INFECTION AND IMMUNITY, Jan. 1983, p. 225–232
0019-9567/83/010225-07$02.00/0

Vol. 39, No. 1

Maturation In Vivo of *Schistosoma mansoni* Schistosomula After Culture In Vitro with Granulocytes and Antibody

ALAIN DESSEIN,* ANTHONY E. BUTTERWORTH,† MATHEW A. VADAS,‡ AND JOHN R. DAVID

Department of Medicine, Harvard Medical School, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115

Received 11 August 1982/Accepted 11 October 1982

Seven experiments were carried out to test the relationship between the morphological assay for damage to schistosomula in vitro with toluidine blue and the loss of the ability of damaged organisms to mature in vivo. Schistosomula were prepared by penetration of rat skin and cultured for 12 to 38 h in the presence of various combinations of purified human eosinophils or neutrophils and heat-inactivated human antischistosomular serum. Samples were scored for microscopically detectable damage, and the remaining organisms were injected intravenously into normal mice. These mice were perfused after 5.5 to 7 weeks, and the recovery of adult worms was determined. After culture of schistosomula in medium alone, between 8.4 and 32.7% of injected organisms matured into adult worms. There was no significant difference in the capacity of freshly prepared and cultured schistosomula to mature in vivo. Schistosomula cultured with antibody alone showed no significant damage in vitro, and in only one of seven experiments was there a significant (35%) reduction compared with the medium controls in their capacity to mature in vivo. Schistosomula cultured with neutrophils alone or eosinophils alone showed no significant damage in vitro and no loss of viability in vivo. Schistosomula cultured with neutrophils and antibody showed a 28% reduction in recovery in one experiment but an increase in recovery (12 and 46%) in two other experiments. In contrast, schistosomula cultured with eosinophils and antibody showed evidence of both marked damage in vitro (22 to 93% dead organisms) and loss of viability in vivo (26 to 98% reduction in recovery) in all seven experiments. These findings justify the use of the toluidine blue morphological assay as an estimate of irreversible damage to schistosomula and confirm that human eosinophils and neutrophils differ markedly in their capacity to mediate antibody-dependent damage in vitro.

During recent years, several groups have shown that a variety of immune effector mechanisms are capable of damaging the young larvae (schistosomula) of *Schistosoma mansoni* in vitro (1–9, 11, 13–15, 17, 18, 21, 22, 24, 28, 29). Such experiments have three marked advantages in comparison with studies in experimental animals in vivo. First, they permit a precise identification and separation of the individual effector mechanisms. Second, they permit a more detailed analysis of the mode of action of each effector mechanism than would be possible in vivo, particularly of the functional properties that are required for the expression of cell-mediated damage (4, 5, 8, 9, 14, 15, 19, 22, 28). Third, and perhaps most important, they allow the use of human materials (1–6, 12, 14, 15, 28, 29) and may therefore eventually be used for the study of immunity in humans.

Such experiments in vitro, however, also bear several intrinsic problems. The most important of these is that it is not possible to claim that any of the effector mechanisms identified in vitro is necessarily operative in vivo, nor that other host responses may not also be required for the expression of immunity (reviewed in reference 23). Moreover, on a more practical level, it is not easy to determine what constitutes irreversible and lethal damage to the target. This is especially true of multicellular and relatively large organisms such as schistosomula: even quite severe but localized damage could theoretically be repaired, and the organism might go on to mature in the normal way. In addition to this, different assays for damage may yield conflicting results: for example, the release of ^{51}Cr from prelabeled schistosomula gives results which are not borne

† Present address: Department of Pathology, Cambridge University, Cambridge CB2 1QP, England.

‡ Present address: Walter and Eliza Hall Institute of Medical Research, Victoria 3050, Australia.

TABLE 1. Assay conditions for adult worm recovery experiments

Expt	Cell donor	% Purity[a]		E/T ratio[b]	Antibody[c]	Culture period (h)[d]	Mouse strain	No. of organisms injected[e]	Time before perfusion (wk)[f]
		Eos	Neutros						
1	A	97	NT	2,000	T (1/15)	24	CF1	150	6
2	B	90	NT	1,500	A1 (1/15)	20	A/J	300	5.5
3	A	90	NT	2,000	A5 (1/10)	28	(C57BL/6 × A/J) F1	425	6
4	A	93	100	3,000 (Eos) 4,000 (Neutros)	Pooled sera (1/12)	38	CBA	300	6
5	C	77	97	4,000	O9 (1/10)	12	CBA	200	6.5
6	D	90	95	4,000	M11 (1/10)	12	CBA	200	7
7	D	100	92	4,000	M11 (1/2, 1/10)	24	CBA	200	7

[a] Eosinophils (Eos) and neutrophils (Neutros) recovered from four different normal individuals. NT, Not tested.
[b] Ratio of effector cells (E) to target schistosomula (T).
[c] Heat-inactivated sera used as antibodies; final dilutions are shown in parentheses.
[d] Incubation period in vitro of schistosomula before assay for damage and injection into mice.
[e] Mice of various strains received 150 to 425 cultured schistosomula.
[f] Perfusions were carried out after 5.5 to 7 weeks.

out by microscopic assays carried out under identical conditions (29). It is therefore necessary to confirm, for any given assay, that the damage observed in vitro is indeed associated with a loss of the ability of the organism to mature in vivo.

Although it is accepted that rat neutrophils kill antibody-coated schistosomula in the presence of complement (13), there is at the moment considerable controversy about the ability of human neutrophils to inflict antibody-dependent damage to schistosomula in vitro. We have consistently found that human eosinophils, but not human neutrophils, can damage schistosomula in the presence of heat-inactivated sera from infected patients (12, 29; A. E. Butterworth and A. Dessein, unpublished observations), whereas other groups have reported that human neutrophils can also inflict damage, which may be as marked as that mediated by eosinophils (1, 18). One of several possible explanations for this discrepancy is that the particular microscopic assay that we use is not sensitive enough to detect a neutrophil-mediated effect, which may yet be sufficient to reduce the viability of the worms.

The aims of the present experiments were therefore to test whether the eosinophil-mediated damage detectable by the toluidine blue morphological assay in vitro was indeed associated with a reduced viability in vivo and to test whether neutrophils could exert an effect which was indetectable microscopically but which was sufficient to reduce the viability of the organisms.

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 (11, 27) into Earle balanced salt solution containing 100 U of penicillin per ml, 100 μg of streptomycin per ml, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.5% lactalbumin hydrolysate, and 10% heat-inactivated fetal calf serum (E-LAC-FCS). After penetration, the organisms were washed three times in E-LAC-FCS and three times in (bicarbonate-free) Eagle minimal essential medium supplemented with antibiotics and HEPES as above (MEM) and 10% heat-inactivated fetal calf serum (MEM-FCS). They were resuspended finally in MEM-FCS at a concentration of 2,000 organisms per ml.

Mice. Mice of strains CF1 (outbred), A/J, CBA, and (C57BL/6 × A/J) F1 were purchased from Jackson Laboratory, Bar Harbor, Maine.

Antisera. Sera from patients with *S. mansoni* infection, either single or in pools, were used as a source of antischistosomular antibodies. Six different serum preparations were used in different experiments at final concentrations ranging from 1/2 to 1/15 (Table 1). 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 (29). Briefly, heparinized peripheral blood was sedimented with 4.5% dextran in phosphate-buffered saline, and the leukocytes recovered from the supernatant were washed twice in MEM-FCS containing 30 mg of DNase per liter. These cells were then layered onto discontinuous gradients of metrizamide (Nyegaard, Oslo) at concentrations of 18 to 25% in Tyrode balanced salt solution containing 0.1% gelatin and 30 mg of DNase per liter. These gradients were centrifuged at 20°C for 45 min at 1,200 × *g*. Cells from each interface were withdrawn and washed twice in MEM-FCS containing 30 mg of DNase per liter before resuspension in MEM-FCS. Cytocentrifuge smears were stained with

TABLE 2. Recovery of adult worms after injection of schistosomula cultured in vitro with granulocytes and antibody

Expt	Schistosomula cultured with[a]:	% Killed in vitro	Total no. recovered in vivo[b]	% Recovery in vivo[c]	% Corrected recovery in vivo[d]	t test[e]
1	Eos + Ab	48	2.4 ± 0.5 (7)	1.6	19	A
	Eos	6	10.2 ± 0.7 (5)	6.8	81	
	Ab	7	11.6 ± 2.0 (7)	7.7	92	
	Medium	6	12.6 ± 1.6 (7)	8.4	100	
2	Eos + Ab	22	72.8 ± 6.5 (5)	24.2	74	B
	Eos	6	103.4 ± 5.7 (5)	34.5	106	
	Ab	2	96.0 ± 9.1 (5)	32.0	98	
	Medium	2	98.0 ± 6.4 (5)	32.7	100	
3	Eos + Ab	93	13.4 ± 1.8 (8)	3.1	17	A
	Eos	3	90.0 ± 10.1 (5)	21.2	118	
	Ab	3	74.8 ± 4.8 (5)	17.6	98	
	Medium	1	76.5 ± 9.1 (6)	18.0	100	
4	Eos + Ab	58	3.9 ± 1.0 (8)	1.3	9	C, D
	Neutros + Ab	15	31.8 ± 2.7 (6)	10.6	71	D, E
	Ab	0	45.0 ± 4.6 (5)	15.0	100	
5	Eos + Ab	50	0.8 ± 0.8 (4)	0.4	2	A, D
	Eos	9	51.8 ± 2.9 (5)	25.9	105	
	Neutros + Ab	12	35.3 ± 3.1 (4)	17.7	72	D, F
	Neutros	7	56.3 ± 4.9 (6)	28.2	114	
	Ab	4	32.2 ± 1.4 (6)	16.1	65	G
	Medium	4	49.4 ± 5.8 (6)	24.7	100	
6	Eos + Ab	54	22.0 ± 2.3 (5)	11	36	D, H
	Eos	0	78.9 ± 6.6 (5)	39.5	130	
	Neutros + Ab	6	89.1 ± 5.9 (5)	44.6	146	D, J
	Neutros	5	108.0 ± 7.1 (5)	54.0	177	K
	Ab	3	64.0 ± 8.1 (5)	32.0	105	
	Medium	0	61.0 ± 8.6 (5)	30.5	100	
7	Eos + Ab 1/10	36	21.1 ± 2.4 (4)	10.6	37	D, H
	Eos	0	78.4 ± 8.4 (5)	39.2	124	
	Neutros + Ab 1/2	0	70.4 ± 11.8 (4)	35.2	111	I
	Neutros + Ab 1/10	10	71.0 ± 2.5 (5)	35.5	112	D, I
	Neutros	6	73.3 ± 8.9 (4)	36.7	116	
	Ab 1/10	5	56.3 ± 13.9 (3)	28.2	89	
	Medium	7	63.2 ± 4.4 (5)	31.6	100	

[a] Eos, Eosinophils; Neutros, neutrophils; Ab, antischistosomular serum.

[b] Mean ± standard error; numbers of mice are in parentheses.

[c] Calculated from the total numbers of organisms originally injected.

[d] Calculated with reference to the medium controls (experiments 1 to 3 and 5 to 7) or the Ab controls (experiment 4).

[e] Summaries of Student's *t* tests: A, Eos + Ab versus Eos, Ab, and medium controls, $P < 0.001$; B, Eos + Ab versus Eos and medium controls, $P < 0.05$; C, Eos + Ab versus Ab control, $P < 0.001$; D, Eos + Ab versus Neutros + Ab, $P < 0.001$; E, Neutros + Ab versus Ab control, $P < 0.05$; F, Neutros + Ab versus Ab control, not significant ($P > 0.05$); G, Ab control versus medium control, $P < 0.02$; H, Eos + Ab versus Eos, Ab, and medium controls, $P < 0.05$; I, Neutros + Ab versus Ab and medium controls, $P < 0.05$; J, Neutros versus medium control, $P < 0.01$; K, Neutros + Ab versus Neutros, Ab, and medium controls, not significant ($P > 0.05$).

Giemsa for immediate examination, and fractions were pooled as appropriate. The purity of each preparation is shown in Table 1: in the case of eosinophils, the contaminating cells were neutrophils; in the case of neutrophils, the contaminating cells were mononuclear cells with occasional eosinophils. Cell concentrations were adjusted to 1.5×10^6 to 4×10^6/ml, yielding effector cell/target schistosomulum ratios of 1,500:1 to 4,000:1 (Table 1).

Culture in vitro and injection into mice. Samples of 1

ml of schistosomula, containing 1,000 organisms, were distributed into 12- by 75-mm round-bottomed plastic tubes (2063; Falcon Plastics, Oxnard, Calif.). To each tube was added 0.1 ml of the appropriate antibody dilution or 0.1 ml of medium as a control and 1 ml of eosinophils or neutrophils or 1 ml of medium as a control. The tubes were incubated in a humidified chamber at 37°C for 12 to 38 h (Table 1). At the end of this period, the contents of each set of identical tubes were pooled, and one to three samples containing 70 to 100 schistosomula were withdrawn for microscopic examination and counting. Slides for microscopic examination were prepared by depositing on each slide 50 to 100 μl of a 0.1% toluidine blue solution in methanol. After a few minutes, the methanol had evaporated, yielding toluidine blue-coated slides. Larvae were examined at a magnification of 100×: organisms were scored as dead if they were immotile and had taken up toluidine blue in an intense and granular fashion (29). The remaining organisms were washed three times and resuspended in MEM for immediate injection into the tail veins of mice, the timing of the injections being randomized among the different experimental groups. Three to eight mice were used for each test condition (Table 2), and each mouse received an equal number of organisms (Table 1), regardless of whether these had been scored as alive or dead in the microscopic assay.

Recovery of adult worms. Adult worms were recovered after 5.5 to 7 weeks (Table 1) by whole-body perfusion according to the technique of Smithers et al. (26). Mice were killed by intraperitoneal injection of 0.2 ml of saline containing 1 mg of sodium pentobarbital (Abbott Laboratories, North Chicago, Ill.) and 100 U of heparin and shaved and rinsed with water, and the abdominal and thoracic cavities were opened. The mice were suspended on a perspex frame over a collecting dish, the hepatic portal vein was cut, and a needle was inserted into the left ventricle. The mice were then perfused through this needle with approximately 100 ml of citrate saline (0.8% sodium citrate, 1.5% sodium citrate), and the intestines were carefully washed with citrate saline to recover worms adhering to the tissue. After perfusion, the livers were squashed between two glass plates and examined for residual worms, which were counted. The perfused worms were washed with citrate saline, concentrated by sedimentation, and counted. Perfusions were carried out blindly and at random among the experimental groups: perfusions which failed to recover 90% of the worm load of the liver were immediately discarded, before allocation to their test groups.

Analysis of results. Mean worm burdens for each experimental group were calculated and compared with the appropriate controls (cells alone, antibody alone, or medium alone) by Student's *t* test. The percentage recovery for each group was calculated from the known number of organisms injected. The percentage corrected recovery was calculated by comparison with control groups cultured with medium alone (experiments 1 to 3 and 5 to 7) or with antibody alone (experiment 4).

RESULTS

Seven experiments were carried out: variations in the experimental conditions are shown in Table 1 and the results of each experiment in Table 2.

Recovery of control organisms. In the first experiment, it was noted that the recovery of adult worms after culture of schistosomula with medium alone was only 8.4% of the number of organisms injected. It was possible that this poor recovery was attributable to the use of a relatively insusceptible outbred mouse strain. In subsequent experiments, therefore, different mouse strains were tested: recoveries of control organisms improved, ranging from 18.0% (experiment 3) to 32.7% (experiment 2). CBA mice consistently gave good recoveries and were used for the later and more extensive experiments. Because of the variability in recovery of control organisms in different experiments, it was necessary, to make comparisons between experiments, to calculate the recovery of test organisms by comparison with the value for control organisms (Table 2, percent corrected recovery). This was achieved by comparison with the medium controls for experiments 1 to 3 and 5 to 7 and with the antibody controls for experiment 4.

An alternative explanation for the poor recovery of control schistosomula could be that such organisms lost viability during culture, even though they were not visibly damaged in the microscopic assay. In two of the seven experiments, therefore, the capacity of freshly prepared and cultured schistosomula to mature in vivo was compared (Table 3). The recovery of

TABLE 3. Recovery of adult worms after injection of freshly prepared or cultured schistosomula

Expt	Schistosomula	Total no. recovered in vivo[a]	% Recovery in vivo	*t* test
2	Freshly prepared	69.8 ± 14.5 (6)	23.3	$P > 0.05$
	Cultured[b]	98.0 ± 6.4 (5)	32.7	
3	Freshly prepared	60.9 ± 11.5 (8)	14.3	$P > 0.05$
	Cultured[b]	76.1 ± 9.1 (6)	18.0	

[a] Mean ± standard error; numbers of mice are in parentheses.
[b] Data for cultured schistosomula are the same as those for the medium controls in experiments 2 and 3 of Table 2.

cultured organisms in both experiments was slightly better than that of freshly prepared organisms, although the differences were not significant. Thus, there was no evidence to indicate that the schistosomula were dying during culture.

Recovery of organisms after culture with antibody alone. Although none of the sera used as a source of antischistosomular antibodies was directly toxic to the schistosomula, as judged by an increased mortality during culture in vitro, it was possible that the antibodies might remain bound to the schistosomula after injection into the mice. Such antibodies might then interact with murine effector cells or complement, leading to further damage to the worm after its injection into the recipient. In all experiments except experiment 4, therefore, the survival of organisms cultured with antibody alone was compared with that of organisms cultured with medium alone. In one experiment (experiment 5), there was evidence for a direct effect of antibody in that experiment organisms cultured with antibody alone showed a significantly lower recovery than those cultured with medium alone, eosinophils alone, or neutrophils alone. In the remaining experiments, in which different sera were used, there were no significant differences in recovery between the antibody controls and the medium controls.

Recovery of organisms after culture with eosinophils alone or neutrophils alone. There was no evidence for direct, antibody-independent damage to schistosomula mediated by either eosinophils or neutrophils. In experiment 6, the recovery of organisms cultured with neutrophils alone was in fact significantly greater that than of the medium controls. A similar effect, although not reaching significant levels, was seen with both eosinophils and neutrophils in several other experiments, possibly suggesting a feeder effect of added cells in the absence of antibody, which enhances schistosomulum viability during culture.

Recovery of organisms after culture with eosinophils and antibody. In six of seven experiments, it was found that schistosomula cultured with eosinophils and antibody in vitro showed a significant or highly significant reduction in their capacity to mature into adult worms in vivo, in comparison with control organisms cultured with medium alone, antibody alone, or eosinophils alone. In the seventh experiment (experiment 2), the effector/target ratio was lower (Table 1), and the degree of damage detected in vitro was slight; nevertheless, the recovery of organisms cultured with eosinophils and antibody was significantly less in this experiment than that of organisms cultured with eosinophils alone or medium alone. The conclusion from these experiments was that the microscopically detectable damage to schistosomula that is induced by eosinophils and antibody in vitro was indeed associated with a loss of the capacity of such organisms to develop into adult worms in vivo.

Recovery of organisms after culture with neutrophils and antibody. Schistosomula cultured with neutrophils and antibody in four experiments underwent significantly less damage, as judged by their ability to mature in vivo, than organisms cultured with eosinophils and antibody. Antibody-dependent damage by human neutrophils was detected after reinjection in vivo in only one of four experiments (experiment 4), and this effect, in comparison with antibody alone, was just significant at $P < 0.05$. In two experiments (experiments 6 and 7), the recovery of organisms cultured with neutrophils and antibody was slightly greater than that of organisms cultured with antibody alone, even when the serum used as a source of antibody was present in concentrations as high as 1/2. The conclusion from these experiments was that neutrophils had, in this assay, either very little or no capacity to damage schistosomula in the presence of antibody, as determined by the ability of cultured organisms to mature in vivo.

DISCUSSION

During culture in vitro, schistosomula are damaged by human eosinophils in the presence of antischistosomular antibodies (2, 14, 15, 29). This damage can be detected by light-microscopic examination as a loss of motility, an increased opacity of the organism, and sometimes gross changes in shape or obvious lesions in the tegumental membrane. These changes are somewhat subjective, but it is also found that such damaged organisms take up toluidine blue, resulting in an intense and granular staining (29). In contrast, intact and motile organisms show no staining. This difference in staining properties of intact and damaged organisms is both reproducible and readily scored in an objective manner and has formed the basis of our microscopic assay. We have now found that the cell-mediated damage detectable by the toluidine blue morphological assay in vitro is associated with a reduced viability of the larvae in vivo. A small proportion of the eosinophil-damaged schistosomula have lost their capacity to mature in vivo but are not recorded as damaged in vitro: this shows that our microscopic in vitro assay identifies the late stages in the death of the organism. In contrast to the findings with eosinophils, the antibody-dependent effect of neutrophils in both the microscopic and the worm recovery assays is absent or marginal: in only one of four experi-

ments was a significant effect observed, and in all experiments, the effect of eosinophils, tested under identical conditions, was markedly greater.

These findings may be compared with those of Kassis et al. (17), who observed a 67% reduction in in vivo recovery of schistosomula cultured for 24 h with murine eosinophil-rich cell preparations and with immune mouse serum, in comparison with organisms cultured with eosinophils and normal mouse serum. Under the same conditions, the percentage of schistosomula showing microscopically detectable damage was 35% for the test preparations, in comparison with 8% for the normal mouse serum controls. In subsequent studies, Kazura et al. (18) reported that schistosomula incubated with human neutrophils, antibody, and complement for 24 h showed a 46% reduction in recovery in comparison with control organisms. This experiment is difficult to evaluate, however, since the recovery of organisms cultured with antibody and complement, antibody alone, or complement alone was not recorded.

Various anticipated problems were encountered during these experiments. First, the recovery of control organisms cultured with medium alone was low, ranging from 8 to 33%. These figures are comparable to those reported in the literature (17–19). The reason for the low recovery of control organisms, especially in our first experiment, is not known. One possibility is that different strains of mice show different susceptibilities to primary *S. mansoni* infection, whether initiated naturally by cercarial challenge or artificially by injection of schistosomula. For this reason, we used CBA mice in later experiments: they are very susceptible to infection and consistently gave adequate control worm recoveries. There is no reason to suppose that the low recoveries of control organisms reflected damage that occurred during the preparation and incubation in vitro and that was not detected by the microscopic assay. Even after cercarial challenge, the proportion of organisms that mature in normal mice is only about 40% (25), whereas James and Taylor (16) have shown that if freshly prepared schistosomula are administered parenterally by various routes, only 6 to 39% mature, depending on the method of preparation and the route of inoculation.

A second potential problem was the introduction of antibody-coated schistosomula into normal mice. It was possible that murine effector cells or complement might interact with the antibody-coated schistosomula after their introduction into the mice: this might lead to death of the worms, even if they had not been damaged in vitro. However, Lewis et al. (20) had shown that when large amounts of human plasma that were known to mediate leukocyte-dependent damage were transferred into normal mice, such mice failed to resist cercarial challenge. This suggested that murine effector mechanisms would fail to mediate damage to schistosomula in the presence of human antibodies. In only one of the seven experiments in the present study was a significant difference observed in the recovery of worms cultured with medium and those cultured with antibody; in the remaining six experiments, in which different human sera were used, there was no such effect.

A third possible event which could have led to erroneous results was that, during culture in vitro, the schistosomula might have acquired into their teguments macromolecules of human origin (26); these macromolecules might have been recognized by the recipient's immune response, with consequent death of the organism. Furthermore, schistosomula cultured with eosinophils and antibody (a process associated with extensive cell adherence) might acquire more macromolecules than schistosomula cultured with eosinophils alone, and this might lead to the appearance of an artificial "antibody-dependent" effect of eosinophils. However, the finding that neutrophils showed a weak or, more usually, absent antibody-dependent effect militates against this argument. Neutrophils adhere to schistosomula in the presence of antibody during the early stages of culture in vitro (29). Moreover, during this adherence, they fuse their membranes with the outer of the two schistosomulum membranes, an event which is likely to be associated with the transfer of neutrophil membrane antigens to the tegument of the schistosomulum (10). Eosinophils, in contrast, fail to make such fusions, and instead deposit their granule contents onto the surface of the organism (10, 14, 15). It is likely, therefore, that if the reduction in recovery of adult worms after culture with eosinophils and antibody had been due to acquisition of eosinophil antigens, then schistosomula cultured with neutrophils and antibody would have acquired neutrophil antigens to the same or greater extent and should therefore have been rejected in the same way.

In light of these comments, it seems reasonable to conclude that the eosinophil-mediated, antibody-dependent damage that is detected by microscopy reflects a true reduction in the viability of the organisms and that neutrophils did not significantly damage antibody-coated schistosomula in our in vitro culture assay. Other explanations must therefore be sought for the discrepancies between our results and those of other workers (1, 18) concerning the relative ability of human eosinophils and neutrophils to damage schistosomula. Studies in progress (A. E. Butterworth, unpublished observations)

indicate that intact schistosomula are able to resist primary attack by human neutrophils under a wide range of experimental conditions, and it is possible that the antibody-dependent damage recorded by others may be attributable to the use of microscopic assays that detect neutrophil-mediated events that are not sufficient to lead to irreversible damage to the schistosomula.

Because of the long duration of the adult worm recovery experiments in vivo and the number of mouse perfusions required for a single datum point, it is not feasible to carry out such studies for each experimental condition that is tested in vitro. However, it was found that the presence of damage detectable in vitro was reflected by a concomitant loss of viability in vivo, even though the data were derived from experiments which varied widely in the nature, duration, and components of the cultures. This supports the view that the microscopic assay is a generally applicable test of the viability of schistosomula and justifies its continued use in experiments in vitro.

ACKNOWLEDGMENTS

This work was supported by grants from the Rockefeller Foundation, the Edna McConnell Clark Foundation, and the Wellcome Trust.

We thank M. Hogan and B. Sherry for excellent technical assistance and A. Sher for his helpful advice and discussion. We gratefully acknowledge Ramona Gonski and Christine Sleiman for manuscript preparation.

LITERATURE CITED

1. **Anwar, A. R. E., S. R. Smithers, and A. B. Kay.** 1979. Killing 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**:628–637.
2. **Butterworth, A. E., J. R. David, D. Franks, A. A. F. Mahmoud, P. H. David, R. F. Sturrock, and V. Houba.** 1977. Antibody-dependent eosinophil-mediated damage to ^{51}Cr-labelled schistosomula of *Schistosoma mansoni*: damage by purified eosinophils. J. Exp. Med. **145**:136–150.
3. **Butterworth, A. E., R. F. Sturrock, V. Houba, and P. H. Rees.** 1974. Antibody-dependent cell-mediated damage to schistosomula in vitro. Nature (London) **252**:503–505.
4. **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**:1456–1471.
5. **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**:221–229.
6. **Capron, A., M. Capron, D. Camus, and A. Vernes.** 1973. Les phenomenes d'hypersensibilite au cours des schistosomiases humaines a *Schistosoma mansoni*. II. Etude in vitro de l'activite lethale des serums de malades pour les schistosomules de *Schistosoma mansoni*. Rapport avec les tests d'hypersensibilite. Pathol. Biol. **21**:1079–1084.
7. **Capron, A., J. P. Dessein, M. Joseph, R. Rousseaux, M. Capron, and H. Bazin.** 1977. Interaction between IgE complexes and macrophages in the rat: a new mechanism of macrophage activation. Eur. J. Immunol. **7**:315–322.
8. **Capron, M., A. Capron, G. Torpier, H. Bazin, D. Bout, and M. Joseph.** 1978. Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG2a antibody and role of mast cells. Eur. J. Immunol. **8**:127–133.
9. **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**:2518–2525.
10. **Caulfield, J. P., G. Korman, A. E. Butterworth, M. Hogan, and J. R. David.** 1980. The adherence of human neutrophils and eosinophils to schistosomula: evidence for membrane fusion between cells and parasites. J. Cell Biol. **86**:46–63.
11. **Clegg, J. A., and S. R. Smithers.** 1972. The effects of immune rhesus monkey serum on schistosomula of *Schistosoma mansoni* during cultivation in vitro. Int. J. Parasitol. **2**:79–98.
12. **David, J. R., M. A. Vadas, A. E. Butterworth, P. A. 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**:1147–1152.
13. **Dean, D. A., R. Wistar, and K. D. Murrell.** 1974. Combined in vitro effects of rat antibody and neutrophilic leukocytes on schistosomula of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. **23**:420–428.
14. **Glauert, A. M., and A. E. Butterworth.** 1977. Morphological evidence for the ability of eosinophils to damage antibody-coated schistosomula. Trans. R. Soc. Trop. Med. Hyg. **71**:392–395.
15. **Glauert, A. M., A. E. Butterworth, R. F. Sturrock, and V. Houba.** 1978. The mechanism of antibody-dependent, eosinophil-mediated damage of schistosomula of *Schistosoma mansoni* in vitro: a study by phase-contrast and electron microscopy. J. Cell Sci. **34**:173–192.
16. **James, E. R., and M. G. Taylor.** 1976. Transformation of cercariae to schistosomula: a quantitative comparison of transformation techniques and of infectivity by different injection routes of the organisms produced. J. Helminthol. **50**:223–233.
17. **Kassis, A. I., M. Aikawa, and A. A. F. Mahmoud.** 1979. Mouse antibody-dependent eosinophil and macrophage adherence and damage to schistosomula of *Schistosoma mansoni*. J. Immunol. **122**:398–405.
18. **Kazura, J. W., M. M. Fanning, J. L. Blumer, and A. A. F. Mahmoud.** 1981. Role of cell-generated hydrogen peroxide in granulocyte-mediated killing of schistosomula of *Schistosoma mansoni* in vitro. J. Clin. Invest. **67**:93–102.
19. **Klebanoff, S. J., E. L. Jong, and W. R. Henderson.** 1980. The eosinophil peroxidase: purification and biological properties, p. 99–114. *In* A. A. F. Mahmoud and K. F. Austen (ed.), The eosinophil in health and disease. Grune & Stratton, New York.
20. **Lewis, F. A., A. Sher, and D. G. Colley.** 1977. Failure of plasma from human schistosomiasis mansoni patients to protect mice from *Schistosoma mansoni* cercarial challenge. Am. J. Trop. Med. Hyg. **26**:723–726.
21. **McLaren, D. J., and F. J. Ramalho-Pinto.** 1979. Eosinophil-mediated killing of schistosomula of *Schistosoma mansoni* in vitro: synergistic effect of antibody and complement. J. Immunol. **123**:1431–1438.
22. **McLaren, D. J., F. J. Ramalho-Pinto, and S. R. Smithers.** 1978. Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of *Schistosoma mansoni* by rat eosinophils in vitro. Parasitology **77**:313–324.
23. **Phillips, S. M., and D. G. Colley.** 1978. Immunologic aspects of host responses to schistosomiasis: resistance, immunopathology, and eosinophil involvement. Prog. Allergy **24**:49–182.
24. **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**:147–156.

25. **Smithers, S. R., and R. J. Terry.** 1965. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. Parasitology **55**:695–700.
26. **Smithers, S. R., R. J. Terry, and D. J. Hockley.** 1969. Host antigens in schistosomiasis. Proc. R. Soc. Lond. B Biol. Sci. **171**:483–494.
27. **Stirewalt, M. A., and A. Uy.** 1969. *Schistosoma mansoni*: cesrcarial penetration and schistosomule collection in an in vitro system. Exp. Parasitol. **26**:77–78.
28. **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**:1441–1448.
29. **Vadas, M. A., J. R. David, A. E. Butterworth, N. T. Pisani, and T. A. Siongok.** 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**:1228–1236.