Increased Resistance to *Taenia crassiceps* Murine Cysticercosis in Qa-2 Transgenic Mice

GLADIS FRAGOSO,1 EDMUNDO LAMOYI,1 ANDREW MELLOR,2 CIRO LOMELI,1 MARISELA HERNÁNDEZ,1 AND EDDA SCIUTTO1*

Department of Immunology, Instituto de Investigaciones Biomédicas, UNAM, México D.F. 04510, México, and Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, Georgia 30912

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We previously reported important differences in resistance to *Taenia crassiceps* murine cysticercosis between BALB/c substrains. It was suggested that resistance might correlate with expression of the nonclassical class I major histocompatibility complex (MHC) Qa-2 antigen; BALB/cAnN is Qa-2 negative and highly susceptible to *T. crassiceps*, whereas BALB/cJ expresses Qa-2 and is highly resistant. In this study, we investigated the role of Qa-2 in mediating resistance to cysticercosis by linkage analysis and infection of Qa-2 transgenic mice. In BALB/cAnN × (C57BL/6J × BALB/cAnN)F2 and BALB/cAnN × (BALB/cJ × BALB/cAnN)F2 backcrosses, the expression of Qa-2 antigen correlated with resistance to cysticercosis. Significantly fewer parasites were recovered from infected Qa-2 transgenic male and female mice than from nontransgenic mice of similar genetic background. These results clearly demonstrate that the Qa-2 MHC antigen is involved in resistance to *T. crassiceps* cysticercosis.

*Taenia solium* cysticercosis is a parasitic disease that seriously affects human health (24) and causes important economic losses in pig farming of developing countries (1) where conditions that favor parasite transmission persist. The essential role of pigs as an obligatory intermediate host in the parasite life cycle offers the opportunity to interfere with transmission by inducing acquired immunity through vaccination (10, 13, 18), by decreasing susceptibility through genetic manipulation (14), or both. Systematic exploration of the role of genetic factors in cysticercosis and the identification of protective immunogens are hampered by the high costs and slow data retrieval involved in studies with pigs. However, another cestode, *Taenia crassiceps*, that naturally infects rodents (3) is highly suitable for experimentation. It shows extensive antigenic cross-reactivity and cross-protective immunity with *T. solium* (7, 21); the antigenic similarity is such that *T. crassiceps* antigens can be used for immunodiagnosis of human cysticercosis (9). Furthermore, *T. crassiceps* and *T. solium* both have a typical two-host taeniid life cycle and morphologically and structurally related larval stages. Since *T. crassiceps* can reproduce asexually, experimental infection is readily attained by infecting the cysticerci in the peritoneal cavity of the mouse (3). Thus, *T. crassiceps* murine cysticercosis has been shown to be a useful experimental model of metacestode infection in the study of genetic factors involved in host resistance (2, 20) and underlying immunological mechanisms (19, 23, 25).

Initial findings showed that genes linked to *H*-2 affect *T. crassiceps* growth in mice (20). Thus, significant differences in the extent of the parasitosis were found between mice carrying the *H*-2d (BALB/cAnN and DBA/2) haplotype, which were the most susceptible, and mice with *H*-2b (*BALB/B, C57BL/6J, and C57BL/10J) or *H*-2a (BALB/K, C3H/HeJ, and C3H/FeJ) haplotype, which were comparatively resistant. Further studies (2)

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* Corresponding author. Mailing address: Departamento de Immunología, Instituto de Investigaciones Biomédicas, UNAM, Apartado Postal 70-228, México D.F. 04510, México. Phone: (525) 622-38-18. Fax: (525) 550-00-48. E-mail: edda@servidor.unam.mx.
3 months after inoculation of 10 cysticerci per mouse as described elsewhere (19, 20). For the evaluation of susceptibility to cysticercosis, mice were injected intraperitoneally with 10 small (2-mm-diameter) nonbudding T. crassiceps larvae suspended in phosphate-buffered saline. Thirty days after infection, mice were sacrificed and T. crassiceps cysts inside the peritoneal cavity were counted as previously described (19, 20).

Production of Q9 transgenic mice. Q9 transgenic mice were derived by introducing the Q9 gene cloned from the C57BL/10 strain (27) into pronuclei of fertilized oocytes from (C57BL/6J × BALB/cAnNF) mice by using standard procedures (4). Transgenic mice were identified by Southern blot analysis, hybridizing a probe from the large intron of the Q9 gene (JBS9; see Fig. 2a) to BamHI-digested DNA extracted from tail biopsy samples. To evaluate the effect of Qa-2 on susceptibility to T. crassiceps, two male transgenic (C57BL/6J × BALB/cAnNF)F2 founder mice (Tg1 and Tg2) were backcrossed to BALB/cAnNF female mice, and their progeny were infected with T. crassiceps as described above. Expression of Qa-2 antigen was determined as described below. For controls, nontransgenic (C57BL/6J × BALB/cAnNF)F2 male mice similarly backcrossed to female BALB/cAnNF mice were used.

Qa-2 antigen detection. Expression of Qa-2 antigen was determined by flow cytometric analysis with a FACScan (Becton Dickinson, Palo Alto, Calif.). For genetic linkage studies, mice were classified as Qa-2 positive (Qa-2+) or null (Qa-2>). For this, peripheral blood lymphocytes from BALB/cAnNF × (C57BL/6J × BALB/cAnNF)F2 and BALB/cAnNF × (BALB/c × BALB/cAnNF)F2 were stained with fluorescein isothiocyanate-conjugated anti-mouse Qa-2 (Pharmingen, San Diego, Calif.). Ten thousand cells were analyzed with a lymphocyte gate. To determine the expression of Qa-2 protein in CD4+ and CD8+ T cells, two-color fluorescence-activated cell sorting (FACS) analysis of thymus, spleen, lymph nodes, and blood cells from transgenic and nontransgenic mice was performed. Cells were stained with monoclonal antibodies for Qa-2, CD4, and CD8. Cell suspensions from 6-week-old Tg1-derived transgenic and nontransgenic mice were stained with fluorescein isothiocyanate-conjugated anti-mouse Qa-2, and biotin-conjugated anti-Qa-2 (all from Pharmingen) followed with streptavidin-conjugated FITC. Cell suspensions were prepared and stained with the antibodies, using standard procedures. Ten thousand cells were analyzed with a lymphocyte gate as defined by light scatter.

Statistical analysis. Statistical comparisons between groups were carried out by the Mann-Whitney and Kruskal-Wallis nonparametric tests.

RESULTS AND DISCUSSION

Linkage analysis between Qa-2 expression and resistance to T. crassiceps. BALB/cAnNF × (C57BL/6J × BALB/cAnNF)F2 and BALB/cAnNF × (BALB/c × BALB/cAnNF)F2 male and female backcross mice were infected to assess the level of resistance to T. crassiceps and simultaneously classified according to their Qa-2 phenotype as Qa-2+ or Qa-2− by flow cytometric analysis. Figure 1 shows that in both backcrosses, Qa-2+ male mice were significantly more resistant to T. crassiceps cysticercosis than null male mice (P < 0.01). Similarly, Qa-2+ female backcross mice harbored fewer parasites than their Qa-2 null littermates (P < 0.01). These data strongly indicate that the presence of the Qa-2 protein correlates with resistance to cysticercosis. Males were less susceptible than females of the same Qa-2 phenotype with the exception of BALB/cAnNF × (BALB/c × BALB/cAnNF)F2 Qa-2+ mice, where females and males showed similar parasite burdens. As can also be seen in Fig. 1, BALB/cAnNF × (C57BL/6J × BALB/cAnNF)F2 mice had lower parasite burdens than their BALB/cAnNF × (BALB/c × BALB/cAnNF)F2 counterparts, probably due to the effect of additional C57BL/6J background genes conferring resistance to the parasitosis (2).

Increased resistance of Qa-2 transgenic mice to T. crassiceps. A genomic clone containing the Q9 gene (Fig. 2a) was used to generate (C57BL/6J × BALB/cAnNF)F1 transgenic mice. Two male transgenic founders (Tg1 and Tg2) were genotyped by Southern analysis (Fig. 2b). A much higher intensity of the band corresponding to the Q9 gene was observed in the Tg1 and Tg2 transgenic mice compared with nontransgenic mice, indicating the presence of multiple copies of the transgene. Increased expression of Qa-2 antigen in T cells of various lymphoid tissues from Tg1- and Tg2-derived mice was found by FACS analysis; no significant changes in the relative size of CD4+ and CD8+ T-cell subpopulations could be detected (Fig. 3).

To investigate the role of Qa-2 in mediating resistance against T. crassiceps, both male and female backcross progeny of Tg1 and Tg2 founder mice were infected with T. crassiceps cysterceri. As shown in Fig. 4, the number of parasites recovered in mice derived from the transgenic founders was significantly lower than that observed in similarly backcrossed nontransgenic mice (P < 0.01). The higher susceptibility of females noted before (2, 20) was maintained.

The data presented here clearly demonstrate that Qa-2 is involved in resistance to T. crassiceps cysticercosis, although the mechanisms underlying the capacity of Qa-2 to control the extent of this parasitosis remain obscure. The role of Qa-2 might be immunologically mediated, since Qa-2 is a class I MHC protein, serves as a peptide receptor (5, 17), and can behave as a transplantation antigen (12). Thus, Qa-2 may be involved in presentation of cysterceral antigens to T cells, including perhaps those expressing y6 receptors or other unique subsets which may participate, probably through the production of cytokines, in the destruction of the larvae at early stages during their cycle, when they are presumably more sensitive to immune attack. It is also plausible that Qa-2 could influence resistance by mediating the selection of relevant effector T cells during intrathymic maturation or by affecting the shaping of
the natural killer cell repertoire during development. Although the mechanism(s) has to be determined, our results constitute strong evidence that expression of Qa-2 in vivo is capable of modifying the course of a parasitic disease and suggest an important biological role for these nonclassical MHC molecules in immunity.

The observed gender-associated differences in susceptibility to *T. crassiceps* reveal that besides Qa-2, other biological factors such as hormonal environment also modulate the outcome of infection. Thus, it has been reported that 17-β-estradiol promotes whereas androgens restrict the growth of cysticerci (8). In addition, preliminary findings suggest that the differential susceptibility between females and males may be immunologically mediated (23) as has been proposed for other diseases (26).

Considering the extensive antigenic cross-reactivity and cross-immunity between *T. solium* and *T. crassiceps* cisticerci, it seems possible that *T. solium* cisticercosis resistance may also be associated to the expression in human and pigs of a protein similar to Qa-2. In this regard, it has been reported that a human nonclassical class I MHC gene product (HLA-G) could be a functional homolog of the mouse Qa-2 antigen (6). Our results suggest the importance of examining the expression levels of HLA-G in cells from cisticercotic and noncisticercotic individuals from an area of endemicity which could reveal genetic differences associated to human cisticercosis. The identification of this resistance gene also suggests possible
practical applications in the production of transgenic pigs with increased resistance to *T. solium* cysticercosis by the transfer of the gene which could have additional potential benefits in pig rearing (28).

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![FIG. 4. Individual numbers of parasites recovered from Qa-2 transgenic and nontransgenic mice. Cysticerci in the peritoneal cavity were counted 30 days after infection. Tg1 and Tg2 transgenic founder mice were (C57BL/6J × BALB/cAnN)F1 males. Both female (F) and male (M) progeny from transgenic and nontransgenic (C57BL/6J × BALB/cAnN)F1 male mice backcrossed to BALB/cAnN females were infected. The bars represent mean parasite numbers for each experimental group. The numbers of cysticerci found in Tg1- and Tg2-derived mice were statistically lower (*P* < 0.01) than those in nontransgenic mice.](http://iai.asm.org/Downloaded from http://iai.asm.org)
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