

Ultrastructure of the Attack of Eosinophils Stimulated by Blood Mononuclear Cell Products on Schistosomula of *Schistosoma mansoni*

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Purified human eosinophils were treated with peripheral blood mononuclear cell supernatants containing eosinophil cytotoxic enhancing activity (ECEA). Schistosomula of *Schistosoma mansoni* which had been coated either with antibody (Ab) from the sera of infected patients or with the lectin concanavalin A (Con A) were incubated with ECEA-treated and untreated cells for 2 minutes to 12 hours and examined ultrastructurally. Killing was assayed at 18 hours. ECEA caused an increase in the killing of Ab-coated worms, but Con-A-coated worms were not killed by either ECEA-treated or untreated cells. Eosinophils began to degranulate on Ab-coated worms within 2 minutes and continued to degranulate, so that by 12 hours about half of the parasites had >50% of their

surface covered by discharge material. The ECEA-treated cells degranulated more than the untreated cells. There was much less discharge material on Con-A-coated worms than on Ab-coated worms. Eosinophils adhered to discharge material on the surface of both Ab- and Con-A-coated parasites. At 3 and 12 hours, lysed cells and cell fragments were also seen adhering to discharge material. In the absence of discharge material the cells adhered to residual glycocalyx or to the tegumental outer membrane. These studies suggest that eosinophils kill schistosomula by progressively degranulating onto their surface over many hours and that the increased toxicity caused by ECEA is due to an increase in discharge. (Am J Pathol 1985, 120:380-390)

MAN IS infected by cercariae of *Schistosoma mansoni* which are released into fresh water from snails, the parasite's intermediate hosts. The cercariae penetrate the host skin, where they transform to larvae called schistosomula. Transformation is accompanied by an alteration in the parasite surface, which is a syncytium called the tegument. The cercarial tegument has a single membrane covered by a 1- μ -thick glycocalyx. The glycocalyx is sloughed, and the single membrane is replaced by a double membrane on the schistosomula.¹ In the skin of an immune host, the larvae induce an inflammatory response in which eosinophils often predominate. The eosinophils are thought to be involved in larval destruction.²

Schistosomula prepared *in vitro* have been used to study antibody-dependent eosinophil-mediated cytotoxicity. In brief, such studies have shown that parasites preincubated with sera from infected patients are killed by human eosinophils and most probably not by neutrophils (see Butterworth et al³ for a review), al-

though others have found that both neutrophils and eosinophils kill schistosomula.^{4,5} Previous morphologic studies have shown that eosinophils degranulate onto the parasite surface and that lesions appear in the tegument beneath the discharge.^{6,7} However, it is not clear why there is such a long interval between the start of degranulation (minutes) and measurable death at 18-24 hours.⁸

More recently, a series of factors have been described which have eosinophil cytotoxicity-enhancing activity. In particular, mast-cell-derived factor(s),^{4,9} mediator(s)

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produced by blood mononuclear cells,¹⁰⁻¹⁴ and possibly colony-stimulating factor (CSF- α)¹⁵ enhance the killing of schistosomula by human eosinophils. However, no ultrastructural study has been done on the mechanism(s) by which these stimulated eosinophils attack schistosomula *in vitro*.

In this study we have examined schistosomula that were preincubated in antibody (Ab) and then cultured with purified human eosinophils treated or untreated with eosinophil cytotoxic enhancing activity (ECEA). These cultures were examined by light and electron microscopy at 2-10 minutes, 2-3 hours, and 12 hours after the start of incubation. Killing was measured at 18 hours. In particular, we wished to know whether the eosinophils mounted a large initial attack which then slowly killed the worm or a sustained progressive assault. Secondly, we wanted to see whether ECEA-treated eosinophils attacked schistosomula in the same fashion as untreated cells and whether they discharged more granule material onto the parasite. Finally, we compared the adherence of eosinophils on Ab-coated worms with eosinophils on concanavalin-A (Con-A)-coated worms to see whether ultrastructural support existed for the two different types of adherence described in the literature.^{16,17}

Materials and Methods

Preparation of Schistosomula

A Puerto Rican strain of *S mansoni* was maintained by passage through outbred mice and *Biomphalaria glabrata* snails. Schistosomula, the larval stage of the organisms, were prepared by two methods. Skin schistosomula were prepared by allowing cercariae to penetrate an excised rat skin into Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate, pH 7 (Flow Laboratories, Inc., Rockville, Md), by methods previously described.^{18,19} Three hours after skin penetration the preparation contained 0-10% organisms with tails and <5% dead organisms, as judged in the light microscope by the absence of both gross movement and flame cell beating.²⁰ Mechanical schistosomula were prepared by a modification of the method of Ramalho-Pinto et al.²¹ Cercarial suspensions were cooled to 4 C in an ice bath, passed through a metal screen for removal of snail debris, concentrated by low-speed centrifugation, and placed in cold minimal essential medium (MEM) GIBCO, Grand Island, NY). The cercariae, 10-50 \times 10³ organisms in 2 ml of MEM in 15-ml Falcon culture tubes, were treated for 1 minute at full speed with a Genie Vortex in order to break the organisms into bodies and tails. The vortexed material

was layered onto 60% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) in cold MEM and centrifuged at 600g for 10 minutes.²² Tails, dead organisms, and snail debris remained on the top of the Percoll and were discarded. The pellet contained cercarial bodies, which were washed three times in MEM for removal of Percoll and incubated for 3 hours at 37 C in RPMI-1640 (Gibco Laboratories) so that the bodies could complete their transformation into schistosomula. Mechanical schistosomula contained 1-5% organisms with tails.

Antisera

Sera from Brazilian patients infected with *S mansoni* were used as a source of antischistosomal antibodies. All sera were heat-inactivated at 56 C for 1 hour.

Purification of Eosinophils and Neutrophils

Neutrophils and eosinophils were recovered from the blood of volunteers by centrifugation of dextran-sedimented blood leukocytes on discontinuous metrizamide gradients.²³ Cytocentrifuge smears of different cell fractions were stained with Wright's Giemsa for judging cell purity, and appropriate fractions were pooled. Purity of both preparations was >95%. In the case of the eosinophils, the contaminating cells were neutrophils; in the case of neutrophils, the contaminating cells were eosinophils. Cells were washed three times and resuspended in minimal essential Eagle's medium supplemented with 25 mM HEPES, 100 U/ml penicillin G, 100 μ g/ml streptomycin, 1% glutamine, 10% fetal calf serum (FCS), and 30 mg/liter deoxyribonuclease.²³

Preparation of Supernatants Containing Eosinophil Cytotoxic Enhancing Activity (ECEA)

Monoclonal cells were purified by centrifugation on Ficoll-Hypaque.¹² Cells that were collected from the interface between plasma and Ficoll were washed (200g for 15 minutes) three times in the serum-free MEM supplemented with 25 mM HEPES. The cells were resuspended (2×10^6 cells/ml) in MEM containing 5 μ g/ml lipopolysaccharide (LPS), 5 mg/ml gentamycin, and penicillin, streptomycin, glutamine, and HEPES in the concentrations used above. Cells were incubated for 24 hours at 37 C in 25-sq cm growth area flasks (Costar, Cambridge, Mass). Subsequently the culture medium was removed and centrifuged (200g for 15 minutes, 4 C). The supernatant was collected and stored at -70 C.

Incubation of Granulocytes With Parasites

Two hundred microliters of purified eosinophils or neutrophils containing 10^6 cells/ml were incubated for 1 hour at 37 C with either 200 μ l of supernatants from LPS-stimulated cultures of mononuclear cells (ECEA-treated cells) or 200 μ l of fresh medium containing 5 μ g/ml LPS (untreated cells). Simultaneously, schistosomula (5×10^3 /ml) were incubated with a 1/5 to 1/20 dilution of human antischistosomal serum (Ab-coated parasites), with 100 μ g/ml of Con A (Con A-coated parasites), or with medium alone (control parasites). Then 200 μ l of the respective larval suspension was added to the ECEA-treated or untreated cells. Cells and larvae were pelleted together by centrifugation (500g for 30 seconds) and were incubated at 37 C for varying times from 2 minutes to 12 hours (morphologic studies) or for 18 hours (killing assay). Reaction in tubes designated for morphologic studies was stopped by adding an equal volume of Karnovsky's fixative.²⁴ The determination of eosinophil helminthotoxicity was done by microscopic examination.²³

Electron Microscopy

Cells and schistosomula were prepared for transmission microscopy as previously described.¹⁸ In brief, cells and parasites were fixed in suspension in an equal volume of Karnovsky's aldehyde fixative²⁴ for 15–30 minutes at room temperature or overnight at 4 C. In some cases the suspensions were reacted for peroxidase by first washing out the aldehyde with RPMI and then 0.05 M Tris, pH 7.6. The suspensions were incubated in 1 ml of diaminobenzidine (0.5 mg/ml) and 10 μ l of 1% H_2O_2 for 15 minutes, and the incubation was repeated with fresh reagents. Suspensions, whether reacted for peroxidase or not, were centrifuged in a microfuge, and the pellets were postfixed in acetate veronal-buffered 1% OsO_4 for 90 minutes at 4 C and stained *en bloc* in buffered 0.5% uranyl acetate. Dehydration and Spurr's embedding were routine.

Thick sections (0.3 μ) were cut with glass knives and stained with azure II–methylene blue. The peroxidase reacted material was viewed unstained. Thin sections with silver to silver-gold interference colors were cut with a diamond knife, picked up on naked or form-

var-carbon-coated copper grids, and stained on grid for 5–10 minutes in uranyl acetate and for 10–30 seconds in lead citrate.²⁵ The grids were examined in a JEOL 100C electron microscope.

Results

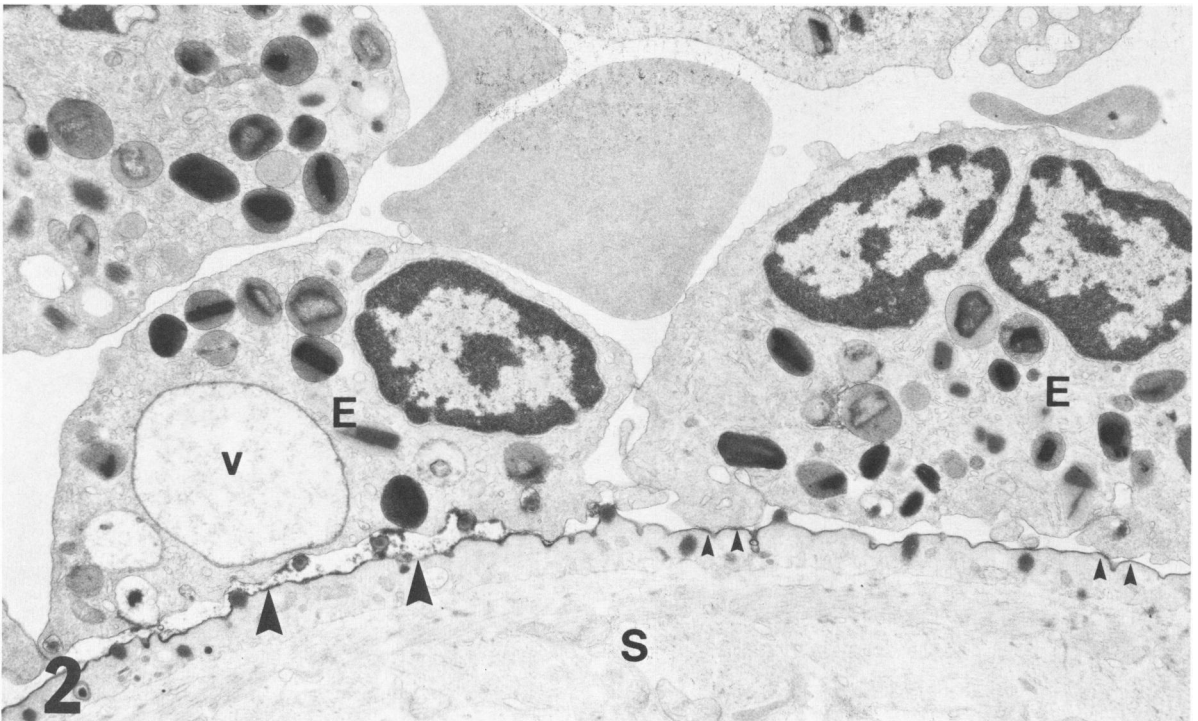
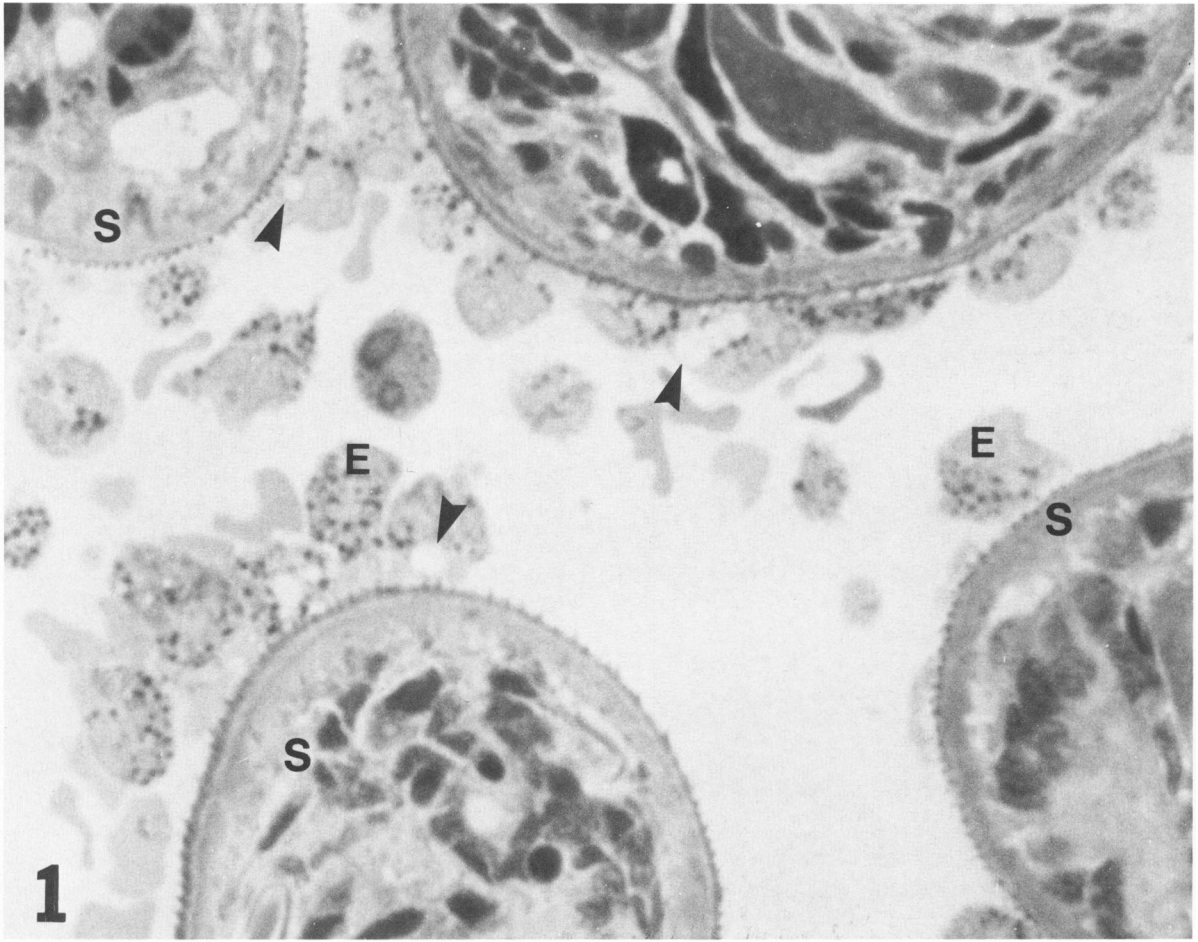
Eosinophils and Ab-Coated Schistosomula

ECEA-treated eosinophils killed $64\% \pm 6\%$ (mean \pm SEM) of the Ab-coated schistosomula with which they were incubated. Untreated eosinophils killed $18\% \pm 6\%$. The differences were significant with $P < 0.0002$ ($n = 8$). ECEA-treated and untreated eosinophils killed $4\% \pm 1\%$ of the control schistosomula. The electron-microscopic appearances of ECEA treated and untreated cells were always similar within a given experiment. However, from experiment to experiment, there were subtle variations in granule structure, none of which could be attributed to ECEA treatment. In addition, eosinophils incubated with ECEA in the absence of schistosomula for 3–12 hours ($n = 3$) had the same appearance as control eosinophils. Because the appearance of ECEA-treated and untreated cells was similar, a single description will serve for both types of cells.

Within 2–10 minutes after cells and Ab-coated schistosomula were mixed, eosinophils adhered to the parasite surface (Figure 1). The adherence was variable from worm to worm in that some worms had only one or two adherent cells and others had a nearly confluent layer of cells on their surface (Figure 1). Still others had clumps of adherent cells where cells were adjoined to cells which were, in turn, adherent to the worm (Figure 1). Vacuoles were present in some of the adherent cells (Figure 1). By low-power electron microscopy the adherent eosinophils either had degranulated onto the worm surface or they had not degranulated and were simply attached to the parasite (Figure 2).

The ultrastructure of eosinophil adherence to the worm depended on the nature of the parasite surface (Figures 3–5). Because the parasites were recently transformed, in some areas they retained cercarial glycocalyx, which was seen as fibrillar material on the outer tegumental membrane (Figure 3). Mechanical schistosomula had more glycocalyx on their surface than skin schistosomula. In either case, the tegument had the

Figure 1—Light micrograph of a 0.3- μ section of ECEA-treated eosinophils incubated with Ab-coated schistosomula for 2 minutes. All four schistosomula (S) have adherent eosinophils (E), although the number and arrangement of cells differ from worm to worm. The parasite at the lower right has a few adherent cells, whereas the worm at the top of the picture has a monolayer of attached cells. Eosinophils on the worm at the lower left form a clump on the surface. Note the vacuoles (arrows) in some of the adherent eosinophils. ($\times 3000$) **Figure 2**—Electron micrograph of ECEA-treated eosinophils (E) adhering to an Ab-coated schistosomulum (S) after 2-minute incubation. The cell on the right has not degranulated and closely approaches the parasite tegumental membrane in a few areas (small arrows). The eosinophil on the left has partially degranulated. There is electron-dense material between the cell and the worm (large arrows), and a large vacuole (v) is present in the cytoplasm. Such vacuoles are formed by the membranes of discharged granules. All micrographs from sections stained 2 minutes in lead citrate and 5 minutes in uranyl acetate. ($\times 16,000$)



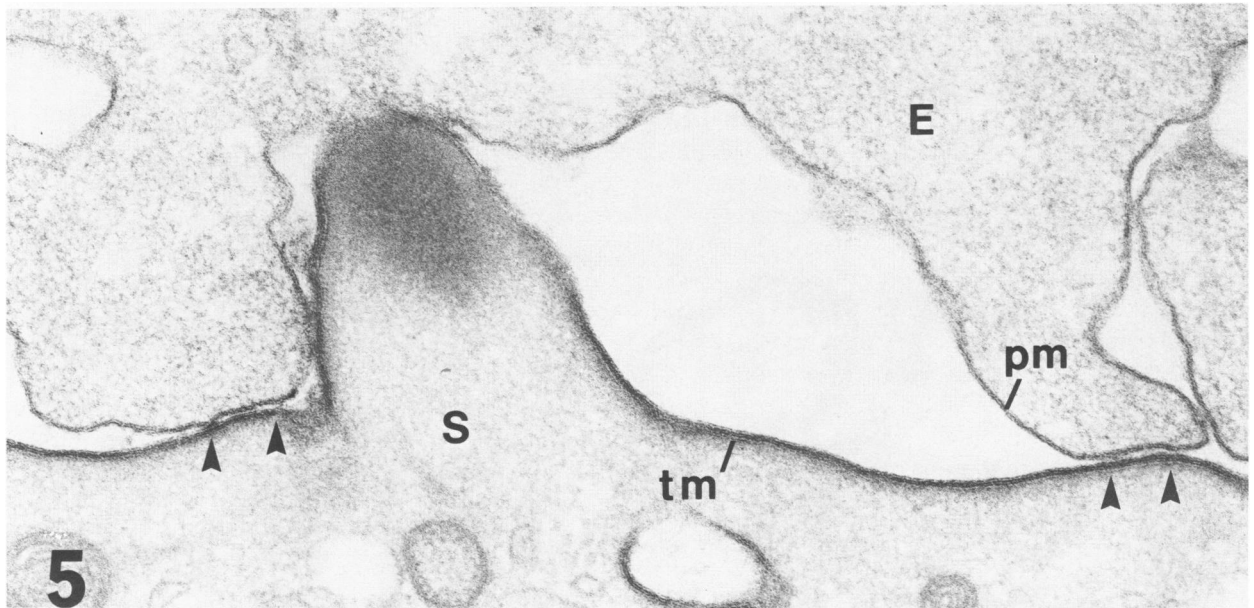
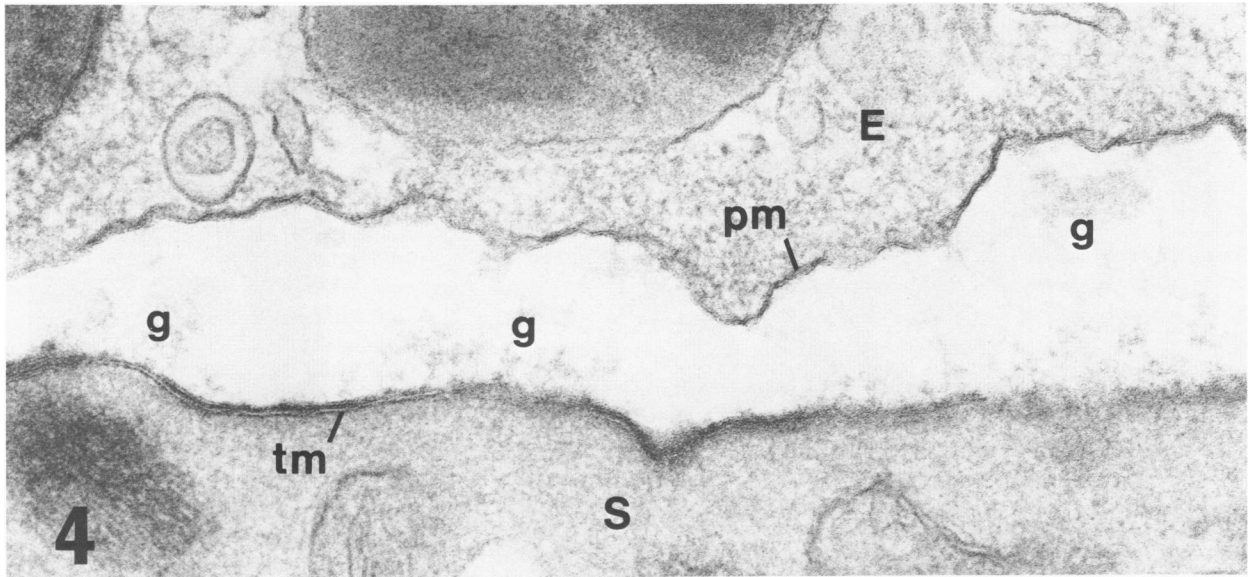
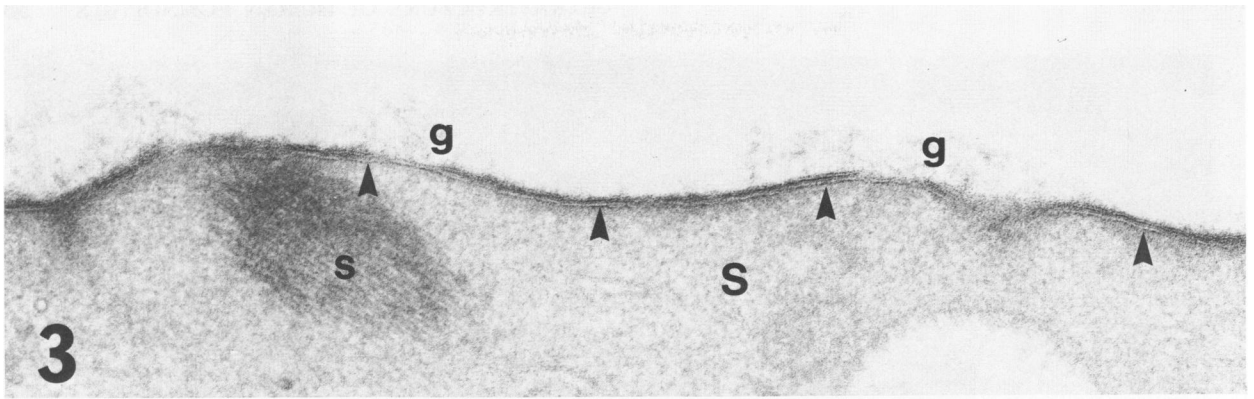


Figure 3—High-power view of the surface of a schistosomulum (S). The electron-dense fibrillar material (g) is the remnant of the cercarial glycocalyx. The tegumental or surface membrane is composed of two opposed unit membranes, which is the characteristic arrangement seen on posttransformational organisms (arrows). The crystalline structure on the left is a spine (s). ($\times 80,000$) **Figure 4**—ECEA-treated eosinophil (E) that has not degranulated adhering to an Ab-coated schistosomulum (S) after 2 minutes' incubation. The eosinophil plasma membrane (pm) is separated from the tegumental membrane (tm) of the worm by fibrillar glycocalyx (g). ($\times 80,000$) **Figure 5**—ECEA-treated eosinophil (E) that has not degranulated adhering to an Ab-coated schistosomulum (S) after 12 minutes' incubation. There is no glycocalyx on the parasite (compare with Figures 3 and 4). Note that the plasma membrane (pm) of the eosinophil approaches within 200 Å of the tegumental membrane (tm) (arrows). ($\times 80,000$)

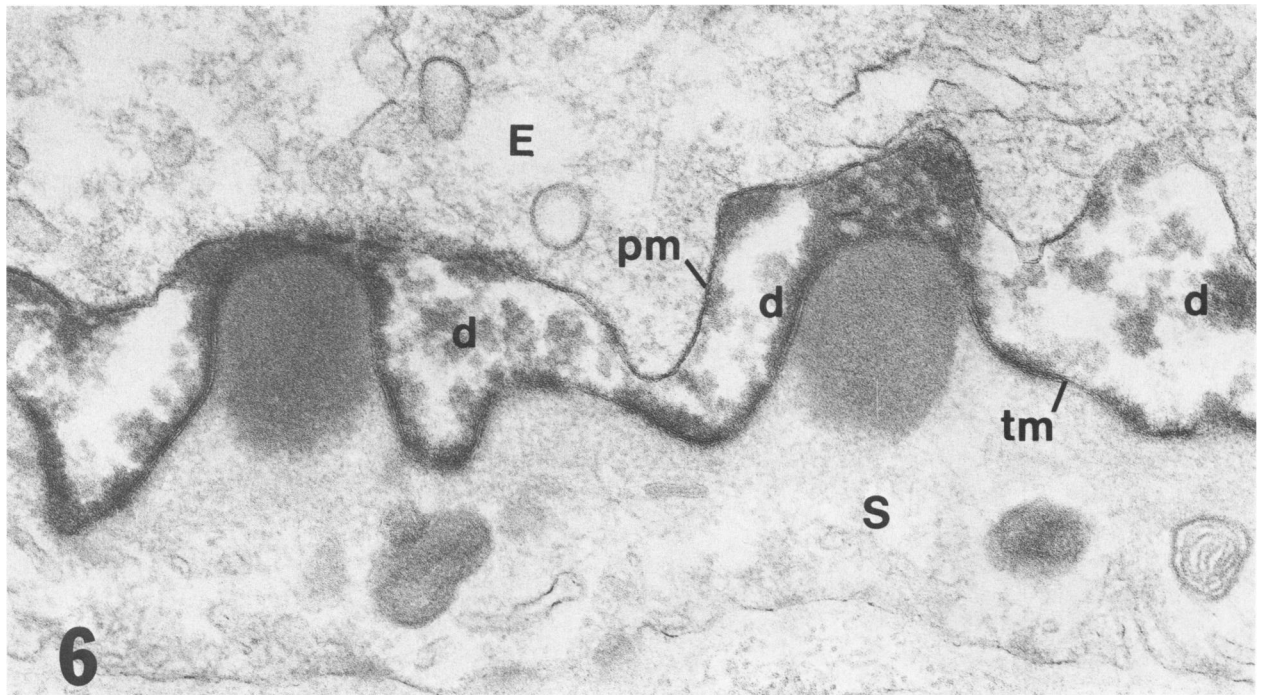


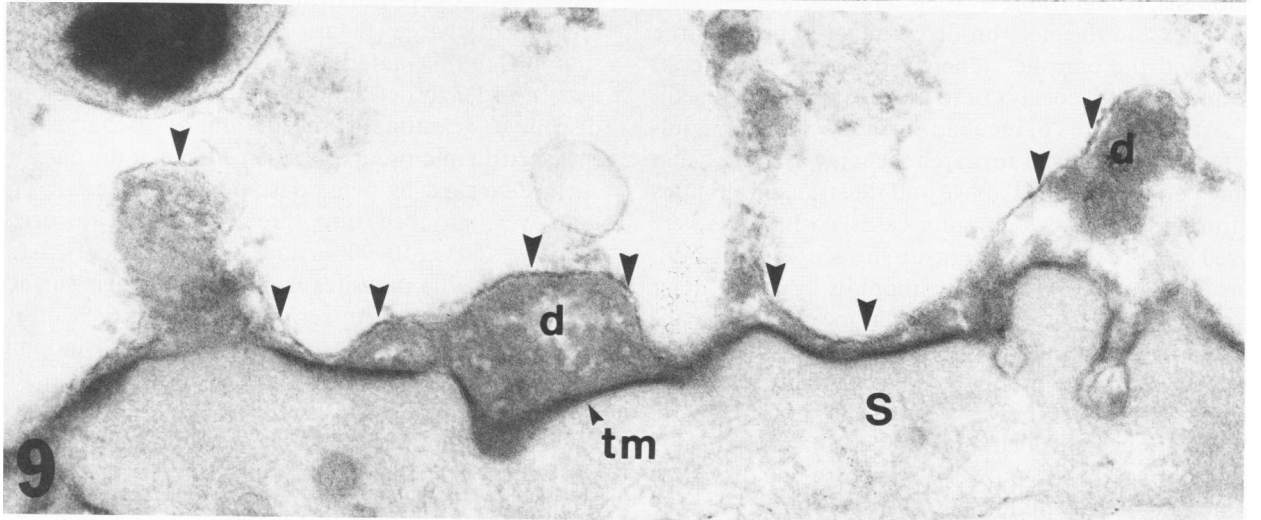
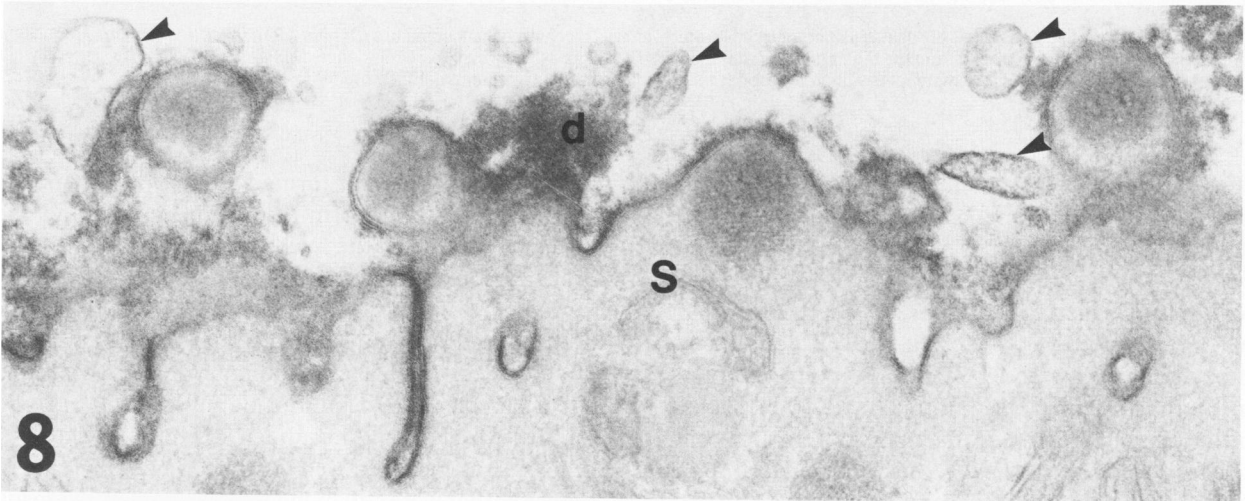
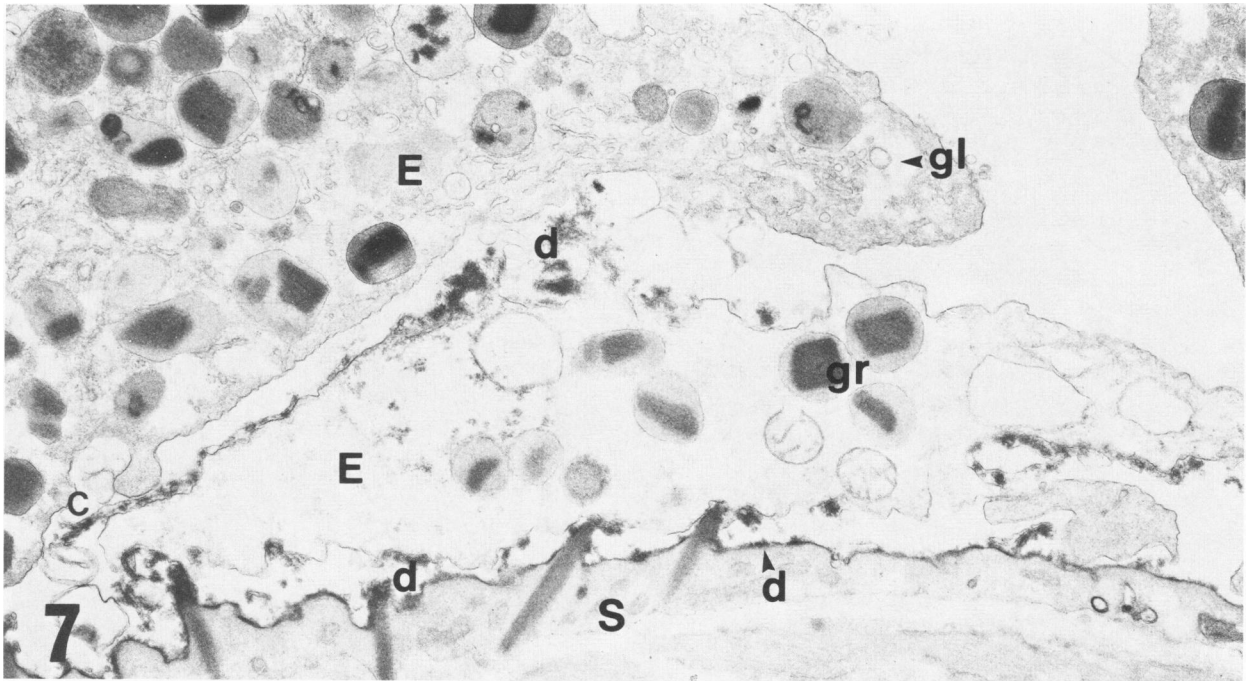
Figure 6—ECEA-treated eosinophil (E) degranulating onto the surface of an Ab-coated schistosomulum (S) after 12 minutes' incubation. The plasma membrane (pm) of the cell is distant from the tegumental membrane (tm) as in Figure 4. However, electron-dense discharge material (d) is seen between the cell and worm. The discharge material is adherent to both the glycocalyx and the tegumental membrane. ($\times 61,000$)

characteristic double membrane of posttransformational organisms (Figure 3). When eosinophils that had not discharged adhered to the surface where the glycocalyx was present, they adhered to the glycocalyx and not to the surface membrane (Figure 4). On the other hand, when the glycocalyx was absent, the cell membrane approached very closely ($\sim 200 \text{ \AA}$) the outer tegumental membrane (Figure 5). Membrane fusions, such as have been described between neutrophils and Ab-coated schistosomula,¹⁸ were not seen. Degranulating eosinophils, recognizable by their large intracytoplasmic vacuoles and the electron-dense material between the cells and the parasite, adhered to discharge material bound to the glycocalyx or to the membrane (Figure 6).

After 2–3 hours of incubation, many eosinophils adherent to the parasite appeared the same as in the acute time period described above and illustrated in Figures 1 through 6. However, in some cases, eosinophils were lysed and broken on the parasite surface (Figures 7–9). Dead cells, identifiable as eosinophils by a few intact granules in their extracted cytoplasm, often were adherent to areas of discharge (Figure 7). Discharge material was also present on the plasma membrane of adherent cells in areas not adjacent to the parasite (Figure 7) and on the parasite in the absence of a recognizable cell (Figures 8 and 9). In the latter case, the discharge material often contained a few vesicular membrane

fragments (Figure 8) or formed a dense layer bounded on one side by the worm tegumental membrane and on the other side by the lysed eosinophil plasma membrane (Figure 9). Up to 3 hours of incubation, no structural changes were seen in the tegument of the parasite even beneath large, $>25 \mu$, areas of discharge.

After 12 hours of incubation many eosinophils and a few parasites were dead. Eosinophils were endocytosing the tegument and invading the bodies of the dead parasites, as has been well described by others.^{6,7} The parasites that were still living were covered by extensive areas of discharge (Figure 10). The adherence of eosinophils to this material was similar to that seen earlier. In order to determine whether ECEA promoted discharge, we estimated the amount of discharge with the electron microscope as a percentage of the parasite surface covered by peroxidase-positive material. The data were grouped into three arbitrary categories, namely, $<50\%$, $50\text{--}90\%$, and $>90\%$ (Table 1). At 3 hours all of the parasites had $<50\%$ of their surface covered by discharge material. At 12 hours 63% of living parasites incubated with ECEA-treated eosinophils had $>50\%$ of their surface covered by discharge material versus 49% of parasites incubated with untreated cells. In addition, 23% of the parasites incubated with the ECEA-treated cells had $>90\%$ of their surface covered, as opposed to 11% of the parasites incubated with



the untreated cells. These differences were statistically significant at a 90–95% confidence limit when analyzed by a paired *t* test. These results suggest that eosinophils have degranulated progressively over the entire 12 hours of incubation and that ECEA caused the eosinophils to increase their discharge.

Eosinophils and Con-A-Coated Schistosomula

In incubations of ECEA-treated and untreated eosinophils with Con-A-coated worms, only 3% ± 1% of the organisms died, which was the same level found with uncoated schistosomula. Discharge material was seen on parasites within 10 minutes, and more material was present at 3 hours than at 10 minutes. When measured at 12 hours, there was less discharge material on Con-A-coated parasites than on Ab-coated parasites (Table 1 and Figure 10). In particular, only about 5–15% of the Con A parasites had more than half their surface covered by peroxidase-positive material, as opposed to 50% in the case of Ab-coated parasites. These differences were significant with 98–99% confidence limits. In addition, the ECEA-treated cells had degranulated slightly more than untreated cells onto Con-A-coated organisms, but the differences were not statistically significant (Table 1).

Ultrastructurally, the cells adhering to Con-A-coated schistosomula were similar to those adhering to Ab-coated organisms. Cells that had not degranulated adhered either to cercarial coat remnants or to the tegumental membrane. Eosinophils, unlike neutrophils,²⁶ did not form membrane fusions with the Con-A-coated parasites. The cells degranulated onto both the membrane and cercarial coat material. At 3 and 12 hours, lysed eosinophils and cell fragments were seen adhering to discharge material on the parasite surface, although much less discharge material was present on the Con-A-coated worms than on the Ab-coated worms.

Neutrophils

In three experiments, neutrophils purified in parallel with the eosinophils used in the above studies were treated with ECEA and incubated with Ab-coated schistosomula. As previously reported,^{23,26} the neutrophils

Table 1—Eosinophil Discharge and Killing

Incubation condition	% of parasite surface covered with peroxidase after 12 hours			% Killed at 18 hours
	<50%	50–90%	>90%	
Con A	95 ± 2*	5 ± 2	0	4.6 ± 3.7†
Con A + ECEA	85 ± 10	13 ± 8	2 ± 2	5.2 ± 2.9
Ab	51 ± 12	38 ± 6	11 ± 7	12 ± 11
Ab + ECEA	38 ± 12	40 ± 10	23 ± 10	48 ± 21

* Each number is the percentage of the intact worms counted ± standard error. Fifteen to 20 worms were counted with the electron microscope in each experiment. Four experiments were done with Con A, and six were done with Ab.

† Killing was assayed by light microscopy.²³ Data are presented only for the experiments in which the percentage of the surface covered by peroxidase was determined.

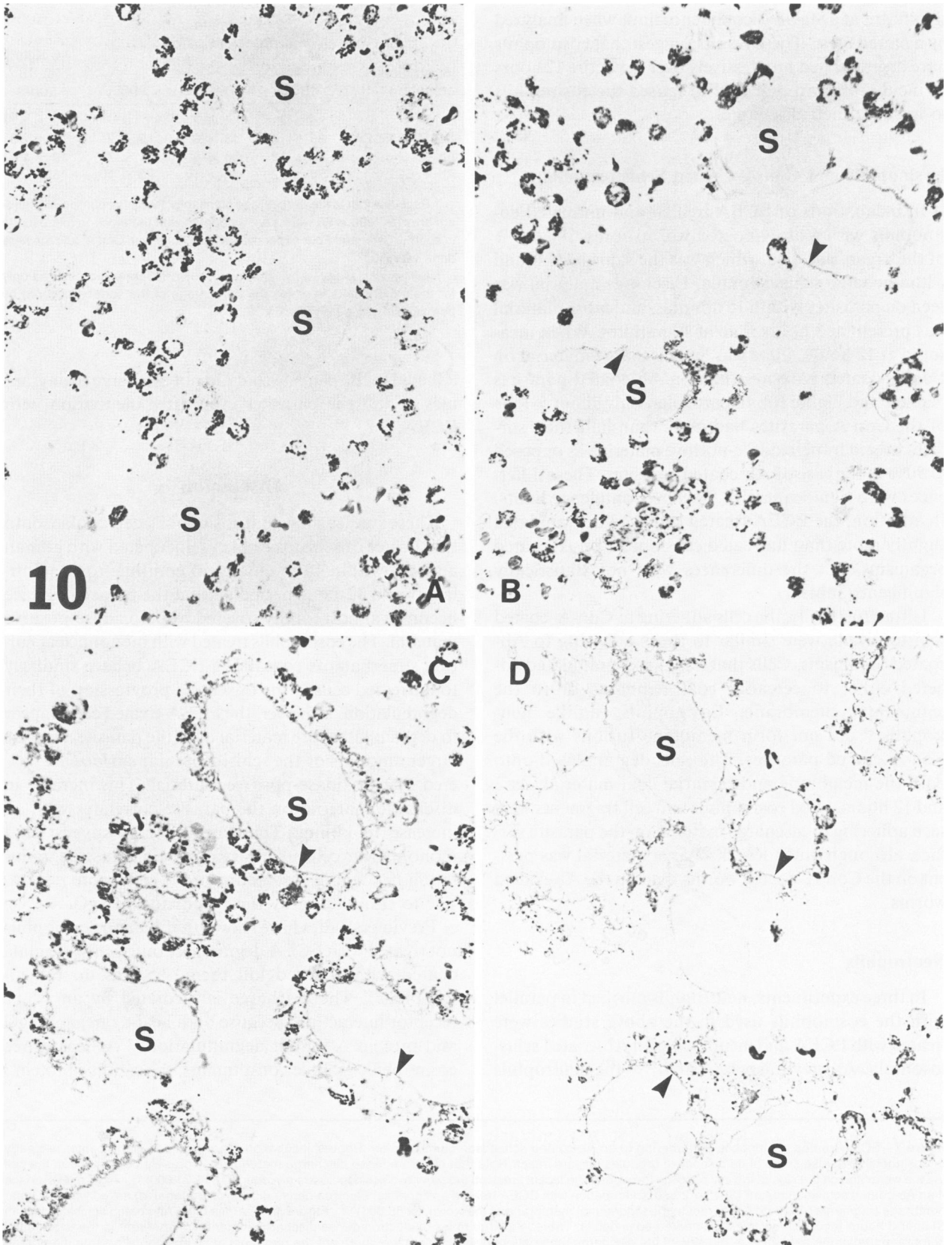
adhered to the parasites but did not discharge their granules or kill the parasites even after incubation with ECEA.

Discussion

These studies show that eosinophils degranulate onto the surface of schistosomula preincubated with human antisera within 10 minutes and continue to degranulate over a 12-hour period so that the parasite surface becomes almost totally covered by peroxidase-positive material. The eosinophils treated with mononuclear culture supernatants containing ECEA behave similarly to untreated cells in the onset and progression of their degranulation. However, the ECEA-treated cells appear to degranulate more material onto the parasite, because larger amounts of the schistosomular surface are covered by peroxidase-positive material. This increase in discharge material on the parasite correlates with an increase in killing. These experiments suggest that mononuclear cell supernatants do not cause degranulation *per se*, but instead make the cells more responsive to stimulation by the Fc portion of IgG.

Previous studies have shown that human eosinophils not treated with ECEA degranulate onto schistosomula coated with Ab and kill them,^{6,7,8,18} as do rat eosinophils.²⁷ The discharge is mediated by an Fc–Fc receptor interaction, because both heat-aggregated IgG and protein A inhibit degranulation.¹⁷ At least three eosinophilic granule constituents, the cationic protein,⁷

←
Figure 7—ECEA-treated eosinophils (E) adhering to an Ab-coated schistosomulum (S) after 3 hours' incubation. The dead cell in the center is clearly an eosinophil, because some of its distinctive granules (gr) are intact. Note that electron-dense discharge material (d) is present both between the cell and the worm and on the side of the cell opposite the worm. The lucent areas on the other two eosinophils are glycogen (gl). (× 21,000) **Figure 8**—Surface of an Ab-coated schistosomulum (S) after 2 hours' incubation with ECEA-treated eosinophils. Electron-dense discharge material (d) mixed with vesicular membrane fragments (arrows) is adherent to the tegumental membrane of the worm. (× 65,000) **Figure 9**—Surface of an Ab-coated schistosomulum (S) after 3 hours' incubation with ECEA-treated eosinophils. There is a large mass of electron-dense discharge material (d) adherent to the tegumental membrane (tm) of the parasite. On the side of the discharge material opposite the parasite, there are the remnants of an eosinophil plasma membrane (arrows) and some membrane fragments. (× 53,000)



major basic protein,²⁸ and peroxidase,²⁹ are lethal to schistosomula *in vitro*. Further, these molecules appear to be bound to the parasite surface when they are effective.^{5,30} The discrepancy, noted by others,⁸ of the long time between the onset of degranulation (minutes) and measurable death (18–24 hours) is explained by these experiments. The parasite is killed by a progressive, massive and sustained attack by many cells over the entire incubation period. Thus, parasites with smaller amounts of discharge material on them such as occurs with Con A are able to escape the attack. Finally, residual glycocalyx may serve to help the parasite escape both by stimulating discharge when the cell is still removed from the parasite membrane and by binding to the discharge material, thus decreasing the amount of material reaching the tegumental membrane.

Eosinophils adhere to the surface of the parasite in two ways. First, they adhere to the cercarial coat material and outer tegumental membrane in the absence of discharge material. Secondly, they adhere to discharge material itself, since the plasma membrane remains adherent to the discharge material even when the rest of the cell has been removed either after lysis or by pipetting.³¹ The dual nature of eosinophil adherence is also suggested by studies in which eosinophil adherence is initially inhibited by protein A and by aggregated IgG and is temperature-independent.^{16,17} After several hours at 37 C, these inhibitors are ineffective.^{16,17} The reversible phase is mediated by an Fc–Fc receptor interaction and corresponds to attachment of the cell to the cercarial coat material or the tegumental membrane.¹⁷ The irreversible phase has been associated with eosinophil degranulation and corresponds to the attachment to discharge material.¹⁷

The behavior of human eosinophils is very different from that of human neutrophils in this assay system.³ Neutrophils also adhere to the Ab or Con-A-coated parasites,^{8,18,23,26,32} but they do not discharge onto or kill them. Others^{4,5,30} have reported that human neutrophils can kill Ab-coated parasites. We have no explanation for the discrepancy in the results from the different laboratories. In our experiments, most neutrophils adhere in a manner similar to the undischarged eosinophils attached to the glycocalyx or the tegumental membrane.^{18,26} However, about 10% of the neutrophils fuse with the outer tegumental membrane of Con-A- or Ab-coated worms to form a hybrid membrane.^{18,26} If the fused cells lyse, the hybrid membranes appear to mix with the normal worm outer membrane

and may be a mechanism by which the parasite acquires host membrane components.^{31,33} ECEA-treated neutrophils do not discharge or behave differently from untreated neutrophils.^{11,13} This suggests that ECEA may have a selective effect on the eosinophil.

Finally, it is not clear how the eosinophils on the parasite surface lyse. They may expend themselves suicidally or damage themselves with their own secretions. Alternatively, the parasite may kill them as appears to be the case with erythrocytes.³⁴ In either case, the discharge material remains on the parasite surface after the cell has died. Whether the various lysosomal enzyme systems remain active after the cell has died and detached is unknown. The experiments in which schistosomula are killed with isolated granule components^{7,28,29,30} would suggest that the discharge material could be active after cell death. In addition, the retention of the eosinophil plasma membrane on the discharge material may help to maintain environmental conditions which augment the lysosomal constituents' attack on the parasite membrane.

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Figure 10—Unstained 0.3- μ sections of 12-hour incubations of eosinophils and schistosomula (S) that were reacted for peroxidase. A—Con-A-coated worms and untreated cells. B—Con-A-coated worms and ECEA-treated cells. C—Ab-coated worms and untreated cells. D—Ab-coated worms and ECEA-treated cells. Discharge is seen as the dark rim on the parasite surface (arrows). Note that there is much less discharge on the Con-A-coated worms than the Ab-coated worms. ($\times 450$)

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