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COMMUNICATION

Newly Transformed Schistosomula Spontaneously Lose Surface Antigens and C3 Acceptor Sites during Culture[1]

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Schistosomula newly transformed from cercariae have been shown to bind antibodies from laboratory hosts immune to *Schistosoma mansoni* (1–3) and to activate complement (C) by the alternative pathway as demonstrated by the surface deposition of C3 (4, 5). In contrast, schistosomula recovered from the lungs of mice 4 to 5 days after host penetration fail to bind anti-parasite antibodies or to activate the alternative pathway (1, 2, 5, 6). Two hypotheses have been proposed to explain this dramatic developmental change in the immunochemical behavior of the worm surface. First, parasite surface antigens and C acceptor sites may be masked by host molecules (7) such as ABH group glycolipids (8), Forssman antigen (9), or products of the major histocompatibility complex (6), all of which appear on the surface of the parasite during the time when the capacity to bind antibody and activate C is lost. Secondly, parasite antigens and C3 acceptor sites could be cleared from the organism's surface as part of its normal development. The latter explanation was suggested by Dean (10) who showed that schistosomula cultured for 3 days in a chemically defined (i.e., host molecule free) medium lose their susceptibility to antibody-dependent C-mediated killing. In this report, we have tested this hypothesis by studying with quantitative immunofluorescence the rate of clearance of both rat anti-schistosome antibody and alternative pathway bound C3 from the surface of schistosomula cultured in chemically defined medium and in medium supplemented with serum. In addition we have measured the ability of precultured schistosomula to bind these molecules. Our results indicate that cultured schistosomula lose both parasite antigens and C3 acceptor sites with exponential kinetics. These changes can also be detected on schistosomula cultured in a chemically defined medium and then labeled and thus must occur in the absence of host molecule uptake by the parasite.

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MATERIALS AND METHODS

Schistosomula were collected 3 hr after transformation of cercariae of *S. mansoni* (Puerto Rican strain) by penetration through rat skin and washed three times in medium (6). Anti-schistosome antibody binding was demonstrated by incubating schistosomula for 60 min at 37°C with a 1/20 dilution of sera pooled from male CD rats (Charles River Laboratories, Wilmington, Mass.) infected 6 weeks previously with 1000 cercariae each (11), followed by washing and then staining for 30 min at 23°C with fluorescein-labeled goat anti-rat IgG (Cappel Laboratories, Cochranville, Pa.). C3 deposition was detected by incubating schistosomula for 60 min at 37°C in a 1/20 dilution of fresh normal rat serum followed by washing and staining for 30 min at 23°C with fluorescein-labeled goat anti-rat C3 (Cappel Laboratories). The control for both antibody and C binding was normal rat serum that had been heat inactivated for 60 min at 56°C. After an additional series of washes, schistosomula (100 to 200/ml) were cultured for 0 to 48 hr at 37°C under 5% CO_2 in the defined medium RPMI 1640 (10) containing penicillin (100 units/ml) and streptomycin (100 μg/ml) in the presence or absence of 20% heat-inactivated normal rabbit serum. In other experiments, the schistosomula were first cultured and then labeled as above. For fluorescence measurements, schistosomula were washed and fixed for 90 min at 4°C in 3% glutaraldehyde and 1% formaldehyde in 0.1 M cacodylate buffer, pH 7.4 (12), mixed 1:1 with the final wash buffer. The fluorescence emission of a 200 μm^2 area of each schistosomula was measured with a Leitz MPV-2 photometer attached to a Leitz Orthoplan microscope equipped with a Ploem illumination system for fluorescein. Fifteen schistosomula were measured at each point, and each experiment was repeated a minimum of three times.

RESULTS

Freshly transformed schistosomula bind rat anti-schistosome antibody and C3 homogeneously over their entire surface as detected by immunofluorescence. The fluorescent labels do not patch or cap on the surface nor do they appear in endocytic vesicles throughout the culture period. Both antibody and C3 are bound uniformly throughout the population of schistosomula (standard deviations = 20 to 25% of the mean values; Fig. 1*A*, *B*). The labeling is also specific, since parasites incubated with heat-inactivated normal serum emit ≤5% of the fluorescence of those labeled with antibody or C3 (Fig. 1*A*, *B*).

During culture at 37°C, anti-schistosome antibody and C3 bound to the surface of fresh schistosomula are lost exponentially with a halftime of 5 hr for both labels (Fig. 1*A*, *B*). The presence or absence of 20% rabbit serum in the culture medium

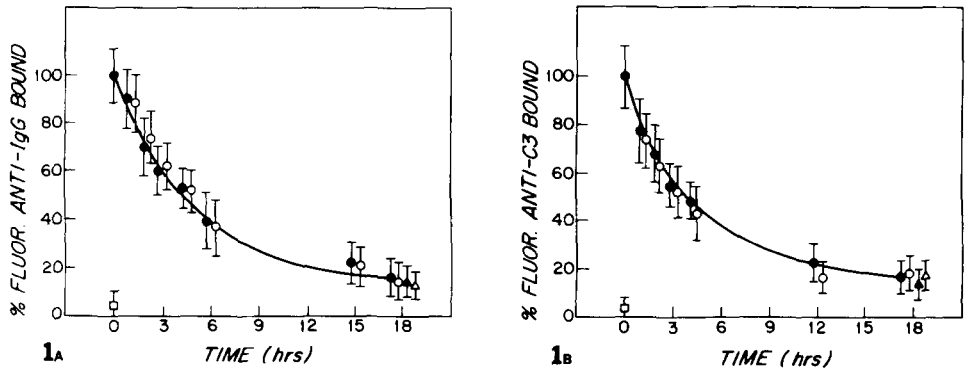

Figure 1. *A* and *B*, A representative experiment showing the binding of fluoresceinated anti-rat IgG (*A*) or fluoresceinated anti-rat C3 (*B*) to the surface of schistosomula. ●, Schistosomula labeled and then cultured in medium containing 20% rabbit serum; ○, schistosomula labeled and then cultured in medium without serum; ▲, schistosomula cultured in medium containing 20% rabbit serum for 18 hr and then labeled; △, schistosomula cultured in medium without serum for 18 hr and then labeled; and □, zero time control with heat inactivated normal rat serum. Values are expressed as a percentage of zero time labeling. Bars indicate standard deviations. The loss of antibody and complement binding from the surface of schistosomula in culture is apparently exponential, because the data from Figures *A* and *B* are well fit (R squared = 0.963) by the equation:

$$\ln \text{Fluor. } (t) = \ln 100 - 0.105 \times t$$

where ln Fluor. (t) = the natural log of the fluorescence expressed as a percentage of zero time labeling at a given time and t = time (hours) in culture. A least squares analysis of the data showed that the coefficient for time (− 0.105) was highly significant (Student's *t*-value = 17.4; $p < 0.001$), whereas the coefficients comparing the rates of loss of the two labels and comparing the rates of loss of the labels under the two culture conditions were small and not statistically significant (t values ≪ 2; p's > 0.3). Further analyses revealed no higher order interactions between time, type of label, and culture condition.

does not affect the rate of loss of either label (Fig. 1*A*, *B*). Furthermore, it was found that schistosomula that were cultured for 18 hr at 37°C in defined medium, or in medium supplemented with serum, and then labeled also fail to bind significant antibody or C3 to their surface (Fig. 1*A*, *B*). In additional experiments, only small amounts of antibody or C3 were detected on schistosomula labeled after culture for 14, 21, and 48 hr in defined medium or to schistosomula recovered from the lungs of mice 2 and 4 days after infection with the parasite (Table I). In contrast, schistosomula incubated at 4°C instead of 37°C for 18 hr showed only a small loss in the ability to bind antibody or C3 (Table I).

TABLE I

Binding of rat immune IgG and C3 to the surface of schistosomula after long-term culture in vitro or recovery in vivo

	Fluor. anti-IgG[a]	Fluor. anti-C3[a]
3-hr schistosomula	100 ± 20	100 ± 22
Control[b]	5 ± 8	9 ± 7
Schistosomula labeled after culture in RPMI-1640 at 37°C:		
14 hr	21 ± 19	19 ± 15
21 hr	10 ± 8	13 ± 11
48 hr	8 ± 6	11 ± 10
Schistosomula labeled after incubation at 4°C:		
18 hr	83 ± 17	80 ± 25
In vivo schistosomula:[c]		
48 hr	4 ± 8	9 ± 9
96 hr	3 ± 7	5 ± 8

[a] Fluorescence (mean ± S.D.) expressed as a percentage of zero time labeling. Standard deviations are large when the fluorescence signal is very weak.

[b] Schistosomula incubated with heat inactivated normal rat serum.

[c] Schistosomula labeled after recovery from the lungs of CF_1 mice (Charles River Laboratories) 2 and 4 days after the injection of 2000 larvae into the tail vein of each animal (6).

DISCUSSION

The experiments reported here show that anti-schistosome antibody and C3 bound to the surface of fresh schistosomula are lost during culture. The loss of both labels follows the pattern of an exponential decay curve, indicating that the process occurs by first order kinetics. The loss does not appear to be induced by the labeling techniques, because schistosomula cultured for 14 to 48 hr in the absence of antibody or C and then labeled also show a decreased binding of antibody or C3. The reduction in the ability to bind antibody or C is apparently not an artifact of *in vitro* culture, because schistosomula recovered from mice 48 or 96 hr after infection also show a decreased ability to bind these reagents.

Since schistosomula kept at 4°C do not lose the ability to bind antibody or C to their surface, it is likely that the loss of immunologically relevant molecules from the parasite's surface is metabolically dependent. These findings parallel those of Tavares *et al.* (13) who were able to block the *in vitro* development of resistance to antibody-dependent C-mediated killing by incubation at 10°C or by the addition of puromycin to cultures. The molecules are probably sloughed, since no endocytosis or aggregation of the fluorescent labels was observed. This interpretation is in agreement with studies showing that surface labels, such as cationized ferritin, are sloughed from schistosomes (14), as well as studies showing that endocytosis does not occur across the organism's tegument (15). It is also possible that these molecules are enzymatically degraded during culture at 37°C. The fact that parasite antigens and C acceptor sites are lost at the same rate is insufficient to establish identity. They may be derived from cercarial coat material, which has also been shown to bind anti-schistosomal antibody and to activate C (16). Alternatively, there may be molecules inserted into the tegumental surface during transformation.

The rate of loss of bound antibody and C3 from schistosomula and the loss of ability to bind these labels is the same in defined

medium as it is in medium supplemented with serum. Therefore, it appears that the loss of surface molecules that bind antibody and activate C is intrinsic to the development of the parasite itself and occurs independently of host molecule absorption. Thus, our data confirm the hypothesis of Dean (10) and argue that developing schistosomula can lose their antigenicity without acquiring host molecules. Whether this process involves a complete loss of all antigenic material from the surface of the parasite or only those recognized by immune serum from *S. mansoni*-infected rats is unclear. Similarly it remains untested as to whether such antigenic material reappears on the surface of later stage organisms. Finally, although our observations argue against a protective function for host molecules with regard to early stage schistosomula, they do not rule out such a role for these molecules during subsequent development of the parasite in the host.

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SUMMARY

The binding of rat anti-schistosome antibody and rat C3 to the surface of newly transformed schistosomula of *S. mansoni* was measured by quantitative immunofluorescence during the first 48 hr of their development *in vitro*. Schistosomula, cultured in media either with or without serum, lost antibody or C3 from their surface exponentially with a halftime of 5 hr for both labels. The loss of the surface molecules is seen in parasites that are labeled and then cultured as well as in parasites cultured and then labeled, indicating that the labeling procedure itself is not inducing the observed change. This immunochemical modification in the schistosomulum surface appears to be independent of host molecule adsorption and intrinsic to the development of the parasite.

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