Brief Definitive Report

DICHOTOMY IN THE TISSUE ORIGIN OF SCHISTOSOME ACQUIRED CLASS I AND CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS

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An intriguing property of schistosome parasites is their surface acquisition of host glycolipids and glycoproteins (1). By masking antigenic determinants on the tegument of the organism, these host molecules may allow the parasite to evade vertebrate immune responses (1, 2). An important set of molecules acquired by schistosomes during murine infection are class I and class II products of the major histocompatibility complex (MHC) (3–5). These glycoproteins can be detected on schistosomula and adult worms *Schistosoma mansoni* during their development in mice (3–6) and have serologic properties and electrophoretic mobilities indistinguishable from the class I and class II MHC molecules expressed on host cells (7). Furthermore, schistosome acquired class I antigens exist in a form recognizable by allogeneic T lymphocytes and able to restrict the recognition of other determinants (haptens, minor histocompatibility antigens) on the parasite surface (8, 9).

Neither the tissue origin nor mechanism of acquisition of schistosome MHC antigens have been defined. Only minimal uptake of these molecules was observed after extensive co-cultivation of schistosomula with lymphoid cells, and no acquisition was observed from mouse serum (3). Caulfield and co-workers (10) observed membrane fusion between cultured blood cells and schistosomula and have speculated that MHC integral membrane glycoproteins might pass from host cells to the outer membrane of the parasite via these fusion sites.

In this study, we have used radiation bone marrow chimeras to explore the origin of schistosome class I and II MHC antigens and, in particular, to determine whether these molecules are acquired from hemapoietically derived cellular elements. Our results indicate that while class II (Ia) antigens are acquired by schistosomes from bone marrow-derived cells, the class I (H-2K and D) antigens expressed on the surface of these worms originate from a different, nonhemapoietic tissue source.

Materials and Methods

Mice. C57BL/10Sn (B10) (H-2^b), B10.A (H-2^a), (B10 × B10.A) or (B6 × A/J)F_1 (H-

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$2^{b/a}$) male mice (2–5 mo old) were obtained from The Jackson Laboratory, Bar Harbor, ME or from the National Cancer Institute breeding facility. Chimeras are designated as bone marrow donor → irradiated recipient with A and B referring to mice bearing either the H-2^a or H-2^b haplotypes, respectively. Recipient mice for A → A × B, B → A × B, A × B → A, A × B → B chimeras were irradiated with 950 rad from a ^{137}Cs source and reconstituted 2–6 h later with 1.5×10^7 bone marrow cells pretreated with rabbit anti-mouse brain serum and complement as previously described (11). The animals were used 2–3 mo later. The hemapoietic cells of mice surviving lethal irradiation and reconstitution for this period have previously been shown to be essentially all of donor bone marrow origin (11). This was confirmed in most experiments by H-2 typing (by indirect immunofluorescence) of spleen cells obtained at the same time as the test parasites.

Lung Stage Schistosomula. Three to five mice in each group were injected intravenously with $3–10 \times 10^3$ *S. mansoni* schistosomula prepared by mechanical transformation (12). Six days later (a time at which we have observed MHC antigen expression by the parasite to be optimal), schistosomula were recovered from the lungs of the animals and partially purified by centrifugation through Percoll (7, 12). The parasites were then washed twice with Hanks' balanced salt solution containing 5% normal rabbit serum (HBSS-NRS) and resuspended in that medium to a concentration of $\sim 10^3$ schistosomula/ml.

Alloantibodies. Alloantibodies directed against the H-2^k haplotype were used to detect antigens encoded by the H-2^a recombinant haplotype ($K^kI^kD^d$). Monoclonal anti-K^k antibody (clone 11.4) was obtained from Becton, Dickinson & Co., Mountain View, CA. Monoclonal anti-I-E^k antibodies 17-3-3S and 14-4-4 in ascites form were generously provided by Dr. David Sachs, National Cancer Institute and used in a 1:1 ratio. Anti-Ia^k alloantiserum (A.TH anti A.TL) was obtained from the Transplantation Division, National Institute of Allergy and Infectious Diseases, and extensively absorbed with C57BL/6J spleen cells to remove anti-Ia^b activity. Normal serum was obtained from C57BL/6J mice.

Detection of Alloantigens. Lung-stage schistosomula (0.1 ml) were incubated in an equal volume of a 1:10 dilution of alloantibody or normal rabbit serum (in HBSS-NRS) for 1 h at 37°C. The parasites were then washed three times by centrifugation in HBSS-NRS and incubated for an additional 30 min at room temperature in a 1:40 final dilution of fluorescein-conjugated rabbit anti-mouse IgG (heavy and light chain) (Cappel Laboratories, Cochranville, PA). After three more washes, the schistosomula were examined for tegumental fluorescence using a Leitz Ortholux fluorescence microscope (E. Leitz, Inc., Rockleigh, NJ) fitted with an MPV compact photometer (7, 13). The fluorescence emission of a 560 μm^2 area of each parasite and an adjacent background field was then quantitated and each parasite examined visually. 10 or more parasites were analyzed in each sample and the mean and standard error of the fluorescence values (worm emission − background emission) calculated. Samples were scored as positive by visual examination if the majority of the worms showed tegumental fluorescence distinct from that of control worms incubated in normal mouse serum.

Results

Schistosomula recovered from the lungs of F_1(A × B) mice reacted strongly (as judged by both visual examination and fluorometry) with the anti-K^k monoclonal antibody whereas worms obtained from control H-2^b mice were negative (Table I). Similarly, positive immunofluorescence was observed with worms recovered from A → F_1, B → F_1, and F_1 → A chimeras. In contrast, in all three experiments performed, schistosomula recovered from F_1 → B bone marrow chimeras were uniformally negative. Thus, in these experiments the expression of K^k class I antigen was determined by the haplotype of the recipient rather than the cell donor.

The expression of class II antigens by lung schistosomula recovered from chimeras was studied using an alloantiserum (A.TH anti-A.TL) directed against the entire I region and a second reagent consisting of a 1:1 mixture of two monoclonal antibodies specific for products of the IE subregion. Both reagents

TABLE I

Expression of K^k Alloantigen by Lung-stage Schistosomula Recovered from Parental and Chimeric Mice

Worm Donors	Experiment 1		Experiment 2		Experiment 3	
	Visual	Fluorescence units*	Visual	Fluorescence units*	Visual	Fluorescence units*
B	−	5.6 ± 0.8	−	9.1 ± 1.4	−	11.1 ± 1.9
F_1(A × B)	+	20.8 ± 3.4	+	19.2 ± 3.5	+	24.5 ± 2.7
B → F_1	+	14.8 ± 2.3	+	17.1 ± 2.3		ND
A → F_1	+	12.5 ± 1.8	+	18.8 ± 3.1		ND
F_1 → B	−	3.9 ± 0.3	−	8.3 ± 1.4	−	12.2 ± 1.6
F_1 → A	+	17.0 ± 3.8	+	29.6 ± 3.5	+	23.0 ± 2.6
Control‡ (normal mouse serum)	−	4.3 ± 0.2	−	8.1 ± 0.9	−	14.0 ± 0.7

* Mean ± standard error of fluorescence readings (parasite emission − background field emission) on 10 or more worms per sample as detected by monoclonal anti-K^k antibody. Quantative variations between experiments reflect different voltage and background settings used on fluorometer. ND, not done.

‡ Mean ± standard error of normal serum control readings performed on all worm preparations within each experiment.

TABLE II

Expression of Ia^k Alloantigens by Lung-stage Schistosomula Recovered from Parental and Chimeric Mice*

Worm donors	Visual	Fluorescence units‡
B	−	5.1 ± 0.4
F_1(A × B)	+	10.8 ± 0.4
B → F_1	−	5.5 ± 0.4
A → F_1	+	15.1 ± 3.2
F_1 → B	+	9.3 ± 2.4
F_1 → A	+	9.1 ± 1.4
Control (normal mouse serum)	−	4.3 ± 0.2

* Detected with B6-absorbed A.TH anti-A.TL antisera.

‡ Mean ± standard error of fluorescence readings (parasite emission − background field emission) on 10 or more worms per sample.

reacted positively with worms from F_1(B × A) mice but not with control parasites from H-2^b animals (Tables II and III). Similarly, both alloantibody preparations recognized schistosomula from A → F_1 chimeras yet failed to react with worms from B → F_1 mice. The latter observation indicated that the expression of class II alloantigens by schistosomula is determined by the haplotype of the donor bone marrow.

Divergent results were obtained analyzing class II antigen expression by parasites from F_1 → parent chimeras. Whereas worms derived from F_1 → A animals reacted with both anti-I region reagents, parasites recovered from F_1 → B bone marrow chimeras stained positively with the anti-A.TL alloantiserum (Table II) but were negative or borderline positive when tested with the anti-I-E^k monoclonals (Table III).

Discussion

The results of our experiments indicate that in murine chimeras schistosomes acquire class I (K region) MHC antigens exclusively from a nonhemapoietic tissue source, whereas the class II (I region) antigens expressed by the parasites are of bone marrow origin. Previous studies have suggested that MHC molecule

TABLE III

Expression of I-E^k Alloantigens by Lung-stage Schistosomula Recovered from Parental and Chimeric Mice*

Worm donors	Experiment 1		Experiment 2		Experiment 3	
	Visual	Fluorescence units‡	Visual	Fluorescence units‡	Visual	Fluorescence units‡
B	−	7.4 ± 1.4	−	12.6 ± 1.8		ND
F_1(A × B)	+	14.0 ± 1.9	+	24.2 ± 3.3	+	61.6 ± 7.6
B → F_1	−	5.9 ± 1.0		ND	−	47.3 ± 5.5
A → F_1	+	11.5 ± 1.2		ND	+	80.9 ± 8.9
F_1 → B	±§	9.3 ± 1.5	−	11.3 ± 0.4		ND
F_1 → A	+	11.3 ± 1.1	+	16.7 ± 3.0		ND
Control (normal mouse serum)	−	8.1 ± 0.9	−	13.0 ± 0.7	−	41.4 ± 5.4

* Detected with a mixture of anti-I-E^k monoclonal antibodies.

‡ Mean ± standard error of fluorescence readings (parasite emission − background field emission) on 10 or more worms per sample. Quantitative variations between experiments reflect different voltage and background settings used on fluorimeter.

§ A proportion of the worms in this sample showed weak positive fluorescence.

acquisition by schistosomes occurs principally in the lungs since these antigens are most readily detected on parasites recovered from this site (3–6). For this reason and for the purpose of eliminating MHC antigen acquisition from other tissue sites, the parasites studied in the present experiments were derived from in vitro prepared (mechanical) schistosomula injected by the intravenous route into the lungs rather than by natural percutaneous infection. In the lungs, the parasites reside principally in microcapillaries, where presumably the only host elements they encounter are plasma, blood cells, and vascular endothelium.

Despite the presence of bone marrow-derived class I antigen-bearing blood cells in their environment, schistosomula were found to acquire these molecules from a nonhemapoietic source (Table I). This result suggests either that the parasite is able to acquire class I antigens only from nonhemapoietic donor cells or alternatively that in the lungs nonhemapoietically derived class I antigens are presented to schistosomula at a higher concentration than bone marrow-derived antigen. The former explanation seems unlikely since the same parasites that fail to express bone marrow-derived class I antigens acquire class II antigens from a hemapoietic source, presumably blood cells. Thus, it is likely that bone marrow-derived class I antigens are not observed on schistosomula because of competition by nonhemapoietically derived antigen.

The two most likely sources of non–bone-marrow-derived class I antigens acquired by lung worms are the plasma and the endothelium. Soluble class I MHC products have been detected in mouse sera (14). However, the tissue origin of these molecules has not been defined. Regardless of their origin, because of their extremely low concentration in blood (14) and the failure of our previous attempts to demonstrate MHC product acquisition by schistosomula cultured in mouse sera (3), it is unlikely that class I antigens are derived from a humoral source. Since schistosomula are in direct contact with capillary endothelium during their residence in the lungs, it is more reasonable to propose that endothelial cells, which are of nonhemapoietic origin, serve as the donor tissue for the class I antigens acquired by the parasite.

In contrast to schistosome acquired class I antigens, the class II antigens

expressed on the parasite appear to be derived exclusively from bone marrow-derived tissue. Thus, whereas worms recovered from B → F_1 and A → F_1 mice both express K^k alloantigens (Table I), only schistosomula recovered from A → F_1 chimeras display detectable I^k alloantigens (Tables II and III). The expression of I^k antigens by parasites derived from F_1 → parent chimeras was less clear-cut. Whereas worms recovered from F_1 → A chimeras, as expected, reacted positively with both the anti-Ia^k and anti-I-E^k test reagents, larvae recovered from F_1 → B chimeras reacted positively with the anti-Ia^k alloantiserum (Table II) but weakly or not at all with the anti-I-E^k antibodies. This discrepancy probably reflects the weak binding activity of the epitope-specific monoclonal anti-I-E^k vs. the polyvalent anti-Ia^k antibodies. Alternatively, efficient acquisition of the IE subregion product by the parasite may depend on MHC compatibility between recipient and donor bone marrow.

Since Ia antigens are expressed principally on bone marrow-derived lymphocytes and cells of the monocyte series, it is likely that these cells serve as the donors for schistosome acquired class II molecules. Although endothelial cells have recently been reported to express class II antigen when stimulated with interferon (15), our data are not consistent with a role for these cells as donors of schistosome acquired Ia molecules.

The finding that schistosomes derive class I and class II MHC antigens from different tissue sources supports previous data suggesting that the acquisition of these host membrane glycoproteins is selective and involves specific interactions between host cells and the parasite (3, 5). Whether, as hypothesized earlier, the uptake of MHC products occurs as a result of host cell-parasite membrane fusions (10) or by absorption of antigen-bearing host vesicles (7, 16), has not been clarified. However, the results reported here suggest that endothelial cells and cells of the lymphocyte-monocyte lineage should be studied as probable donors of these host molecules.

Summary

Schistosoma mansoni schistosomula recovered from the lungs of mice have previously been shown to express host-derived class I and class II major histocompatibility complex (MHC) antigens. To investigate the tissue origin of parasite-acquired MHC products, lung-stage schistosomula were obtained from a series of parent → F_1 and F_1 → parent bone marrow chimeras and the parasites typed by immunofluorescence for the presence of haplotype-specific K region and I region MHC determinants. The results of these experiments indicated that, despite their intravascular residence in the host, schistosomula derive all of their class I antigen from a nonhemapoietic tissue source. In contrast, the class II antigens expressed on the surface of schistosomula were found to originate from bone marrow-derived donor cells. These results support the hypothesis that MHC product acquisition by schistosomes involves selective and specific interactions with host tissue and, in the case of class I antigens, suggest that the endothelium may be a major site of host molecule uptake for the parasite.

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