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Chronic Giardiasis in B-Cell-Deficient Mice Expressing the *xid* Gene

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The role of antibody in immunity to *Giardia muris* infection was investigated by studying B-cell-deficient CBA/N mice expressing the *xid* gene. After gastric administration of infective *G. muris* cysts, CBA/N male and female mice developed prolonged *G. muris* infection, whereas BALB/c mice eliminated their infection in 6 to 8 weeks. Male F_1 progeny obtained from matings between female CBA/N mice and male BALB/c mice expressed the *xid* gene and developed prolonged infections. In contrast, all other F_1 progeny of CBA/N and BALB/c matings, which did express the *xid* gene, eliminated *G. muris*. The link between the *xid* gene and prolonged infection was confirmed by studies of C57BL/6 mice congenic for the *xid* gene. When compared with BALB/c or F_1 mice, CBA/N mice produced large quantities of immunoglobulin A (IgA) anti-*G. muris* antibody in serum and gut secretions during prolonged infection. Serum IgG anti-*G. muris* antibody levels were reduced in CBA/N and F_1 male mice that expressed the *xid* gene. The inability of *xid* mice to eliminate *G. muris* is consistent with the importance of antibody in the development of immunity to *G. muris*. We hypothesize that mice bearing the *xid* gene fail to produce IgA antibody of appropriate specificity to an antigen or antigens whose recognition by antibody is critical for successful elimination of the parasite.

Infection with the protozoan parasite *Giardia muris* occurs in the lumen of the upper small bowel of mice, and in most strains of mice this parasite is eliminated in 6 to 8 weeks (3, 4, 25, 32). Evidence that immune factors are important for elimination was first obtained when Roberts-Thomson and Mitchell (25) reported that congenitally athymic mice failed to eliminate a primary infection. Nude mice reconstituted with syngeneic spleen cells displayed a pattern of infection intermediate between those of unreconstituted nude mice and intact heterozygous littermates. A recent study indicates that T cells of the helper-inducer phenotype ($L3T4^+$) are necessary for expulsion of the parasite (13).

Resolution of infection in resistant strains of mice is accompanied by antibody production that features immunoglobulin A (IgA) anti-*G. muris* antibody secreted into the intestinal lumen as well as IgA and IgG anti-*G. muris* antibody in serum (32). The importance of antibody-mediated mechanisms was highlighted by studies of anti-IgM-treated mice. These mice developed prolonged *G. muris* infection and failed to produce anti-*G. muris* antibody in serum and intestinal secretions during chronic infection (31). Since *G. muris* exists predominantly in the lumen of the gut (20), the evidence points to an important role for IgA antibody in the elimination of a primary *G. muris* infection.

In this communication, we report experiments designed to examine the course of *G. muris* infection and specific antibody production in mice that have the X-linked immunodeficiency associated with the expression of the *xid* gene (8, 27). Numerous reports indicate reduced antibody production by these mice to systemically administered T-cell-independent antigens (2, 17, 29, 30) and some T-cell-dependent antigens (5, 23, 24, 28). Female mice that are homozygous or male mice that are hemizygous for *xid* have reduced resistance to systemic bacterial, protozoan, and viral infections linked to deficient antibody production (10, 14, 16, 18, 19). In this study, we compared mice expressing the *xid* gene with phenotypically normal mice to test our hypothesis that antibody is important for normal elimination of *G. muris*.

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MATERIALS AND METHODS

Mice. Male and female CBA/N mice and BALB/c mice were obtained from Jackson Laboratory (Bar Harbor, Maine). C57BL/6N.Xid mice were a generous gift of Alfred Steinberg (National Institutes of Health, Bethesda, Md.). F_1 mice were obtained by reciprocal matings of CBA/N and BALB/c mice carried out in the Department of Laboratory Animal Science at the University of Toronto. All mice were housed in that facility in cages with filter tops and in a filtered-air hood and received standard rodent chow and water.

As a control for the expression of the *xid* gene in these mice, their sera were assayed by an immunoradiometric assay for IgM as described below. Low levels of IgM in serum are a phenotypic trait of *xid* mice (1). Sera from CBA/N (male and female), (CBA/N × BALB/c) F_1 male mice hemizygous for the *xid* gene, and C57BL/6N.Xid mice had less than 50 μg of IgM per ml, compared with sera from normal control mice which had 200 to 500 μg/ml.

Parasite source and infection procedure. *G. muris* was obtained originally from Ian C. Roberts-Thomson (Hall Institute, Melbourne, Australia) and was maintained by constant passage of infection through nude mice. To infect mice, we isolated *G. muris* cysts (see below) from the feces of infected nude mice and inoculated groups of mice with 5,000 cysts per mouse by esophageal intubation.

Isolation and quantitation of *G. muris* cysts and trophozoites. *G. muris* cysts isolated from fecal matter by low-speed centrifugation on 1 M sucrose were washed twice in saline and counted in a hemacytometer (26). Trophozoites were isolated in the pellets of centrifuged samples of gut washings obtained to measure intestinal antibody (see below). The trophozoites were suspended in 0.15 M NaCl–10 mM potassium phosphate (pH 7.4) (phosphate-buffered saline) and counted in a hemacytometer.

Collection of sera and gut washings. Sera were collected from mice before cyst inoculation and at 4 and 9 weeks after

inoculation. Mice were bled from the orbital plexus, or at the end of each experiment (week 9) cardiac bleeds were performed under ether anesthesia. A sample of gut luminal contents was obtained from individual mice at week 9 of infection by a procedure in which excised small bowel was filled with 4 ml (4°C) of phosphate-buffered saline containing enzyme inhibitors, briefly massaged, and then emptied, as described previously (32). This material was analyzed for IgA anti-*G. muris* antibody. Previous experiments had indicated that the gut wash procedure did not result in degradation of intestinal antibody and reflected secreted antibody with no serum protein contamination (31).

Measurement of IgM in serum. The amounts of IgM in sera from different groups of mice were measured by using a solid-phase sandwich-type immunoradiometric assay. The details of specificity, sensitivity, and procedures for this assay are given elsewhere (31, 32). A standard curve of counts per minute bound versus nanograms of IgM per milliliter was produced by using a purified myeloma protein standard (MOPC 104E) and used to estimate the concentration of IgM in test sera. Standard curve analysis and dose interpolation for test sera were done by using a (2 + 2) radioimmunoassay program of Davis et al. (9).

Measurement of IgG and IgA anti-*G. muris* antibody in sera and gut washings. Solid-phase immunoradiometric assays were performed to measure *G. muris*-specific IgA and IgG antibody in gut washings and sera of infected mice. Details of the procedures and the specificity and sensitivity of these assays were published previously (31, 32). In brief, the procedure involved coating microtiter wells with an antigen preparation from sonicated *G. muris* trophozoites, incubation with dilutions of sera or gut washings, and final incubation with ^{125}I-labeled anti-IgA or anti-IgG. A standard curve of specific counts per minute bound versus arbitrary units of antibody per milliliter was produced for each assay with a standard serum. Each standard serum was chosen from the serum of the mouse strain which gave the highest specific counts per minute bound and the most sensitivity. Preliminary results indicated that CBA/N IgA serum antibody and BALB/c IgG serum antibody provided the best standard curves for the respective assays. Antibody values for the test sera or gut wash were calculated from the specific counts per minute bound with dilutions of that serum, provided the specific counts per minute fell in the linear portion of each standard curve.

Statistical analysis of data. The immunoglobulin and antibody data from various groups of mice were compared by the Student's *t* test or the Mann-Whitney nonparametric test, as appropriate. Differences between means of various groups of data were considered significant at $P < 0.05$ when tested for the null hypothesis in a two-sided test.

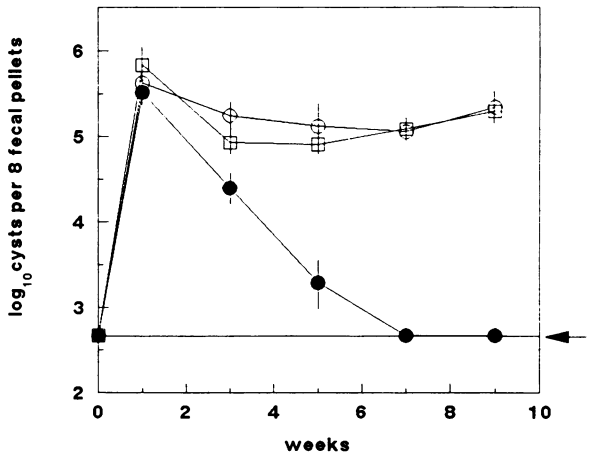

FIG. 1. Course of *G. muris* infection in male (□) and female (○) CBA/N mice and female BALB/c mice (●). Mice were inoculated at week 0, and cysts were counted ($\log_{10}$, *y* axis) at various time points up to 9 weeks. Each point is the mean of $\log_{10}$ cyst counts for groups of 6 to 10 mice. Arrow marks the limit of detection. Error bars show standard error of the mean (SEM).

RESULTS

Course of infection in mice expressing the *xid* gene. *G. muris* infections in CBA/N and BALB/c mice are compared in Fig. 1. BALB/c mice are resistant to *G. muris*, having no detectable infection by 7 to 9 weeks of infection (25, 32). Both male and female CBA/N mice developed prolonged infections (>9 weeks) and had high numbers of excreted cysts relative to BALB/c mice. *G. muris* trophozoites were counted in intestinal washes obtained at week 9 of infection. CBA/N mice had a mean of 2.0×10^6 trophozoites per mouse, but BALB/c mice had no detectable trophozoites (assay detection limit was 500 trophozoites). Prolonged infections in CBA/N mice were observed in more than five separate experiments.

We next compared the response of F_1 progeny from reciprocal matings of CBA/N and BALB/c mice. Expression of the *xid* gene is recessive. Therefore, only the F_1 male mice produced from female CBA/N and male BALB/c matings (CBA/N × BALB/c) exhibit the Xid phenotype, since they are hemizygous for the *xid* gene. The (CBA/N × BALB/c)F_1 males had prolonged infection when compared with littermate females or reciprocal (BALB/c × CBA/N)F_1 males. Cyst excretion at week 9 of infection is presented in Table 1. The (BALB/c × CBA/N)F_1 females did not develop prolonged infections (data not shown).

Linkage of prolonged infection with the *xid* gene(s) was demonstrated further by analysis of *G. muris* infection in

TABLE 1. Cyst counts at week 9 of infection from progeny of matings of BALB/c and CBA/N mice

Expt	Mice	Mean no. of cysts (10^3)[a]	Range of SEM[b]	No. of mice infected/ total no. tested
1	F_1 female (CBA/N × BALB/c)	1	<0.5–2.2	1/5
	F_1 male (*xid*) (CBA/N × BALB/c)	135	115–158	6/6
2	F_1 male (*xid*) (CBA/N × BALB/c)	24	16–36	5/5
	F_1 male (BALB/c × CBA/N)	1	0.7–2.5	1/5

[a] Mean of log values of cysts per eight fecal pellets (converted to linear values); limit of the assay was 500 cysts per sample.
[b] SEM of log values was added to and subtracted from that mean to obtain the upper and lower values of the range of SEM. Those log values were then converted to linear values.

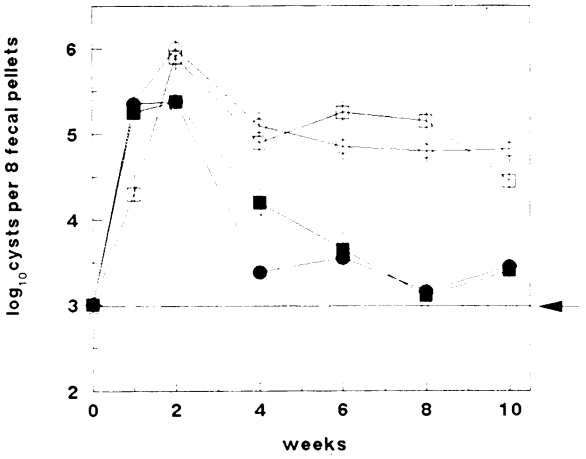

FIG. 2. Course of infection in male (■) and female (●) C57BL/6 mice and male (□) and female (○) C57BL/6N.Xid mice. Other details are described in the legend to Fig. 1.

congenic C57BL/6N.Xid mice (Fig. 2). Both male and female C57BL/6N.Xid mice excreted large numbers of cysts for up to 10 weeks. Normal C57BL/6 mice had barely detectable numbers of cysts beyond 6 weeks of infection, as we observed previously (32). Results similar to those in Fig. 2 were obtained in three different experiments.

Other investigators have reported that CBA mice display moderately prolonged infection with *G. muris* (4, 12, 25). To examine the influence of any genes in the CBA background on resistance to *G. muris*, we examined the course of infection in BALB/c, CBA/J, and (CBA/J × BALB/c)F_1 mice (Fig. 3). Although female CBA/J mice had higher cyst excretion rates than BALB/c mice at weeks 3 and 5 of infection, neither CBA/J mice nor the F_1 mice (male or female) had detectable cysts by week 9 of infection.

Anti-*G. muris* antibody production by *xid* mice. Immunoradiometric assay for IgA anti-*G. muris* antibody revealed that CBA/N mice had significantly higher amounts of IgA antibody compared with BALB/c mice in serum (Fig. 4) and in intestinal secretions (mean IgA antibody levels in secretions from CBA/N mice were 850 U/ml versus 400 U/ml in secretions from BALB/c mice, $P < 0.01$). Similar results

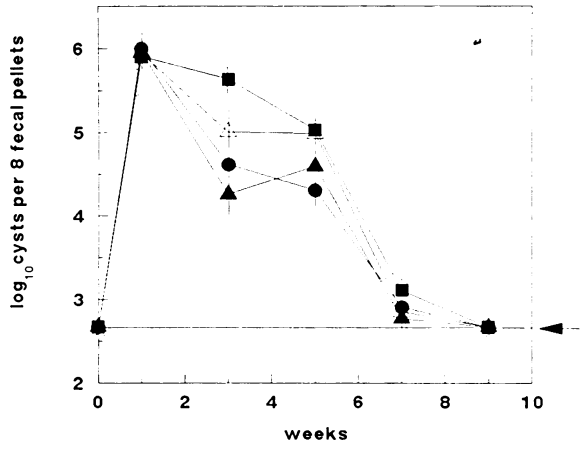

FIG. 3. Course of *G. muris* infection in female CBA/J mice (■), female BALB/c mice (●), and male (△) or female (▲) (CBA/J × BALB/c)F_1 mice. Other details are described in the legend to Fig. 1.

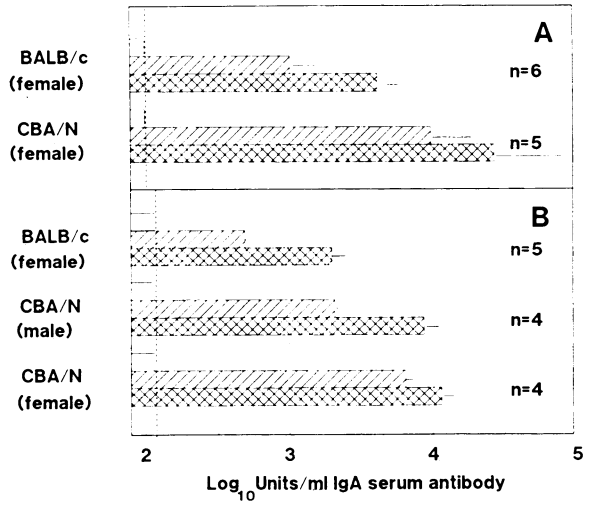

FIG. 4. IgA anti-*G. muris* antibody previous to (□) and at weeks 4 (▨) and 9 (▩) after cyst inoculation in the sera of CBA/N and BALB/c mice. Panels A and B show separate experiments. Each bar shows mean of $\log_{10}$ units of antibody per milliliter with SEM. Numbers of mice per group are shown (*n*). When compared at weeks 4 and 9, CBA/N mice had significantly more antibody than BALB/c mice ($P < 0.01$).

were observed in three separate experiments. However, sera from all infected F_1 male and female progeny of reciprocal matings of BALB/c and CBA/N mice had similar levels of serum IgA anti-*G. muris* antibody (Fig. 5).

IgG anti-*G. muris* antibody levels were lower in sera from infected CBA/N or (CBA/N × BALB/c)F_1 male mice, which express the *xid* gene, than in sera of BALB/c mice or (BALB/c × CBA/N)F_1 male mice, which do not have the *xid* gene (Fig. 6).

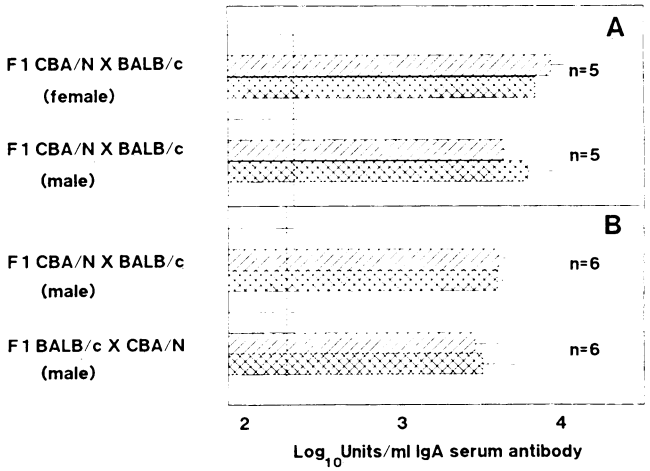

FIG. 5. IgA anti-*G. muris* antibody previous to (□) and at weeks 4 (▨) and 9 (▩) after cyst inoculation in the sera of F_1 progeny of BALB/c and CBA/N mice. Panels A and B show separate experiments. Each bar shows the mean of $\log_{10}$ units of antibody per milliliter with SEM. Numbers of mice per group are shown (*n*). There were no significant differences ($P > 0.2$) between the male and female progeny of CBA/N × BALB/c matings (A) or between the (CBA/N × BALB/c)F_1 males and the (BALB/c × CBA/N)F_1 males (B).

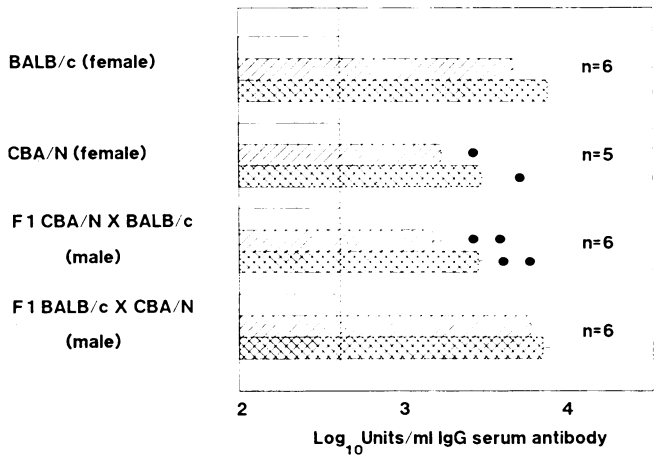

FIG. 6. IgG anti-*G. muris* antibody previous to (□) and at weeks 4 (▨) and 9 (▩) after cyst inoculation in the sera of CBA/N, BALB/c, and F_1 male progeny. Each bar shows the mean of $\log_{10}$ units of antibody per milliliter with SEM. Numbers of mice per group are shown (*n*). When compared at weeks 4 and 9, CBA/N mice had significantly less antibody than BALB/c mice (●, $P < 0.01$) and the (CBA/N × BALB/c)F_1 males had significantly less antibody than the (BALB/c × CBA/N)F_1 males (●●, $P < 0.02$).

DISCUSSION

The results of experiments presented here indicate that mice which bear the *xid* gene in a homozygous or hemizygous state develop a chronic primary *G. muris* infection. Susceptibility to infection was linked to the *xid* gene by the observation that chronic infections occurred only in the (CBA/N × BALB/c)F_1 male mice that were hemizygous for *xid* and not the other progeny of matings between CBA/N and parasite-resistant BALB/c mice. Susceptibility of the congenic strain C57BL/6N.Xid to chronic infection compared with resistance of normal C57BL/6 mice indicated a close linkage of susceptibility to infection with the expression of the *xid* gene(s).

Since *xid* mice display a defect in B cells and antibody formation, we interpret these findings as indicating an important role for B cells and antibody in the elimination of a primary *G. muris* infection. This is consistent with our previous experiments with B-cell-deficient mice produced by anti-IgM antibody treatment from birth (31). The precise cellular basis for the antibody deficiency in *xid* mice has not been elucidated. However, a variety of studies have indicated a marked inability of *xid* mice to synthesize antibody to bacterial antigens, particularly polysaccharides (2, 5, 11). This in turn is associated with the inability of *xid* mice to develop immune resistance to infection with extracellular microorganisms (10, 14, 19). In normal mice, antibodies to polysaccharide antigens are preferentially produced within the IgM and IgG3 isotypes (21, 30). Mice which express the *xid* gene are deficient in circulating levels of IgM (50% of normal) and IgG3 (<3% of normal) (1, 22), which may reflect a primary defect in their ability to respond to polysaccharide antigens rather than a defect leading to a generalized decrease in IgM and IgG3 levels.

In the experiments reported here, *xid* mice developed chronic infection with *G. muris* concomitant with the production of normal to elevated levels of IgA antibodies to sonicated whole trophozoites. Our hypothesis is that *xid* mice fail to make some specific IgA antibody response to a particular antigen(s). Responses to that antigen(s) may represent a small fraction of the total IgA antibody response, and therefore the difference would be undetectable in our system, which measures IgA antibody to whole trophozoites. In the absence of this crucial antibody, *xid* mice may develop a chronic infection accompanied by substantial IgA antibody production to antigens not primarily involved in stimulating a protective response. Others have noted a normal level of IgA antibody response to sheep erythrocytes by *xid* mice, but did not report information on the fine specificity of that antibody (15).

Clearly, at least two other interpretations of our data are possible. One possibility is that IgG antibody is important for elimination of this parasite, since we observed lower levels of IgG antibody in serum in *xid* mice than in normal controls. We consider this unlikely since we have been unable to detect IgG antibody in the secretions of the gut lumen, where the *G. muris* trophozoites are located (20), and since twice-weekly administration of serum containing IgG antibody from resistant CBA/J mice (D. Skea and B. J. Underdown, manuscript in preparation) did not render CBA/N mice resistant to a primary infection with *G. muris*. A second possibility is that *xid* mice have a deficiency in cell-mediated immunity. However, such defects, if they exist in vivo, do not appear to impair cell-mediated immune elimination of other infectious agents by *xid* mice (6, 7, 19). Studies are in progress to document more explicitly the cellular defect in *xid* mice which leads to chronic *G. muris* infection, as well as the nature of the putative antibody specificity defect.

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