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Fine Specificity of the Genetically Controlled Immune Response to Native and Recombinant gp15/400 (Polyprotein Allergen) of *Brugia malayi*

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Polyprotein allergens are a family of structurally homologous molecules from parasitic nematodes which induce specific immunoglobulin E in infected individuals. We show here that both *H-2* and non-*H-2* factors determine the ability of mice to generate T- and B-cell responses to the filarial polyprotein allergen (*Brugia malayi* gp15/400). Further, *H-2* and non-*H-2* genes can complement one another to overcome nonresponsiveness to this molecule. However, these genetic restrictions govern only responses to the native glycoprotein and all strains of mice respond equivalently when immunized with a recombinant polypeptide. Overlapping fragments of gp15/400 were constructed to compare the T-cell and antibody responses to native versus recombinant gp15/400 in responder (BALB/c $H\text{-}2^d$) and nonresponder (B10.D2 $H\text{-}2^d$, CBA $H\text{-}2^k$, and BALB.K $H\text{-}2^k$) strains. BALB/c mice generated T-cell responses to the same fragment (positions 89 to 133 and 1 to 21) whether immunized with native or recombinant material, although the antibody responses differed in fine specificity. $H\text{-}2^k$ mice, unresponsive to the native molecule, generated T cells responsive to the centrally located peptide (positions 57 to 100) only when immunized with the recombinant. Antibody responses in $H\text{-}2^k$ mice were directed at the peptide (positions 11 to 67) which is glycosylated in the native molecule. Our findings suggest that recognition of gp15/400 is affected by modifications that occur in the parasite but are absent when the molecule is produced in bacteria. This study provides a detailed evaluation of the immune response to an important nematode antigen as a start to the unraveling of the complex interaction of these multicellular parasites with mammalian hosts.

Over 75 million people in the tropics are infected with the causative agents of lymphatic filariasis: *Brugia malayi*, *Brugia timori*, and *Wuchereria bancrofti* (34). These parasitic nematodes are transmitted by mosquitoes and reside in the lymphatics, where adult females produce large numbers of microfilariae that circulate in the bloodstream. Infection with filarial parasites leads to a spectrum of host immune responses, ranging from a remarkable absence of responsiveness to severe immunopathological conditions, such as elephantiasis (19, 23). The way in which the immune system recognizes and responds to these complex multicellular organisms determines whether infection leads to protective immunity, tolerance, or disease development (13, 19, 23). Information regarding immune recognition in most nematode infections, including lymphatic filariasis, has been largely restricted to crude extracts of whole parasites. However, the ability to recognize individual parasite molecules is under host genetic control and it is these antigen-specific responses that may determine whether the host response is appropriate or detrimental. Thus, to begin to understand immune recognition of parasitic nematodes it is imperative to look at individual antigens and to combine studies of antibody reactivity with an analysis of the T-cell responses that are elicited.

gp15/400 is a surface-associated molecule found in each of the *Brugia* life cycle stages and is broadly distributed in all tissues of the worm (29). It is released into culture medium in vitro and is a key component of the excretory-secretory material produced by the parasite (29). Although highly immunogenic in many infected individuals, gp15/400 does not elicit an antibody response in some people with otherwise elevated immune responses to *Brugia* organisms. Interestingly, there is a considerably lower frequency of the DR2 major histocompatibility complex (MHC) class II allele in gp15/400 nonresponders than in the rest of the population (35). Differential antibody isotype responses to gp15/400 have also been reported among individuals in separate clinical categories. Elephantiasis patients have consistently strong immunoglobulin E (IgE) responses to this molecule, in contrast to patently infected but asymptomatic persons (24). Asymptomatic individuals tend to have low IgE levels together with high IgG4 levels, which may function to block the damaging effects of IgE (10). Consistent with the heterogeneity of the immune response in humans, a striking restriction of the antibody response to gp15/400 has been observed in mice (15).

gp15/400 is a member of a phylogenetically conserved family of molecules termed the nematode polyprotein allergens (NPA) (6, 21, 25). These molecules frequently elicit IgE responses in infected hosts, as seen in *Brugia* infection. NPA are composed of a series of direct repeats with regularly spaced proteolytic cleavage sites such that the molecules are processed into multiple polypeptides of approximately 15 kDa from a large 400-kDa precursor (21, 32). In *Brugia* sp., this cleavage is incomplete and a "ladder" of polypeptides in molecular mass multiples of 15 kDa is observed when the polypeptides are separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Each repeat of *Brugia* gp15/400 con-

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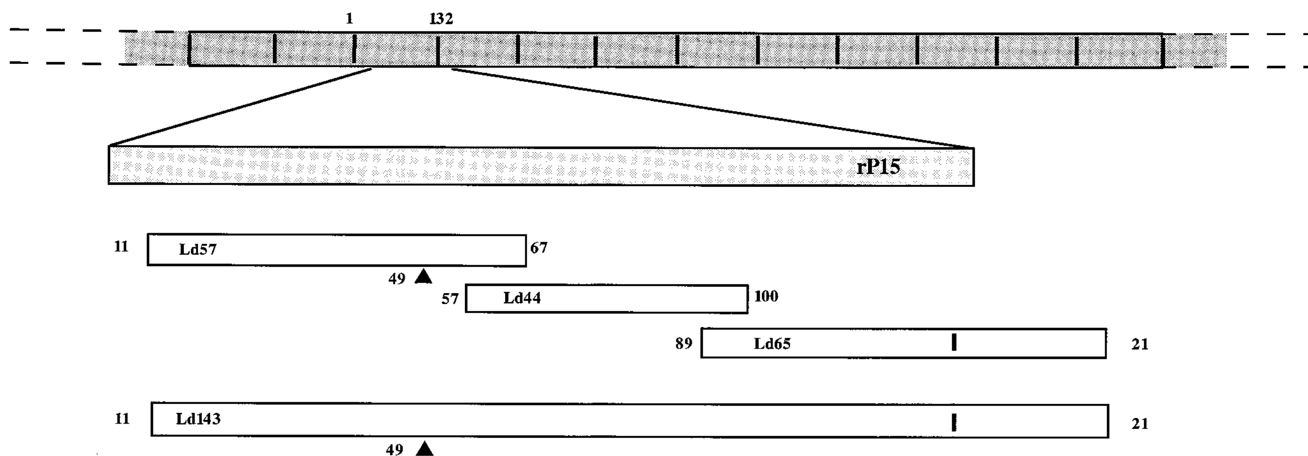

FIG. 1. Map of recombinant proteins used to evaluate T- and B-cell responses to gp15/400. The number of repeats in the precursor molecule has been estimated to be around 20. The Ld set of recombinant proteins are fusions with MBP. rP15 has a polyhistidine tail (24). All MBP fusion proteins were made by PCR amplification of genomic DNA and directional cloning into the pMAL-c vector. The dark triangles indicate glycosylation sites.

tains a single site for N-linked carbohydrate attachment which is glycosylated in the native molecule (32).

The NPA family was defined by the prototype molecule in *Ascaris* sp., ABA-1, which is the major constituent of the body fluid and is responsible for observed allergic responses to this organism (4, 20, 30). Interestingly, the antibody response to ABA-1 is also under strict genetic (*H-2*) control in mice and there is distinct polymorphism among human ascariasis cases, with most individuals producing no antibody to ABA-1 (12). The capacity of NPA to elicit potentially damaging IgE responses may lead to modulation of the immune response to this molecule during both *Brugia* and *Ascaris* infections. These mechanisms of control may differ in *Brugia* and *Ascaris* spp. but lead to the same consequence: that many individuals are prevented from responding in the context of natural infection.

In the absence of a good animal model for lymphatic filariasis, we have chosen to study the response to gp15/400 by implantation of live worms into the peritoneal cavities of mice, as well as by adjuvant-assisted immunization. In all of the mouse strains used in this study, adult worms survive for more than 8 weeks in the peritoneal cavity, with the female parasites continuously producing viable microfilariae. Although it is not the natural context of infection, the ability to use defined murine reagents and inbred strains for analysis of genetic responsiveness provides the most suitable starting point to unravel immune responses to individual parasite antigens. In this study, we analyzed T- and B-cell responses to both the native molecule and an *Escherichia coli* recombinant in detail not previously attempted for antigens from nematode parasites. We found that several inbred mouse strains completely fail to recognize the native molecule and that this failure is controlled by both *H-2* and non-*H-2* genes. Interestingly, we found that these restrictions are invoked only when an animal is exposed to the native molecule whereas genetic control of the anti-gp15/400 response is lost when the recombinant form is presented. By using the recombinant protein, we mapped B- and T-cell determinants on this antigen to examine why sites on the native molecule may not be accessible to the immune system.

MATERIALS AND METHODS

Parasite material. Live adult worms were obtained from *B. malayi*-infected jirds (*Meriones unguiculatus*) purchased from TRS Laboratories, Athens, Ga. Adult worms were harvested from the peritoneal cavities of the jirds. *B. malayi* antigen (Bma) was prepared by homogenization of adult worms in phosphate-buffered saline (PBS) on ice followed by centrifugation at 10,000 $\times$ *g* for 20 min. The supernatant was passed through a 0.2-μm-pore-size filter, and the protein concentration was determined by Bradford analysis. A parasite extract enriched for gp15/400 was made by incubation of 50 adult worms in 200 μl of 5% 2-mercaptoethanol in PBS at 37°C for 30 min followed by a single wash with 150 μl of PBS (18). The 2-mercaptoethanol strip and wash were pooled and used for immunizations.

Amplification and cloning of gp15/400 fragments. Overlapping fragments of the gp15/400 gene were obtained by PCR amplification. The absence of introns in this gene (32) permitted use of genomic *Brugia* DNA prepared from microfilariae as the template DNA. Oligonucleotides were synthesized with 5′ *Eco*RI sites and 3′ *Xba*I sites for directional, in-frame cloning into the pMAL-c vector from New England Biolabs (Beverly, Mass.). Downstream primers included an in-frame stop codon. PCR amplification and cloning into the expression vector were performed by using standard techniques (27). Fusion proteins represent three overlapping fragments encompassing slightly more than a single repeat unit of gp15/400. The fragments overlap by at least 10 amino acids, and Ld143 contains all three subfragments. Recombinant P15 (rP15) has been described previously (24) and contains one full repeat unit and a polyhistidine tail. A map of these constructs is shown in Fig. 1.

Selection of pMAL-c recombinants and verification of expression were done as described by the manufacturer (New England Biolabs). We produced Ld57 with primers LAD1 and LAD2, Ld44 with LAD3 and LAD4, Ld65 with LAD5 and LAD6, and Ld143 with LAD1 and LAD6. Oligonucleotide sequences used for amplification were as follows: LAD1, 5′-GAC-GAA-TTC-CGG-ACG-CAT-CTA-AGT-3′; LAD2, 5′-CCT-TCT-AGA-TTA-ACG-ACA-ACC-TCC-ACG-3′; LAD3, 5′-GGA-GAA-TTC-GAA-GCC-GGT-GAG-AAA-CT-3′; LAD4, 5′-TGT-TCT-AGA-ATT-TAA-TCT-ACT-TTA-GCT-3′; LAD5, 5′-AAA-GAA-TTC-GGA-CTC-GGT-CAG-G-3′; and LAD6, 5′-TTC-TCT-AGA-TTA-GGC-ATC-CGT-AAG-CCA-3′. The correct amino acid sequence of each clone was verified by sequencing of plasmid DNA with the malE sequencing primer purchased from New England Biolabs.

P-RUNG was constructed by PCR amplification and cloning into pET-15b (Novagen, Madison, Wis.) with an intermediate step of cloning into pBluescript (Stratagene, La Jolla, Calif.). The upstream oligonucleotide was designed with an *Nco*I site which generated a methionine immediately prior to *Asp*-1 of the gp15/400 repeat. The cloned product represents one repeat of the ladder beginning with Asp-1, the first amino acid following the proteolytic cleavage site, and ending with Arg-132 (32). Oligonucleotides used for amplification were 5′-GGA-TCG-ACA-TAC-CAT-GGA-TAA-CCA-TGA-GCA-TA-3′ and 5′-GCT-CCC-TAG-GTT-ATC-GTT-TAT-GTC-GAT-CCT-CAT-AGA-3′. The PCR product was amplified from genomic *Brugia* DNA and cloned into *Sma*I-digested pBluescript by blunt-end ligation. The P-RUNG sequence was then subcloned into pET-15b with *Nco*I and *Xho*I. All cloning steps used the TG2 host cells in which the recombinant was maintained. For expression, the plasmid was transferred into host strain BL21(DE3) as described below. The recombinant pET-15b, designated pET.Bm.RUNG, was confirmed by sequence analysis.

Growth and purification of gp15/400 recombinant molecules. Cloning and purification of rP15 have been described previously (24). The expressed protein contains one full repeat unit with a polyhistidine tail and was purified by nickel affinity chromatography (24). Maltose-binding protein (MBP) fusion proteins Ld57, Ld44, Ld65, and Ld143 were purified as described by the New England Biolabs protocols, except that no azide was used in any of the buffers to preclude

interference with T-cell assays. Briefly, *E. coli* lysates were passed over an amylose resin column which was washed extensively and the purified fusion proteins were eluted with 10 mM maltose. P-RUNG was produced by transformation of pET.Bm.Rung into expression host BLD21(DE3). A single transformant was used to inoculate 100 ml of Luria broth with ampicillin. Expression was induced with isopropyl-β-D-thiogalactopyranoside (IPTG), and the host cells were lysed by lysozyme treatment and sonication as described by the manufacturer (Novagen). The supernatant was then dialyzed against 20 mM Tris (pH 8) prior to purification by fast protein liquid chromatography (FPLC) with an anion-exchange column (Mono Q HR 5/5; Pharmacia, Uppsala, Sweden). P-RUNG eluted at 50 mM NaCl. All purified recombinant proteins were dialyzed against PBS for use in immunological assays. Protein purity was assessed by SDS-PAGE.

Immunization of mice. Male BALB/c, CBA/Ca, B10.D2, and BALB.K mice were obtained from Harlan-UK (Bicester, Oxfordshire, England) at 6 to 8 weeks of age. BALB.K males were bred with B10.D2 females. The F_1 offspring were used at 8 weeks of age.

(i) Adjuvant-assisted immunization. The mice were immunized by subcutaneous injection with 10 μg of Bma emulsified in complete Freund's adjuvant (CFA). Four to five weeks later, they were boosted in the footpad with a 2-mercaptoethanol strip of adult worms (see above) emulsified in incomplete Freund's adjuvant. Sera were taken, and the draining popliteal lymph nodes were removed 10 days after the boost.

(ii) Infection with live worms. The peritoneal cavities of mice were implanted with six viable adult *B. malayi* females and three to six adult males. Sera were taken and spleens were removed 3 to 4 weeks following implantation.

(iv) Immunization with rP15. Mice were given a single subcutaneous immunization with 10 μg of rP15 emulsified in CFA, and sera were taken 3 to 7 weeks later.

Preparation of T-cell lines. BALB/c and CBA mice were immunized subcutaneously with a preparation of rP15 purified by separation on SDS–12% PAGE and transfer to nitrocellulose. The nitrocellulose paper was dissolved in dimethyl sulfoxide and mixed with CFA for the primary immunization. Six weeks later, mice were boosted with 10 μg of rP15 (affinity but not gel purified) in incomplete Freund's adjuvant. Popliteal lymph nodes were taken 10 days following the boost and stimulated in vitro with 20 μg of rP15 per ml. Five days following primary stimulation, the cells were washed and "rested" for 7 days by addition of mitomycin C-treated splenocytes (feeder cells) and 10% mouse-derived concanavalin A-stimulated medium. This was followed by additional stimulation with antigen and feeder cells for 6 days and another 4-day rest with mouse-derived concanavalin A-stimulated medium and feeder cells. The third in vitro stimulation was done with FPLC-purified P-RUNG for 5 days followed by a 1-week rest prior to assay.

T-cell assays. (i) Primary assays. Popliteal lymph node cells were cultured in complete medium at 5×10^5 cells per well in U-bottom microtiter plates with 10 μg of antigen per ml. At 65 h, supernatants were removed for cytokine analysis. Production of cytokines was assessed by measuring the proliferation of the NK cell line (31), which is responsive to both interleukin 2 (IL-2) and IL-4.

(ii) Assay of T-cell lines. T cells were cultured in complete medium at 1×10^5 cells per well in U-bottom microtiter plates with 10 μg of antigen per ml and 5×10^5 mitomycin C-treated splenocytes per well as antigen-presenting cells. Cytokine analysis was performed with the NK cell line by addition of neutralizing antibody to IL-2 (S4B6) or IL-4 (11B11) and comparison to a standard curve determined with recombinant IL-2 and IL-4 as described previously (16).

Enzyme-linked immunosorbent assay (ELISA). Nunc Maxisorp Immuno plates were coated by incubation overnight at 4°C with 10 μg of Bma per ml or 5 μg of purified fusion protein per ml in 0.1 M carbonate buffer (pH 9.2). Plates were blocked with 5% bovine serum albumin for 30 min at 37°C. Mouse sera were diluted 1/100 in PBS–0.05% Tween 20 and incubated for 3 h at 37°C. Plates were washed with PBS–0.05% Tween 20 and then incubated with peroxidase-conjugated goat anti-mouse IgG (gamma chain specific; BioRad, Richmond, Calif.) diluted 1/2,000 in PBS–0.05% Tween 20. Reactions were detected with ABTS substrate (KPL Biotechnology, Gaithersburg, Md.). The isotype ELISA was performed as described previously (16).

RESULTS

The antibody response to native gp15/400 is controlled by factors both within and outside the *H-2* complex. Previous experiments with mice implanted with live adult worms and using immunoprecipitation of native antigen (15) have shown that BALB/c mice make antibody to gp15/400 whereas B10.D2 mice do not. This demonstrated that the antibody response to this molecule is governed by a non-*H-2*-encoded factor(s), as both strains of mice share the *H-2*^{d} gene complex. In addition, B10.BR and CBA mice (*H-2*^{k}) do not make antibody (15). We first extended these studies by implanting BALB.K (*H-2*^{k}) mice, in addition to BALB/c, CBA, and B10.D2 mice, with adult worms for 4 weeks. Sera were tested against recombinant Ld143 (Fig. 1) by ELISA with a gamma chain-specific second antibody to detect all T-helper cell-dependent antibody isotypes. As demonstrated in Fig. 2A, only BALB/c mice were able to make antibody to gp15/400 following implantation with live adult worms. All animals gave equivalently high (optical density, >0.8) responses to Bma, demonstrating that the absence of response in CBA, BALB.K, and B10.D2 mice was not due to a general immunosuppressive component produced by the parasite. The inability of BALB.K mice to make antibody to gp15/400 showed that the response requires both an appropriate *H-2* allele (*H-2*^{d}) and a background gene represented in the BALB congenic strains.

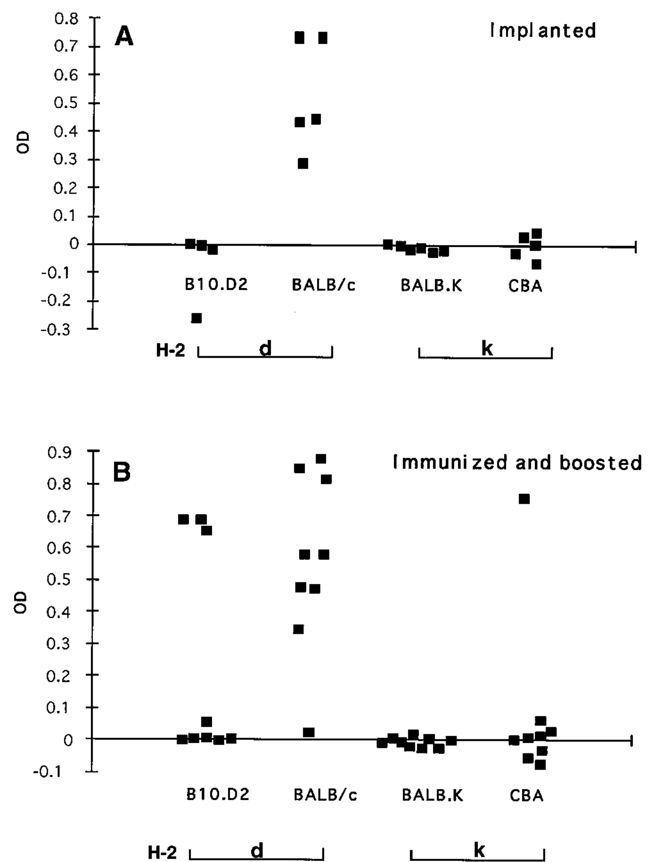

FIG. 2. Antibody responses to native gp15/400 in four strains of mice. The antibody responses to Ld143 for individual mice are shown for mice whose peritoneal cavities were implanted with live adult parasites for 4 weeks (A) or mice immunized with Bma in CFA and boosted with a preparation enriched for gp15/400 (B). The sera were tested by ELISA with a horseradish peroxidase-conjugated anti-murine gamma chain second antibody. The results are shown as optical densities (OD) with the response to MBP subtracted from the response to Ld143.

We also evaluated the abilities of these strains of mice to make antibody to gp15/400 when immunized with *Brugia* extract emulsified in CFA and boosted with a preparation enriched for gp15/400. The results are shown in Fig. 2B. The response was similar to that of mice experiencing live infection except that adjuvant-assisted immunization and boosting generated a response in some B10.D2 mice. This suggests that the factors outside the *H-2* that determine antibody responses are less stringent than those imposed by the MHC itself.

Analysis of the sera by immunoprecipitation of ^{125}I-labeled adult parasite extract gave an identical pattern of results (data not shown). Therefore, absence of a response by recombinant-

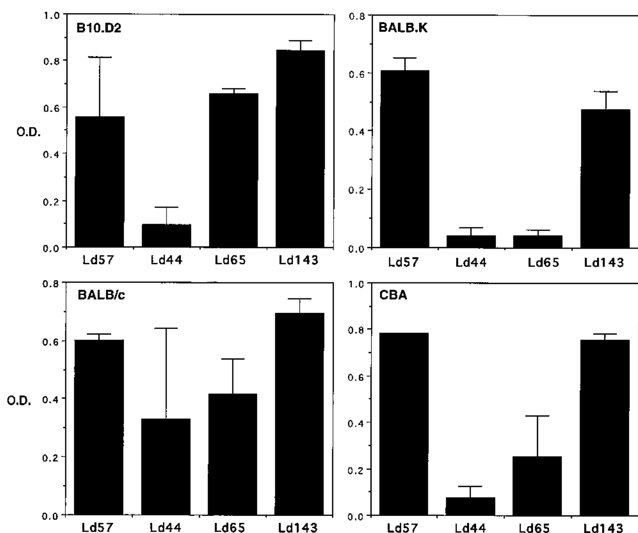

FIG. 3. Antibody responses to subfragments in four strains of mice immunized with rP15. The antibody responses to individual fusion proteins in mice immunized with rP15 were tested by ELISA. The response to MBP was subtracted. Standard deviations between individual mice are shown and represent the responses of two to four mice per group. No specific antibody was detected in sera from control mice immunized with CFA alone. O.D., optical density.

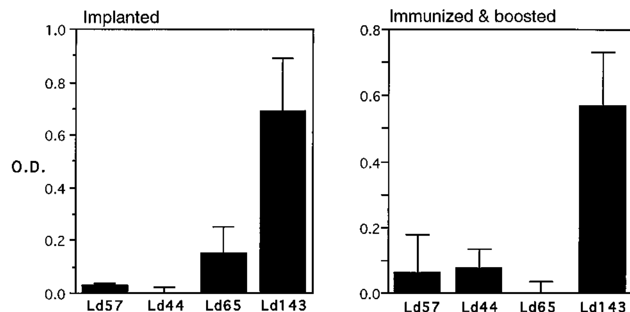

FIG. 5. Antibody responses to subfragments in BALB/c mice exposed to native antigen. The antibody responses to individual fusion proteins in animals either implanted with live worms or immunized with Bma and boosted with a gp15/400-enriched fraction were tested by ELISA. The response to MBP was subtracted. Standard deviations between individual mice are shown and represent the responses of six mice. No specific antibody was detected in sera from control mice. O.D., optical density.

based ELISA was not due to specificity of the antisera for native determinants or insensitivity of the assay system.

All mouse strains can make antibody following immunization with rP15. A striking contrast to the response seen following exposure to native antigen was found when mice were given a single subcutaneous immunization with purified rP15. All strains of mice were able to make a significant IgG response to Ld143 3 to 7 weeks following the immunization (Fig. 3). This demonstrated that the inability of CBA and BALB.K mice to mount an antibody response to the native molecule was not due to an absence of T-cell determinants recognizable by H-2^k strains and suggested that a structural or contextual feature of native gp15/400 blocks H-2^k responsiveness. In addition, the non-*H-2*-controlled mechanism that prevents B10.D2 mice from responding to gp15/400 in the context of infection is overcome by immunization with the recombinant molecule. An isotype analysis of the response to rP15 is shown for CBA mice in Fig. 4. IgG1, IgG2a, and IgG2b were the most abundant antigen-specific isotypes generated, verifying that the production of antibody to the recombinant required T-cell help.

The recombinant molecule elicits antibody to the amino-terminal fragment, while native gp15/400 generates antibody to conformational determinants. *E. coli*-derived fusion proteins representing three overlapping fragments of the gp15/400 repeat unit (Fig. 1) were used to analyze the fine specificity of the immune response to native and recombinant antigens. When the subfragments were used to map the antibody response to rP15, all four strains made antibody to Ld57, the amino-terminal fragment which is glycosylated in the native molecule (Fig. 3). This was particularly striking in H-2^k mice (BALB.K and CBA), which responded poorly to the other

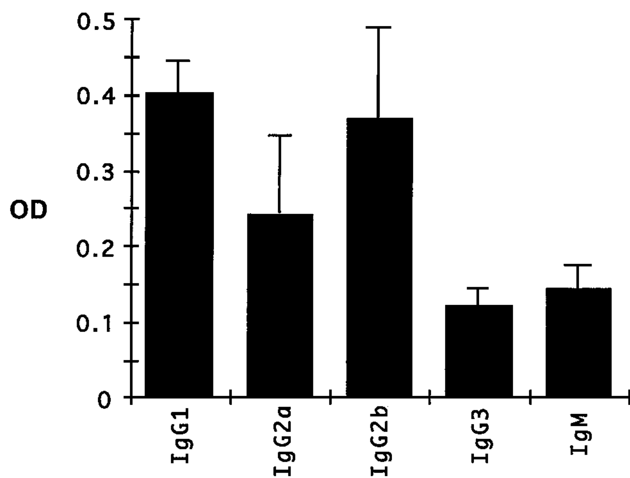

FIG. 4. Analysis of the rP15-specific antibody isotype response in CBA mice. Sera from mice immunized with rP15 were tested against Ld143 by ELISA. Peroxidase-conjugated antibodies against murine immunoglobulin subclasses were used to evaluate the sera. The results are shown as optical densities (OD) with the response to MBP subtracted from the response to Ld143. The standard deviation represents the responses of three mice per group.

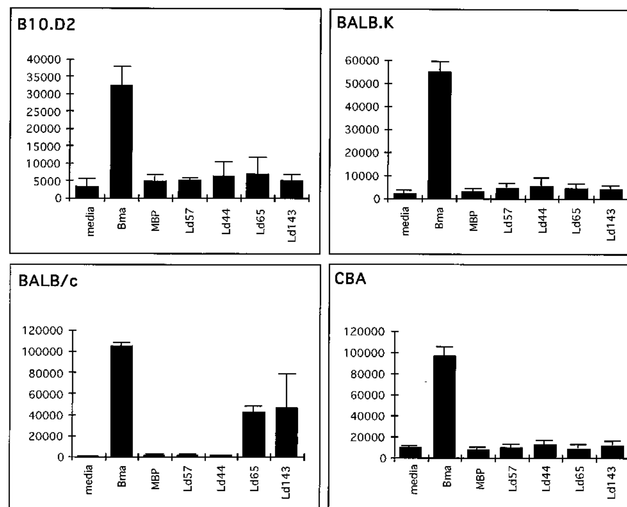

FIG. 6. T-cell responses to native gp15/400 in four strains of mice. T-cell responses to individual fusion proteins and Bma were measured after mice were immunized with Bma and boosted with a gp15/400-enriched preparation. Responses (shown in cpm) were measured by proliferation of the IL-2–IL-4-sensitive NK cell line (31) in response to T-cell assay supernatants. Standard deviations between individual mice are shown and represent the responses of three BALB/c, three CBA, five B10.D2, and five BALB.K mice.

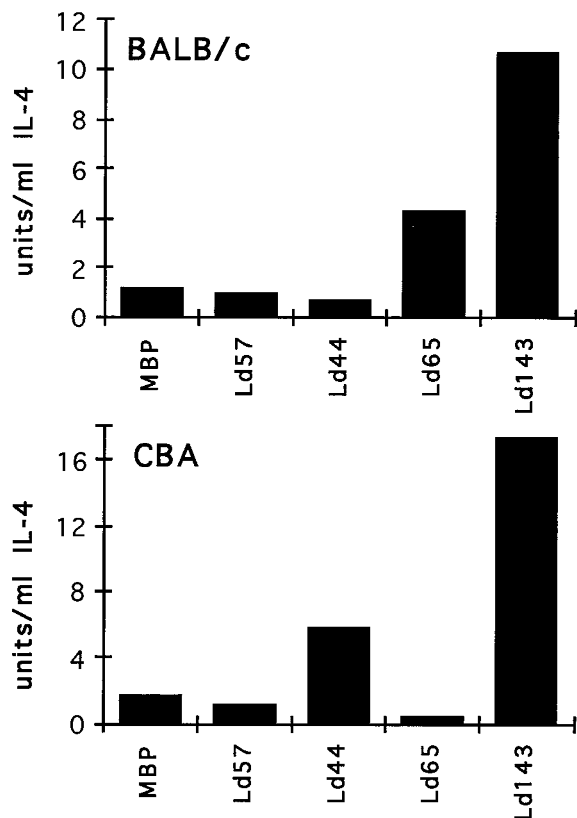

FIG. 7. T-cell responses to subfragments in CBA and BALB/c mice immunized with rP15. T-cell responses to individual fusion proteins were evaluated with rP15-specific T-cell lines. Results are shown as units of IL-4 produced per milliliter as determined with the indicator NK cell line sensitive to IL-2 and IL-4. No antigen-specific IL-2 was produced. The standard deviation between triplicate wells was <10%.

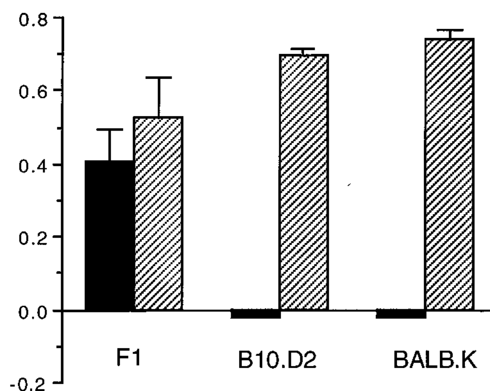

FIG. 8. Antibody responses in B10.D2, BALB.K, and B10.D2 × BALB.K F_1 mice following implantation with adult parasites. Antibody responses of implanted mice to MBP, Ld143 (solid bars), and Bma (hatched bars) were tested by ELISA. The response to MBP was subtracted. Standard deviations between individual mice (four per group) are shown. No specific responses were detected in control unimmunized mice. The numbers are optical densities.

fragments. *H-2^d* mice (BALB/c and B10.D2) responded to both Ld57 and Ld65, the fragment which contains the T-cell determinant recognized by these strains (see below).

A different pattern was observed when the subfragments were used to map the antibody response to native gp15/400 from responder BALB/c mice which had been either implanted with adult worms or immunized with Bma and boosted with a gp15/400-enriched fraction (Fig. 5). The antibody response was found to be directed almost entirely to Ld143 (and rP15; data not shown) and not to the individual subfragment peptides. The structures of NPA, including gp15/400, are predicted to form a four-helix bundle and are known to be extremely stable and resistant to denaturation (21). Full-length recombinant proteins Ld143 and rP15 are apparently able to take on this structure (11), whereas the individual fragments almost certainly do not. Thus, the ability to respond only to full-length constructs suggests that animals exposed to native gp15/400 make an antibody response that recognizes conformational rather than linear epitopes.

T-cell responses following exposure to native gp15/400 are genetically restricted. The inability of BALB.K, CBA, and most B10.D2 mice to make antibody was reflected in the absence of gp15/400-specific T-cell cytokine responses when mice were immunized with Bma and boosted with a preparation enriched for gp15/400 (Fig. 6). The cells from the draining lymph node were evaluated for specific T-cell responses to gp15/400-derived fusion proteins. T-cell responsiveness was assessed by measurement of T-cell-derived cytokines in response to specific antigen by using a cell line highly sensitive to both IL-2 and IL-4 (31). Despite strong, specific responses to Bma in all four strains of mice, only BALB/c mice showed significant reactivity to any of the gp15/400-derived fusion proteins. BALB/c mice gave a strong response to Ld143. In addition, this result defined the carboxyl-terminal fragment, Ld65, as the target of the T-cell response in BALB/c mice. gp15/400-specific cytokine production by BALB/c mice was predominantly IL-4 (data not shown).

T-cell lines to rP15 define *H-2^d* and *H-2^k* T-cell sites on gp15/400. Analysis of the T-cell response to the recombinant molecule demonstrated that BALB/c (*H-2^d*) and CBA (*H-2^k*) mice recognize distinct domains and epitopes. T-cell lines were derived by rP15 immunization of mice and culture of the lymphocytes with rP15 for two rounds of stimulation and rest. These T-cell lines were then stimulated with another gp15/400 recombinant, P-RUNG, which represents a single repeat of the ladder with no polyhistidine tail. Selection on this FPLC-purified molecule reduced background responses to *E. coli* contaminants. Specific cytokine production by these recombinant-specific T-cell lines shows that BALB/c mice recognize Ld65, the same determinant recognized by gp15/400-specific T cells, whereas CBA mice recognize the central fragment, Ld44 (Fig. 7). Generation of the optimal stimulatory peptides may require full-length Ld143, as the response to the entire repeat was greater than those to the individual subfragments. The cytokine responses of these T-cell lines determined by NK cell analysis were exclusively IL-4 with no antigen-specific IL-2 production. The strictly Th2 cytokine profile of these T-cell lines was probably due to the IL-4-rich conditioned medium in which they were maintained but may have been influenced by the nature of the antigen.

Complementation of *H-2* and non-*H-2* genes in the antibody response to gp15/400. Nonresponder BALB.K and B10.D2 mice were genetically crossed, and the F_1 generation was implanted with live parasites. The antisera from these mice were tested by ELISA and showed excellent antibody responses to gp15/400 (Fig. 8). Thus, as would be expected from the responsiveness of BALB/c mice, the BALB background was able to present antigen effectively in the context of *H-2^d*, showing that non-*H2* genes controlling the inability of B10.D2 mice to respond are recessive.

DISCUSSION

Nematode parasites frequently establish long-lived chronic infections in their vertebrate hosts that are characterized by the ability of the parasites to both modulate and evade the host immune response. Numerous strategies of immune evasion, from masking, shedding, and changing of target antigens to production of anti-inflammatory factors, have been identified (2, 17). Additional mechanisms, such as interference with antigen processing have been proposed as means of immune modulation, but evidence remains sparse (3, 33). In infections such as filariasis, a spectrum of clinical manifestations is seen, as well as a wide variation in the duration of parasite survival. This suggests that host genetics can greatly influence the efficacy of these evolutionary strategies to alter or avoid immune recognition. Studies with inbred mice have verified that the host genetic background can profoundly alter the outcome of infection with parasitic nematodes (2, 8, 9, 22).

The gp15/400 molecule from *B. malayi* evokes a heterogeneous response in the human population (24) that is reflected in a striking restriction of the antibody response in mice (15). Thus, it provides a model system with which to assess in detail the influence of host genetics on the B-cell and T-cell responses to an important nematode antigen. In this study, four inbred strains of mice were exposed to gp15/400 in three contexts: by infection with live parasites, by adjuvant-assisted immunization with the native molecule, and by immunization with a recombinant molecule. It is not practical to purify sufficient quantities of native gp15/400 from the parasite, and therefore, reactivity to the native molecule was studied by immunization with whole-parasite extracts or by infection. Our results demonstrated that the antibody and T-cell responses to the native molecule are controlled by factors both within and outside the *H-2* complex but that these restrictions are overcome by immunization with an *E. coli*-derived recombinant. This suggests that recognition of gp15/400 by T cells, and therefore B cells, is affected by secondary or posttranslational modifications that occur in the parasite but are absent when the molecule is produced in bacteria. These may include N-linked glycosylation, dimer formation, cofactor binding, or the mode of protein folding.

Recognition of *Brugia* gp15/400 may be critically influenced by the presence of an N-linked carbohydrate on the amino-terminal portion of the repeat unit. Strains of mice which are nonresponsive to native gp15/400 (CBA and BALB.K) make antibody to the amino-terminal fragment (Ld57) when primed with the recombinant molecule and generate a T-cell response to a fragment adjacent to the glycosylation site (Ld44). It is possible that the presence of the carbohydrate blocks the ability of this molecule to be processed or appropriately recognized by T cells and thus prevents effective antibody production. Our data show that BALB/c mice which respond to a more distant site (Ld65) are capable of generating effective T- and B-cell responses irrespective of glycosylation in the amino-terminal domain. Glycosylation of antigenic peptides may affect T-cell responses at the level of antigen uptake, peptide processing, MHC binding, or TCR recognition. *N*-Glycanase treatment of Bma, although effectively deglycosylating gp15/400, did not stimulate a B- or T-cell response in BALB.K mice (data not shown). However, *N*-glycanase, in addition to removing the N-linked carbohydrate, converts the asparagine on the amino acid backbone to an aspartic acid. This change may alter the site for cleavage by lysosomal enzymes and prevent the generation of appropriately processed peptides.

Other aspects of the higher-ordered structure of this molecule could influence T-cell recognition. For example, human chorionic gonadotrophin, a dimer of noncovalently associated subunits, cannot be processed and appropriately presented by antigen-presenting cells in native form, yet the free subunits are effectively recognized (26), demonstrating that the quaternary structure of an antigen can be important in antigen processing. Although recombinant gp15/400 is known to form a homodimer of the 15-kDa subunit (11), as is the native ABA-1 molecule of *Ascaris suum* (4), it is not certain whether this is structurally identical to native gp15/400. In addition, gp15/400 is known to be a fatty acid- and retinoid-binding protein (11) and the natures of the molecules bound to the native and recombinant forms of gp15/400 may differ. Thus, dimer formation or cofactor binding by native gp15/400 may alter its fate in the antigen-processing pathway and prevent access to the relevant T-cell sites.

Antibody mapping of the BALB/c response to the native molecule demonstrated poor reactivity to linear determinants, indicating that the antibody response is predominantly to conformational determinants. Receptor-mediated uptake by antigen-specific B cells can influence which peptides are made available for MHC binding by protecting certain fragments from proteolytic degradation (7). Thus, the inability to respond to native gp15/400, although apparent at both the B- and T-cell levels, may be caused by inability of B cells to recognize or process the appropriate determinants because of constraints on antibody recognition by tertiary-quaternary structure. More specifically, in $H\text{-}2^k$ mice, recognition of the amino-terminal portion (Ld57) by specific immunoglobulin receptors on antigen-presenting B cells may be required for appropriate processing and presentation of the immunogenic peptide in the central fragment.

The polyprotein nature of gp15/400 may also play a role in T-cell recognition. However, high levels of IgM to gp15/400 are not observed (data not shown), and thus, the classic model of a repetitive antigen diverting the immune response by inducing a T-independent reaction (28) seems unlikely. In addition, the molecules that elicit T-independent responses, such as the circumsporozoite protein of malaria, contain much smaller repeats able to cross-link B-cell receptors and induce production of T-independent antibodies (28). Further, although the precursor exists as a polyprotein, *B. malayi* secretes large amounts of gp15/400 that have been cleaved to single or double repeats. The polyprotein nature of gp15/400 and the other NPA may be related more to the function of the molecule than to a mechanism of immune evasion.

The finding that non-MHC genetic factors control the ability to make antibody is unusual. Most restricted antibody responses to individual proteins have been linked directly to the *H-2* complex. The inability of B10.D2 mice to respond following implantation with live worms demonstrates that the processing or recognition of gp15/400 is influenced by non-*H-2* genes. This response can be partially overcome when an adjuvant is used, in a manner analogous to the response seen with *A. suum*, in which immunization of mice with native ABA-1 in CFA circumvents the MHC restriction (5). Further, when T-cell lines to whole *Brugia* extract were derived from B10.D2 mice implanted with live parasites, a small response to Ld65 and Ld143 was generated (1). This demonstrated that these animals have the capacity to generate a T-cell response to gp15/400, but at a very low level.

The mechanism of the non-*H-2*-controlled response to gp15/400 is more difficult to interpret than the *H-2* response, as the background genetic factors are less well characterized and more widely distributed than MHC genes. Differences may occur in the endosomal-lysosomal proteases used to process antigenic peptides or the type of antigen-presenting cells re-

cruited in response to an antigenic challenge. We have recently demonstrated that C57BL/10 (H-2^b) mice are able to make a vigorous antibody response following implantation with live worms (1). B10.D2 mice are a congenic strain derived from C57BL/10 but carrying the H-2^d locus (14). This suggests that the C57BL/10 background is able to process appropriately the gp15/400 for presentation by H-2^b but not H-2^d class II molecules. Complementation experiments, in which the ability of BALB.K mice genetically crossed with B10.D2 mice to make antibody demonstrated that the B10.D2 nonresponder phenotype is a recessive characteristic. This result excludes the possibility that B10.D2 nonresponsiveness is due to tolerance induced by recognition of gp15/400 in the context of H-2^d as self or degradation of the H-2^d epitope by B10 processing enzymes.

The results of this study illustrate that use of a recombinant antigen rather than a parasite-derived antigen can have significant consequences for the nature of the immune response that is generated. Basic immunological studies, in particular, vaccine-based research, are frequently founded on the presumption that responses to peptides reflect the response seen with the native molecule. This can be a misleading assumption. Whether or not gp15/400 emerges as a vaccine target, differences in response to native antigen versus recombinant antigen have significant consequences for vaccine strategies in general and emphasize the importance of considering the response to critical antigens both in the context of infection and with regard to the native structure of the molecule.

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