESP (39–41).

Patients with heavy schistosome infections and/or hepatosplenomegaly do not regulate ECEA production as do patients with subclinical forms of the disease. A possible alteration of the regulation of the immune response in patients with hepatosplenomegaly has been the subject of several studies (42–45). No clear conclusion has been reached so far.

In a first group of studies (42, 43), patients with clinical and those with subclinical schistosomiasis were found to be similar in their delayed skin reaction to schistosome

antigens. A second group of studies (44, 45) reported that patients with hepatosplenomegaly had a higher reactivity to parasite antigen by skin testing and in the *in vitro* proliferation assay. The Camus *et al.* study (45) is probably the most relevant to the observations reported here because it has been carried out in the same geographic area with patients in the same age range and because it also suggests the higher reactivity of the patients with heavy infections. Hepatosplenomegaly in this area and others has been shown to develop primarily in heavily infected young individuals such as those included in this report (46, 47).

As previously suggested by studies in murine schistosomiasis (48, 49), failure to reduce the inflammatory reaction growing around the eggs may be an important factor contributing to the development of hepatosplenomegaly. The injury caused to the hepatic tissues by the products of the inflammation aggravates liver fibrosis and portal hypertension. Eosinophils may take a significant part in this deleterious process since they are numerous in the early granuloma and release substances toxic for the tissue (7, 8), as well as mediators of the inflammation. Other reports have suggested that eosinophils may cause severe tissue damage in individuals affected by hypereosinophilic syndromes (3–6). It is, therefore, possible that the failure to suppress ECEA leads to enhanced eosinophil activity and aggravates tissue injury. Further studies on the effect on ECEA on inflammation associated with the presence of eosinophils are being carried out.

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