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# Quantitative and Temporal Analyses of Murine Antibody Response in Serum and Gut Secretions to Infection with *Giardia muris*

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**We analyzed the appearance and level of *Giardia muris*-specific antibody of immunoglobulin A (IgA), IgG, and IgM isotypes, at weekly intervals, over the course of a 7-week infection in BALB/c and C57BL/6 mice. Using sensitive immunoradiometric assays, we observed that IgA antibody was the only detectable anti-*G. muris* antibody in intestinal secretions throughout the course of infection. No secreted IgG or IgM anti-*G. muris* antibody was detected even in concentrated intestinal secretions. The expulsion of *G. muris* by the mice was associated closely with the appearance and increasing levels of secreted anti-*G. muris* IgA antibody. Both IgG and IgA serum antibody to *G. muris* were detected, but no serum IgM antibody was detected. Serum IgA and IgG anti-*G. muris* antibody remained at high levels up to 10 weeks following clearance of the parasite. An interesting observation indicated that serum IgA antibody to *G. muris* developed more slowly in response to infection than secreted IgA antibody. An analysis of the molecular weight distribution of total serum IgA in infected mice determined that infection produced a transient but significant shift in serum IgA to high-molecular-weight (≥dimeric IgA) forms. The results indicate that a substantial IgA antibody response occurs in sera and in gut secretions of *G. muris*-resistant mice and that IgA antibody is the dominant and possibly the only effector antibody active in intestinal secretions during *G. muris* infection in mice.**

Information concerning the antibody response to *Giardia muris* in infected mice has appeared in several reports over the last few years. Both immunoglobulin A (IgA) and IgG antibody to *G. muris* have been detected in serum by immunoradiometric assays (IRMA) (1, 14, 16). IgA antibody was found in serum and milk by indirect immunofluorescence of whole fixed parasites (2, 5). Apart from our own recent report (14), there has been only one other report (1) of the detection of IgA anti-*G. muris* antibody in intestinal secretions. In the latter report, there was no analysis of IgG or IgM antibody in gut secretions. Since the gut lumen is the site of infection by *G. muris* (12), a more detailed examination of secreted antibody is required.

Previously, we reported a study which strongly implicates a role for B cells and antibody in the clearance of a primary *G. muris* infection in mice (14). We observed, using sensitive IRMA, that mice treated from birth with anti-IgM antiserum were unable to expel *G. muris* and had no detectable IgG or IgA anti-*G. muris* antibody in serum or gut secretions. However, those gut secretions were sampled at only one time point, following the clearance of the parasite from the gut of the untreated infected mice.

Since we observed chronic *G. muris* infections in antibody-deficient mice (14), but information on the parameters of the antibody response to *G. muris* is sketchy, we have examined precisely the time course of the antibody response to *G. muris* in serum and gut secretions of two parasite-resistant strains of mice. In doing so, we have compared the rate of clearance of *G. muris* with both the quantitative and temporal characteristics of anti-*G. muris* antibody responses of different immunoglobulin isotypes. In addition, we examined response parameters such as serum IgA molecular weight distribution and total IgA in gut secretions to determine how they reflected the nature of the IgA anti-*G. muris* response.

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## MATERIALS AND METHODS

**Mice.** Female of BALB/c and C57BL/6 mice were obtained from Charles River Canada (St. Constant, Quebec) at 5 to 6 weeks of age. They were housed in the Medical Sciences Building at the University of Toronto in an air-filtered environment and in cages with filter tops. The mice were maintained on standard rodent chow and sterilized water ad libitum.

**Experimental protocol.** One week after arrival, groups of mice from each strain were infected by esophageal intubation with 5,000 *G. muris* cysts. Other mice were left uninfected as normal controls. In one experiment, infected and noninfected age-matched BALB/c and C57BL/6 mice were sacrificed in groups of four to five. Samples of serum and gut secretions were taken from noninfected mice prior to the experiment and then from noninfected and infected mice at weeks 1 to 5 and 7 postinfection. Parasite loads were assessed by counting cysts immediately before sacrifice and trophozoites at the time of sacrifice. In other experiments, single groups of infected or noninfected BALB/c mice were bled from the orbital plexus at intervals of up to 18 weeks post-cyst inoculation.

**Isolation of *G. muris* cysts and trophozoites.** *G. muris* cysts were isolated from the feces of infected mice by sucrose gradient centrifugation in a manner similar to that described by Roberts-Thomson et al. (13) and counted. Cysts used to infect mice were isolated from the stools of infected nude mice, through which the parasite was constantly passaged. *G. muris* trophozoites were isolated from pelleted intestinal

material following gut wash procedures as outlined previously (14). Counts of both cysts and trophozoites were made with a hemacytometer. The minimal count was 500 cysts per eight fecal pellets or 500 trophozoites per ml of intestinal contents.

**Collection of sera and gut secretions.** Sera were collected from groups of mice of both strains via cardiac bleeding under ether anesthesia at various times during the infection. Some groups of BALB/c mice were bled from the orbital plexis under ether anesthesia, using capillary pipettes. Between 150 and 400 μl of serum per animal was obtained after clotting of blood (at 4°C) and centrifugation (8,000 × *g*).

Gut secretion sampling was done following exsanguination of the mice, which had been deprived of food for 2 to 4 h before isolation of the gut secretion material. In brief, the whole small intestine was excised from the animal and ligated within 4 cm of the end of the ileum. A solution of 4 ml of ice-cold phosphate-buffered saline containing 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and 0.05 U of trypsin inhibitor, Aprotinin (Sigma Chemical Co., St. Louis, Mo.) per ml was injected into the duodenal end, and the tissue was massaged for 1 min. The fluid contents of the bowel were then allowed to drain out into a 15-ml conical tube that was then centrifuged at 800 × *g* for 10 min at 4°C. The resulting supernatant was frozen at −20°C until use. About 3.5 ml of solution, referred to as "gut secretion material," was usually recovered.

To test whether the gut secretion sampling procedure and subsequent treatment of gut secretion material may have resulted in degradation of IgG, IgM, or albumin, normal sampling procedures were carried out on separate groups of normal noninfected BALB/c mice. Trace amounts of $^{125}$I-labeled IgM, IgG, or albumin or $^{131}$I-labeled dimeric IgA were injected individually with the sampling solution. The resulting gut secretion samples were then treated as usual and analyzed by high-pressure liquid chromatography to determine percent degradation of the labeled protein. The results indicated that no significant degradation of any protein occurred and that IgG, IgM, and albumin showed no greater degradation under these conditions than did IgA (data not shown).

**IRMA for immunoglobulins in serum and gut secretions.** We have previously detailed the development of highly sensitive and specific IRMA for mouse IgM, IgG, and IgA (14). Solid-phase sandwich-type IRMA were performed with affinity-purified antibodies to immunoglobulin isotype heavy chains, labeled with $^{125}$I. Each labeled anti-isotype antibody demonstrated <3% cross-reactivity to other isotypes and serum proteins such as albumin and transferrin. The sensitivity of each assay for purified myeloma standard proteins was <1 ng. To measure the serum levels of each isotype, sera were diluted 1/1,000 for IgA, 1/4,000 for IgM, and 1/10,000 for IgG in 0.01 M potassium phosphate–0.15 M sodium chloride, pH 7.4 (phosphate-buffered saline), containing 1 mg of bovine serum albumin (BSA) per ml. These dilutions of serum were chosen because they represented the point of 50% maximum binding on the slopes of the serum dilution curves for each of the assays. The slope of the dilution curve of sera from both strains of mice varied by <10% from that of the myeloma protein standard dilution curve for each of the isotypes measured. Immunoglobulins in gut secretions were measured in a similar manner, with dilutions (in phosphate-buffered saline-BSA) of 1/5 for IgM and IgG and 1/60 for IgA.

**IRMA for *G. muris*-specific antibody.** We developed IRMA to detect anti-*G. muris* antibody (14) in a manner similar to that of Anders et al. (1). The same labeled anti-isotypic reagents used in the immunoglobulin assays were used to examine the binding of IgG, IgA, and IgM antibody to *G. muris* antigen that was coated onto polyvinyl microtiter plate wells. A standard serum (pool from each mouse strain after 7 weeks of infection) was used to develop a standard dilution curve of antibody activity in units per milliliter. Similarly, standard curves were developed with a concentrated pool (approximately fourfold) of gut secretion material taken at week 7 of infection from mice of each strain.

To measure specific anti-*G. muris* antibody, the wells of the assay plate were coated with *G. muris* antigen that was prepared by sonication of *G. muris* trophozoites isolated from infected nude mice. Details of the procedures for these assays are outlined elsewhere (14). Antigen-negative wells were coated with "sham antigen" isolated from noninfected nude mice or with BSA. The average counts per minute bound to BSA-coated wells were not significantly less than those bound to sham antigen-coated wells. "Specific counts per minute bound" were determined as counts bound to antigen-coated wells minus those bound to antigen-negative wells. Sera from noninfected mice had low but significant levels of specific counts per minute bound.

Antibody specific to *G. muris* and produced during infection was assessed by subtracting the specific counts per minute bound by pooled sera (or gut secretions) of noninfected mice from those bound by the sera or gut secretions of infected mice. The resulting difference was referred to as "net counts per minute bound." The standard curves of antibody activity were based on net counts per minute bound by dilutions of the standard serum (or gut secretions), given a value of 10,000 U/ml. The net counts per minute bound by individual sera (or gut secretions) were then converted to units of antibody per milliliter by interpolation on the standard curves. Individual sera or gut secretions from noninfected mice occasionally showed low but significant net counts per minute bound. The antibody levels calculated for those mice are indicated in the results along with the antibody levels in the infected mice.

Sera or gut secretion samples from both infected and noninfected, age-matched controls were examined at two to three dilutions in duplicate in phosphate-buffered saline containing 1 mg of BSA per ml. Minimal dilutions of sera were set at 1/20 for IgA antibody, 1/40 for IgG antibody, and 1/20 for IgM antibody, after analysis of the nonspecific binding of components of normal mouse serum to antigen-negative wells (14). Gut washes for testing were diluted 1/2 and 1/4.

**Analysis of immunoglobulin and antibody data.** Dose interpolation and curve fitting were performed for all IRMA, using the curve-fitting programs of Davis et al. (7) adapted to a TI-59 calculator or to BASIC programming on a microprocessor in our laboratory. Unless otherwise stated, the data are presented as geometric mean and standard error of the mean (SEM).

**High-pressure liquid chromatography-IRMA analysis of molecular weight distribution of immunoglobulin and antibody.** Sera and gut secretion samples were examined by high-pressure liquid chromatography in conjunction with IRMA to determine the molecular weight distribution of IgA antibody. The details of procedures for this analysis were outlined previously (14). In brief, 10- to 60-μl samples of sera were precipitated in 45% ammonium sulfate, and the resulting precipitate was suspended in and dialyzed against 0.01 M Tris–0.15 M NaCl–0.02% sodium azide, pH 7.0 (TBS). This material (nominal volume of 200 μl) was loaded onto a 60-cm

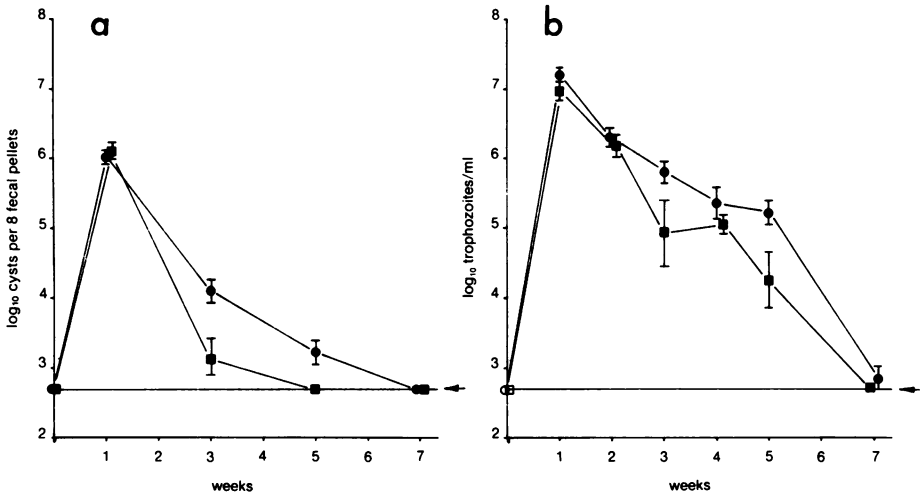


FIG. 1. Course of infection in *G. muris*-resistant strains of mice. The levels of (a) cysts and (b) trophozoites are compared between infected C57BL/6 (■) and BALB/c (●) mice. Mice were inoculated with 5,000 *G. muris* cysts at week zero. Animals of each strain that were not inoculated did not have *G. muris* cysts or trophozoites (data not shown). Each point represents the geometric mean of counts of cysts per eight fecal pellets or trophozoites per milliliter of gut secretion material for groups of four to five mice. The arrows show the limit of detection of each assay. Error bars are SEM.

TSK-G3,000 column (LKB-Produckter, Bromma, Sweden) equilibrated in TBS. Sample fractions of column effluent were collected directly onto microtiter plates prepared for immunoglobulin or anti-*G. muris* antibody analysis. Plates were processed by the normal procedures of each assay type, and standard curves were run simultaneously with the chromatographed samples. The amount of IgA in paired fractions was displayed as a percentage of the total IgA detected. Anti-*G. muris* IgA antibody in paired fractions was displayed as a percentage of the total net counts per minute bound. For IgA antibody molecular weight distribution in serum, 60 μl of pooled serum was used, while 300 μl

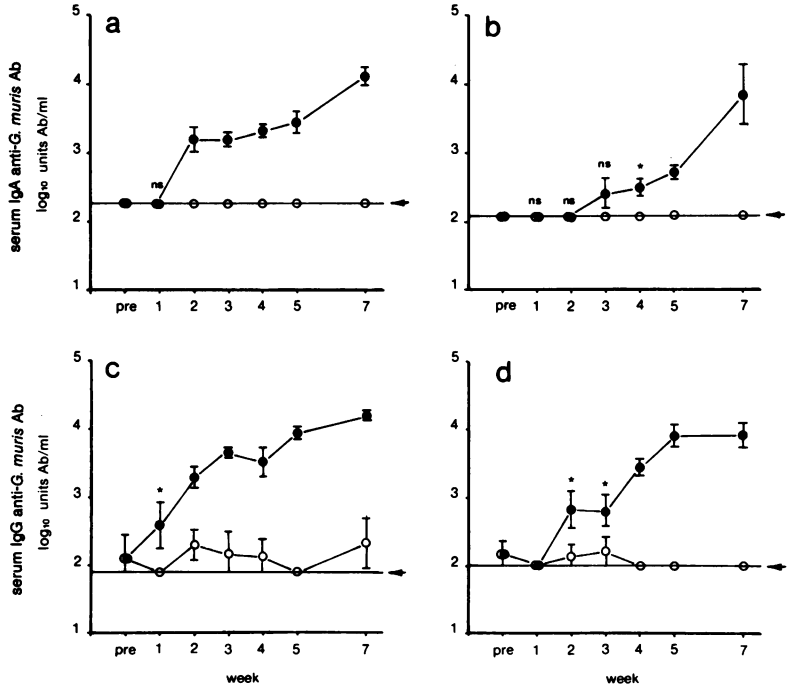


FIG. 2. IgA and IgG anti-*G. muris* antibody detected in sera of BALB/c and C57BL/6 mice. IgA antibody was measured in serum samples taken prior to and up to 7 weeks following cyst inoculation of (a) BALB/c and (b) C57BL/6 mice. Groups of infected (●) or noninfected (○) age-matched mice were used. Each point is a geometric mean of values from four to five mice. Similarly, serum IgG anti-*G. muris* antibody was measured for (c) BALB/c and (d) C57BL/6 mice. Error bars represent SEM. The difference between infected and noninfected mice was significant at $P < 0.005$, except as marked: $P < 0.05$ (*) or not significant (ns). The arrow shows the limit of detection for each assay. These limits differ chiefly because separate standard sera and controls for each strain were used (see Materials and Methods).

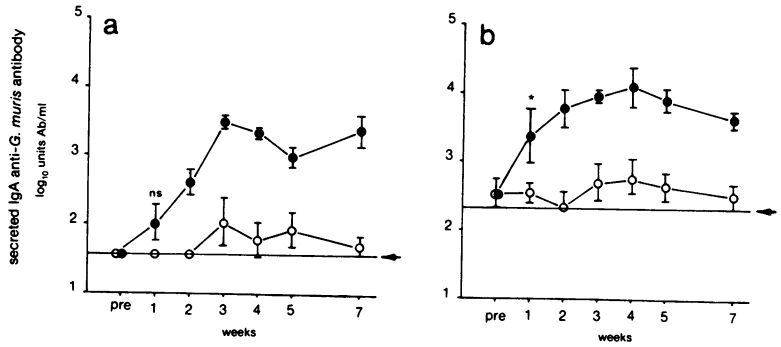


FIG. 3. IgA anti-*G. muris* antibody detected in gut secretions of (a) BALB/c and (b) C57BL/6 mice. Groups of infected (●) or noninfected (○) age-matched mice were used. See Fig. 2 legend for details.

of pooled and dialyzed (in TBS, 18 h, 4°C) gut secretion material was used for analysis of secreted IgA antibody.

**Albumin assays and immunoglobulin secretion estimation.** Albumin and immunoglobulin levels were assayed in gut secretions and serum to differentiate transudation from secretion of different immunoglobulin isotypes into the gut. A radial diffusion assay was developed which was similar to that of Mancini et al. (9) and sensitive to 1 μg of albumin. Details of the assay appeared in a previous publication (14). A standard curve was produced, using purified mouse albumin. Immunoglobulin levels in gut secretion material and sera were determined by IRMA and then normalized to albumin levels (immunoglobulin/albumin ratio). The quotient derived when the fraction immunoglobulin/albumin (in secretion) is divided by the fraction immunoglobulin/albumin (in serum) represents a measure of secretion of that immunoglobulin into the gut (15). Values of >1.0 indicate secretion, while those <1.0 are considered less than that expected by transudation.

**Statistical analysis.** The Mann-Whitney nonparametric test or Student's *t* test was used, where appropriate, to determine the statistical significance of apparent differences between means of various groups of data.

## RESULTS

**Analysis of the course of *G. muris* infection by cyst and trophozoite counts.** In two preliminary studies (data not shown), we observed that during a primary infection C57BL/6 female mice eliminated *G. muris* more quickly than BALB/c females, as assessed by cyst excretion levels. Figure 1 shows the numbers of cysts and trophozoites isolated from both strains of mice over a 7-week infection period. The results indicate that C57BL/6 mice had lower numbers of parasites than BALB/c mice, especially at weeks 3 and 5. Both strains had cleared *G. muris* by week 7. These

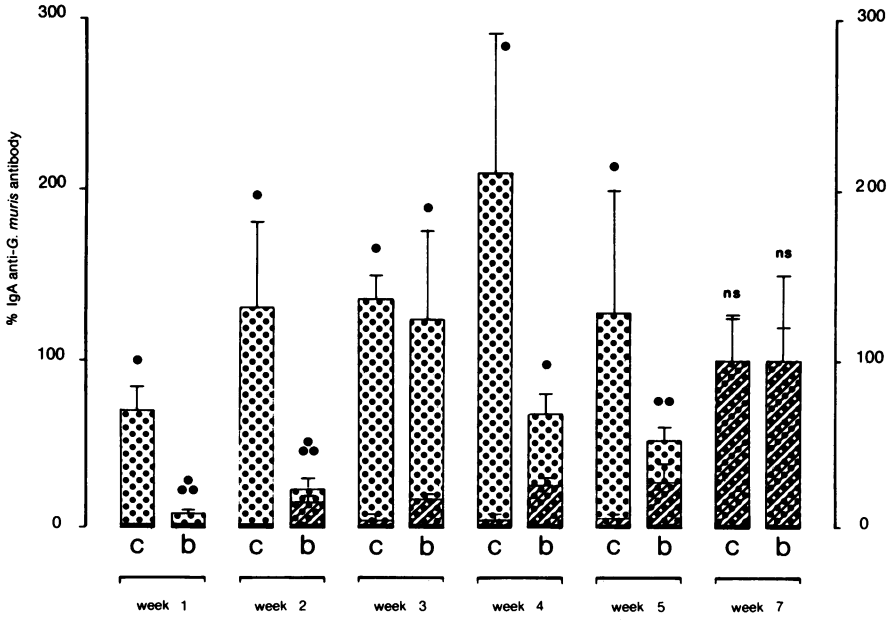


FIG. 4. Comparison of serum (▨) and secreted (▩) anti-*G. muris* IgA antibody produced by BALB/c (b) and C57BL/6 (c) mice during infection. Amounts of antibody are expressed as a percentage of the mean amount of antibody (units per milliliter) at week 7 of infection. Mean percentages and SEM (error bars) were then calculated for each group. Minimum assay detection was ≈2% (▬). Differences between serum and secreted antibody were significant, as follows: $P < 0.005$ (•); $P < 0.05$ (••); $P < 0.09$ (•ns•); or not significant (ns).

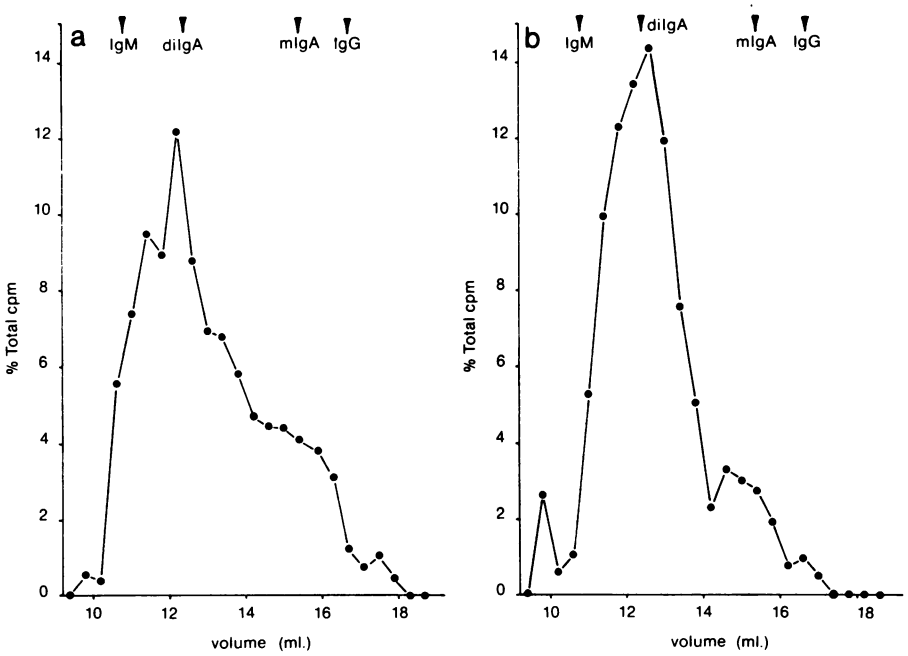


FIG. 5. IgA anti-*G. muris* antibody molecular weight profile in (a) serum and (b) gut secretions of C57BL/6 mice. The total net counts per minute bound were ≈13,000 for serum IgA antibody and ≈2,000 for secreted antibody. The arrows mark the peak elution positions of various mouse immunoglobulin standards: IgM, MOPC 104E; diIgA, MOPC 460 dimer; mIgA, MOPC 460 monomer; IgG, normal mouse serum IgG.

small differences are similar to those reported previously by Belosevic et al. (4).

**Time course of the *G. muris*-specific antibody response in serum.** Figure 2 indicates the levels of anti-*G. muris* IgA andIgG antibody in the sera of BALB/c and C57BL/6 mice during the course of infection. IgA antibody was first present in BALB/c sera by week 2 of infection (four of four mice) and in C57BL/6 sera (three of four mice) by week 3 of infection (Fig. 2a and b). For 2 to 3 weeks thereafter, a constant, low level of serum IgA antibody was found in both strains of mice. This was followed by a sharp increase in the level of serum IgA antibody between weeks 5 and 7 in both strains.

BALB/c mice (two of four at week 1 and four of four at week 2) had serum IgG antibody at early points in the infection. At week 2, three of five C57BL/6 mice had serum IgG anti-*G. muris* antibody. The level of serum IgG antibody in the sera of both strains of mice peaked by weeks 4 to 5 of infection (Fig. 2c and d).

In a separate experiment, the levels of IgG and IgA anti-*G. muris* antibody in serum were monitored to week 18 of infection in BALB/c mice. The results indicated that, by 18 weeks following cyst inoculation, substantial amounts of IgG and IgA antibody were still present in the sera of BALB/c mice. For example, IgG antibody was 3,500 U/ml at week 6 and 2,200 U/ml at week 18, while IgA antibody was 1,500 U/ml at week 6 and 3,200 U/ml at week 18. Week 18 was approximately 10 weeks after clearance of *G. muris* (data not shown).

IgM antibody in serum was not detected by IRMA, even during the early weeks of infection, when IgM responses might have been expected. The labeled anti-IgM reagent was capable of detecting <1 ng of myeloma IgM.

**Time course of the *G. muris*-specific antibody response in gut secretions.** Both strains of mice demonstrated IgA antibody specific to *G. muris* in gut secretions beginning at week 1 of infection (Fig. 3). From week 2 to the end of the experiment all infected mice secreted IgA anti-*G. muris* antibody. IgG antibody was not detected in gut secretions, at various time points, even when secretions were pooled and concentrated (up to eightfold) before analysis for antibody (data not shown). No attempt was made to measure IgM antibody, since IgM in gut secretions was barely detectable (see below).

A comparison of Fig. 2a and b with Fig. 3 indicates that secreted IgA antibody was detected 1 to 2 weeks prior to serum IgA antibody and that secreted IgA antibody reached peak levels before serum IgA antibody. This is shown more clearly in Fig. 4, where IgA antibody levels in serum and gut secretions are expressed as a percentage of week 7 antibody levels.

**Size of *G. muris*-specific antibody in serum and gut secretions.** Figure 5 shows the size distribution of IgA anti-*G. muris* antibody in pooled serum and gut secretion material of C57BL/6 mice at week 7 of infection. Serum IgA antibody was predominantly of high molecular weight (≥dimeric IgA), although some antibody appeared to be monomeric in size. Secreted IgA anti-*G. muris* antibody was almost entirely of a size ≥dimeric IgA. IgA antibody detected in BALB/c serum and secretions was of a size similar to that shown here for C57BL/6 and to our previously published results (14).

**Size distribution of IgA in serum during and after clearance of *G. muris* infection.** We observed previously (14) that at week 11 of infection BALB/c mice had a substantial increase in high-molecular-weight (≥dimeric) serum IgA relative to low-molecular-weight (≤monomeric) IgA. This contrasted with noninfected mice in which the serum IgA was predominantly monomeric in size. In the experiments presented here we studied the size distribution of serum IgA at various times during infection. The results indicated that during the course of infection in C57BL/6 mice (Fig. 6a) an increase occurred in the amount of IgA of ≥dimeric size relative to

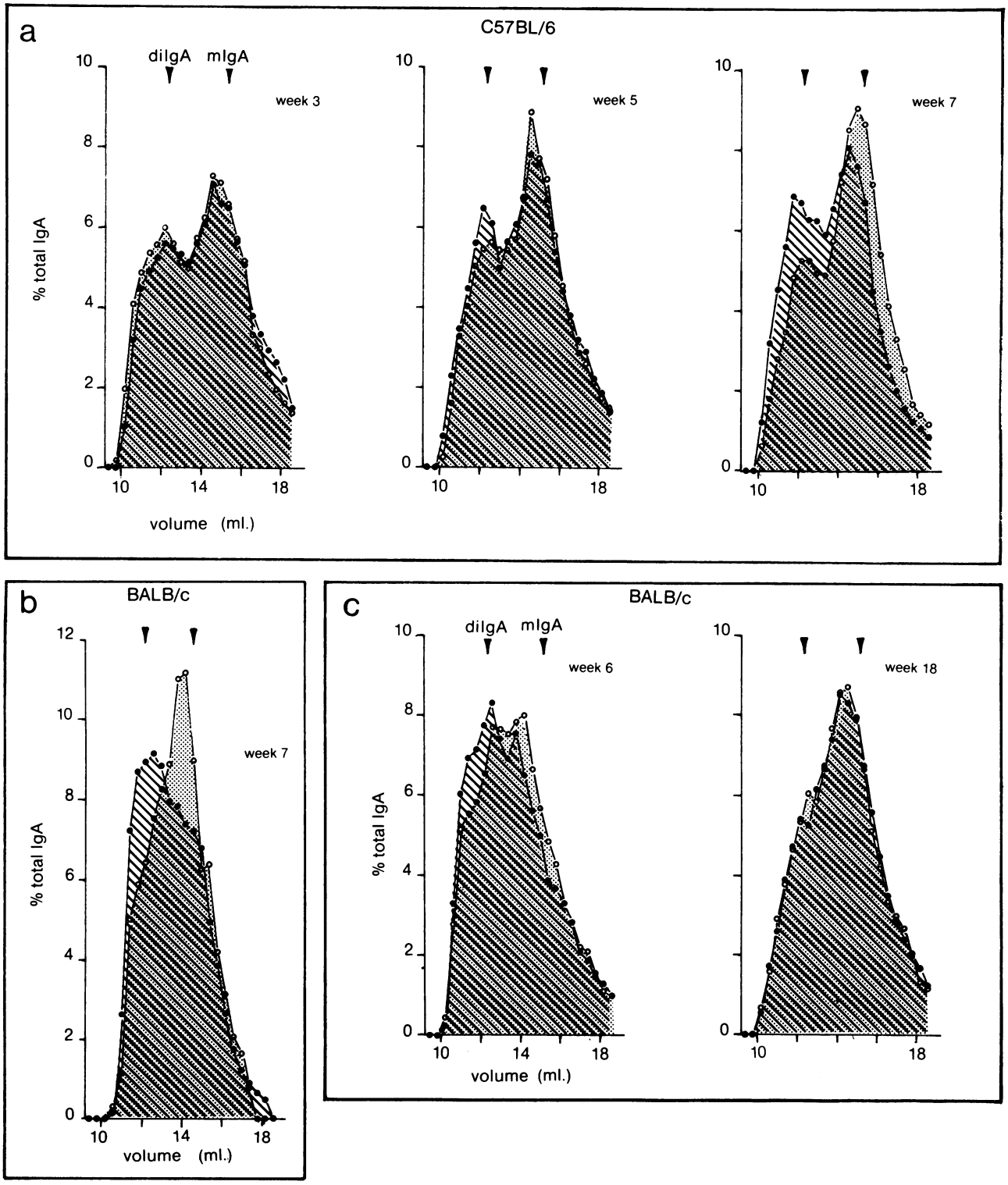


FIG. 6. Serum IgA molecular weight profile of pooled sera from infected (▨) and noninfected (▩) age-matched mice. Each point is the amount of IgA in two sequential fractions, presented as a percentage of the total IgA measured in the entire eluate. Arrows mark the peak elution position of dimeric IgA MOPC 460 (dilgA) and monomeric MOPC 460 (mIgA). Panel a shows data from C57BL/6 mice at weeks 3, 5, and 7 of infection, panel b shows data from BALB/c mice at week 7, and panel c shows data from BALB/c mice at weeks 6 and 18 of infection. The total IgA measured in the serum samples of infected and noninfected mice was between 2 and 5 μg.

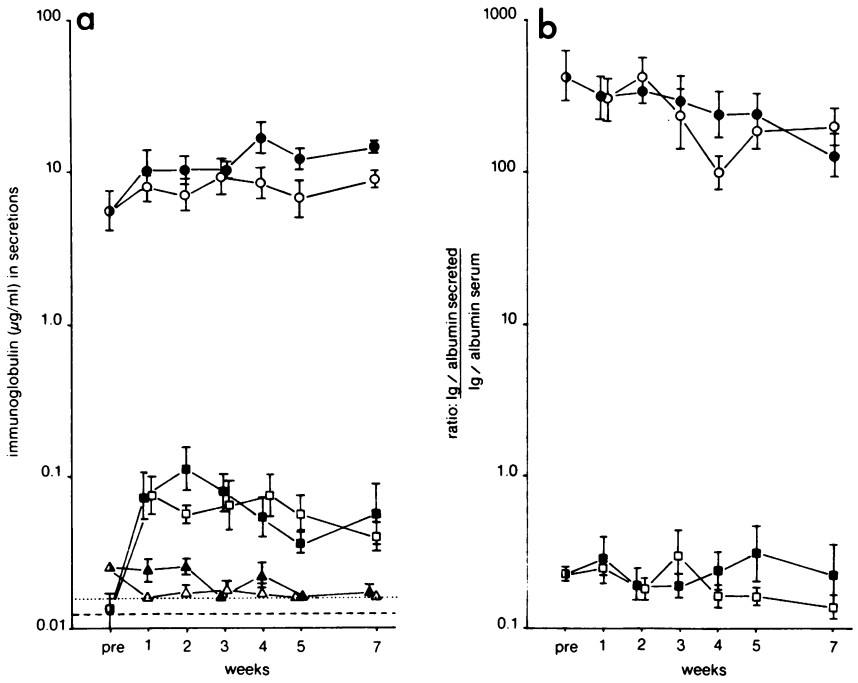


FIG. 7. Levels of secreted immunoglobulin in infected and noninfected C57BL/6 mice during a 7-week infection period. (a) IgA levels in noninfected (○) and infected (●) mice, IgG levels in noninfected (□) and infected (■) mice, and IgM levels in noninfected (△) and infected (▲) mice. Each point is the geometric mean of values from four to five mice. Error bars are SEM. The dotted line is the limit of detection for IgM and the dashed line is the limit of detection for IgG. (b) Secretion values for IgA and IgG were calculated by using the formula: (secreted immunoglobulin/secreted albumin)/(serum immunoglobulin/serum albumin). Mean secretion values are for IgA of infected (●) and noninfected (○) mice and for IgG of infected (■) and noninfected (□) mice. Values of >1.0 indicate selective secretion of the particular immunoglobulin. Error bars are SEM.

monomeric size in serum. Similar results were again observed in BALB/c mice (Fig. 6b). To determine the duration of this effect, sera were examined from a separate group of BALB/c mice at weeks 6 and 18 postinfection (Fig. 6c). By week 18 of infection the serum distribution of IgA in the previously infected mice was the same as that in age-matched mice that had no previous infection.

Analysis of the total amount of serum IgA by IRMA indicated marginal increases in serum IgA during infection: BALB/c infected, 42 to 112 μg/ml; noninfected, 43 to 88 μg/ml, at all time points. There was no significant statistical difference ($P > 0.2$ at all time points) between the serum IgA levels of infected and noninfected mice.

**Levels of immunoglobulin and albumin in gut secretions.** We were interested in determining whether a substantial increase in total immunoglobulin (especially IgA) or albumin levels occurred in gut secretions over the course of infection. Increased immunoglobulin secretion might reflect an antibody response to infection. In addition, an increased level of albumin in the gut could indicate increased intestinal permeability or egress of serum proteins (8). Figure 7a shows that IgA is the dominant immunoglobulin in gut secretions of both infected and noninfected C57BL/6 mice at all time points. There was little difference in the amount of secreted IgA between the infected and noninfected mice ($P > 0.2$ at all time points). IgG levels were <1% of IgA at all time points and in all mice regardless of the presence of *G. muris* infection. Infected mice had no significant increase in IgG at any time point ($P > 0.3$). IgM levels were consistently low in all gut secretion samples. BALB/c mice examined at the same time showed results nearly identical to those with the C57BL/6 mice.

Figure 7b shows the ratio of IgA and IgG levels to albumin levels in gut secretion samples and serum of C57BL/6 mice. The data indicate the selective secretion of IgA (secretion values of >100) into the gut and indicate that no IgG secretion occurred, since the IgG secretion values were <1.0. Similar results were obtained for BALB/c mice. No significant change in the amount of albumin in the gut occurred during infection of either strain of mice. Albumin levels in gut secretions of noninfected mice (range, 7.1 to 12.6 μg/ml) were not different from those of infected mice (range, 8.2 to 11.4 μg/ml).

## DISCUSSION

In a previous paper we presented strong evidence that a role exists for *G. muris*-specific antibody in the expulsion of a primary *G. muris* infection in mice (14). We demonstrated that the treatment of mice from birth with rabbit anti-mouse IgM antisera caused such mice to develop a chronic *G. muris* infection in association with immunoglobulin and *G. muris*-specific antibody deficiency in serum and gut secretions. However, that study did not define fully the in vivo antibody response in normal *G. muris*-resistant mice and examined secreted antibody at only one time point. Anders et al. (1) examined antibody production in serum and gut secretions of mice during *G. muris* infections, but did not analyze immunoglobulin isotypes other than IgA in gut secretions. The interpretation of the relative roles of serum or gut antibodies in the clearance of the parasite has been hindered by the incomplete analysis that has been done to date. Also, there has been no direct analysis of the relationship of the serum antibody response with the secreted antibody re-

sponse, a point of interest since serum antibody has been used as a measure of antibody response to *G. muris*, despite the fact that the parasite is a gut luminal-dwelling organism (12, 16). We therefore set out in the present set of studies to document carefully the characteristics and response patterns of serum and secreted antibody in two strains of mice that were resistant to *G. muris*.

The analysis of gut secretions showed that *G. muris*-specific antibody of the IgA isotype was the only detectable antibody in gut secretions during the entire course of infection in both BALB/c and C57BL/6 mice. This gut IgA antibody response was detected early during infection, probably reflecting the preferential stimulation of the mucosal IgA response via the Peyer's patches (6, 10, 11). IgG and IgM anti-*G. muris* antibodies were not detected in gut secretions, even after concentration of gut secretion material and the use of sensitive IRMA. The method of antibody collection allowed measurement of all immunoglobulin isotypes and albumin without apparent degradation of any of these proteins (see Materials and Methods). We detected very little IgG in gut secretions, and it was apparent that IgG was not actively secreted into the gut. Thus the gut secretion samples had little contamination with serum antibody.

The secretion of IgA antibody specific to *G. muris* occurred during the same period of time in which trophozoite levels had begun to drop noticeably in both strains of mice. Thus, a good temporal association was indicated between *G. muris* expulsion and the amount of IgA antibody in secretions. This association, together with the lack of detectable IgG and IgM antibodies in secretions and the observation of chronic infection in mice with mucosal antibody deficiency (14), leads us to conclude that IgA antibody is the most likely effector antibody in the gut during murine giardiasis.

IgG antibody to *G. muris* was detected in serum but not in gut secretions. Like secreted IgA antibody, serum IgG antibody was detected early during infection. Its maximal production also coincides with the expulsion of the parasite. However, since no IgG anti-*G. muris* antibody was detected in gut secretions it appears that very little or no IgG antibody reaches the intestinal location of the parasite, and its role in the expulsion of *G. muris* remains uncertain.

The origin of the serum anti-*G. muris* IgA antibody cannot be determined directly from the data presented here. Nevertheless, two observations indicate that the serum IgA antibody may reflect IgA antibody produced in the gut mucosa. First, the IgA antibody in serum was mostly of high-molecular-weight (polymeric) form(s), similar to IgA antibody produced and secreted into the gut (Fig. 5). Second, there was a transient shift in serum IgA to high-molecular-weight forms, in association with the *G. muris* infection. This effect was most noticeable when serum IgA antibody levels were highest (cf. Fig. 2b and 6a). It therefore appears that during *G. muris* infection serum IgA antibody reflects antibody produced in the gut mucosa that has entered the circulation. This would be consistent with the work of Bazin et al. (3), indicating the contribution of mucosal IgA to serum IgA.

Serum IgA antibody was detected later and rose to peak levels later than secreted IgA antibody. This phenomenon was demonstrated in both strains of mice. The differences between serum and secreted IgA antibody in the time of appearance and peak levels are not necessarily inconsistent with a mucosal origin of serum IgA antibody, but demand additional mechanisms for explanation.

The observation of the shift in the molecular weight character of the total IgA in serum, in association with *G. muris* infection, is in itself intriguing. The indirect evidence suggests that the increase in polymeric size IgA is a result of the production of polymeric IgA anti-*G. muris* antibody which makes its way into the serum. At the same time no significant increase in total serum IgA levels occurred. In addition, while the shift to increased polymeric IgA in serum is transient, serum levels of anti-*G. muris* IgA antibody remained high for an extended period after infection. Such a pattern of IgA response in serum may be common to a variety of gut mucosal infections, but we do not know of any studies that describe this phenomenon in other gut infections.

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