

Antibody Responses Induced by Immunization of Inbred Mice Susceptible and Resistant to African Trypanosomes

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We tested the ability of inbred mice that were either susceptible (strain A/J) or resistant (strain C57BL/6 and A/J × C57BL/6 hybrids) to African trypanosomes to produce specific antibodies to trypanosome antigens in the absence of living parasites. This experiment was carried out to eliminate the influence of trypanosome growth or metabolism on immune responsiveness. Mice were immunized with keyhole limpet hemocyanin or solubilized *Trypanosoma brucei gambiense*, and serum antibodies were measured in solid-phase radioimmunoassays after primary and challenge injections. Both susceptible and resistant mice showed increases in keyhole limpet hemocyanin-specific or trypanosome-specific immunoglobulin M and immunoglobulin G after immunization. When immunized with trypanosome antigens, resistant mice made qualitatively and quantitatively superior specific immunoglobulin M responses, particularly to the trypanosome major variable surface glycoprotein. Susceptible A/J mice produced good specific antibody responses, although these were predominantly of the immunoglobulin G isotypes. These results show that A/J and C57BL/6 mice respond differentially in terms of immunoglobulin isotype and repertoire in response to injected antigens. The possibility that this differential antibody response influences susceptibility to African trypanosomes is discussed.

A range in susceptibility of various inbred mouse strains infected with African trypanosomes (4, 8, 17, 20, 26) has been observed. Although some variability is seen with the parasite strain, in general A strain (A/J) mice are found to be the most susceptible and B strain (C57BL/6, C57BL/10, and congenic lines) the most resistant. Differences in susceptibility do not correlate with initial parasite infectivity but are reflected in parasitemia profiles and survival times.

Trypanosome resistance in mice is under multigenic control. Several autosomal and at least one X-linked gene appear to be involved (10, 18, 20), and it is clear that resistance is not strictly H-2 linked (17, 20). Resistance is inherited as a dominant trait. Thus, B₆AF₁ (A/J × C57BL/6J) mice survive chronic *Trypanosoma congolense* infections as well as the resistant parent (20).

Resistance to African trypanosomes appears to be related in part to the capacity to limit numbers of bloodstream forms of the parasite (20). Although the exact mechanism(s) by which successive waves of trypanosomes are cleared from the blood is still not understood, it is evident that antibody responses are critical in limiting parasite numbers. Active and passive immunization studies have revealed that anti-

bodies specific for trypanosome variable surface glycoproteins (VSGs) protect against challenge with the homologous parasite (reviewed in reference 18). Campbell et al. (6) showed that mice depleted of B lymphocytes by neonatal anti- μ treatment were unable to control parasite numbers in the first peak when infected with *Trypanosoma rhodesiense* or to resist challenge with the homologous organism. These results show that VSG-specific antibody responses are an important aspect of trypanosome resistance. It follows that the quantity, affinity, and isotype of antibodies produced to trypanosome surface VSGs would influence the parasitemia profiles and perhaps the susceptibility of an infected animal to trypanosomes. The virulence of a given clone of trypanosomes within a strain (8) and the various susceptibility differences among mouse strains (17, 20) could thus reflect the ability of the host animal to produce effective antibodies. We decided to compare, in the simplest terms, the ability of trypanosome-susceptible and -resistant mouse strains to produce antibodies to trypanosome antigens without the complicating variables of antigenic overload and immunodepression which accompany active infection. To do this, we injected mice with solubilized trypanosomes in the absence of adjuvant;

thus, the animals received the full complement of antigens they would experience during an active infection without the metabolic influences from living normal or irradiated parasites.

MATERIALS AND METHODS

Animals. A/J and C57BL/6J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. B₆AF₁ (A/J × C57BL/6J) mice were bred in the animal quarters of the Department of Biochemistry and Microbiology (University of Victoria, Victoria, British Columbia, Canada) from parental stock recently purchased from Jackson Laboratories. BALB/c mice and Long Evans rats were bred from stock purchased from Canadian Breeding Farms and Laboratories Ltd., St. Constant, Quebec, Canada. All mice were males aged 3 to 5 months at the onset of experiments. Male rats aged 2 months were used to grow trypanosomes for antigen preparation.

Antigens. (i) **Malarial and normal erythrocyte antigens.** Malarial schizont antigens prepared by detergent (Triton X-100, 2%) extraction of Rhesus monkey erythrocytes infected with *Plasmodium knowlesi* clone W962 and normal Rhesus monkey erythrocytes solubilized by the same procedure were gifts from Russell Howard, National Institutes of Health, Bethesda, Md.

(ii) **KLH.** Keyhole limpet hemocyanin (KLH) was obtained from Calbiochem-Behring, La Jolla, Calif., and dissolved in sterile physiological saline or phosphate-buffered saline (pH 7.4).

(iii) **Trypanosomes and trypanosome antigens.** *Trypanosoma* (Trypanozoon) *brucei gambiense* TTrT-1, a triply cloned organism derived from the Wellcome (TS) strain (24), was obtained from J. R. Seed, Department of Parasitology and Laboratory Practice, University of North Carolina, Chapel Hill, in July 1981. After immediate passage in BALB/c mice, the parasite was preserved as a cryostabulate in liquid nitrogen in our laboratory.

Soluble antigens from lysed trypanosomes. A Long Evans rat was infected by intraperitoneal injection of 10⁷ *T. gambiense* TTrT-1 organisms. Five days later, the rat was exsanguinated, and trypanosomes were isolated from heparinized blood on DEAE-cellulose columns by the method of Lanham and Godfrey (16). Column-purified trypanosomes were washed extensively with ice-cold phosphate-buffered saline-glucose (16) and then resuspended to 10⁹ parasites per ml in PSG, (pH 8.0) containing 0.017% phenylmethylsulfonyl fluoride-0.0005% pepstatin (Sigma Chemical Co., St. Louis, Mo.) to inhibit proteolysis. The trypanosome suspension was sonicated for 5 min (1-min bursts at 60 W in a model W185D Sonifier cell disruptor; Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) on ice and then subjected to three freeze-thaw cycles in liquid nitrogen and a 100°C water bath. Complete disruption of trypanosomes was verified microscopically, and the suspension was centrifuged for 1 h at 20,000 × g. The protein concentration of the supernatant was estimated from the absorbance at a wavelength of 280 nm and adjusted to 5 mg/ml in phosphate-buffered saline-glucose (pH 8.0) containing the proteolysis inhibitors, phenylmethylsulfonyl fluoride and pepstatin. This preparation was determined by two-dimensional polyacrylamide gel electrophoresis

(1, 2) to contain more than 300 protein gene products.

Major VSG. A partially purified preparation of *T. gambiense* TTrT-1 VSG was obtained by gently agitating 10¹⁰ column-purified, washed trypanosomes for 14 h in 0.15 M sodium phosphate (pH 5.5) containing 1% glucose. This procedure gently extracts surface proteins from the trypanosome without obviously altering the structural integrity of the parasite (3, 23). After incubation, the trypanosomes were pelleted by centrifugation at 600 × g at room temperature for 15 min. The supernatant, containing proteins removed from the trypanosome surface, was dialyzed against 0.5 M NaCl for 48 h at 4°C and then cleared of particulate material by centrifugation at 30,000 × g for 1 h at 4°C. The supernatant was then passed through a concanavalin A (ConA)-Sepharose affinity column (1 by 20 cm; 2 mg of ConA per ml of Sepharose 4B) equilibrated with 0.01 M sodium phosphate (pH 8.0) containing 0.5 M NaCl and 0.01% NaN₃. Glycoproteins binding to ConA were eluted with 10% α-methyl-D-mannoside in 0.01 M phosphate (pH 8.0)-0.5 M NaCl. Eluted material was dialyzed extensively against distilled water at 4°C and then lyophilized and stored at -20°C. This preparation is referred to as ConA-VSG. Two-dimensional polyacrylamide gel electrophoresis (1, 2), in which sensitive silver staining was used, and gel permeation and reverse-phase high-pressure liquid chromatographic analysis revealed that the affinity-purified VSG contained several minor contaminants in addition to the putative VSG and that this preparation was as pure as several other VSGs purified by conventional isoelectric focusing procedures described by Cross (9).

Immunization protocols. (i) **Primary immunizations.** Three to five mice of each strain were injected intraperitoneally with 200 μg of soluble *T. gambiense* antigens or KLH in 0.2 ml of sterile physiological saline without adjuvant. Three to five mice of each strain (normal controls) received 0.2 ml of saline intraperitoneally. Seven to ten days later, 0.2 ml of tail blood was collected from each mouse, allowed to clot overnight at 4°C, and then microfuged for 5 min. The sera from mice in each immunization group were pooled and stored at -20°C (1° immune sera). Control mice were also bled and their sera were pooled (normal mouse sera).

(ii) **Secondary and tertiary immunizations.** Seven to ten days after primary immunization, mice were challenged by intraperitoneal injection with 50 μg of the respective antigen in sterile saline without adjuvant. The animals were tail-bled 7 to 10 days later, and the sera were pooled and stored at -20°C (2° immune sera). After the second bleeding, mice were further challenged with 50 μg of antigen by the same procedure and then bled 7 to 10 days later (3° immune sera).

Antisera and antibodies for solid-phase radioimmuno-metric assays. Antisera containing antibodies specific for murine immunoglobulin light chains were produced by immunizing goats with F(ab')₂ portions of DEAE-purified normal BALB/c mouse immunoglobulin G (IgG) (obtained from Neil Barclay, Medical Research Council, Oxford, England) emulsified in complete Freund adjuvant. Antibodies to light chains were affinity purified by passage of whole serum through a mouse IgG-Sepharose 4B column. F(ab')₂ portions were produced by pepsin digestion by the method of

Jensenius and Williams (15). The anti-light-chain-specific reagent was designated anti-F(ab')₂.

Affinity-purified goat anti-murine IgM heavy chains (anti- μ) and goat anti-murine IgG heavy chains (anti- γ) were purchased from Kirkegaard-Perry Laboratories, Gaithersburg, Md.

Iodination and specificity testing of antibodies. Antibodies were iodinated by the Chloramine-T method described by Jensenius and Williams (15) and tested for specificity in solid-phase radioimmunoassays, with myeloma proteins or monoclonal antibodies of a known isotype used as antigens.

Solid-phase radioimmunoassays. (i) **Specific antibody determinations.** Solid-phase radioimmunoassays were carried out as described by Tsu and Herzenberg (25) with the following modifications. Wells of 96-well polyvinyl chloride microtiter plates (U-bottom serocluster vinyl plate no. 2797, Costar, Cambridge, Mass.) were coated for 18 h at 4°C with 100 μ l of KLH or malarial or erythrocyte, or trypanosome solubilates (adjusted to 25 μ g/ml in 0.015 M carbonate [pH 9.6] containing 0.02% NaN₃). For all antigen mixtures tested, the optimum coating concentration (25 μ g/ml) had previously been determined in chessboard titration assays with known positive and negative sera. Antigen solutions were removed, wells were washed with phosphate-buffered saline-Tween (0.01 sodium phosphate [pH 7.4], 0.15 M NaCl containing 0.05% Tween 20, 0.01% NaN₃), and serial 10-fold dilutions of normal or immune sera (in phosphate-buffered saline-Tween) were added in 100- μ l samples

to antigen-coated wells. After 2 h of incubation at room temperature, the sera were removed, the wells were washed as described above, and 100 μ l of ¹²⁵I-labeled second antibody (diluted to 100,000 cpm/100 μ l in phosphate-buffered saline-Tween) was added to each well for a further 2 h of incubation. The plates were then washed and cut with a hot wire, and individual wells were counted for 1 min in an LKB/282 Compugamma Universal gamma counter (Wallach Oy, Turku, Finland).

RESULTS

Specific-antibody responses in mice immunized with KLH. To determine whether A/J and BL/6 mice were innately different in their abilities to mount an antibody response to a single purified protein antigen, mice were immunized with 200 μ g of KLH and challenged twice at 10-day intervals with 50 μ g of the same antigen. The total antibody response of A/J mice to a single injection of KLH was elevated over that observed in BL/6 mice. When multiply challenged with KLH, A/J mice produced large amounts of specific antibodies, whereas titers in BL/6 mouse sera did not change appreciably until the third exposure to KLH (Fig. 1A). Figure 1B and C show the specific-isotype responses of these mice. After primary immunization, A/J mice

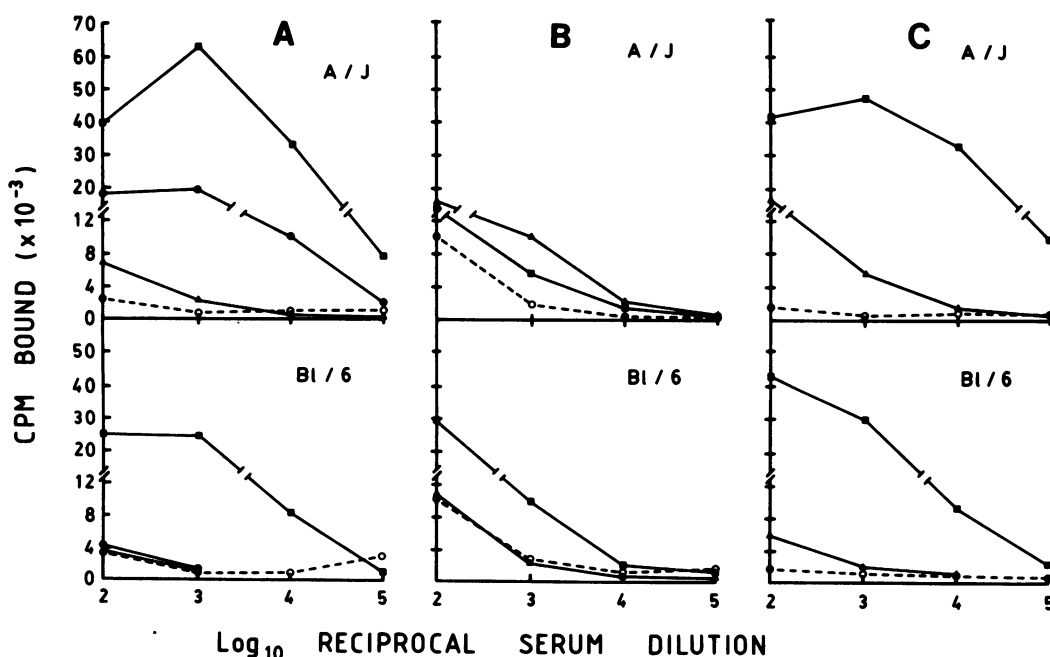


FIG. 1. Specific-antibody responses of mice immunized with KLH. Sera were titrated in solid-phase radioimmunoassays with 25 μ g of KLH per ml. Variance between replicates ranged from 3 to 5%. (A) Antibodies of all isotypes; (B) IgM antibodies; (C) IgG antibodies. Symbols: ○---○, sera from normal mice; ▲---▲, sera after primary immunization with KLH; ●---●, sera after secondary immunization with KLH; ■---■, sera after tertiary immunization with KLH.

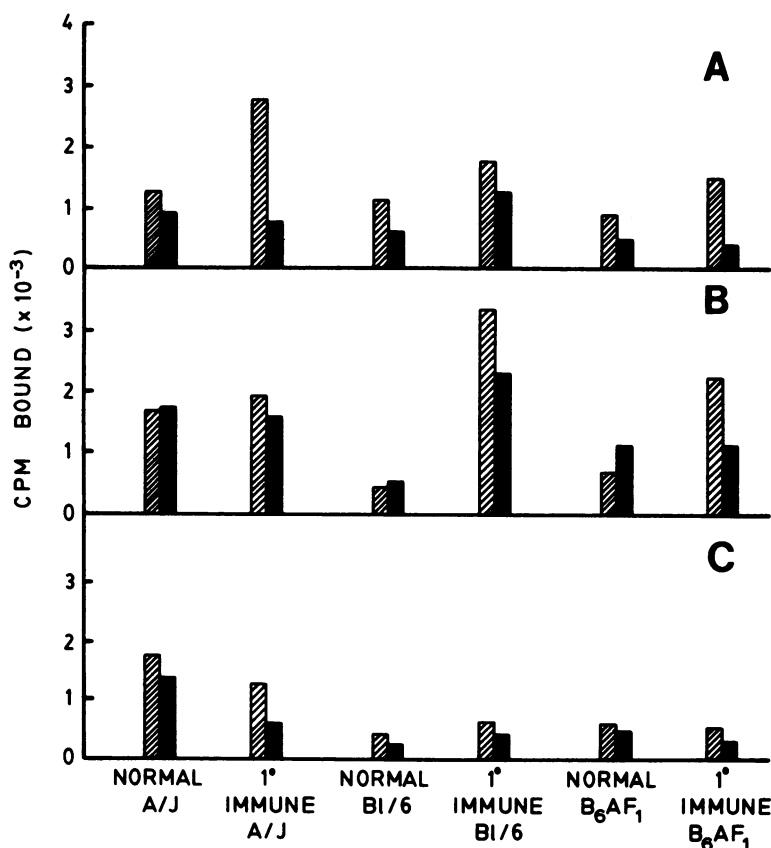


FIG. 2. Binding of normal and primary *T. gambiense*-immune mouse sera to parasite antigens. Normal mouse sera and sera from mice immunized by a single injection of 200 μ g of soluble *T. gambiense* antigens (1° immune) were tested at 10^{-2} dilutions for binding to soluble *T. gambiense* antigens (▨) or soluble *P. knowlesi* schizont antigens (■) in solid-phase radioimmunometric assays. (A) Antibodies of all isotypes; (B) IgM antibodies; (C) IgG antibodies.

produced higher titers of KLH-specific antibody of both isotypes than did BL/6 mice. After two challenge injections, IgM titers in A/J mice declined, whereas those in BL/6 mice were significantly elevated.

Specific-antibody responses to trypanosome antigens. (i) **Primary-antibody responses.** After immunization with solubilized *T. gambiense* antigens, all three mouse strains produced specific antibodies (Fig. 2). The light-chain-specific reagent was used to detect antibodies of all classes in these assays (Fig. 2A). A/J mice produced antibodies which bound more strongly to *T. gambiense* antigens than those produced by BL/6 or B₆AF₁ mice. Levels of antibodies binding to *P. knowlesi* schizont antigens were not significantly increased in A/J and B₆AF₁ mice, although a slight increase was seen in 1° immune BL/6 serum.

When the isotype distribution of the antibody response to primary immunization with *T. gam-*

biense antigens was analyzed, it was found that A/J mice produced poor specific IgM responses (Fig. 2B). Only BL/6 and B₆AF₁ mice showed significant increases in levels of IgM antibodies binding to trypanosome antigens. As expected after a primary immunization, the IgG response of all strains was negligible (Fig. 2C).

(ii) **Antibody responses after challenge immunizations with soluble *T. gambiense* antigens.** After challenge with soluble *T. gambiense* antigens, the highest antibody titers were seen in A/J and B₆AF₁ mice (Fig. 3). The margin of specificity had also increased, with low, if any, binding above background to malarial antigens. A further challenge with soluble *T. gambiense* antigens increased the titers of trypanosome-specific antibodies in all strains without altering the hierarchy observed between the strains.

Isotype-specific second antibodies were used to further analyze the anti-trypanosome response after challenge immunizations (Fig. 4 and

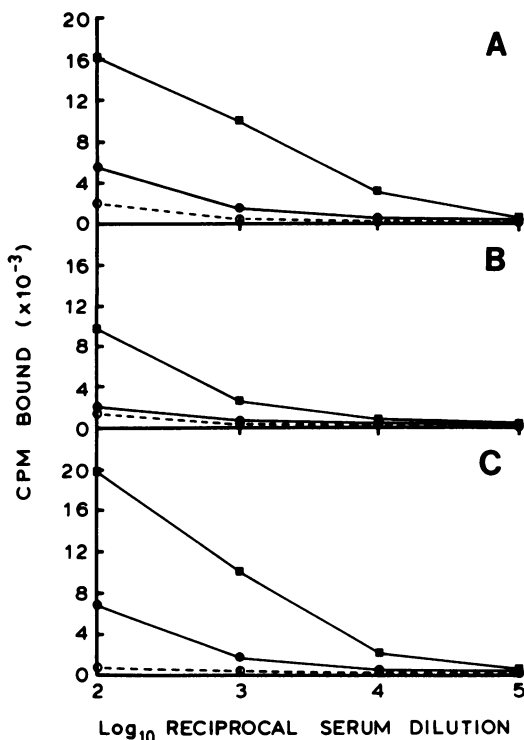


FIG. 3. Binding of normal and *T. gambiense*-immune mouse sera to soluble *T. gambiense* antigens, all isotypes. Antibodies of all isotypes were measured in solid-phase radioimmunoassays in which *T. gambiense* soluble antigens were used at a concentration of 25 $\mu\text{g/ml}$ to coat microplate wells. The developing antibody was ^{125}I -labeled anti-F(ab')₂. (A) A/J mice; (B) BL/6 mice; (C) B₆AF₁ mice. Symbols: ○---○, normal sera; ●—●, sera after secondary immunization; ■—■, sera after tertiary immunizations with *T. gambiense* soluble antigens.

5). The IgM titers increased in all strains. Trypanosome-specific IgG levels in BL/6 mice did not increase upon first challenge, although after a subsequent challenge, titers finally increased. In A/J and B₆AF₁ mice, specific-IgG levels increased markedly after both challenge injections with soluble *T. gambiense* antigens.

(iii) **Antibody responses to trypanosome variable surface glycoprotein.** When ConA-VSG from the homologous parasite was used as a target antigen, only BL/6 and B₆AF₁ mice had increased titers after primary immunization (Fig. 6). A/J mice did not produce significant amounts of VSG-specific antibodies on primary immunization but, on repeated challenge, produced amounts comparable to those of BL/6 and B₆AF₁ mice. However, titers of BL/6 mice, although comparable to those of A/J and B₆AF₁ mice after a single challenge with trypanosome antigens (2^o immunization), did not rise to the

levels observed in A/J and B₆AF₁ mice upon repeated challenge (3^o immunization). When the isotype distribution of the VSG-specific antibody response was determined, it was found that BL/6 and B₆AF₁ mice were superior to A/J mice in specific IgM production (Fig. 7A). All strains produced good IgM antibody responses to tertiary challenge. All mouse strains showed poor IgG responses to primary injection. Both A/J and B₆AF₁ mice produced substantial amounts of IgG binding to ConA-VSG when repeatedly challenged with solubilized trypanosomes, whereas IgG titers were considerably lower in BL/6 mice (Fig. 7B).

DISCUSSION

It has long been known that antibodies specific for trypanosome surface antigens are protec-

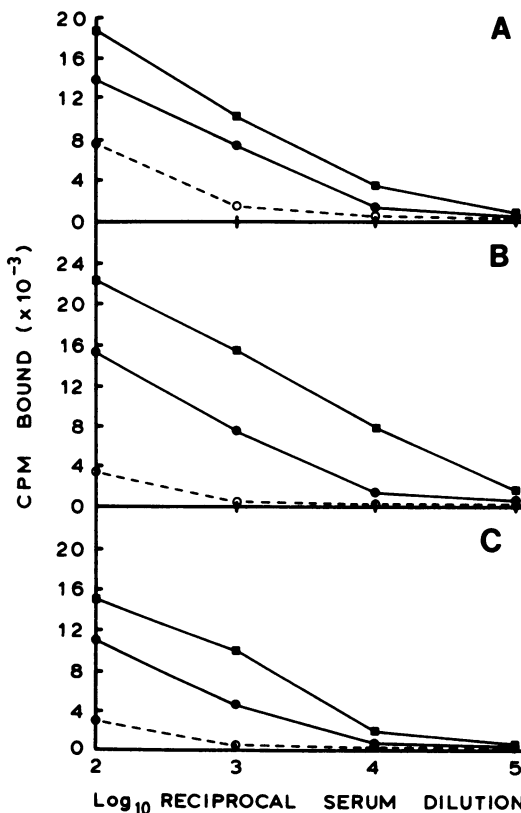


FIG. 4. Binding of normal, 2^o, and 3^o *T. gambiense*-immune mouse sera to soluble *T. gambiense* antigens, IgM antibodies. Specific IgM antibodies were measured in solid-phase radioimmunoassays in which *T. gambiense* soluble antigens were used at a concentration of 25 $\mu\text{g/ml}$ to coat microplate wells. The developing antibody was ^{125}I -labeled anti- μ chain. (A) A/J mice; (B) BL/6 mice; (C) B₆AF₁ mice. Symbols: ○---○, normal sera; ●—●, sera after secondary immunization; ■—■, sera after tertiary immunizations with *T. gambiense* soluble antigens.

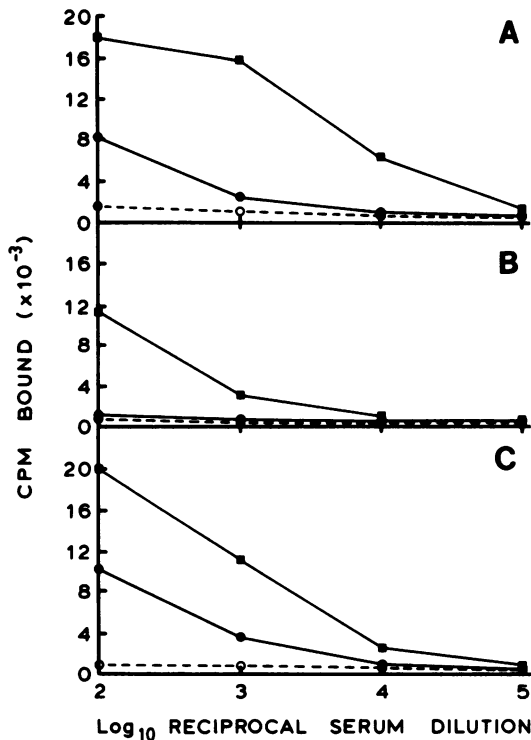


FIG. 5. Binding of normal, 2° and 3° *T. gambiense*-immune mouse sera to *T. gambiense* soluble antigens, IgG antibodies. Specific IgG antibodies were measured in solid-phase radioimmunoassays in which *T. gambiense* soluble antigens were used at a concentration of 25 µg/ml to coat microplate wells. The developing antibody was ¹²⁵I-labeled anti-γ chain. (A) A/J mice; (B) BL/6 mice; (C) B₆AF₁ mice. Symbols: ○-○, normal sera; ●-●, sera after secondary immunization; ■-■, sera after tertiary immunization with *T. gambiense* soluble antigens.

tive when administered passively, provided that homologous parasites are used for infection. In addition, IgM antibodies have been shown to be more effective than IgG antibodies (reviewed in reference 18). These findings are not surprising. The contribution that antibodies make to differential mouse strain susceptibility, however, has not been established. Clearly, it is necessary to measure immune responses in mouse strains which differ in susceptibility to African trypanosomes in order to establish the role of the immune system in trypanosome infections. As a first step in this direction, we chose to measure the specific antibody responses to trypanosome antigens in highly susceptible (strain A/J) and resistant (strains BL/6 and B₆AF₁) inbred mice in the absence of infection. To do this, we performed primary, secondary, and tertiary immunizations with a purified protein antigen (KLH) and solubilized *T. gambiense* antigens

and measured specific-antibody responses. Our choice of trypanosome (*T. gambiense* TTrT-1, a clone which shows no differential virulence for different mouse strains) and the use of trypanosome lysate as immunogen at first appear illogical for an attempt to elucidate the mechanisms involved in causing differential strain susceptibility to infection. However, we wanted simply to measure the innate ability of susceptible and resistant mice to produce specific antibodies in a situation in which the parasite itself did not influence the immune response by its metabolism or other processes. Thus, we did not use

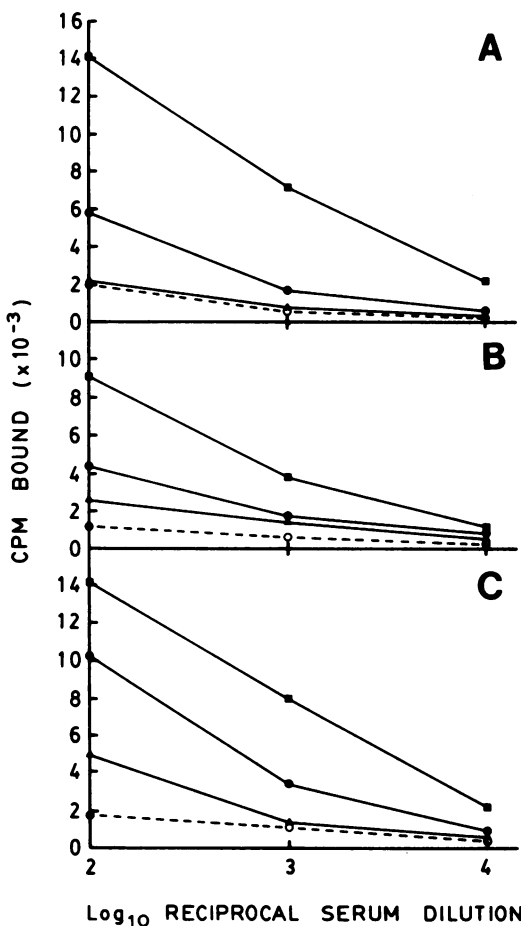


FIG. 6. Binding of normal and *T. gambiense*-immune mouse sera to *T. gambiense* VSG, antibodies of all isotypes. (A) A/J mice; (B) BL/6 mice; (C) B₆AF₁ mice. Sera taken from normal mice (○-○) and from mice after primary (▲-▲), secondary (●-●), and tertiary (■-■) immunizations with *T. gambiense* soluble antigens were tested in solid-phase radioassays for their abilities to bind to ConA-affinity-purified major VSG from the homologous parasite. ¹²⁵I-labeled anti-F(ab')₂ was used to detect antibodies of all isotypes.

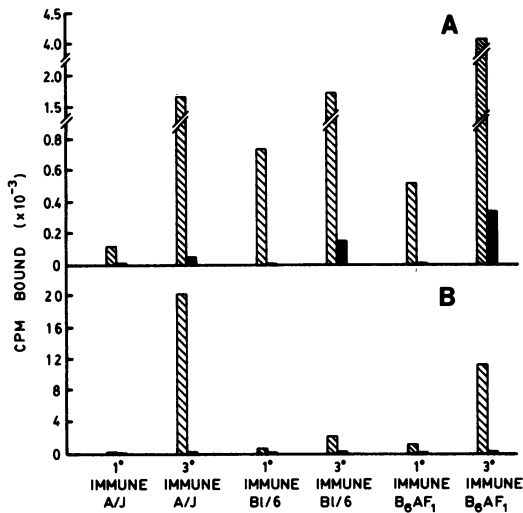


FIG. 7. Binding of *T. gambiense*-immune mouse sera to *T. gambiense* VSG. Sera from mice immunized with 200 μ g (1° immune) and challenged twice at weekly intervals with 50 μ g (3° immune) of soluble *T. gambiense* antigens were tested in solid-phase radioassays for binding to affinity-purified VSG from the homologous parasite (▨) or to soluble *P. knowlesi* schizont antigens (■). IgM (A) and IgG (B) antibodies were detected with ¹²⁵I-labeled anti- μ and ¹²⁵I-labeled anti- γ , respectively. Results are expressed as specific counts per minute bound above normal serum background.

irradiated trypanosomes (which are still viable and metabolizing) nor a trypanosome strain which shows differential virulence. Our results showed that A/J mice produce excellent antibody responses when immunized with purified protein antigens (KLH) or complex antigenic mixtures (solubilized *T. gambiense*). Outwardly, these responses appear to be both qualitatively and quantitatively superior to those of BL/6 mice immunized by the same regimens. However, distinct differences in the isotype distribution and specificity of the antibodies generated in A/J and BL/6 mice immunized with solubilized whole *T. gambiense* suggest that these animals may differ in their antigen recognition capacities or their abilities to regulate class switches and antibody levels, or both.

When specific antibody responses were measured, it was found that the response of A/J mice to a single pure antigen (KLH) was stronger than that of BL/6 mice in both primary and challenge immunizations. A/J mice produced good IgG responses to primary immunization (suggesting early activation of immunoglobulin isotype switching), whereas the bulk of the anti-KLH response of BL/6 mice belonged to the IgM class.

When immunized with a complex mixture of antigens (soluble *T. gambiense*), A/J mice appeared to be better responders, producing high titers of IgG antibodies binding to trypanosome solubilates. Specific-IgG responses of B₆AF₁ mice were even better than those of the A/J parent, again suggesting that class switching may be accentuated in these strains. Also, the relative strength (counts per minute bound) of A/J and B₆AF₁ responses suggests that the immune systems of these animals may be capable of recognizing more antigens present in the complex mixture used for immunization or of producing antibodies of higher affinity or avidity. The relationship of the relatively strong antiparasite IgG response in these strains to resistance or susceptibility to trypanosomiasis is unknown.

Although A/J mice produced excellent IgG responses when immunized with *T. gambiense* antigens, it is clear from the present study that BL/6 mice injected with this complex antigenic mixture produced predominantly IgM antibodies (specific for both trypanosome solubilates and partially purified VSG) which were qualitatively superior in both titer and avidity to those of A/J mice. B₆AF₁ mice produced intermediate amounts of parasite-specific IgM; however, these antibodies had avidities similar to those of the BL/6 parent. Most trypanosome infections, whether natural or experimental, are characterized by significant elevations in serum IgM levels, which are thought to occur as a result of polyclonal activation of B lymphocytes (11). Although much trypanosome-induced IgM is thought to be nonspecific and the source of various heterophile antibodies, rheumatoid factors, and autoantibodies described in both infected humans and animals (18), amounts of VSG-specific IgM occurring throughout an infection have not been adequately determined (owing to the sheer difficulty of measuring antibodies to the mixtures of phenotypically distinct parasites in sequential parasite peaks). However, it is known from active and passive immunization studies (reviewed in reference 18) that VSG-specific IgM antibodies are the most active and protective. Studies conducted by Jayawardena and Waksman (13) and Campbell et al. (5) have shown that T cell-deprived mice not only survive *T. brucei* and *T. rhodesiense* infections as well as do normal mice, but also experience lower parasite burdens. These observations indicate that T cell-independent anti-VSG IgM responses may be the most important antibodies for controlling parasite levels.

The ability to produce good VSG-specific IgM responses may be a significant factor in the resistance of BL/6 and B₆AF₁ mice to African trypanosomiasis. Although A/J mice produce

substantial amounts of IgM antibody binding to trypanosome solubilates, much of this antibody is nonspecifically adherent to plastic (uncoated wells) or nonrelated antigens in solid-phase radioassays, whereas those of BL/6 and B₆AF₁ mice are not.

Thus, the current study shows that, in the absence of active infection, resistant mice (strains BL/6 and B₆AF₁) produce better trypanosome-specific IgM responses than do susceptible mice (strain A/J). Whether this observation will be made in mice with an active infection (*T. congolense*) is currently under study. VSG-specific IgM levels have been found to be higher in BL/6 mice than in highly susceptible C3H mice infected with *T. rhodesiense* (John Mansfield, personal communication). Total IgM has also been shown to increase in BL/6 mice to levels higher than those found in other strains (7). Immune depression and suppression occur during infections with African trypanosomes (21, 22). Mansfield and Bagasra (19) have shown that T cell-dependent IgG responses in *T. rhodesiense*-infected mice are rapidly lost but that T cell-independent IgM responses persist and may even be enhanced in the course of chronic infections. Therefore, it would be expected that the persistence of T cell-independent antitrypanosome IgM responses permit the survival of BL/6 and B₆AF₁ mice in the face of progressive immune depression in active infections. Our results show that A/J mice are probably more efficient at immunoglobulin isotype switching, thus supporting this hypothesis.

We have observed (unpublished data) that immune depression and suppression parallel mouse strain susceptibility to *T. congolense*. Are the antibody responses in infected mice linked to immune depression? Jayawardena et al. (14) have shown that antigenic overload may lead to a wave of T cell help being rapidly followed by nonspecific suppression. Our results show that A/J mice may have efficient T cell help for antitrypanosome IgG responses as compared with BL/6 mice. Thus, A/J mice may become rapidly immunosuppressed and unable to produce effective antitrypanosome responses. There is a way to test whether differential strain susceptibility is due to differences in the degree of immunosuppression or simply to the quality of antitrypanosome antibodies. A/J mice should be as resistant as BL/6 mice if immunized (in the absence of infection) and challenged with homologous trypanosomes, provided that antibody quality is similar between strains. This is not likely to be the case, as our results show that BL/6 and B₆AF₁ mice produce better antitrypanosome IgM responses.

Finally, it must be considered that antibody responses are only partially responsible for sus-

ceptibility differences in experimental African trypanosomiasis and that other mechanisms, such as differential macrophage activity (12) or as yet undefined mechanisms (S. J. Black and C. N. Sendashonga, *Parasite Immunol.*, in press), are involved.

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