Differential V β T-Cell Receptor Usage during Chronic Experimental Schistosomiasis Corresponds with Distinct Pathological Presentations

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CBA/J male mice with chronic *Schistosoma mansoni* infections display either moderate splenomegaly syndrome (MSS) or hypersplenomegaly syndrome (HSS). As MSS and HSS mice differ in several immunologic characteristics, we investigated T-cell receptor V β usage. The groups had significantly different expression of several V β s, suggesting a relationship between the T-cell repertoire and schistosomiasis pathology.

Chronic (20-week) infection of CBA/J male mice with *Schistosoma mansoni* results in development of two distinct pathologies that closely parallel those of humans with chronic schistosomiasis (4). Moderate splenomegaly syndrome (MSS) is analogous to the less severe human intestinal form of the disease. Hypersplenomegaly syndrome (HSS) in mice resembles the more severe human hepatosplenic disease form and is characterized by periportal liver fibrosis, extreme splenomegaly, profound anemia, cachexia, ascites, and thymic atrophy.

In addition to these differences in pathology, mice with MSS and HSS disease forms also differ extensively in regard to their immune responses. HSS mice produce more tumor necrosis factor alpha and less antigen-specific interleukin 10 (1, 2), produce lower levels of Th1-associated antibody isotypes specific for schistosome antigens (3, 7), and display higher levels of cell activation markers (3) than MSS mice. However, the most compelling immunologic evidence linking this murine model to responses in human schistosomiasis is the parallel production of certain anti-schistosome soluble egg antigen (SEA) antibodies in mice and in humans with the less severe disease form (6). These distinct antisoluble egg antigen antibodies express characteristic idiotypes (Id) that are produced by MSS (but not HSS) mice and by humans with the intestinal disease form (but not those with the hepatosplenic disease form). These Id also are also immunologically stimulatory, inducing T-cell proliferation and differential cytokine production (5, 7). Furthermore, anti-idiotypic sera produced against Id from human patients with the intestinal form of the disease cross-react with Id produced from the sera of MSS mice (3, 6). We recently demonstrated that injection of neonatal mice with this cross-reactive Id alters future immune responses and the development of pathology in mice when they are subsequently infected with *S. mansoni*. We believe this may in part explain the pathological differences in disease development between persons born to mothers in areas where schistosomiasis is endemic versus those

born where the disease is nonendemic (8, 9). The presence of T-cell-stimulatory cross-reactive Id could alter the developing immune response, perhaps by their effect on the T-cell repertoire.

To address this possibility, we investigated whether MSS and HSS animals display differential T-cell receptor (TCR) V β usage. As the V β gene product expressed by a given TCR is a major determinant of the antigen specificity of that T cell, differences in V β TCR usage could imply that different antigens are being predominantly recognized or that different regulatory T cells are engaged in the differentiation of MSS and HSS mice. Male CBA/J mice used in these experiments were obtained from The Jackson Laboratory, housed in the American Association for Accreditation of Laboratory Animal Care-approved animal care facilities of the Centers for Disease Control and Prevention, and cared for in compliance with institutional guidelines and federal regulations. Mice were infected by subcutaneous injection of 45 cercariae of a Puerto Rican strain of *S. mansoni* that had been maintained in *Biomphalaria glabrata* snails. At 20 weeks of infection, animals were sacrificed and classified as having MSS or HSS, based on percent spleen body weight and gross pathological appearance. Single-cell suspensions were made from the spleens and erythrocytes were lysed with a red blood cell lysing buffer (Sigma Chemical Co., St. Louis, Mo.). Spleen cells were stained with a panel of fluorescein isothiocyanate-conjugated anti-TCR V β antibodies (catalogue no. 0143KK; PharMingen, San Diego, Calif.) and phycoerythrin-conjugated anti-CD3 antibodies (catalogue no. 01085B; PharMingen) according to previously published methods (3). Flow cytometric data were acquired with a FACScan flow cytometer (Becton-Dickinson, San Jose, Calif.) and analyzed with Cell Quest software (Becton-Dickinson).

TCR V β usage was calculated as the percent CD3⁺ cells positive for the various TCR V β markers. MSS and HSS mice (two to five mice per group) from five different infection dates were used to alleviate any effect that may be peculiar to a given infecting inoculum. An uninfected control mouse was included in each experiment. Percentages of the various TCR V β gene products expressed on CD3⁺ cells were averaged, and

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FIG. 1. Mean percent of CD3 cells positive for various TCR V β s in spleens of noninfected (grey bars, $n = 5$), MSS (white bars, $n = 17$), or HSS (black bars, $n = 15$) mice. An asterisk indicates statistical significance ($P < 0.05$) in group means as determined by ANOVA.

means were compared by analysis of variance (ANOVA) with the InStat II statistics package (GraphPad Software, San Diego, Calif.). Results are presented in Fig. 1. We found significant differences ($P < 0.05$) in 11 of 15 V β TCRs tested, with HSS spleen cells making up a larger percentage of 8 V β TCRs and MSS spleen cells making up a larger percentage of 3 V β TCRs. Interestingly, for almost every specific V β TCR tested, the percentage of MSS spleen cells more closely resembled that of the uninfected mouse spleen cells than that of the HSS spleen cells. These findings are consistent with those of Vella and Pearce (10), who found that TCR V β usage among CD4 $^{+}$ cells of 9-week-infected C57BL/6 mice did not differ significantly from that of uninfected C57BL/6 mice. One experiment in this study, in which cells were stained with anti-TCR V β antibodies and anti-CD4 antibodies, showed a pattern of TCR V β usage in MSS and HSS mice that was similar to, albeit less

dramatic than, that obtained when total T cells were assessed (data not shown).

We also performed regression analysis comparing the degree of splenomegaly in chronically infected mice with the percent CD3 $^{+}$ cells that were positive for the various V β TCRs (Table 1). There were significant correlations between the percent spleen body weight of individual HSS mice and the percent positive cells for all 11 V β TCRs that showed differences between means of uninfected, MSS, and HSS groups of mice by ANOVA as well as 1 additional V β TCR. As these correlation results are somewhat predictable from the ANOVA, we also performed correlative analyses within the HSS group alone and found a significant relationship between the degree of splenomegaly and the percent positive cells for six V β TCRs. There was a significant positive correlation for four V β s and a significant inverse correlation for the other two (Table 1). Positive correlations suggest preferential expansion of certain V β $^{+}$ T cells in response to antigenic stimulation. However, it is not clear whether the negative correlations represent a selective down-regulation, or even elimination, of T cells positive for these V β TCRs or are simply the result of a more prominent expansion by T cells expressing other V β TCRs.

The variation of TCR V β usage between MSS and HSS animals indicates that a different T-cell repertoire develops in MSS versus HSS mice and, by implication, recognition of different antigen sets by these groups of chronically infected animals. We are currently addressing this question. We also do not know whether the differences in TCR V β usage of HSS mice are a cause or effect of the severe pathology in these animals or whether the thymic atrophy observed in HSS mice is related to the altered TCR V β usage of these animals. These questions are the topic of ongoing research.

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TABLE 1. Correlations between percent spleen body weight and percent V β -positive CD3 cells^a

V β specificity	Regression analysis of:			
	All infected mice ($n = 32$)		HSS mice only ($n = 15$)	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
2	0.6168	0.0002	0.5627	0.0290
3	0.5959	0.0003	0.2721	0.6265
4	0.6711	<0.0001	0.6847	0.0049
5.1, 5.2	0.6307	0.0001	0.6354	0.0196
6	0.5975	0.0003	0.4273	0.1122
7	0.1887	0.3092	0.1409	0.6321
8.1, 8.2	0.6870	<0.0001	0.6769	0.0156
8.3	-0.6249	0.0001	-0.4118	0.1272
9	-0.0700	0.7081	-0.2075	0.4580
10b	-0.6742	<0.0001	-0.5617	0.0293
11	0.6417	<0.0001	0.3196	0.2455
12	0.5166	0.0025	0.3861	0.1552
13	0.0795	0.6654	0.1800	0.5209
14	-0.6870	<0.0001	-0.6482	0.0122
17a	0.6135	0.0005	0.4541	0.1191

^a Boldface values are indicative of significant correlations.

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