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Immunology

Modulation of *Schistosoma mansoni* egg-induced granuloma formation: *I-J* restriction of T cell-mediated suppression in a chronic parasitic infection

(suppressor T lymphocytes/immune regulation/major histocompatibility complex/congeneic mice)

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ABSTRACT **Newly formed hepatic granulomas around *Schistosoma mansoni* eggs become progressively smaller during the chronic (≥15 weeks after infection) phase of the disease. This reduction in granuloma size, termed "modulation," is known to be caused in part by a T lymphocyte that can adoptively transfer modulation to 6-week-infected mice. The present study examines a possible role for the *I-J* locus in regulating the suppressor T lymphocyte aspects of modulation. Adoptive transfer between congeneic B10.A(3R) and B10.A(5R) mice (differing at the *I-J* locus) indicated that optimal suppression is dependent upon homology at the *I-J* locus. *In vivo* treatment of chronically infected mice with microliter amounts of antiserum specific for the recipient's I-J determinant blocked modulation during chronic infection and prevented adoptive transfer of suppression to 6-week-infected mice. The *in vivo* regimen of anti-I-J had no effect on anti-schistosomal egg antigen titers during chronic infection. These results demonstrate an *I-J* restriction for suppression. It appears that the suppressor T lymphocyte circuit responsible for this aspect of modulation requires an I-J-positive lymphocyte.**

In the mouse, *Schistosoma mansoni* produces a chronic disease state in response to the constant production of eggs by adult worm pairs (1). A large number of these eggs do not reach the intestinal lumen; instead, they are carried to the liver via the portal blood flow where they lodge in the presinusoidal capillaries and induce granulomatous reactions (1). The granulomatous response is primarily a delayed type, T lymphocyte-mediated response (1, 2) to soluble egg antigens (SEA) released by the schistosome egg (3). Maximal granuloma size is observed at 8 weeks after infection (4), which is 3–4 weeks after egg production begins. Newly formed granulomas observed after the eighth week of infection become progressively smaller as the length of infection increases (5–7). The phenomenon of decreased granuloma size during chronic infection has been termed "modulation" (6, 8).

The modulatory effect can be transferred from chronically infected mice (≥15 weeks duration) to 6-week-infected mice with spleen cells (9, 10) but not serum (9). The adoptive transfer of modulation is detected by a decrease in granuloma size 2 weeks later (i.e., 8 weeks after infection). The cell mediating this suppressive effect is sensitive to *in vitro* treatment with anti-Thy 1.2 and complement (10–12) or anti-Ia and complement (10) prior to transfer and produces antigen-specific suppression (10). Adult thymectomy at 6 weeks after infection (13) or *in vivo* treatment of chronically infected mice with low doses of cyclophosphamide (12), rabbit anti-mouse thymocyte antiserum, or hydrocortisone acetate (13) partially blocks the expression and adoptive transfer of modulation. These observations have been interpreted to indicate a role for an antigen-specific suppressor T lymphocyte (T_s) circuit in the modulatory process.

Several circuits or network systems of T-cell sets have been hypothesized for suppressor expression (14–20). In many cases, determinants coded for in the *I-J* locus of the major histocompatibility complex have been implicated as being critical to the suppressor system (15, 16, 19, 21–24). Greene *et al.* (22) have developed a procedure involving the *in vivo* administration of microliter quantities of anti-I-J antiserum to block the development or expression of T lymphocyte-mediated suppressor activity (T_s) in a tumor system.

The current study was undertaken to identify possible *I-J* restrictions in granuloma modulation by using the anti-I-J procedure of Greene *et al.* (22) as well as adoptive transfer of splenic suppressors between congeneic strains of mice differing at the *I-J* subregion. These experiments have indicated that granuloma modulation is sensitive to *in vivo* anti-I-J treatment and that identity at the *I-J* locus between chronic suppressor donors and recipient animals is necessary for optimal adoptive transfer of suppression.

MATERIALS AND METHODS

Animals and Infections. Male CBA/J mice were obtained from The Jackson Laboratory. Congeneic B10.A(3R) and B10.A(5R) breeding pairs were kindly provided by E. A. Boyse under the auspices of the National Institutes of Health congeneic mouse supply program and subsequently were maintained and bred in the animal care facility of the Veterans Administration Medical Center. Animals were infected at 7–9 weeks of age with *S. mansoni* cercariae (30 per mouse). All mice had access to commercially prepared rodent chow and acidified water.

Antisera. Anti-I-J^b and anti-I-J^k antisera were graciously provided by D. C. Shreffler. These sera were prepared by using HTT anti-9R (anti-I-J^k) and (5R × A) anti-3R (anti-I-J^b) immunizations. These sera have the demonstrated capacity to absorb soluble suppressor factors. *In vivo* administration of antiserum was essentially as described by Greene *et al.* (22). Briefly, the antiserum was diluted in phosphate-buffered saline and the equivalent of 2 μl of undiluted serum was administered in 0.5 ml at each injection. Diluted antiserum was administered intravenously and intraperitoneally on alternating days for a total treatment period of 10–17 days immediately prior to sacrifice.

Adoptive Transfers. Spleen cell suspensions from chronically infected mice (15–20 weeks after infection) were prepared

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Abbreviations: SEA, soluble egg antigens; T_s, suppressor T lymphocyte; T_s Aux, auxiliary suppressor T lymphocyte.

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in RPMI-1640 (Flow Laboratories, Rockville, MD) by teasing with fine forceps. The cells were passed through two layers of sterile gauze, washed once by centrifugation (190 × *g* for 10 min at 4°C) and resuspended in RPMI-1640 at 80 × 10^6 nucleated cells per ml. Spleen cell suspension (0.5 ml) was injected intravenously into each recipient mouse at 6 weeks after infection. Control mice received 0.5 ml of saline or RPMI-1640. Two weeks after adoptive transfers (8 weeks after infection), the recipients were sacrificed and granulomas were measured.

Granuloma Assay. Granuloma size in the livers of either chronically infected or adoptively transferred 8-week-infected mice was evaluated exactly as reported (12). Briefly, livers were removed, fixed in 10% neutral buffered-formalin, processed, stained with hematoxylin and eosin, and coded so that the examiner was unaware of their identity. Twenty-five newly formed granulomas were located in the liver sections of each mouse, and their diameters were measured across perpendicular axes by using an ocular micrometer. The mean volume of each granuloma was calculated, based on its mean diameter and the generally spherical nature of the lesions (25). Mean granuloma volumes were calculated for each animal.

Anti-SEA Passive Hemagglutination. Circulating antibody levels against SEA (3) were determined by a passive hemagglutinating assay using sheep erythrocytes coated with SEA (12). The $\log_2$ of the reciprocal of the highest dilution of serum (preabsorbed with sheep erythrocytes) which gave positive agglutination of SEA-coated erythrocytes was recorded as the highest titer.

Statistical Analysis. Analysis of experiments containing two groups was based on the Student *t* test. For three or more groups, analysis was based on a multiway analysis of variance. Multiple comparisons between means were made by using two-sided confidence intervals about the difference between means. The *a priori* Bonferroni multiple comparison method (26) based on an experiment-wise error rate, the error mean square, was routinely used. When several duplicate experiments were performed with mice from different infections, the final data were often pooled for a single value. To determine the validity of pooling, a single analysis of variance was performed on the data from the duplicated experiments. If no significant difference was found between the individual experiments, pooling was permissible. In all cases in which data were pooled, levels of significance for differences between groups was not changed by pooling.

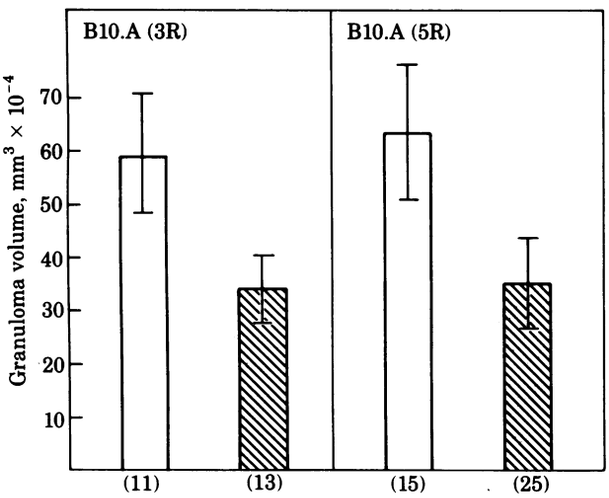

FIG. 1. Mean (±SD) *S. mansoni* egg-induced hepatic granuloma volumes in B10.A(3R) and B10.A(5R) congeneic mice at 8 weeks (open bar) and 15 weeks (hatched bar) after infection. Data have been pooled from three separate infections. The number in parentheses below each bar indicates the number of mice contributing to each mean. For both strains, 8 week ≠ 15 week ($P < 0.01$).

RESULTS

Granuloma Modulation in B10.A(3R) and B10.A(5R) Congeneic Mice. The modulation of hepatic granulomas to *S. mansoni* eggs has been described in several strains of mice. However, to confirm that similar modulation occurs in the B10.A(3R) and B10.A(5R) mice, newly formed granulomas were measured in 8- and 15-week-infected mice of these strains (Fig. 1). The 8-week (maximal) granulomas observed in the B10.A(3R) and B10.A(5R) mice were only about half the size of those routinely observed in CBA/J mice (for example, see Fig. 4, group A). In spite of this strain difference, Fig. 1 demonstrates that B10.A(3R) and B10.A(5R) mice do modulate the size of their granulomas during chronic (15-week) infections.

Reciprocal adoptive transfer experiments were performed between B10.A(3R) and B10.A(5R) mice. These mice differ at the *I-J* subregion, and a requirement for I-J recognition should be detected as a failure to adoptively transfer suppressor activity between the two strains. The adoptive transfer of suppression was successful only when there was homology at the *I-J* locus—i.e., only chronic B10.A(3R) spleen cells suppressed B10.A(3R) recipients and only chronic B10.A(5R) spleen cells suppressed B10.A(5R) recipients (Fig. 2). These results indicate that recognition of some *I-J* encoded determinants is likely. Adoptive transfers across the *I-J* barrier produced a somewhat decreased granuloma volume but this difference was never significant. However, the consistency of this observation may suggest that more than one mechanism can be operative in modulation.

Loss of Suppression After Treatment with Anti-I-J Antiserum. Chronically infected mice were treated (2 μl/day) with normal mouse serum, anti-I-J^b antiserum, or anti-I-J^k antiserum for 10–17 days prior to their sacrifice. For both the CBA/J (I-J^k) and B10.A(5R) (I-J^k) mice, only the anti-I-J^k antiserum blocked modulation; for the B10.A(3R) mice, only the anti-I-J^b antiserum abrogated modulation (Fig. 3). These data suggest that an I-J-positive cell or factor is involved in the modulation of granulomas during chronic infection.

Failure of Spleen Cells from Anti-I-J Treated, Chronically Infected Mice To Adoptively Transfer Suppression. Spleen cells from the anti-I-J treated chronic animals (described in Fig. 3) were adoptively transferred to syngeneic recipients. The re-

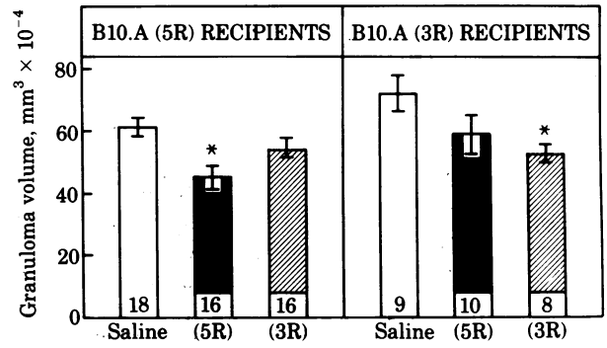

FIG. 2. Mean (±SEM) *S. mansoni* egg-induced hepatic granuloma volumes in 8-week-infected B10.A(5R) and B10.A(3R) mice which, at 6 weeks after infection, received saline (open bar) or spleen cells from chronically infected B10.A(5R) mice (solid bar) or B10.A(3R) mice (hatched bar). Data have been pooled from three and two separate infections for B10.A(5R) and B10.A(3R) mice, respectively. The number within each bar indicates the number of mice contributing to that mean. *, Significant difference ($P < 0.05$) from saline control.

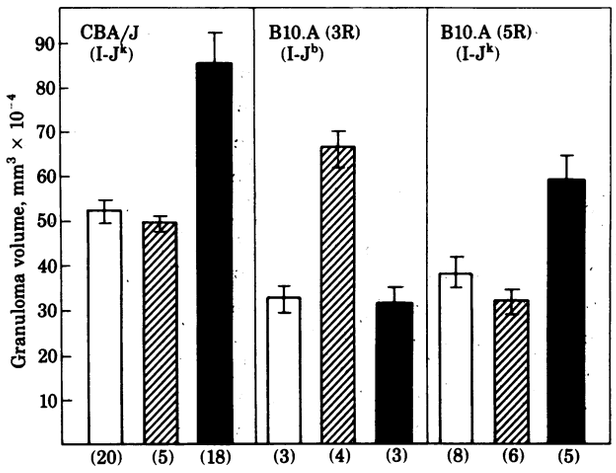

FIG. 3. Mean (±SEM) *S. mansoni* egg-induced hepatic granuloma volumes in three strains of mice treated (2 μl/day) with normal mouse serum, (open bar), anti-I-J^b antiserum (solid bar), or anti-I-J^k antiserum (hatched bar) for 10–17 days prior to sacrifice at 15 weeks after infection. Data for normal mouse serum and anti-I-J^k treatment and CBA/J and B10.A(5R) mice have been pooled from three separate infections. Data for anti-I-J^b treatment and B10.A(3R) mice represent a single infection. The number in parentheses below each bar indicates the number of mice contributing to each mean. For each panel, the group treated with homologous anti-I-J antiserum had a mean granuloma volume different from either of the other treated groups ($P < 0.01$).

sults for a representative experiment with CBA/J mice are presented in Fig. 4 and indicate that treatment with anti-I-J^k antiserum prevented the transfer of suppression. Limited data for the B10.A(3R) and B10.A(5R) mice are presented in Fig. 5. Because only a single preliminary experiment has been performed with these congeneic mice and only small numbers of infected recipients survived in some of the groups, data points representing mean granuloma volumes for individual animals are indicated. For the B10.A(3R) mice, anti-I-J^b antiserum

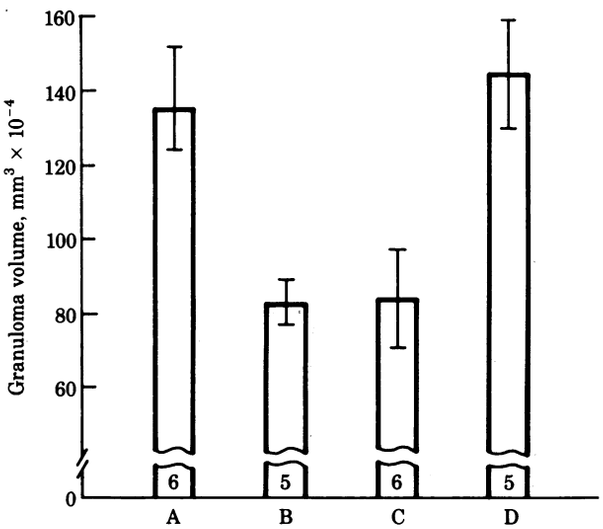

FIG. 4. Mean (±SEM) *S. mansoni* egg-induced hepatic granuloma volumes in 8-week-infected CBA/J mice which, at 6 weeks after infection, received spleen cells from 15-week-infected mice that had been treated with: B, normal mouse serum; C, anti-I-J^b antiserum; D, anti-I-J^k antiserum. Controls (A) received saline. The number within each bar indicates the number of mice contributing to that mean. Groups A and D are significantly different from groups B and C ($P < 0.01$).

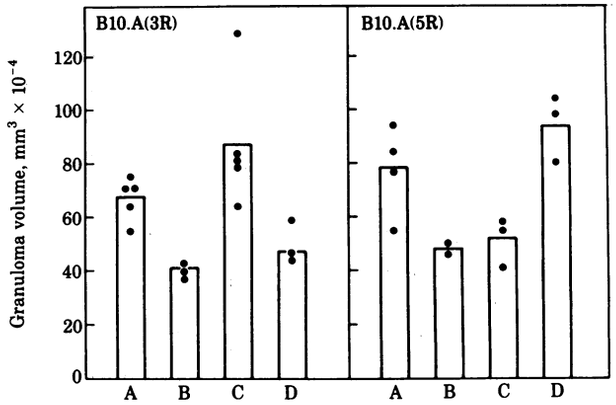

FIG. 5. Mean *S. mansoni* egg-induced hepatic granuloma volumes in 8-week-infected B10.A(3R) and B10.A(5R) mice which, at 6 weeks after infection, received spleen cells from 15-week-infected mice that had been treated with: B, normal mouse serum; C, anti-I-J^b antiserum; D, anti-I-J^k antiserum. Controls (A) received saline. Dots represent mean granuloma volumes for each mouse in that group.

blocked the adoptive transfer of suppression; anti-I-J^k antiserum did so in B10.A(5R) mice as it had in CBA mice.

Anti-SEA Antibody Titers in the Absence of Granuloma T$_s$ Activity. To evaluate the effect of anti-I-J treatment on humoral responses in chronic mice, anti-SEA titers were determined for animals from the experiments shown in Fig. 3. The results (Table 1) indicate that anti-I-J treatment had no effect on the humoral responses to SEA.

DISCUSSION

The results of this investigation demonstrate that granuloma modulation during chronic schistosomiasis (*S. mansoni*) is largely regulated by the *I-J* locus of the major histocompatibility complex. Transfer of suppressive chronic spleen cells between B10.A(3R) and B10.A(5R) congeneic strains has shown a requirement for homology at the *I-J* locus between T$_s$ donor and recipient cells for suppression to occur. Furthermore, granuloma modulation in chronic mice and the adoptive transfer of suppression to 6-week-infected mice both are sensitive to *in vivo* treatment with microliter amounts of anti-I-J. These data are compatible with those reported by Chensue and Boros (10, 27) implicating an *Ia(ABJ)*-bearing cell in modulation. Because the adoptive transfer of suppression was also sensitive to anti-I-J treatment of cell donors, it is most likely that the target of the antiserum is an I-J determinant on a lymphocyte within the suppressor circuit and not on a released I-J-bearing factor. Chensue *et al.* (28) have recently reported that suppression of lymphokine production may be contributory to modulation. An Ly2$^+$ I-J- and I-C-bearing T cell found in chronic (20-week-in-

Table 1. Anti-SEA titers

Mouse strain	Mean (±SEM) titer, log$_2$		
	Normal mouse serum	Anti-I-J^b antiserum	Anti-I-J^k antiserum
CBA	9.6 ± 0.3 (n = 5)	9.0 ± 0.3 (n = 5)	8.7 ± 0.4 (n = 5)
B10.A(3R)	9.0 ± 0.6 (n = 3)	8.5 ± 0.6 (n = 4)	8.3 ± 0.6 (n = 3)
B10.A(5R)	9.1 ± 0.5 (n = 8)	9.5 ± 0.5 (n = 6)	9.4 ± 0.6 (n = 5)

Mice received the indicated antiserum (2 μl/day) for 10–17 days prior to sacrifice at 15 weeks after infection. No differences between groups are significant. n, Number of mice in the mean.

fected) spleen cell populations has been shown to suppress the SEA-induced macrophage inhibition factor production by 8-week-infected spleen cells.

In vitro antibody responses have also been reported (29) to be sensitive to anti-I-J treatment. In those experiments, F_1 macrophages cultured with anti-I-J antiserum (without complement) specific for one parental haplotype were incapable of cooperating with untreated helper T cells of the same haplotype. Cooperation with helper T cells of the other parental haplotype was unaffected, suggesting that anti-I-J blocked cell–cell communication. As shown in Table 1, the humoral response to SEA was not affected by anti-I-J treatment in the present investigation. Such differences in effects on humoral responses may be due to *in vivo* versus *in vitro* treatment with the antiserum or to the dose of anti-I-J used [Niederhuber and Allen (29) used anti-I-J at a final concentration of 10%].

Microliter quantities of anti-I-J have been used *in vivo* to ablate suppressor activity in tumor (22) and primary antibody response (30) systems. In the tumor system, Perry *et al.* (31) recently ruled out the direct interaction of anti-I-J with tumor cells or contaminating antiviral antibody as possible mechanisms of action. Little more is known about the possible mechanism by which such small quantities of specific antibody produce such a profound effect. It remains likely that the anti-I-J binds to *I-J* encoded cell surface receptors or factors and thus interferes with the cell–cell communication required for suppression.

The fact that granuloma modulation during chronic schistosomiasis is mediated by a T cell (11, 12, 27) which is $Ia(ABJ)^+$ and antigen specific in action (10) and *I-J* restricted strongly points to the involvement of a T_s circuit or feedback system (15–19). The granuloma model being investigated here makes comparison with reported T_s circuits difficult. The major obstacle is the massive antigen exposure (several hundred eggs per day) which is continuous after the fourth or fifth week of infection. The generation of suppressor activity, and its adoptive transfer, weeks after antigen exposure has started makes analogies with more experimentally defined models less clear.

Some limited comparisons are warranted, however. Eardley *et al.* (18) described a feedback inhibition circuit in which antigen-stimulated $Ly1^+$ cells activated a resting set of $Ly1,2,3^+$ cells to become T_s. Cantor *et al.* (14) characterized this inhibition circuit as being sensititve to adult thymectomy or low-dose cyclophosphamide therapy. The defect in the circuit after either of these treatments was due to a decrease in $Ly1,2,3^+$ cell pool. The modulation of egg-induced granulomas has also been shown to be reversed by adult thymectomy or hydrocortisone treatment (13) or low-dose cyclophosphamide treatment (12). These results suggest the involvement of a recently thymus derived ($Ly1,2,3^+$?) cell in the suppressive process. Although the feedback inhibition model was described in a humoral rather than cell-mediated system, it may still provide applicable analogies. Indeed, recent evidence reported by Doughty *et al.* (32) from an adotive transfer/lung schistosomal egg granuloma model has indicated that $Ly1^+$ and $Ly2,3^+$ T cell sets are involved in recruitment processes leading to modulation.

The *I-J* restriction of granuloma suppression and sensitivity to anti-I-J treatment is compatible with many other T_s systems. *I-J* bearing T_s cells have been reported (24, 33–36) as well as I-J-bearing T_s factors (16, 19, 21, 23, 37). In particular, the contact sensitivity system described by Sy *et al.* (15) involves an $I\text{-}J^+$ cell in a suppressor circuit with characteristics that are comparable to egg-induced granuloma modulation. These investigators described an auxiliary suppressor cell termed "T_sAux." These cells are found in immune lymph node cell populations, yet they are necessary for adoptively transferred T_s cells to produce suppression. The T_s Aux is sensitive to adult thymectomy, cyclophosphamide, and anti-brain associated θ serum as is the granuloma T_s (12, 13) and is $I\text{-}J^+$. However, in the present study, removal of a T_sAux-like cell by anti-I-J would not explain the failure to transfer suppression adoptively. Because infected recipient animals were exposed to eggs for about 1 ½ weeks prior to the cell transfer, one would anticipate an abundance of T_s Aux-like cells and thus a reexpression of the suppressor activity. In terms of similarity, in regard to chronic antigenic exposure and continuing processes involving the *I-J* subregion, the granuloma system in this parasitic disease most closely resembles the tumor response and its suppression studied by Benacerraf, Greene, and colleagues (22, 31, 37).

Future examination of the suppressor system responsible for granuloma modulation in experimental schistosomiasis (*S. mansoni*) should provide a more complete phenotypic and functional characterization of the cell responsible for transfering suppression. Such characterization will hopefully identify the critical cell(s) in the modulatory process so that a cellular interaction sequence may be hypothesized in this chronic murine schistosomiasis model.

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