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The Binding of Human Low-Density Lipoproteins to the Surface of Schistosomula of *Schistosoma mansoni* Is Inhibited by Polyanions and Reduces the Binding of Anti-Schistosomal Antibodies

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Host molecules such as serum lipoproteins, blood group glycolipids, and histocompatibility antigens may bind to schistosomes and thereby prevent immune recognition of the parasite. This study examines the kinetics of lipoprotein binding, the ability of polyanions to inhibit lipoprotein binding, the binding of anti-schistosomal antibodies to worms that have previously bound low-density lipoprotein (LDL), and the distribution of lipoproteins bound to the parasites. Lipoproteins in human serum (HS) and purified LDL, very low-density lipoprotein (VLDL), and apolipoprotein B (apo B) in defined media were demonstrated on the surface of schistosomula of* Schistosoma mansoni *by fluorescence and immunoelectron microscopy using a polyclonal goat anti-human apolipoprotein B antibody (anti-apo B). By fluorophotometric microscopy, lipoprotein binding began within 15 minutes and was largely completed within 3 hours of exposure. Lipoprotein binding saturated at 10% HS or 20 μg protein/300 μl of purified LDL. Suramin inhibited LDL binding by 59% in a dose-dependent fashion. In the absence of LDL in the medium, 2 mM suramin dissociated 41% of bound LDL from the worm surface within 15 minutes and 10 mg/ml heparin dissociated 36%. The binding of human anti-schistosomal antibodies to schistosomula was inhibited by bound LDL. By fluorescence microscopy, serum or purified lipoproteins were distributed over the entire surface of the parasite with focal areas of high intensity. Ultrastructurally, reaction product was seen on the outer leaflet of the outer tegumental membrane and in aggregates and surrounding vesicular structures varying in diameter from 13 to 83 nm. These studies demonstrate that lipoproteins bind to the surface of schistosomula. The binding of lipoproteins is partially inhibited by polyanions, reduces the binding of human anti-schistosomal antibodies, and may help the parasite escape the immune response. (Am J Pathol 1989, 134:1007–1018)

One of the central problem areas in schistosomiasis is the parasite's avoidance of the host immune response. In humans, for example, the parasite can survive 10 to 20 years in the hepatic portal system despite high levels of circulating antibodies against a variety of parasite antigens. Many mechanisms have been proposed to explain the parasite's survival, eg, immunosuppression of the host, protection of the surface syncytium or tegument by two lipid bilayers, and the acquisition of host antigens. In particular, schistosomes acquire host membrane components such as ABO blood group glycolipids,[1,2] Forssman antigen,[3] as well as histocompatibility antigens.[4–8] The glycolipid antigens may be acquired either from the serum or from cells.[1–3] These host antigens may mask parasite antigens. Thus, exposure to serum has been shown to confer resistance to the parasites *in vitro* in antibody-dependent cell and/or complement-mediated cytotoxic reactions.[9–12] The components in serum responsible for this protection and the mechanism of action are not well defined, however.

Rumjanek and coworkers[13] have shown that iodinated

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LDL bind to schistosomula apparently via a receptor that migrates as a 45 kd doublet on SDS-PAGE. The expression of these proteins on the parasite surface is dependent on exposure to human serum (HS), as measured by iodination.[12] Parasites exposed to HS are protected *in vitro* during complement-mediated and rat eosinophil-mediated cytotoxic reactions,[11] but parasites exposed to delipidated serum are not,[12] suggesting that the binding of serum lipids may inhibit antibody-dependent cytotoxicity. These studies have not yet demonstrated that LDL binds to the parasite surface directly, although the trypsin sensitivity of LDL binding suggests this.[13] Further, the concentration of HS that saturates the surface binding as well as the kinetics of lipoprotein binding have not been determined.

In this study we have examined the distribution of lipoproteins bound on the surface of the schistosomula by fluorescence and immunoelectron microscopy. Parasites were cultured in HS or defined media containing purified human high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), or apolipoprotein B (apo B). Apolipoproteins were detected with polyclonal goat anti-human apolipoprotein A-I (anti-apo A-I) or anti-apo B. The time dependence and concentration dependence of the binding of lipoproteins in HS or purified LDL to schistosomula in defined medium was measured by fluorophotometric microscopy. The ability of the polyanion suramin to inhibit the lipoprotein binding as well as the ability of suramin and heparin to dissociate bound LDL from the parasites also were tested. Finally, inhibition of human anti-schistosomal antibody binding to schistosomula by purified LDL also was shown by fluorophotometry.

Materials and Methods

Materials

RPMI 1640, penicillin, streptomycin, L-glutamine, and HEPES buffer were purchased from GIBCO Laboratories, Grand Island, NY; bovine serum albumin fraction V (BSA), and 3,3′-diaminobenzidine (DAB), from Sigma Chemical Co., St. Louis, MO; fetal calf serum (FCS), from Hyclone Laboratories, Inc., Logan, UT; mouse serum and normal goat serum, from Jackson Immunoresearch Laboratories, Inc., West Grove, PA; purified human VLDL, LDL, HDL, apo B in 10 mM deoxycholate, polyclonal anti-apo B, and anti-apo A-I, from Chemicon International, Inc., El Segundo, CA; rhodamine conjugated rabbit anti-goat IgG (rhodamine conjugated anti-IgG) and fluorescein conjugated $F(ab)_2$ fragments of rabbit anti-human IgG (fluorescein conjugated anti-IgG), from Cappel Organon Teknika Corporation, West Chester, PA; peroxidase conjugated swine anti-goat IgG (peroxidase conjugated anti-IgG), from Boehringer Mannheim Biochemicals, Indianapolis, IN; rabbit anti-goat IgG conjugated with 5 nm gold particles (gold conjugated anti-IgG), from Janssen Life Sciences Products, Olen, Belgium; and suramin, from Mobay Corporation, FBA Pharmaceuticals, NY. HS was obtained from 10 healthy donors and inactivated at 56 C for 30 minutes. Human anti-schistosomal antiserum pooled from patients with schistosomiasis was provided by Dr. Donald Harn, Harvard School of Public Health.

Parasites

Schistosoma mansoni (Puerto Rican strain) was maintained in *Biomphalaria glabrata* snails and in CBA/J mice purchased from the Jackson Laboratories, Bar Harbor, MA. Schistosomula were prepared from cercariae by vortexing.[14] The larvae were separated from the tails by centrifugation on a Percoll gradient.[15] The parasites were approximately 3 hours posttransformation at the beginning of incubation with serum or lipoproteins.

Culture Conditions

To test if lipoproteins could be detected by fluorescence microscopy on the surface of schistosomula cultured in sera, 1000 parasites were cultured in a 1.5 ml polypropylene centrifuge tube in 1 ml of RPMI 1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 20 mM HEPES buffer (RPMI 1640-PS) with 1%, 10%, or 50% HS under 5% CO_2/95% air at 37 C for 18 hours. As a control, worms were cultured in 10% FCS, 10% mouse serum, or 1% BSA.

To determine whether purified human HDL, LDL, VLDL, and apo B adhered to the surface of schistosomula and whether this adherence was dependent on preexposure to HS, parasites were pretreated with 10% HS for 5 minutes, washed 3 times in RPMI 1640-PS, and then incubated with 300 μl of RPMI 1640-PS with 1% BSA plus 10.5 μg HDL, 37.5 μg, 75 μg or 180 μg LDL, 15 μg VLDL, and 7.5 μg or 37.5 μg apo B for 18 hours. The lower amount of each compound corresponds to about 10% of the serum concentration. Note that the amounts of lipoprotein are expressed throughout as micrograms of protein. Alternatively, organisms were cultured in the same conditions but without pretreatment with HS.

Experiments were designed to determine the serum or LDL concentration that saturated the surface binding of lipoproteins to schistosomula, the time course of lipoprotein acquisition, the inhibition of lipoprotein binding by suramin, the dissociation of bound LDL by suramin and heparin, and whether the purified human LDL inhibited the

binding of human anti-schistosomal antibodies to worms. In all cases, the bound lipoproteins or human anti-schistosomal antibodies were measured by fluorophotometry. In general, 1000 schistosomula in each tube were cultured in 300 μl of RPMI 1640-PS with various concentrations of either HS or LDL in 1% BSA. RPMI 1640-PS with 1% BSA was used as a negative control. For determining the serum or LDL concentration that saturated the surface binding of lipoproteins to schistosomula, worms were cultured in 1 to 100% of HS or 5 to 100 μg/300 μl of LDL for 18 hours. For the time course of lipoprotein acquisition, worms were incubated in either 10% HS or 40 μg/300 μl LDL in 1% BSA for 15 minutes to 18 hours.

For inhibition of lipoprotein binding by suramin, worms were cultured in either 10% HS or 40 μg/300 μl LDL in 1% BSA along with 0 to 2 mM of suramin for 18 hours. For dissociation of bound lipoproteins from schistosomula by suramin in the presence of lipoproteins in the medium, worms were cultured overnight in either 10% HS or 40 μg/300 μl LDL in 1% BSA. The next morning, 2 mM suramin was added to each tube for 15 minutes to 3 hours. To test the ability of suramin and heparin to release the bound LDL from the worm surface in the absence of LDL in the medium, parasites were cultured in 40 μg/300 μl LDL in 1% BSA overnight. The next morning, worms were washed 3 times and then 2 mM suramin or 10 mg/ml heparin was added to the medium for 15 minutes to 2 hours. Worms were also washed and then incubated at 37 C for 1 or 2 more hours to see whether the bound LDL dissociated spontaneously. In addition, 2 mM suramin or 10 mg/ml heparin was added to some of these washed and cultured worms at the end of 1 or 2 hours of incubation for 30 minutes. For inhibition of human anti-schistosomal antibody binding by LDL, parasites were cultured in 1% BSA with 0 to 100 μg/300 μl LDL for 3 hours, and then incubated with 200 μl of 1:40 dilution of human anti-schistosomal antiserum or normal HS (as a control) for 30 minutes.

The culture conditions for electron microscopy were similar to those for fluorescence microscopy; however, the worm number was 3000 to 4000 per tube instead of 1000 per tube and the volume of the culture medium was 1 ml instead of 300 μl.

Immunostaining

In general, two-step indirect immunostaining was used to detect the lipoproteins on worm surface. The general staining procedure for fluorescence microscopy and fluorophotometry was as follows: cultured schistosomula were washed three times in phosphate buffered saline with 1% BSA (PBS/BSA), incubated with primary antibody for 20 minutes at room temperature (RT), washed three times in PBS/BSA, incubated with secondary antibody for 20 minutes at RT, and washed three times in PBS/BSA.

For staining of lipoproteins, the primary antibodies were 400 μl of 1:200 dilution of anti-apo A-I to detect HDL and anti-apo B to detect VLDL, LDL, or apo B. Normal goat serum was used as a control. The secondary antibody was 200 μl of 1:40 dilution of rhodamine conjugated anti-IgG. The binding of human anti-schistosomal antibodies on worms was detected directly by 200 μl of 1:40 dilution of fluorescein conjugated rabbit anti-human IgG.

For electron microscopy, cultured schistosomula were washed three times in PBS/BSA, incubated with 1.4 ml of 1:200 dilution of primary antibody (anti-apo B, anti-apo A-I, or normal goat serum as a control) for 30 minutes at RT, and washed three times in PBS/BSA. Antigen was demonstrated with both peroxidase and colloidal gold conjugated antibodies. For the peroxidase reaction, the parasites were incubated with 200 μl of 1:10 dilution of peroxidase conjugated anti-IgG for 30 minutes at RT, washed 3 times in PBS/BSA, fixed in Karnovsky's aldehyde fixative (KAF)[16] consisting of 3% glutaraldehyde and 1% formaldehyde in 0.1 M cacodylate buffer, pH 7.4, for 15 minutes at RT, and washed three times in 0.1 M cacodylate buffer. The parasites were incubated with 1 ml of 0.05% DAB. After 5 minutes, 10 μl of 1% H_2O_2 was added and the incubation continued for another 10 minutes. For immunogold staining, the secondary antibody was 200 μl of 1:5 dilution of gold conjugated anti-IgG. In some experiments, specimens were fixed in KAF for 30 minutes at RT before applying the primary antibody.

Light Microscopy

Immunostained parasites were observed with a Leitz Orthoplan microscope equipped with a Ploem illumination system containing an H2 cube for fluorescein and an N2 cube for rhodamine. Parasites were photographed with Kodak Tri-X film exposed at ASA 1600 and developed with Diafine developer.

For measuring the amount of lipoproteins or human anti-schistosomal antibodies bound on worms, fluorescence of a 200 sq μ area between the head and the ventral sucker of schistosomulum was measured with a Leitz MPV compact photometer attached to the microscope. In each experiment, the fluorescence was generally set at 70 to 100 units for the brightest sample and all readings were measured relative to this value. The duration of each reading was 0.25 seconds. For each condition, the readings of 15 organisms were averaged.

Electron Microscopy

After immunostaining and primary fixation, organisms were washed three times in cacodylate and postfixed with

Table 1. *Summary of Lipoprotein Binding Under Various Culture Conditions*

Schistosomula cultured in RPMI 1640-PS plus	Primary antibody		
	anti-apo A-I	anti-apo B	NGS (control)
1% BSA	−*	−	−
1% HS	−	+	−
10% HS	−	+	−
50% HS	−	+	−
10% FCS	−	−	−
10% MS	−	−	−
1% BSA + HDL	−	−	−
1% BSA + LDL	ND	+	−
1% BSA + VLDL	ND	+	−
1% BSA + apo B	ND	+	−

* Positive or negative reactions were determined either by immunofluorescence or immunoelectron microscopy.
ND, not done.

1% OsO_4 in acetate veronal buffer for 1 hour at 4 C, stained *en bloc* in 0.5% uranyl acetate for 1 hour at RT, dehydrated, infiltrated, and embedded in conical Beem capsules in Epon 812.

Thick sections (0.3 μ) were cut with glass knives and stained with azure II-methylene blue. Thin sections showing silver-gold interference colors were cut on a diamond knife, picked up on naked copper grids, stained with 2% uranyl acetate for 5 minutes and lead citrate for 1 minute, carbon coated, and examined in a JEOL 100C/ASID electron microscope operating at 80 kV.

Repetition of Experiments

Each experiment was repeated at least three times.

Results

Lipoproteins bound to the surface of schistosomula cultured in medium containing HS, but not FCS, mouse serum, or BSA, as detected by anti-apo B (Table 1). Even when cultured in as little as 1% HS, parasites had lipoproteins on the surface (Figure 1a). Purified LDL, VLDL, and apo B also adhered to the surface of the parasites (Figures 1c to e). HDL in HS or purified HDL in 1% BSA did not bind to the worm surface as detected by anti-apo A-I (Table 1). Because purified lipoproteins in RPMI 1640-PS containing 1% BSA bound to the worms, exposure to human serum was obviously not required for binding.

By fluorescence microscopy, lipoproteins detected by anti-apo B and rhodamine conjugated anti-IgG were distributed over the entire surface (Figure 1a to e). However, the intensity varied, with bright punctate areas overlying diffuse weaker fluorescence. Fluorescence was not seen inside the parasite where cell bodies connected to the tegument were located. Control schistosomula cultured with pure lipoproteins in medium or with HS and reacted with normal goat serum and rhodamine conjugated anti-IgG did not fluoresce (Figure 1f).

Fluorophotometry

The binding of serum lipoproteins as well as purified LDL to schistosomula occurred in a concentration-dependent and saturable manner (Figure 2). After 18 hours of culture, in 1% serum there was about 50% of the binding seen in 100% serum. However, in 10 to 80% serum the relative fluorescence averaged 86% of the value at 100% serum, suggesting that the binding of lipoproteins was largely saturated at 10% serum. Similarly, with purified LDL there was a sharp increase in the binding of LDL up to 20 μg/300 μl and saturation above this. Thus, saturation appears to occur at 10% HS or 20 μg of purified LDL in 300 μl of medium.

Schistosomula bound serum lipoproteins or purified LDL in medium in a time-dependent way (Figure 3). Within the first 15 minutes in culture, parasites acquired about 42 or 50% of lipoproteins bound to 18-hour cultured worms in 10% HS or 40 μg/300 μl LDL in defined medium, respectively. By 3 hours, parasites had about 87 or 86% of the fluorescence of 18-hour worms in 10% HS or 40 μg/300 μl LDL in 1% BSA, respectively. From 3 to 18 hours, parasites only bound 13 to 14% more of the total bound lipoproteins. Thus, the parasites acquired most lipoproteins within the first 3 hours.

Suramin inhibited the lipoprotein binding in a concentration-dependent manner (Figure 4). At a concentration of 0.2 mM, suramin inhibited about 26 or 29% of lipoprotein binding to schistosomula cultured for 18 hours in 10% HS or 40 μg/300 μl LDL in 1% BSA, respectively. At concentrations above 1 mM, suramin inhibited the binding of lipoproteins to worms to a constant level of 52 or 41% of the control worms, which were cultured in 10% serum or 40 μg/300 μl LDL without suramin, respectively. Suramin also dissociated the bound lipoproteins from schistosomula quickly. In the presence of lipoproteins in the medium, about 30% of the lipoproteins were removed from the worm surface by suramin within 15 to 30 minutes (Figure 5). At a concentration of 2 mM, suramin dissociated the bound lipoproteins from the worm surface to a constant level of 68% of control by 15 minutes in 10% HS or to a constant level of 70% of control by 30 minutes in 40 μg/300 μl LDL in defined medium (Figure 5).

In the absence of LDL in the medium, 2 mM suramin released 41% of bound LDLs from schistosomula within 15 minutes and 10 mg/ml heparin released 36% (Figure 6). There was no additional effect found if the incubations were continued for 2 hours (Figure 6). Control worms that

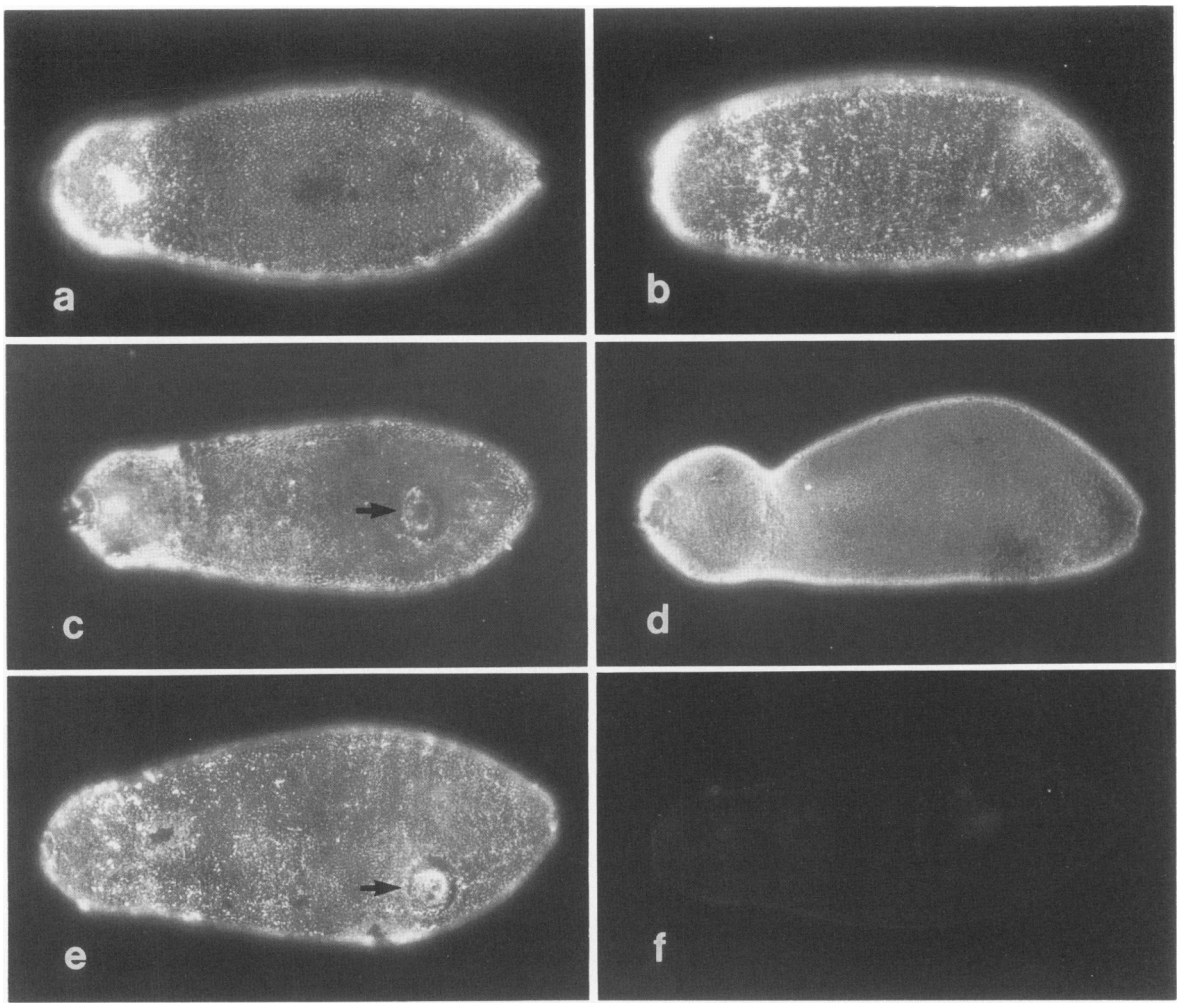
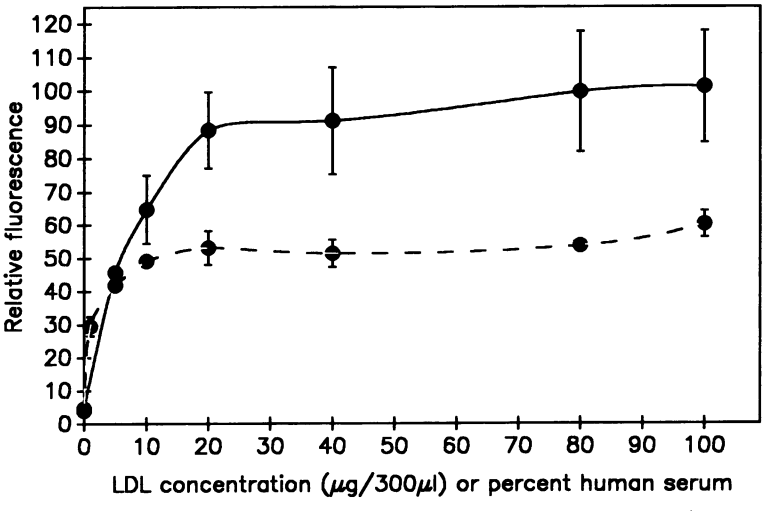


Figure 1. *Light micrographs of schistosomula cultured in* (a) *1% HS,* (b) *10% HS,* (c) *1% BSA + 15 μg VLDL,* (d) *1% BSA + 37.5 μg LDL,* (e) *1% BSA + 7.5 μg apo B, and* (f) *1% BSA + 75 μg LDL for 18 hours either with HS pretreatment (f) or without HS pretreatment (c to e). In panels a to e, lipoproteins detected by anti-apo B and rhodamine conjugated anti-IgG were distributed over the entire surface of the parasite with focal areas of high intensity. The bright area on the body (arrows in panels c and e) is due to staining of the acetabulum or ventral sucker located on the other side of the worm. In panel f, worm cultured in LDL and reacted with normal goat serum and rhodamine conjugated anti-IgG does not fluoresce (×250).*

Figure 2. *Lipoprotein binding to schistosomula at varying concentrations of HS and LDL. Worms were cultured for 18 hours in varying concentrations of HS (dashed line) and LDL (solid line) as indicated, stained with anti-apo B and rhodamine conjugated anti-IgG, and measured by fluorophotometry. Each point is the average of 3 separate experiments in which readings were taken from 15 different organisms and averaged. The error bars are the standard deviations. The error around the points that do not appear to have error bars is less than the diameter of the points themselves.*



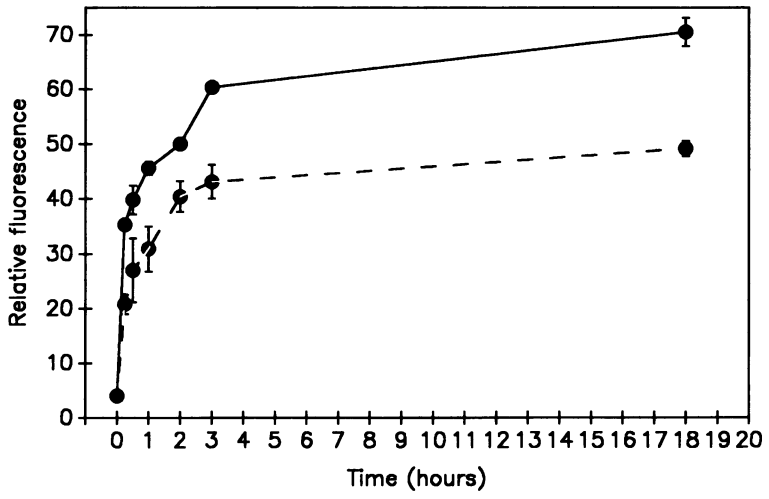


Figure 3. *Time course of lipoprotein binding to schistosomula. Worms were cultured in either 10% HS (dashed line) or 40 μg/300 μl LDL (solid line) in 1% BSA for 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, or 18 hours. The immunostaining, fluorophotometry, and data expression were the same as those described in the legend of Figure 2.*

were washed but not exposed to suramin or heparin did not lose LDL from the surface during 2 hours of incubation (Figure 7). When 2 mM suramin or 10 mg/ml heparin was added to the control washed worms immediately after washing or at the end of 1 or 2 hours of incubation, either drug dissociated the same amount of LDL from the parasites after 30 minutes (Figure 7). The distribution of lipoproteins on suramin and heparin-treated worms was similar to that shown in Figure 1 but of lower intensity. These results suggest that polyanions can partially inhibit the binding of lipoproteins to schistosomula and can partially displace the bound lipoproteins from the parasite.

LDL also inhibited the human anti-schistosomal antibody binding to schistosomula in a concentration-dependent manner (Figure 8). After 3-hour culture in medium with 0 to 20 μg/300 μl LDL, the human anti-schistosomal antibody binding to worms was inhibited progressively. About 61% of anti-schistosomal antibody binding in control was detectable in worms cultured in LDL concentrations above 20 μg/300 μl. The results here parallel the LDL binding shown in Figure 2, because saturation of LDL binding occurred above 20 μg/300 μl as did inhibition of human anti-schistosomal antibody binding.

Electron Microscopy

In most immunocytochemistry experiments, the worms were fixed after incubation with both anti-apo B and peroxidase or gold conjugated anti-IgG. The incubations and washes lasted about 1 hour and were done at RT. In all cases the reaction product was confined to the parasite surface including its invaginations or pits (Figures 9a, b, and 10a to c). There was no evidence of reaction product deeper in the tegument or in the cell bodies connected to the tegument but lying beneath the muscle layers (Figure 9a, b). Thus, there appeared to be no endocytosis of the lipoproteins.

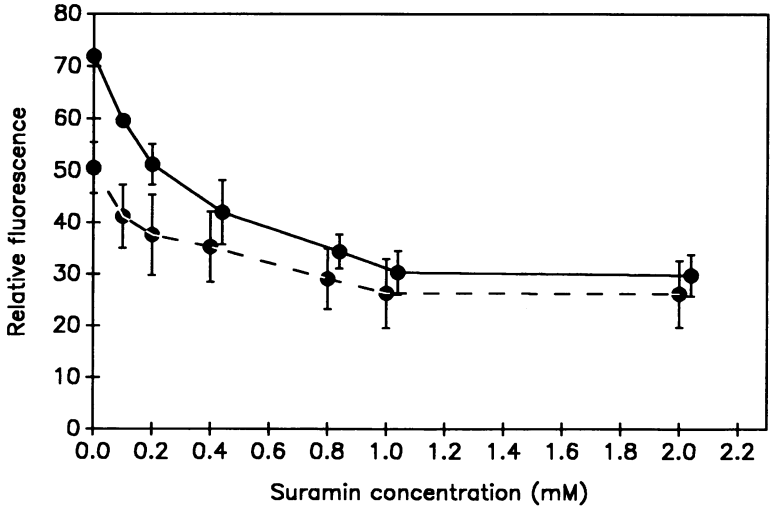


Figure 4. *Inhibition of lipoprotein binding to schistosomula by suramin. Worms were cultured for 18 hours in either 10% HS (dashed line) or 40 μg/300 μl LDL (solid line) in 1% BSA with 0 to 2 mM of suramin as indicated. The immunostaining, fluorophotometry, and data expression were the same as those described in the legend of Figure 2. To prevent the overlapping of the error bars, the points ≥ 0.4 mM were offset.*

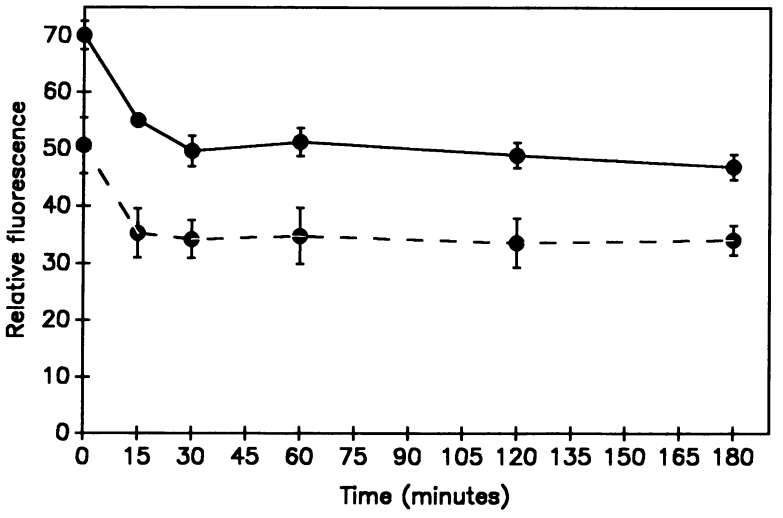


Figure 5. *Dissociation of bound lipoproteins from schistosomula by suramin. Worms were cultured overnight in either 10% HS (dashed line) or 40 μg/300 μl LDL (solid line) in 1% BSA. The next morning, 2 mM suramin was added to each tube for 15 minutes to 3 hours as indicated. The immunostaining, fluorophotometry, and data expression were the same as those described in the legend of Figure 2.*

By immunoelectron microscopy, the appearance of the lipoproteins depended on the concentration of the serum or purified lipoproteins. More reaction product was seen on the parasite surface after incubation in 10% HS than after 1% (Figure 9a, b). After incubation in serum, reaction product was present on the tegumental membrane and surrounded large aggregates of various sizes and vesicular structures (Figure 9a, b). In experiments with purified lipoproteins, the distribution of the reaction product varied depending on the species of lipoprotein used. Thus, after incubation with VLDL there were large numbers of vesicular structures, measuring 53 ± 30 nm, surrounded by reaction product (Figure 10a). After incubation with LDL, the vesicles were smaller, measuring 21 ± 8 nm, but the reaction product was densely heaped on the outer tegumental membrane (Figure 10b), where it formed a 21 nm thick layer (Figure 10d). After incubation with apo B, few vesicular structures or aggregates were seen, but reaction product was more uniformly distributed along the membrane (Figure 10c). After incubation in serum or purified lipoproteins, there were small areas of the membrane not covered by any reaction product (Figures 9a, b, and 10a, b).

Lipoproteins detected by anti-apo B and gold conjugated anti-IgG also appeared as aggregates and vesicular structures surrounded by colloidal gold (Figure 10e). Occasional binding of the colloidal gold to the outer tegumental membrane also was observed (Figure 10e). The control schistosomula cultured in HS or purified lipoproteins reacted with normal goat serum and either peroxidase or gold conjugated anti-IgG had only scant nonspecific staining (Figure 9c).

Discussion

Three major conclusions can be drawn from this study. First, apo B-containing lipoproteins in HS as well as puri-

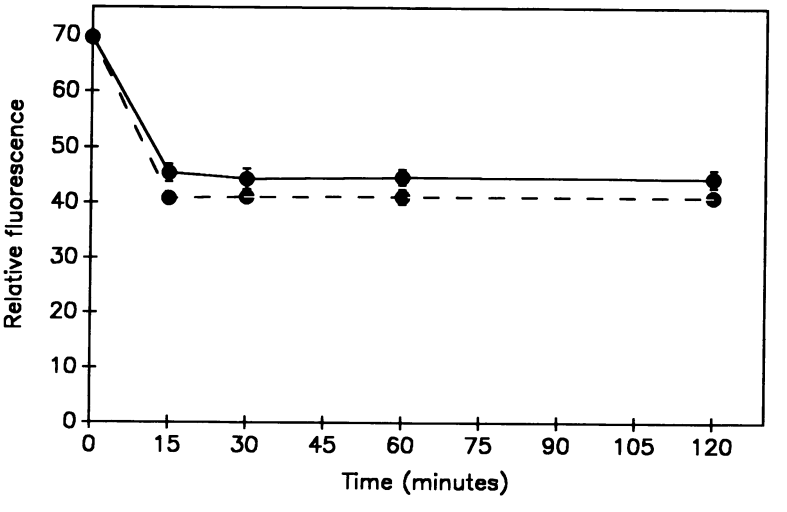


Figure 6. *Dissociation of bound LDL from schistosomula by suramin and heparin. Schistosomula were cultured overnight in 40 μg/300 μl LDL in 1% BSA. The next morning, worms were washed three times in RPMI 1640-PS with 1% BSA, then 2 mM suramin (dashed line) or 10 mg/ml heparin (solid line) was added to each tube for 15 minutes to 2 hours as indicated. The immunostaining, fluorophotometry, and data expression were the same as those described in the legend of Figure 2.*

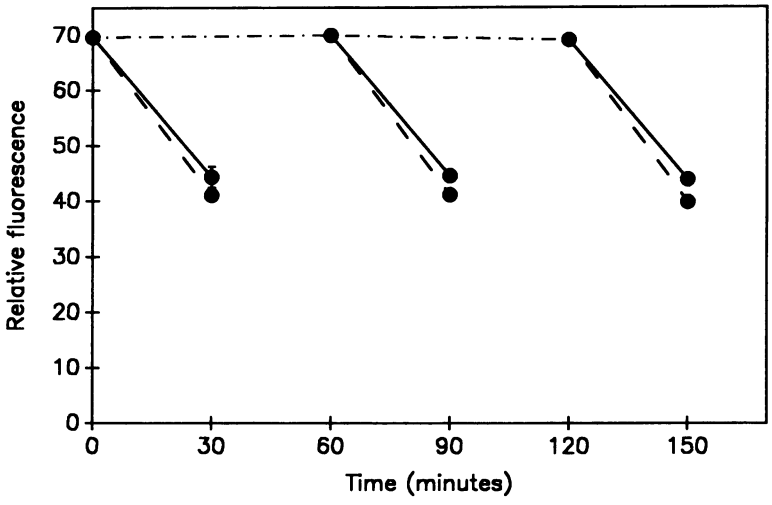


Figure 7. *Dissociation of bound LDL from schistosomula by suramin and heparin. The culture condition and washes were the same as those described in Figure 6. The control worms (dashed-dotted line) were stained immediately after washing at 0 time or after 1 or 2 hours of culture. Alternatively, 2 mM suramin (dashed line) or 10 mg/ml heparin (solid line) was added to washed organisms at 0 time or after 1 or 2 hours of culture. Staining was performed 30 minutes later. The immunostaining, fluorophotometry, and data expression were the same as those described in the legend of Figure 2.*

fied LDL, VLDL, and apo B in defined media bind to the surface of schistosomula of *S. mansoni*. Apo A-I-containing lipoproteins or HDL do not bind. The binding of lipoproteins to worms begins within 15 minutes of exposure to either purified lipoproteins or HS. Second, the binding of LDL to schistosomula is saturable, time dependent, and partially inhibited by suramin. The bound LDL can be dissociated from the parasites by suramin and heparin, suggesting that the LDL binding to worms is in part charge-dependent. Third, the LDL inhibits the binding of human anti-schistosomal antibodies to schistosomula.

By fluorescence and immunoelectron microscopy, lipoproteins in the HS or purified VLDL, LDL, or apo B in defined media bound diffusely to most of the schistosomular membrane (Figures 1a to e, 9a, b, and 10). Ultrastructurally, the lipoproteins in 1% HS and apo B in defined medium were distributed fairly uniformly along the parasite membrane (Figures 9a and 10c). However, when worms were cultured in 10% HS (Figure 9b) or defined media with VLDL or LDL (Figure 10a, b) aggregates of various sizes were seen. The brighter punctate areas seen by fluorescence probably corresponded to these aggregates (Figure 1a–c, e). The punctate fluorescent areas were not seen in every experiment, however (Figure 1d), suggesting that the reagents may have aggregated before binding to the parasite. Finally, by both light and electron microscopy the bound LDL appears to remain on the parasite surface for a long period of time.

The diffuse surface distribution of lipoprotein without endocytosis is very different from the fate of lipoproteins bound to human cells such as fibroblasts and macrophages. In human fibroblasts, LDL binds to LDL-receptor located in the coated pits, which are internalized rapidly (minutes) in coated vesicles, and delivered to lysosomes where the apolipoprotein is degraded and cholesterol esters are de-esterified. The free cholesterol in turn suppresses *de novo* synthesis of cholesterol.[17-19] In schistosomula, bound lipoproteins are not endocytosed (Figures 1a to e, 9a, b, and 10). In fact, endocytosis has never been demonstrated on the surface of the parasite, al-

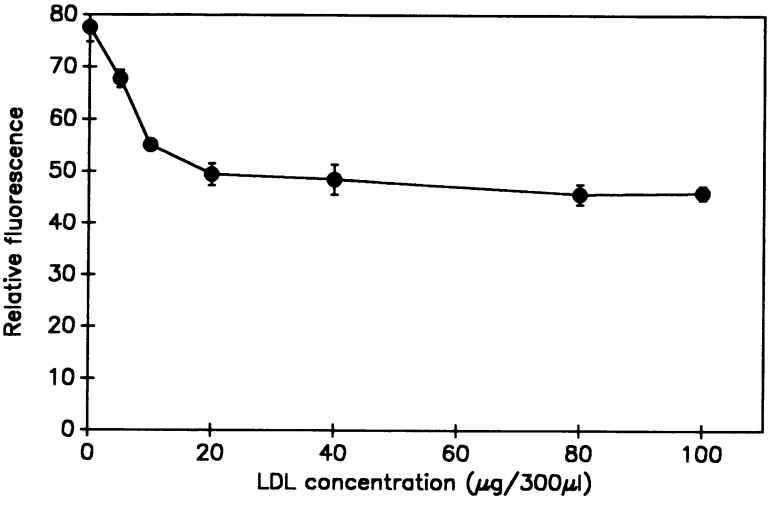


Figure 8. *Inhibition of human anti-schistosomal antibody binding to schistosomula by LDL. Worms were cultured in 5, 10, 20, 40, 80, or 100 μg/300 μl LDL in 1% BSA for 3 hours, incubated with human anti-schistosomal antiserum (experiment group) or normal HS (control group) for 30 minutes, stained with fluorescein conjugated anti-IgG, and measured by fluorophotometry. Each point expressed here is the average of three separate experiments. Each experimental value was obtained by subtracting the fluorescence reading of the control group from that of the corresponding experimental group, and each fluorescence reading is the average of readings of 15 different organisms. The error bars are the standard deviations. The error around the points that do not appear to have error bars is less than the diameter of the points themselves.*

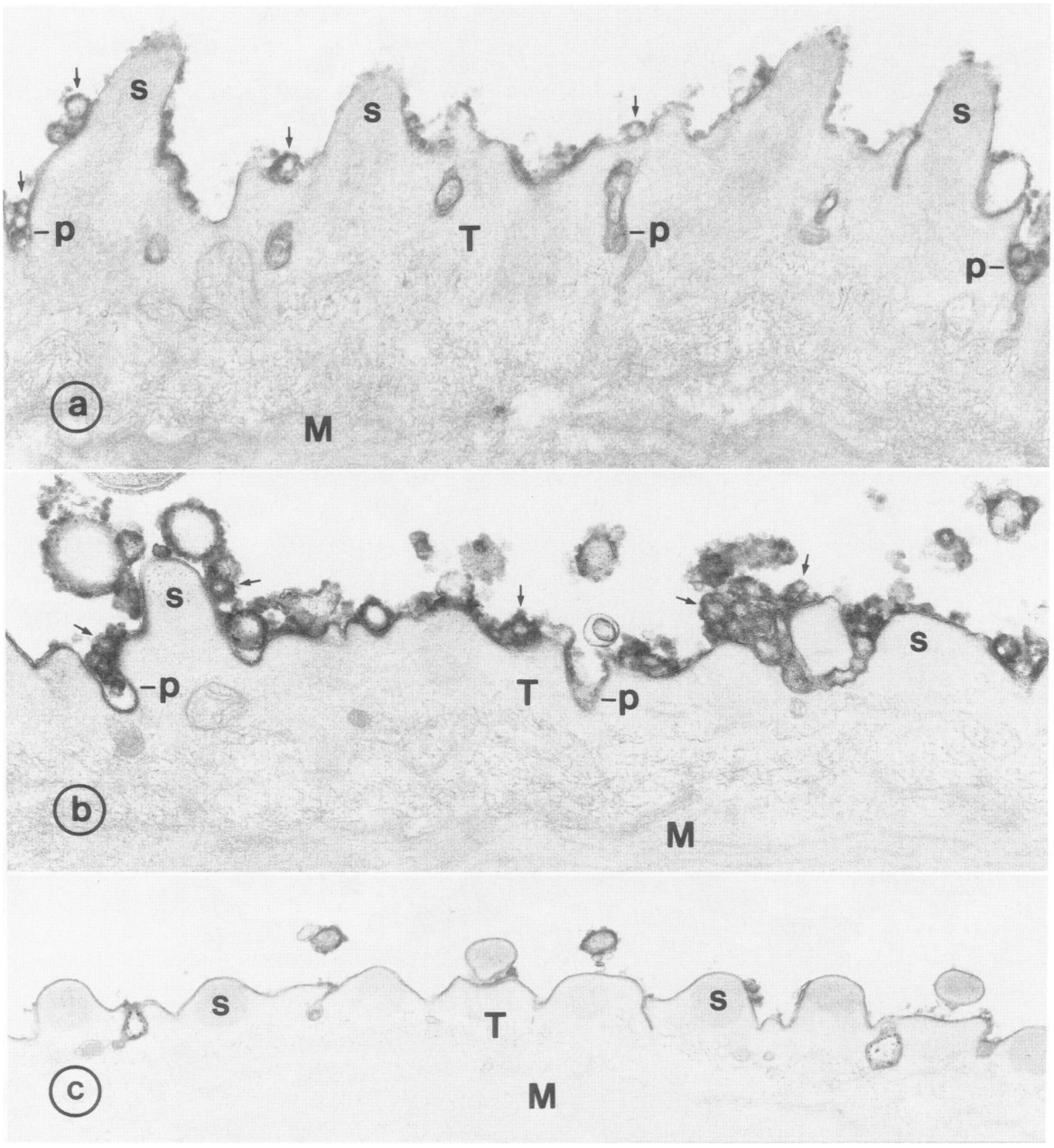


Figure 9. *Transmission electron micrographs of schistosomula cultured in* (a) *1% HS, and* (b) *and* (c) *10% HS for 18 hours. In panels a and b, lipoproteins detected by anti-apo B and peroxidase conjugated anti-IgG appear as single or clumped vesicular structures (arrows) found both on the spines (s) and in the pits (p). Reaction product is on the tegumental membrane and surrounds the vesicular structures. Controls reacted with normal goat serum and peroxidase conjugated anti-IgG have scant reaction product on the surface (c). T, tegument; M, muscle (a, ×30,000; b, ×36,000; c, ×23,000).*

though many multivalent ligands such as lectins and antibodies bind to it.[20] Thus, any contribution that bound lipoproteins make to parasite lipid metabolism must take place on the worm surface. Further, worm sterol metabolism differs from the human in that the worm cannot synthesize cholesterol *de novo,*[21] so there can be no suppression of this pathway. Whether the parasite can deesterify cholesterol ester, degrade apolipoprotein, or use the phospholipid in the lipoprotein are unknown. It has been suggested, however, that exposure to serum alters parasite lipid composition.[11]

In the human fibroblast system, lipoprotein binding to the LDL receptors is well characterized. Apo B or apolipoprotein E binds to the receptor through charge interactions.[22-27] The nature of the lipoprotein binding to schistosomula is not totally clear. Because apo B-containing lipoproteins, LDL and VLDL, and pure apo B, but not HDL, bind to the parasites, most binding should be due to apo

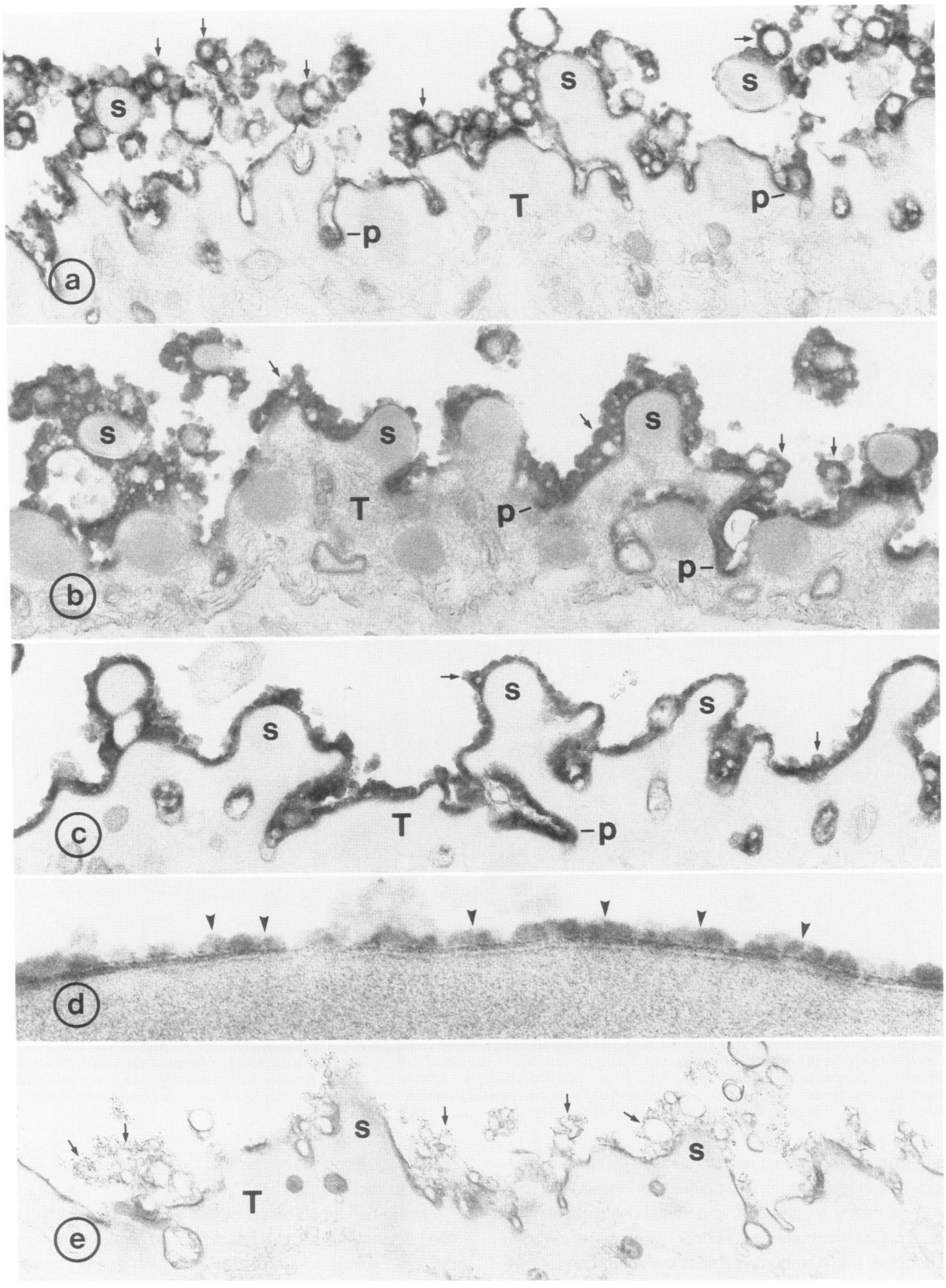


Figure 10. *Transmission electron micrographs of schistosomula cultured for 18 hours in 1% BSA with* (a) *15 μg VLDL,* (b) *37.5 μg LDL,* (c) *7.5 μg apo B, and* (d) *180 μg LDL, and* (e) *in 50% HS. In panels a to c, lipoproteins detected by anti-apo B and peroxidase conjugated anti-IgG appear as aggregates and vesicular structures (arrows) surrounded by reaction product. Note that more vesicular structures are present on worms cultured in VLDL than LDL and in LDL than apo B. Reaction product is also found on the outer leaflet of outer tegumental membrane (arrowheads in panel d). In panel e, lipoproteins detected by anti-apo B and gold conjugated anti-IgG appear as vesicles surrounded by colloidal gold (arrows). T, tegument; s, spine; p, pit (a to c, ×30,000; d, ×168,000; e, ×28,000).*

B. Lipoprotein binding to worms is probably partly ionic, because the binding is inhibited by suramin (Figure 5) and the bound LDL can be released from the schistosomula by suramin and heparin (Figures 6 and 7). Heparin, suramin, and dextran sulfate release LDL from the high-affinity receptors on human fibroblasts by binding to the positively charged domain of apo B via ionic interaction.[25] In other systems, suramin (2 mM) dissociates about 95% of ^{125}I-LDL from bovine adrenal receptor[28] and heparin (10 mg/ml) dissociates about 80% of bound LDL from fibroblast receptor.[25] Here the same concentrations of suramin and heparin release about 41 and 36% of LDL from schistosomula, respectively (Figure 7). Thus, at least some of the lipoprotein binding to worms is mediated by charge but whether all the binding is so mediated must be determined. However, other polyanions such as chondroitin sulfate B also produce only partial inhibition in the human system.[25] Finally, the kinetics in the human and schistosome are similar, namely the lipoprotein binding is rapid and saturable at approximately the same serum concentration.

Bogitsh has shown that 0.7 mM suramin (1 mg/ml) is toxic to 12-day-old larval schistosomes.[29] The drug induces ultrastructural changes in the parasite gut beginning at 8 hours and progressing to 48 hours when the worms die. The effects of suramin on lipoprotein binding shown here are probably not due to a systemic effect of the drug on the parasite because the parasites were viable throughout the longest exposure to the drug, 18 hours, and the dissociation of bound LDL was rapid (15 minutes) compared with the development of toxicity (>8 hours). Further, the gut is largely nonfunctional in the newly transformed schistosomula used here as opposed to 12-day organisms. We do not know whether heparin is toxic to the parasite at other stages.

Rumjanek and coworkers[12] have identified 45 kd protein doublets that they claim are LDL receptors on schistosomula.[13] The expression of these doublet proteins on the parasite surface depends on exposure to HS.[12,30] Moreover, the expression occurs when a dialysis membrane separates the parasite and HS, suggesting a low molecular weight for the inducing moiety.[30] The 45 kd proteins bind to LDL during affinity chromatography specifically and in a calcium-dependent manner.[13,30] The present experiments disagree with a central prediction of these authors' work, namely that LDL binding should not occur in the absence of HS. Here purified LDL, VLDL, and apo B in defined medium all bind to worms never exposed to HS (Figures 1c to e, 10a to d). However, the criterion used by Rumjanek et al[12,13,30] to define surface expression, namely the ability to iodinate the 45 kd proteins, may not reflect the exposure of a binding site on the proteins to the ligand in the medium. It remains to be seen whether these doublet proteins are the receptors or are the only receptors, for LDL, what the specificity of the binding is, and whether binding induces specific physiologic effects in the parasite.

The result that LDL binding to worms can block antigenic sites and thus inhibit the human anti-schistosomal antibody binding agrees with the work of Rumjanek and McLaren,[11] who showed that schistosomula cultured in HS have greater protection against both complement-mediated and eosinophil-mediated cytotoxicity. However, parasites in delipidated HS do not.[12] Evidence that the lipoprotein binding may be an immune protective mechanism on the schistosomes is also supported by other experimental systems, in which lipoproteins have been shown to inhibit the attachment of complement to membranes and complement-mediated lysis.[31,32] Furthermore, monomeric C9 contains a domain homologous to the LDL receptor domain that binds to apo B.[17,23,33,34] Therefore, apo B in LDL or VLDL on the parasites might react with C9 and block its insertion into the membrane.[35] Thus, lipoproteins, especially the LDL and VLDL, may help the young schistosome to evade antibody and complement-dependent immune effector mechanisms in the host.

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