

ENHANCEMENT OF HUMAN BLOOD EOSINOPHIL
CYTOTOXICITY BY SEMI-PURIFIED EOSINOPHIL
COLONY-STIMULATING FACTOR(S)*

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Eosinophils are a major component of the host-immune response to helminth infections (1, 2), and factors modulating their helminthotoxic capacity are now receiving considerable attention. Lymphokines secreted by cells form *Schistosoma mansoni* egg granulomas (3) and eosinophil chemotactic factor of anaphylaxis tetrapeptides released by mast cells (4-6) potentiate the ability of eosinophils to destroy helminths in vitro; the release of these factors in vivo probably results in the local activation of tissue eosinophils.

Recent studies suggest that circulating eosinophils isolated from the blood of eosinophilic patients are activated: they have a low surface charge, high levels of certain lysosomal and membrane enzymatic activities (7), and enhanced helminthotoxicity in vitro (8). Also, blood eosinophils in eosinophilic patients are often vacuolated and degranulated (9-11), and certain substances normally stored in their granules have been found in abnormal concentrations in the blood of some of these patients (12, 13). It has been suggested that these cells, unlike eosinophils in normal individuals, release their granule content in the blood in response to unknown stimuli. Some of these eosinophil-derived substances are toxic for mammalian cells (14, 15), and they could be responsible for the tissue damage observed in some patients with hypereosinophilic syndrome (12, 16-18).

The mechanisms causing these changes in the properties of blood eosinophils are not known. Because they occur in association with eosinophilia, it is possible that some eosinopoietic factors induce them. Colony-stimulating factors (CSF)¹ could probably have this dual function because they have been reported to stimulate progenitor cells (19) as well as mature cells (20-22).

We tested this hypothesis using a human placental conditioned medium that is a source of human eosinophil CSF (23, 24). The data show that a material contained in the human placental conditioned medium markedly enhances eosinophil cytotoxicity.

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¹ Abbreviations used in this paper: Ab, antibody; Con A, concanavalin A; CSA, colony-stimulating activity; CSF, colony-stimulating factors; D, deoxyribonuclease; HPCM, human placental conditioned medium; MEM, minimum essential medium.

This material copurifies with eosinophil CSF on phenylsepharose and Sephadex G-100 columns, suggesting that eosinophil CSF might be the molecule(s) responsible for the enhancement of eosinophil cytotoxicity. Studies on the mechanism of this enhancement suggest that normal blood eosinophils develop, after a short time incubation with this factor(s), properties that resemble some of the properties presented by circulating eosinophils in hypereosinophilic patients.

Materials and Methods

Life Cycle of S. mansoni. A Puerto Rican strain of *S. mansoni* was routinely maintained by passage through outbred mice and *Biomphalaria glabrata* snails. Schistosomula were prepared by allowing cercariae to penetrate an isolated preparation of rat skin in vitro (25, 26).

Antisera. Sera from patients with *S. mansoni* infection, either single or in pools, were used as a source of antischistosomal antibodies. All sera were heat-inactivated at 56°C for 1 h and had previously been tested for their ability to mediate microscopically detectable eosinophil-dependent damage to schistosomula in vitro.

Effector Cells. Neutrophils and eosinophils were recovered from the blood of normal individuals by fractionation on metrizamide gradients as previously described (27). Cytocentrifuge smears of different cell fractions were stained with Wrights Giemsa for immediate examination, and fractions were pooled as appropriate. Purity of cell preparation is indicated in figure legends; in the case of eosinophils, the contaminating cells were neutrophils; in the case of neutrophils, the contaminating cells were mononuclear cells with occasional eosinophils. Cells and schistosomula were washed and resuspended in minimal essential Eagle's medium supplemented with 25 mM 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 (27) (MEM/FCS/D). Cell concentrations were adjusted to 2 or 8 × 10⁶ cells/ml, yielding effector cell-to-target schistosomulum ratios of 1,000:1 (adherence assay) or 4,000:1 (killing assay). Because neutrophils also adhere to antibody (AB)-coated schistosomula, the eosinophil adherence assays were performed with eosinophils that were >98% pure.

Adherence Assay. Aliquots containing 100 schistosomula (50 µl), eosinophils (50 µl), and appropriate dilutions of antischistosomal antiserum (50 µl) and a fraction of placental conditioned medium (50 µl) were incubated for 1–6 h in humidified airtight boxes at 37°C. At the end of the incubation period, schistosomula and cells that had sedimented 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 adherent cells on each organism was then counted at a magnification of 100×. In most experiments, results are recorded as the percentage of schistosomula bearing >20 cells. This threshold of 20 cells per schistosomula was chosen because it corresponds usually to the degree of eosinophil adherence required to kill the schistosomula when incubation is prolonged up to 24 h for the killing assay. Concanavalin A (Con A)-dependent eosinophil adherence is weaker than antibody-dependent eosinophil adherence at 37°C (28). Therefore, Con A-dependent eosinophil adherence was recorded as the percentage of parasites bearing >10 cells.

Killing Assay. 100 schistosomula, 4 × 10⁶ eosinophils, and appropriate dilutions of antischistosomal antiserum and placental conditioned medium were incubated in plastic tubes as indicated for the adherence assay. Damage was determined after 24 h of culture. Larvae were scored as dead if they were immotile and had taken up toluidine blue in an intense and granular fashion (27). Separate experiments have shown that schistosomula considered dead by these criteria are unable to mature into adult worms when reinjected into mice.²

Inhibition of Eosinophil Protein Synthesis by Puromycin. Eosinophils (93% pure) were resuspended (8 × 10⁶ cells/ml) in methionine-free Dulbecco's medium supplemented with 20 mM Hepes, 20 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.01 mM [³⁵S]methionine (sp act, 1,000 Ci/mmol) with or without 5 µg/ml puromycin (63178; Sigma Chemical Co., St. Louis, MO). After 3 h incubation, 10⁶ cells were deposited on a filter paper (Whatman, 3 mM)

² Dessein, A. J., A. E. Butterworth, M. A. Vadas, and J. R. David. Maturation of *Schistosoma mansoni* after culture in vitro with granulocytes and antibody. Manuscript submitted for publication.

that was immediately immersed in 10% boiling trichloroacetic acid; the cells on the filters were then washed three times in 10% trichloroacetic acid and twice in 90% ethanol, dried, and counted. ^{35}S incorporation (10^6 cells) was as follows: cells incubated without puromycin, $63,000 \pm 5,000$ cpm; cells incubated with puromycin, $22,000 \pm 3,000$ cpm; and cells kept at 4°C , $25,000 \pm 4,000$ cpm.

Preparation of Conditioned Media. Human placental conditioned medium was prepared as described previously (24). Briefly, pieces of fresh human placenta were incubated for 7 d in RPMI 1640 medium containing 5% FCS. The supernatant was then collected, pooled, and tested for CSF activity.

Purification of CSF from Human Placental Conditioned Medium (HPCM)

GEL FILTRATION ON SEPHADEX G-100. HPCM was concentrated 10-fold using an Amicon DC-2A apparatus (Amicon Corp., Scientific Sys. Div., Lexington, MA) with a H1P10 hollow filter cartridge and dialyzed against distilled water. It was then adsorbed to calcium phosphate gel and eluted with 0.05 M sodium phosphate buffer as described previously (29). This concentrated material (25 ml) was then applied to a column of Sephadex G-100 (29) (LKB-Produkter, Bromme, Sweden), 2.6×100 cm, and eluted at a flow rate of 15 ml/h with phosphate-buffered (0.02 M, pH 7.3) saline (0.15 M) containing polyethylene glycol 6,000 (0.005% wt/vol). Fractions of 5 ml were collected and assayed separately before pooling.

FRACTIONATION ON PHENYL SEPHAROSE CL-4B. Samples of HPCM (either calcium phosphate eluates or active fractions from gel filtration) were applied to a column of phenyl sepharose CL-4B, 2.6×20 cm, (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated in phosphate-buffered saline (29). The column was eluted with the same buffer until eluate absorbance reached background level, and then the eluate was changed to 60% (vol/vol) ethylene glycol in distilled water. CSF failing to bind to the resin in phosphate-buffered saline was designated fraction- α , and that eluting with ethylene glycol was designated fraction- β .

Results

Enhancement by HPCM of the Antibody-dependent Eosinophil-mediated Killing of Schistosomula. Schistosomula were incubated with purified human blood eosinophils with or without HPCM and human antischistosomular serum. In the presence of HPCM there was a 4- to 10-fold increase in parasite death scored after 20 h of culture. This enhancement of eosinophil cytotoxicity was observed with eosinophils from the blood of all 15 volunteers tested (eosinophil count between 1 and 15%) and with all 10 human antischistosomular sera assayed. Table I shows the details of such experiments performed with eosinophils from the blood of five different individuals. HPCM without eosinophils was not toxic to the larvae; it should be noted, however, that it allowed a modest antibody-independent eosinophil-mediated killing of schistosomula.

Neutrophils adhered to antibody-coated larvae but failed to damage them in our assay (27). HPCM did not stimulate neutrophils to kill the larvae (Table I).

Enhancement of eosinophil cytotoxicity was maximum at antibody concentrations that allowed a marginal killing of schistosomula by control eosinophils (Fig. 1). Moreover, in the presence of HPCM, eosinophils required 5 to 10 times less antibodies than control eosinophils to demonstrate a similar killing ability (Fig. 2).

Enhancement of eosinophil cytotoxicity was proportionate to the dilution of HPCM (Fig. 2). This effect was observed at dilutions of up to 1/500. Small but significant enhancement of the antibody-independent eosinophil killing of schistosomula is observed in most experiments at dilutions up to 1/100.

Enhancement by HPCM of the Complement-dependent Eosinophil-mediated Killing of Schistosomula. Purified human eosinophils kill schistosomula coated with human complement (30, 31). This antibody-independent damage is maximum with mechanically

TABLE I
Enhancement by HPCM of the Ab-dependent Eosinophil-mediated Damage to Schistosomula

Patient	HPCM	Percent dead schistosomula				
		Ab	Eosinophils + Ab			Neutrophils + Ab
		$\frac{1}{40}$ *	$\frac{1}{40}$	$\frac{1}{120}$	0‡	$\frac{1}{6}$
1	-	4 ± 2 (A)§	14 ± 2	ND	3 ± 1	ND
	+	3 ± 3	62 ± 5¶	ND	14 ± 2¶	ND
2	-	2 ± 2 (B)	70 ± 12	30 ± 2	3 ± 2	ND
	+	3 ± 4	98 ± 2¶	85 ± 2¶	20 ± 2¶	ND
3	-	7 ± 4 (C)	20 ± 3	5 ± 2	7 ± 3	8 ± 4
	+	7 ± 3	85 ± 10¶	48 ± 4¶	13 ± 5	10 ± 5
4	-	5 ± 3 (C)	12 ± 5	4 ± 2	5 ± 2	9 ± 3
	+	6 ± 4	90 ± 2¶	38 ± 10¶	7 ± 4	8 ± 4
5	-	3 ± 3 (A)	21 ± 4	6 ± 3	1 ± 1	6 ± 4
	+	4 ± 1	79 ± 4¶	49 ± 5¶	10 ± 4	9 ± 3

Eosinophils and neutrophils were purified (>90% pure) from the blood of five different patients (blood eosinophil count 1-8%), and their ability to kill schistosomula in the presence of antischistosomular Ab was tested as described in Materials and Methods. HPCM ($\frac{1}{100}$) was added at the beginning of the culture. Numbers represent arithmetic means of duplicate determinations ± SD obtained in five separate experiments (one experiment for each patient).

* Dilutions of human antischistosomular antiserum.

‡ No antibody.

§ Letters in parentheses refer to the human antischistosomular serum used in the corresponding experiment.

|| Not determined.

¶ Values that differ significantly from their controls (incubations without HPCM) $P < 0.01$.

prepared schistosomula; skin-prepared schistosomula are much less susceptible (32). HPCM caused a 4- to 10-fold enhancement of the complement-dependent eosinophil-mediated killing of skin-prepared schistosomula. Eosinophils incubated with HPCM killed schistosomula at fresh normal human serum concentrations that were 5 to 10 times lower than those required by control eosinophils (Fig. 3).

Copurification of Eosinophil Cytotoxicity Enhancing Activity with Eosinophil Colony-stimulating Activity (CSA). Chromatography on phenyl-Sepharose columns resolves the HPCM into two major fractions, α and β (29). Both fractions have granulocyte-macrophage-CSA, but only fraction- α has eosinophil CSA; when tested in the killing assay, only fraction- α enhanced the antibody-dependent eosinophil-mediated killing of schistosomula (Table II). As was found with unfractionated HPCM, fractions α and β were unable to convert antibody-dependent neutrophil adherence to schistosomula into a killing reaction.

It was also found that eosinophil cytotoxicity-enhancing activity and eosinophil colony-stimulating activity are associated with molecule(s) having a similar apparent molecular weight (~30,000). The HPCM was filtered on Sephadex G-100 (29), and eosinophil CSA-containing fractions were pooled and tested in the killing assay (Table II). These fractions enhanced antibody-dependent eosinophil-mediated killing of

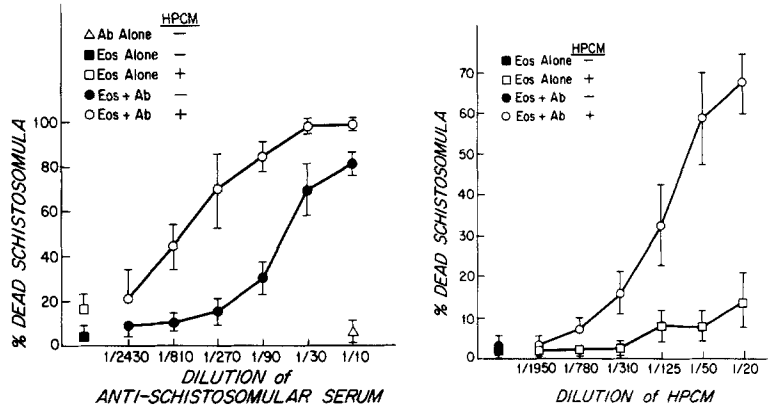


FIG. 1. (left) Enhancement by HPCM of the Ab-dependent eosinophil-mediated killing of schistosomula at various Ab concentrations. Schistosomula were incubated with eosinophils (>89% pure from donors with blood eosinophil counts of 3–13%) and grading concentrations of human antischistosomular antisera. HPCM (1/100) was added to the culture at the beginning of the incubation period. Each point represents the arithmetic mean \pm SD of determination in three separate experiments.

FIG. 2. (right) Enhancement by HPCM of the Ab-dependent eosinophil-mediated killing of schistosomula at various HPCM concentrations. Schistosomula were incubated with eosinophils (>85% pure from donors with blood eosinophil counts of 4–10%). HPCM was added to the culture at the beginning of the incubation period. To reveal a maximum HPCM-mediated enhancing effect, antischistosomular sera (from three different patients) were used at dilutions that corresponded to the thresholds that permit eosinophil-mediated killing of schistosomula. Each point represents the mean \pm SD of duplicate determinations of the percentage of dead schistosomula observed in three separate experiments.

schistosomula. They did not allow neutrophils to damage Ab-coated larvae (Table II).

All subsequent experiments were performed with the G-100 and phenyl-Sepharose-purified fraction of HPCM. This fraction will be referred to as CSF- α .

Effect of CSF- α on Eosinophil Adherence to Schistosomula. The next experiments were carried out to investigate the mechanism of the enhancement of the eosinophil-mediated killing of schistosomula by CSF- α . It was found that CSF- α enhances eosinophil adherence to antibody-coated schistosomula (Fig. 4). This enhancement was observable 90 min after the addition of CSF- α to the culture. At that time, eosinophils incubated with CSF- α adhered to the larvae twice as well as control eosinophils. Maximum cell adherence was reached after 5–6 h incubation and was 3–10 times higher with CSF- α incubated cells than with control eosinophils. Schistosomula mortality recorded after 20 h was enhanced to the same extent (Fig. 4).

Eosinophils that had been preincubated with CSF- α and then washed and added to antibody-coated schistosomula demonstrated an increase of adherence as early as 30–45 min after addition of the cells to the parasites (Fig. 5). A similar degree of adherence was reached 45–60 min later by eosinophils that had been in contact with CSF- α in the second culture only. This shows that the enhancing effect of CSF- α on cell adherence requires a minimum of 45–60 min to be detectable.

Enhancement of Eosinophil Adherence by CSF- α Occurs in the Absence of Protein Synthesis. Enhancement of eosinophil adherence by CSF- α occurs in the presence of doses of puromycin that totally inhibit eosinophil protein synthesis (see Materials and

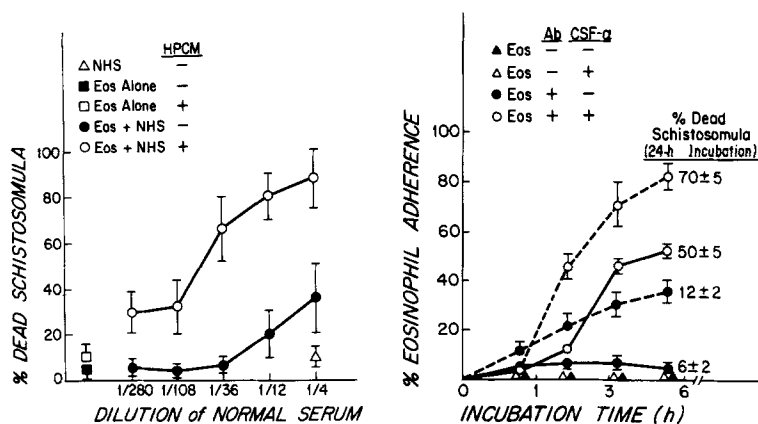


FIG. 3. (left) Enhancement by HPCM of the complement-dependent eosinophil-mediated killing of schistosomula. Schistosomula were incubated with eosinophils (>88% pure from donors with blood eosinophil counts of 4 and 9%) and various dilutions of fresh normal human serum (as source of complement). HPCM (1/100) was added to the culture at the beginning of the incubation period. Each point represents the arithmetic mean \pm SD of the schistosomula mortality observed in two separate experiments.

FIG. 4. (right) Effect of CSF- α on Ab-dependent eosinophil adherence to schistosomula. Schistosomula were incubated with human eosinophils (98% pure; donor eosinophilia, 8 and 7%) and antischistosomal serum ($1/160$ or $1/800$ dilutions). CSF- α was added to the culture ($1/160$) at the beginning of the incubation period. Eosinophil adherence (two experiments) is recorded as the percentage \pm SD of organisms bearing >20 cells. Killing was scored after 24 h incubation. ---, Ab $1/120$; —, Ab $1/800$.

TABLE II
Purification of Eosinophil Cytotoxicity-enhancing Activity by Filtration through Phenyl-Sepharose and Sepharose G-100 Columns

HPCM fraction	Percent dead schistosomula			
	Ab	Eosinophils	Eosinophils + Ab	Neutrophils + Ab
None	6 \pm 3	5 \pm 2	18 \pm 5	7 \pm 3
Phenyl-Sepharose fractions				
α	4 \pm 1	15 \pm 4*	70 \pm 5*	4 \pm 5
β	ND	8 \pm 3	20 \pm 2	6 \pm 4
None	5 \pm 3	8 \pm 5	16 \pm 4	8 \pm 3
G-100 (fraction 30,000 mol wt)	7 \pm 4	10 \pm 3	73 \pm 10	7 \pm 4

HPCM was fractionated by filtration through phenyl-Sepharose and Sephadex G-100 columns as described in Materials and Methods, and fractions were added ($1/100$ dilution) to the culture at the beginning of the incubation period. Eosinophils (>89% pure) and neutrophils (99% pure) were obtained from the blood of five patients (blood eosinophil count, 2–15%). Numbers represent arithmetic means of duplicate determinations \pm SE obtained in six experiments (upper part of the table) and three experiments (lower part of the table).

* These values differ significantly from vertically adjacent values ($P < 0.01$).

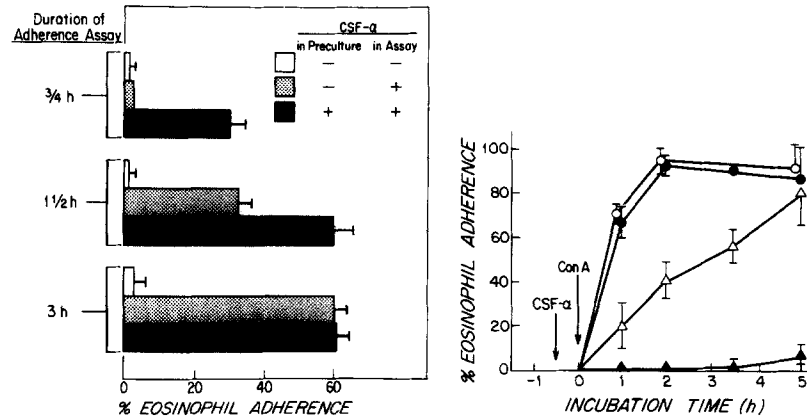


FIG. 5. (left) Time required for enhancement of eosinophil adherence by CSF- α . Eosinophils (95% pure from patients with blood eosinophil counts of 6 and 12%) were incubated in MEM/FCS/D \pm CSF- α for 60 min, then Ab-coated schistosomula were added to the culture, and cell adherence was scored at regular time intervals (3/4, 1 1/2, and 3 h). Control eosinophils were kept at 37°C in MEM/FCS/D during the preincubation period, and their ability to adhere to Ab-coated schistosomula in the presence or absence of CSF- α (1/100) was tested as above. Eosinophil adherence is expressed as the percentage of schistosomula bearing >20 cells. Each bar represents the arithmetic mean of duplicate determinations \pm SD (two experiments).

FIG. 6. (right) Effect of CSF- α on Con A-dependent eosinophil adherence to schistosomula. Eosinophils (2×10^5 per tube) were incubated 1/2 h at 37°C in MEM/FCS/D \pm CSF- α (1/100 dilution), then schistosomula (100/tube) and Con A (100 μ g/ml) were added and incubation forwarded at 37°C. At regular time intervals (1, 2, 3 1/2, and 5 h), four tubes in each group were removed from the incubator and α CH₃ mannoside (2×10^{-1} M) was added to two of them. After gently mixing, these tubes were incubated at 37°C for a further 30 min, then eosinophil adherence was scored. Eosinophil adherence is expressed as the percentage of larvae bearing >10 cells. Very few larvae (<5%) bore >20 cells. Each point represents the mean of duplicate determinations in two (1, 2, and 3 1/2 h) and four (5 h) separate experiments. Bar represents standard deviations between experiments. ●, Eos + Con A; ▲, Eos + Con A + α CH₃ mannoside; ○, Eos + CSF- α + Con A; △, Eos + CSF- α + Con A + α CH₃ mannoside.

Methods). As is shown in Table III, this occurs whether puromycin was present during the activation period only (experiments 1 and 2) or during the whole adherence assay (experiments 3 and 4). No effect of puromycin on the enhancement by CSF- α of the eosinophil-mediated killing of schistosomula was detected (Table III, experiments 1 and 2) when puromycin was added during a short activation period. Enhancement by CSF- α of the eosinophil-mediated killing of the larvae was also observed when puromycin was present during the complete (20 h) assay (experiments 3 and 4) but was less dramatic in tubes with puromycin than in tubes without. Puromycin also inhibited the killing reaction in the absence of CSF- α (experiment 5). This phenomenon has been reported for other antibody-dependent cell-mediated cytotoxicity reactions (33) and might reflect the need for a minimum level of protein synthesis for maintenance of the cells in killing reactions (33) rather than a true requirement for newly synthesized proteins for the killing process itself.

Stage of the Killing Reaction Affected by CSF- α . Adherence of eosinophils to antibody-coated schistosomula is a two-step process (28, 34). The first step is a temperature-independent reaction via Fc receptors, whereas the second step is a temperature-dependent reaction, possibly involving cell degranulation that makes the cell adherence irreversible.

TABLE III
Effect of Puromycin on Enhancement by CSF- α of the Ab-dependent Eosinophil Adherence and Eosinophil-mediated Damage to Schistosomula

Experiment	Time with agent		Percent eosinophil adherence*			Percent dead schistosomula \ddagger
	CSF- α	Puromycin	60 min	90 min	150 min	20 h
1	0	0		8	17	19 \pm 2
	0	30 min		13	10	17 \pm 5
	10 min	0		77	79	41 \pm 6
	10 min	30 min		64	70	33 \pm 7
2	0	0	23	13	26	13 \pm 2
	0	60 min	5	6	4	17 \pm 3.5
	40 min	0	52	86	60	43 \pm 7
	40 min	60 min	41	64	40	34 \pm 1.5
3	0	0	5	21	30	6 \pm 2
	0	20 h	4	12	13	3 \pm 2
	20 h	0	38	40	65	44 \pm 2
	20 h	20 h	56	59	50	17 \pm 1 \S
4	0	0		9		15 \pm 5
	0	20 h		2		10 \pm 3
	20 h	0		57		52 \pm 5
	20 h	20 h		43		43 \pm 6
5	0	0				75 \pm 13
	0	20 h				43 \pm 5 \S

Eosinophils were incubated in MEM/FCS/D with or without puromycin for 10 min at 37°C, then CSF- α was added at $\frac{1}{100}$ dilution. In experiment 1 and 2, cells were washed three times 10 or 40 min later and resuspended for a further 10-min incubation period in MEM/FCS/D \pm puromycin; then cells were washed again three times, and their ability to adhere to and to kill Ab-coated larvae was tested as described in Materials and Methods. In experiments 3 to 5, cells were kept during the whole assay in MEM/FCS/D \pm puromycin. Eosinophils from four different patients (blood eosinophil count, 4–12%) were used. Eosinophil purity was >90% when eosinophil adherence was tested, and >87% when eosinophil-mediated killing was assayed (see Materials and Methods). Puromycin concentrations were 5 μ g/ml in experiment 1, 10 μ g/ml in experiments 2, 3, and 4, and 15 μ g/ml in experiment 4.

* Single determinations in experiments 1 and 2 and arithmetic mean of duplicate determinations in experiments 3 and 4 (SD, <7%).

\ddagger Arithmetic mean of duplicate determinations (experiments 1, 2, 3, and 4) and arithmetic mean of duplicate determinations in three separate experiments (experiment 5).

\S These values differ significantly from vertically adjacent values ($P < 0.02$).

Eosinophils that had been preincubated for 2 h at 37°C with CSF- α did not show enhanced adherence to Ab-coated schistosomula when the adherence assay was performed at 4°C (Table IV). This shows that CSF- α does not affect the temperature-independent stage of eosinophil adherence that is mediated by Fc receptors. As soon as the adherence assay was warmed to 37°C, CSF- α preincubated eosinophils adhered much better than control eosinophils to Ab-coated larvae (Table IV in experiments 1 and 3).

CSF- α Incubated Eosinophils Adhere Irreversibly to Con A-coated Schistosomula. The results of the above experiments show that CSF- α affects the temperature-dependent stage of eosinophil adherence possibly by promoting the mechanism(s) that make eosinophil

TABLE IV
CSF- α Enhances the Temperature-dependent Stage of Eosinophil Adherence to Ab-coated Schistosomula

Experiment	CSF- α	Adherence assay		Schistosomula bearing*				
		$^{\circ}\text{C}$	<i>h</i>	0-5 cells	>5 cells	>10 cells	>20 cells	
1	-	37	1½	53	49	21	ND	
	+			10	90	50‡	ND	
	-	4	4	76	24	0	0	
	+			79	21	0	0	
			then					
	-	37	½	44	57	36	ND	
+	0			100	78‡	ND		
2	-	4	12	85	15	0	0	
	+			88	11	0	0	
3	-	4	14	72	22	0.5	0	
	+			83	5	0	0	
		then						
	-	37	½	13	87	63	23	
+	5			95	90	62‡		

Eosinophils (>96% pure) were incubated (37°C, 2 h, 2×10^5 /tube) in assay medium with or without CSF- α , then tubes were either kept at 37°C (experiment 1, first two lines) or transferred at 4°C, and 100 schistosomula that had been coated with human antischistosomular antibodies (dilution 1/6, 30 min at 37°C) were added to each tube. Eosinophil adherence was scored after a further 1½ h at 37°C (experiment 1, first two lines) or 4-14-h incubation period at 4°C. Tubes remaining at 4°C (experiments 1 and 3) were then retransferred at 37°C, and eosinophil adherence was scored ½ h later.

* Eosinophil adherence is expressed as the percentage of larvae bearing 0-five cells, >5, 10, or 20 cells. Numbers are arithmetic means of duplicate determinations, and standard deviations were <8%.

‡ Values that differ significantly from their controls (incubations without CSF- α), $P < 0.02$.

adherence irreversible. That hypothesis was tested in the Con A-dependent eosinophil adherence assay. Eosinophils adhere to Con A-coated schistosomula, and this adherence is fully reversible by addition of α -methyl mannoside (28). We found that adherence of eosinophils to Con A-coated schistosomula becomes irreversible when CSF- α is added to the culture (Fig. 6). Maximum irreversibility of the Con A-dependent eosinophil adherence was achieved after 5 h incubation with CSF- α , corresponding to the period required for maximum adherence of CSF- α -activated eosinophils to antibody-coated schistosomula (Fig. 4). In four experiments, CSF- α incubated eosinophils, like normal eosinophils, failed to kill Con A-coated schistosomula (data not shown).

Discussion

The observation that eosinophils from eosinophilic patients have an enhanced helminthotoxicity (8) suggested that the functional activity of mature blood eosinophils might be regulated by factors that also control the proliferation and maturation of eosinophil progenitor cells.

CSF are required for the growth and differentiation of granulocyte and macrophage colonies in vitro (19). They have also been shown to increase RNA (20) and protein synthesis (21) in mature cells and to enhance the killing of leishmania promastigote parasites by macrophages (22). Finally, granulocyte and macrophage CSF serum

levels fluctuate sharply under conditions that involve altered rates of polymorphonuclear cell and monocyte production, such as in granulocyte leukemia (35) and in viral (36) and bacterial (37) infections. This suggests that colony-stimulating factors could have the dual function of regulating granulopoiesis and of controlling the activity of mature granulocytes.

The data presented here show that human placental conditioned medium that contains eosinophil CSF does, in fact, significantly enhance human blood eosinophil helminthotoxicity. This enhancement represents a 4–10-fold increase in the eosinophil schistosomicidal activity and allowed eosinophils to kill Ab- or complement-coated larvae at Ab or complement concentrations ten times lower than the Ab or complement concentrations required by normal eosinophils to damage schistosomula. The activity that enhances eosinophil helminthotoxicity and the eosinophil colony-stimulating activity of HPCM are eluted in the same fraction after chromatography on Sephadex G-100 and phenylsepharose columns, suggesting that both activities might be associated with the same molecule.

The conditions required for colony stimulation and for activation of mature eosinophils are different. Removal of CSF from bone marrow cultures causes an immediate cessation of granulocyte proliferation (38, 39), whereas washing eosinophils a few minutes after the addition of CSF- α does not prevent full activation. Moreover, eosinophil activation when assessed by enhancement of eosinophil adherence, in contrast to cell proliferation and maturation, does not require protein synthesis. This suggests that eosinophil CSA and the activity that enhances eosinophil helminthotoxicity act on eosinophil progenitors and on mature eosinophils by different mechanisms.

We attempted to determine how CSF- α enhances the killing reaction. It was first observed that CSF- α enhances antibody-dependent adherence of eosinophils to the larvae, and the subsequent increase of parasite death was proportional to this enhancement. Because the killing of schistosomula by eosinophils requires the attachment of the cells to the parasite tegument, it is reasonable to assume that the effect of CSF- α on eosinophil adherence accounts, at least partially, for the increase in killing.

CSF- α could modify eosinophil adherence in several ways. First, as Anwar et al. (40) and Capron et al. (5) have reported for eosinophil chemotactic factor of anaphylaxis-activated eosinophils, CSF- α could increase the number of eosinophil CR (40) and Fc receptors (5). This is probably not the case here because the temperature-independent stage of eosinophil adherence that is probably dependent only on the interaction between Fc receptors and Ab bound to schistosomula (28, 34) is not enhanced by CSF- α . Moreover, the number of Fc receptors detectable in a rosetting assay is less on CSF- α -activated eosinophils than on normal cells (J. F. Jakubowitz and M. H. Vadas, manuscript in preparation).

CSF- α does affect, however, the temperature-dependent stage of the eosinophil adherence to Ab-coated larvae. Thus, eosinophils incubated with CSF- α , which adhere as normal eosinophils at 4°C, have enhanced adherence within a few minutes of reaching 37°C. Eosinophil adherence to Ab-coated schistosomula becomes irreversible at 37°C, and it is thought that irreversibility of eosinophil adherence is achieved during cell degranulation. This notion is supported by experiments showing that eosinophil adherence mediated by ligands (like Con A) that fail to trigger eosinophil degranulation is reversible unless degranulating agents are added to the reaction (28). It is shown here that CSF- α incubated eosinophils adhere irreversibly

to Con-A-coated larvae, suggesting that CSF- α affects the mechanism, possibly cell degranulation, that insures irreversibility of cell attachment. This interpretation is consistent with the observation that a small but significant number of eosinophils adhere to schistosomula in the absence of Ab if these cells have been incubated with high doses of CSF- α . Normal eosinophils adhering to Con A-coated schistosomula kill the parasite when the calcium ionophore A23187 is added (28), whereas CSF- α -activated eosinophils do not. This suggests that irreversibility of cell adherence is achieved by mechanism(s) that do not necessarily damage the larvae.

In conclusion, this study suggests that poietic molecules like eosinophil CSF could be the factors that enhance cytotoxicity of blood eosinophils in eosinophilic patients. CSF- α effects on the cell degranulation-associated events that insure irreversibility of eosinophil adherence suggest that these factors could also be responsible for the abnormal tendency of eosinophils to degranulate in the blood of these patients (12, 13). A more definitive demonstration of the involvement of eosinophil CSF-like factors in the regulation of blood eosinophil functions would require, however, a greater purification of eosinophil CSF and a more definite demonstration of its role in eosinopoiesis in vivo.

Summary

Purified human blood eosinophils, when incubated in human placental conditioned medium (a source of colony-stimulating factors [CSF]) demonstrate an enhanced ability to damage antibody- or complement-coated schistosomula. This enhancement represents a 4- to 10-fold increase of eosinophil schistosomicidal ability and a 10-fold lowering of the threshold for antibody or complement required in the killing reaction. The activity that enhances eosinophil cytotoxicity and the eosinophil colony-stimulating activity in the placental conditioned medium are eluted in the same fraction (CSF- α) after chromatography on Sephadex G-100 and phenyl-Sepharose columns, suggesting that these two activities might be associated with the same molecule.

CSF- α enhances the adherence step of the killing reaction: antibody-coated larvae were frequently found covered by several layers of eosinophils in tubes containing CSF- α . Such a degree of adherence was rarely seen in control tubes lacking CSF- α . This enhancement of the eosinophil adherence is detectable 45–60 min after addition of CSF- α to the culture. It is not affected by washing the cells after a short time of preincubation with CSF- α , and it occurs in the absence of protein synthesis, whereas colony-stimulating activity requires continuous protein synthesis and ceases when CSF is removed from the culture. Finally, CSF- α enhances the temperature-dependent reaction that insures the irreversibility of eosinophil attachment to schistosomula.

These observations suggest that eosinopoietic factors could be responsible for some of the modified properties of blood eosinophils in eosinophilic individuals.

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References

1. Ehrlich, P., and A. Lazarus. 1898. Die Anamie. *In* *Specielle Pathologie and Therapie*. H. Nothnagel, editor. Vienna, Holder. English translation *In* *Histology of the Blood: Normal*

- and Pathological. 1900. W. Myers, editor. Cambridge University Press, Cambridge, England.
2. Butterworth, A. E. 1977. The eosinophil and its role in immunity to helminth infection. *Curr. Top. Microbiol. Immunol.* **77**:127.
 3. James, S. L., and D. G. Colley. 1978. Eosinophil-mediated destruction of *Schistosoma mansoni* eggs. III. Lymphokine involvement in the induction of eosinophil functional abilities. *Cell. Immunol.* **38**:48.
 4. Anwar, A. R. E., J. R. McKean, S. R. Smithers, and A. B. Kay. 1980. Human eosinophil- and neutrophil-mediated killing of schistosomula of *Schistosoma mansoni* in vitro. I. Enhancement of complement-dependent damage by mast cell-derived mediators and formyl methionyl peptides. *J. Immunol.* **124**:1122.
 5. Capron, M., A. Capron, E. J. Goetzl, and K. F. Austen. 1981. Tetrapeptides of the eosinophil chemotactic factor of anaphylaxis (ECF-A) enhance eosinophil Fc receptors. *Nature (Lond.)* **289**:71.
 6. Capron, M., J. Rousseaux, C. Mazingue, H. Bazin, and A. Capron. 1978. Rat mast cell-eosinophil interaction in antibody-dependent eosinophil cytotoxicity to *Schistosoma mansoni* schistosomula. *J. Immunol.* **121**:2518.
 7. Bass, D. A., W. H. Grover, J. C. P. Szejda, L. R. Dechatelet, and C. E. McCall. 1980. Comparison of human eosinophils from normals and patients with eosinophilia. *J. Clin. Invest.* **66**:1265.
 8. David, J. R., M. A. Vadas, A. E. Butterworth, P. Azevedo de Brito, E. M. Carvalho, R. A. David, J. C. Bina, and Z. A. Andrade. 1980. Enhanced helminthotoxic capacity of eosinophils from patients with eosinophilia. *N. Engl. J. Med.* **303**:1147.
 9. Saran, R. 1973. Cytoplasmic vacuoles of eosinophils in tropical pulmonary eosinophilia. *Am. Rev. Respir. Dis.* **108**:1283.
 10. Blatt, P. M., G. Rothstein, H. L. Miller, and W. J. Cathey. 1974. Löffler's endomyocardial fibrosis with eosinophilia in association with acute lymphoblastic leukemia. *Blood.* **44**:489.
 11. Tai, P. C., and C. J. F. Spry. 1976. Studies on blood eosinophils. I. Patients with a transient eosinophilia. *Clin. Exp. Immunol.* **24**:415.
 12. Olsson, I., and P. Venge. 1979. The role of the eosinophil granulocyte in the inflammatory reaction. *Allergy.* **34**:353.
 13. Wassom, D. L., D. A. Loegering, G. O. Solley, S. B. Moore, R. T. Schooley, A. S. Fauci, and G. J. Gleich. 1981. Elevated serum levels of the eosinophil granule major basic protein in patients with eosinophilia. *J. Clin. Invest.* **67**:651.
 14. Butterworth, A. E., D. L. Wassom, G. J. Gleich, D. A. Loegering, and J. R. David. 1979. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *J. Immunol.* **122**:221.
 15. Gleich, G. J., E. Frigas, D. A. Loegering, D. L. Wassom, and D. Steinmuller. 1979. Cytotoxic properties of the eosinophil major basic protein. *J. Immunol.* **123**:2925.
 16. Spry, C. J. F., and P. C. Tai. 1976. Studies on blood eosinophils. II. Patients with Löffler's cardiomyopathy. *Clin. Exp. Immunol.* **24**:423.
 17. Guerra-Caceres, J. G., A. D. M. Bryceson, I. Quakyi, and C. J. F. Spry. 1980. Studies on the mechanisms of the adverse reactions produced by diethylcarbamazine in patients with onchocerciasis—the Mazzotti reaction. *Parasite Immunol. (Oxf.)* **2**:121.
 18. Spry, C. J. F. 1981. Alterations in blood eosinophil morphology, binding capacity for complexed IgG and kinetics in patients with tropical (filarial) eosinophilia. *Parasite Immunol. (Oxf.)* **3**:1.
 19. Metcalf, D. 1978. The control of neutrophil and macrophage production at the progenitor cell level. In *Experimental Hematology Today*. S. J. Baum and G. D. Ledney, editors. Springer Verlag, Berlin.

20. Burgess, A. W., and D. Metcalf. 1977. The effect of colony-stimulating factor on the synthesis of ribonucleic acid by mouse bone marrow cells in vitro. *J. Cell. Physiol.* **90**:471.
21. Burgess, A. W., and D. Metcalf. 1977. Colony stimulating factor and the differentiation of granulocytes and macrophages. In *Experimental Hematology Today*. S. J. Baum and G. D. Ledney, editors. Springer Verlag, New York. 135.
22. Handmann, E., and A. W. Burgess. 1979. Stimulation by granulocyte-macrophage colony-stimulating factor of *Leishmania tropica* killing by macrophages. *J. Immunol.* **122**:1134.
23. Burgess, A. W., E. M. A. Wilson, and D. Metcalf. 1977. Stimulation by human placental conditioned medium of hemopoietic colony formation by human marrow cells. *Blood.* **49**:573.
24. Nicola, N. A., D. Metcalf, G. R. Johnson, and A. W. Burgess. 1978. Preparation of colony stimulating factors from human placental conditioned medium. *Leuk. Res.* **2**:313.
25. Stirewalt, M. A., D. R. Minnick, and W. A. Fregeau. 1966. Definition and collection in quantity of schistosomula of *Schistosoma mansoni*. *Trans. R. Soc. Trop. Med. Hyg.* **60**:352.
26. Clegg, J. A., and S. R. Smithers. 1972. The effect of immune rhesus monkey serum on schistosomula of *Schistosoma mansoni* during cultivation in vitro. *Int. J. Parasitol.* **2**:79.
27. Vadas, M. A., J. R. David, A. E. Butterworth, N. T. Pisani, and T. A. Siongonk. 1979. A new method for the purification of human eosinophils and neutrophils and a comparison of the ability of these cells to damage schistosomula of *Schistosoma mansoni*. *J. Immunol.* **122**:1228.
28. Butterworth, A. E., M. A. Vadas, D. L. Wassom, A. Dessein, M. Hogan, B. Sherry, G. J. Gleich, and J. R. David. 1979. Interactions between human eosinophils and schistosomula of *Schistosoma mansoni*. II. The mechanism of irreversible eosinophil adherence. *J. Exp. Med.* **150**:1456.
29. Nicola, N. A., D. Metcalf, G. R. Johnson, and A. W. Burgess. 1979. Separation of functionally distinct human granulocyte-macrophage colony-stimulating factors. *Blood.* **54**:614.
30. Ramalho-Pinto, F. J., D. J. McLaren, and S. R. Smithers. 1978. Complement-mediated killing of schistosomula of *Schistosoma mansoni* by rat eosinophils in vitro. *J. Exp. Med.* **147**:147.
31. Anwar, A. R. E., S. R. Smithers, and A. B. Kay. 1979. Killing of schistosomula of *Schistosoma mansoni* coated with antibody and/or complement by human leukocytes in vitro. Requirement for complement in preferential killing by eosinophils. *J. Immunol.* **122**:628.
32. Dessein, A., J. C. Samuelson, A. E. Butterworth, M. Hogan, B. A. Sherry, M. A. Vadas, and J. R. David. 1981. Immune evasion by *Schistosoma mansoni*. Loss of susceptibility to antibody or complement-dependent eosinophil attack by schistosomula cultured in medium free of macromolecules. *Parasitology.* **83**:357.
33. Perlmann, P., and J. C. Cerottini. 1981. Cytotoxic lymphocytes. In *The Antigen*. M. Sela, editor. Academic Press, Inc., New York. 174.
34. Vadas, M. A., A. E. Butterworth, B. Sherry, A. Dessein, M. Hogan, D. Bout, and J. R. David. 1980. Interactions between human eosinophils and schistosomula of *Schistosoma mansoni*. I. Stable and irreversible antibody-dependent adherence. *J. Immunol.* **124**:1441.
35. Metcalf, D., S. H. Chan, F. W. Gunz, P. Vincent, and R. B. M. Ravich. 1971. Colony stimulating factor and inhibitor levels in acute granulocytic leukemia. *Blood.* **38**:143.
36. Metcalf, D., and B. Wamren. 1968. Bone marrow colony-stimulating activity of sera in infectious mononucleosis. *Brit. Med. J.* **3**:99.
37. Trudgett, A., T. A. McNeill, and M. Killen. 1973. Granulocyte-macrophage precursor cell and colony-stimulating factor responses of mice infected with *Salmonella typhimurium*. *Infect. Immunol.* **8**:450.
38. Metcalf, D., and R. Foster. 1967. Behavior on transfer of serum stimulated bone marrow colonies. *Proc. Soc. Exp. Biol. Med.* **126**:758.

39. Paran, M., and L. Sachs. 1968. The continued requirement for inducer for the development of macrophages and granulocyte colonies. *J. Cell. Physiol.* **72**:247.
40. Anwar, A. R. E., and A. B. Kay. 1978. Enhancement of human eosinophil complement receptors by pharmacologic mediators. *J. Immunol.* **121**:1245.
41. Ruscetti, F. W., R. M. Cypess, and P. A. Chervenick. 1976. Specific release of neutrophilic- and eosinophilic-stimulating factors from sensitized lymphocytes. *Blood.* **47**:757.