Antibody Response to Phenolic Glycolipid I in Inbred Mice Immunized with Mycobacterium leprae

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The level of circulating antibody to phenolic glycolipid I of Mycobacterium leprae was determined in 18 inbred strains of mice after immunization with M. leprae organisms. By using a solid-phase radioimmunoassay with phenolic glycolipid I as test antigen, a continuous distribution of antibody levels ranging from high to low was observed. The level was found to be controlled by multiple genes, including both H-2 complex- and Igh allotype complex-linked genes. Low antibody response to phenolic glycolipid I was shown to be inherited as a dominant trait in three combinations of high × low responder F1 progeny.

Mycobacterium leprae, the causative agent of leprosy, is a remarkably nontoxic intracellular parasite capable of reaching extremely high numbers in various tissues without resulting in damage or inciting an inflammatory response (28, 30). In human infection, polar tuberculoid leprosy, the high-resistance form, is characterized by few lesions, low numbers of bacilli, high lymphocyte transformation tests, and low titers of anti-M. leprae antibodies (11, 12, 24, 40). At the other end of the clinical spectrum, polar lepromatous leprosy, the low-resistance form, is exemplified by large numbers of disseminated bacilli, reduced reactions in lymphocyte transformation tests, and high levels of anti-M. leprae antibodies. Between the two ends of this spectrum is a wide range of clinical-pathological symptoms with various cutaneous and immunological manifestations known as borderline leprosy. The results of host immune reactions to bacillary antigens (30), rather than variations in the M. leprae organism itself (27, 32), are believed to be responsible for most of the clinical-pathological spectrum of the disease. Furthermore, the expression of the clinical spectrum may be due in part to human major histocompatibility complex (HLA) and non-HLA controlled host immune responses to M. leprae (31, 38).

Studies examining immune responses to whole M. leprae and M. leprae antigens in humans may not discriminate between naive responses and altered responses secondary to the infectious process. On the other hand, studies in noninfected inbred mice, with well-defined genetic compositions, allow meaningful analysis of the host genetic factors involved in governing immune responsiveness to antigens of M. leprae. Comparisons between the magnitude of genetically controlled humoral and cellular immune responses in different infected and uninfected mouse strains also provide data pertinent to the hypothesis that the different clinical-pathological expressions of leprosy result from differences in the magnitude of the two types of immune response, a consequence of imbalanced T-cell immunoregulation (21–23, 36).

An M. leprae-specific glycolipid antigen, triglycosyl phenolic diacyl phthiocerol (phenolic glycolipid I [PGL-I]), has recently been isolated, purified, and structurally characterized (13, 14). It is distinguished by a species-specific trisaccharide [3,6-di-O-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-O-methyl-α-D-rhamnopyranosyl-(1→2)-3-O-methyl-α-D-rhamnopyranosyl] glycosidically linked to a genus-specific phenolic diacylphthiocerol. PGL-I has been shown to be antigenic in animals (1, 13, 14) and leprosy patients (2, 4, 14, 25, 43). By using solid-phase immunoassays, immunoglobulin G (IgG) and IgM antibodies to PGL-I were demonstrated in the majority of untreated leprosy patients of all clinical-pathological classifications. Patients with lepromatous leprosy had higher levels of anti-PGL-I antibodies than did patients with tuberculoid leprosy (2, 4, 43). PGL-I was also found to induce nonspecific suppressor T-cell function in vitro (21).

PGL-I represents ca. 2% of the organism mass and is present in large quantities in infected tissues from which the bacilli have been removed (13). Since it may represent the major interface between host defense mechanisms and the microorganisms, we have undertaken a series of studies to examine the genetic control of immune responses to PGL-I in mice immunized with M. leprae organisms.

MATERIALS AND METHODS

Mice. The following strains of inbred female mice and F1 hybrids were used: A/J, A.SW/SNj, B10.BR/SgSnj, BALB/cByJ, CBA/J, C57BL/6J, C57BL/10J, DBA/2J, DBA/2J, SJL/J, SWR/J, (C57BL/6J × A/J)F1, (SWR/J × A/J)F1, (SJL/J × CBA/J)F1 (Jackson Laboratory, Bar Harbor, Maine) and BALB.10, BALB.K, BALB.Igα (N-20), BALB.Igα (N-10), BALB.Igα (N-18), BALB.Igα (N-11), F-3, (N-11) (Noel Warner and Ed Walker, University of New Mexico School of Medicine, Albuquerque, N.M.). All mice were 8 to 20 weeks of age at the start of the experiment.

Immunization. M. leprae was isolated from irradiated infected armadillo livers (13). Purified M. leprae was emulsified in incomplete Freund adjuvant (Marcol:Arlacel, 4:1). Primary and secondary immunizations with 0.1 ml of emulsion containing 50 μg of M. leprae were carried out intraperi-
The bleeding 2 weeks before plates (Dynatech coated that of PGL-I to phate-buffered saline postimmunization sera. The drying. Samples (gel-EDTA-PBS). Added to duplicate wells and incubated for 3 h. Plates were then washed rapidly three times with gel-EDTA-PBS, followed by a 5-min wash with gel-EDTA-PBS and a 5-min wash with 1% gamma globulin-free bovine serum albumin (BSA) in PBS (BSA-PBS). An amount of 100,000 cpm of 125I-sheep anti-mouse kappa (125I-SaMK) or 125I-protein A (125I-PA) diluted in 50 μl of BSA-PBS was added and incubated for 3 h at room temperature. Each well was washed three times, for 5 min each time, with 150 μl of 0.3% gelatin (gel) dissolved in phosphate-buffered saline (PBS; pH 7.2) containing 1 mM EDTA (gel-EDTA-PBS). Samples of 50 μl of preimmune and day-30 postimmunization sera diluted 1:5 in gel-EDTA-PBS were added to duplicate wells and incubated at room temperature for 3 h. Plates were then washed rapidly three times with gel-EDTA-PBS, followed by a 5-min wash with gel-EDTA-PBS and a 5-min wash with 1% gamma globulin-free bovine serum albumin (BSA) in PBS (BSA-PBS). An amount of 100,000 cpm of 125I-sheep anti-mouse kappa (125I-SaMK) or 125I-protein A (125I-PA) diluted in 50 μl of BSA-PBS was added and incubated for 3 h at room temperature. Each well was washed three times, for 5 min each time, with 150 μl of BSA-PBS, cut from the plate with a hot wire, and counted in α-spectrometer. All sera were also studied in dilutions of 1:50 to ensure that the differences observed in uptake of 125I at 1:5 dilutions was not due to a pro-zone effect. The paired t-test was used to determine the statistical significance of antibody present in serum on day 30 after immunization. The specific antibody response for each mouse represents the difference in counts per minute obtained by subtracting

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**FIG. 1.** Levels of circulating anti-PGL-I antibodies detected by a solid-phase radioimmunobinding assay with 125I-SaMK in different inbred strains of mice immunized with 100 μg of nonviable *M. leprae* organisms. A continuous distribution in the level of circulating anti-PGL-I antibodies was observed. Mouse strains are arbitrarily grouped into high, intermediate, and low responders. Data expressed as means ± standard error.

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<table>
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<tr>
<th>Strain</th>
<th>H-2 Type</th>
<th>Ig Allotype</th>
<th>No. Animals</th>
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**FIG. 2.** The levels of circulating anti-PGL-I antibodies detected by a solid-phase radioimmunobinding assay with 125I-SaMK in H-2 congenic mouse strains. The H-2 haplotype was associated with a high antibody response on the BALB/c background. NS, Not statistically significant. Data expressed as means ± standard error.
preimmune counts per minute from postimmunization counts per minute. The mean ± standard error of the mean of the antibody responses for each mouse strain were calculated, and the data analyzed by the Student t-test for comparisons between strains.

RESULTS

Strain distribution of anti-PGL-I antibody response. All mouse strains tested produced significant anti-PGL-I antibodies (P < 0.05) after two intraperitoneal injections of 50 µg of nonviable M. leprae emulsified in incomplete Freund adjuvant (Fig. 1). Similar results were obtained with 1:5 dilutions (data not shown). A continuous distribution in the level of antibody, indicative of polygenic control (7), was observed. Nevertheless, the animals could be arbitrarily grouped as high-, intermediate-, and low-responder strains (Fig. 1).

Role of H-2 in controlling the anti-PGL-I antibody response. Possible H-2 influence in controlling the level of anti-PGL-I antibody response was suggested by the finding that mice possessing the H-2^a, H-2^b, and H-2^d haplotypes were generally high to intermediate responders (BALB.B10 mice being an exception), whereas mice possessing the H-2^k and H-2^d haplotypes were low responders (Fig. 1). Additional support came from studies with the H-2 congenic resistant strains of BALB/c (H-2^d); BALB.K (H-2^a) and BALB.B10 (H-2^d) (Fig. 2). The H-2^d haplotype was associated with a high antibody response when present on the BALB/c background (P < 0.005), whereas the H-2^k and the H-2^d haplotypes were both low responders. This was not observed with C57BL/10 H-2 congenic mice, however (Fig. 2).

Role of Igh allotype complex-linked genes in anti-PGL-I antibody response. To examine the influence of IgH allotype complex-linked genes on the anti-PGL-I antibody response, BALB/c immunoglobulin allotype congenic mice were studied. BALB/c mice possessing the Ig^a, Ig^c, and Ig^e allotypes demonstrated significantly higher levels of anti-PGL-I antibodies (P < 0.002, < 0.005, and < 0.001, respectively) than BALB/c mice possessing the Ig^b, Ig^a, and Ig^e allotypes (Fig. 3).

Inheritance of anti-PGL-I antibody response. The mode of inheritance of the anti-PGL-I antibody response was studied in three sets of F1 progeny of high or intermediate × low parental phenotypes: (CBA/J × SJL/J)F1, (C57BL/6J × A/J)F1, and (SWR/J × A/J)F1. In all three, the anti-PGL-I antibody response was below that of the low-responder parental strain, suggesting that low anti-PGL-I antibody responsiveness is inherited as a dominant genetic trait. Data expressed as means ± standard error.

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**Fig. 3.** Levels of circulating anti-PGL-I antibodies detected by a solid-phase radioimmunobinding assay with ^125I-SaMK in BALB/c IgH allotype congenic mice. BALB/c mice possessing the Ig^a, Ig^c, and Ig^e allotypes produced significantly higher levels of anti-PGL-I antibodies. NS, Not statistically significant. Data expressed as means ± standard error.

**Fig. 4.** Levels of circulating anti-PGL-I antibodies detected by a solid-phase radioimmunobinding assay with ^125I-SaMK in parental strains and F1 progeny of high × low responder strains of mice. In all three groups of F1 progeny the anti-PGL-I antibody response was below that of the low-responder parental strain, suggesting that low anti-PGL-I antibody responsiveness is inherited as a dominant genetic trait. Data expressed as means ± standard error.
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parental strain (Fig. 4). The results suggest that low anti-PGL-I antibody response is inherited as a dominant genetic trait.

Anti-PGL-I antibody isotype. To study the antibody isotype of the anti-PGL-I antibody response, sera were examined by using 125I-PA in place of 125I-SaMK in the solid-phase radioimmunoassay. PA reacts predominantly with murine IgG2 and to a lesser degree with IgG3 (18); thus, PA-binding antibodies represent mainly antibodies of IgG2 or IgG3 isotypes, or both. 125I-PA counts per minute were higher than 125I-SaMK counts per minute in only two strains of mice, BALB.K and CBA/J (Fig. 5). Taking into consideration the finding that PA is more sensitive than SaMK in detecting anti-PGL-I antibodies (data not shown), it appears that the antibody response of C57BL/6, C57BL/10, and DBA/1 strains was composed mainly of non-PA-binding immunoglobulins (Fig. 5), whereas PA-binding antibodies constituted a significant portion of the total response in SWR, CBA, B10.BR, and BALB.K mice (Fig. 5). In the other strains, including the F1 progeny, the level of antibody response determined by PA correlated with the level determined by SaMK.

We next examined the PA-binding anti-PGL-I antibody responses in the H-2 and IgH allotype congenic mice mentioned above. The results again clearly demonstrate that the H-2k haplotype is associated with high responsiveness (Fig. 6). Unlike the results from the study on antibodies detected with SaMK, the PA-binding antibody response of B10.BR was significantly higher than that of C57BL/10, indicating that the PA-binding anti-PGL-I antibody response is governed by H-2k haplotype linked genes (Fig. 6). These results suggest that the anti-PGL-I antibody response may be isotype restricted, which in turn is under the control of H-2. By identifying the isotype(s) of the anti-PGL-I antibody response in all mice tested, it should be possible in future studies to determine whether or not the anti-PGL-I antibody response is indeed isotype restricted (26).

FIG. 5. Comparison of the levels of circulating anti-PGL-I antibodies detected by a solid-phase radioimmunobinding assay with 125I-SaMK and 125I-PA in high- and intermediate-responder strains of mice. SWR, B10.BR, CBA, and BALB.K mice responded with significant levels of PA-binding anti-PGL-I antibodies, whereas the anti-PGL-I antibody response of C57BL/6, C57BL/10, and DBA/1 mice consisted mainly of non-PA-binding immunoglobulins. Data expressed as means ± standard error.

FIG. 6. Levels of circulating anti-PGL-I antibodies in H-2 congenic strains of mice detected by a solid-phase radioimmunobinding assay with 125I-PA. Higher levels of PA-binding anti-PGL-I antibody are associated with the H-2k haplotype on both the BALB/c and C57BL/10 background. Data expressed as means ± standard error.

DISCUSSION

In this study, the antibody response to an M. leprae-specific glycolipid antigen, PGL-I, was examined in inbred mouse strains after immunization with nonviable organisms. The results of this study demonstrate a continuous distribution of antibody response, which is presumably linked to the genetic characteristics of each mouse strain. Similar results have been observed in mice immunized with synthetic antigens poly-L-(Tyr, Glu)-poly-D,L-Ala-poly-L-Lys (20), and terpolymer poly(Glu137 Lys14 Ala1) (17) with group A streptococcal polysaccharide (5), staphylococcal nuclease (16), sperm whale myoglobin (41) and ferritin (42). Continuous distributions in antibody response may indicate multigene control, with Ir genes operating at the various levels of cellular interactions (7). An alternative explanation is based on the cross-tolerance hypothesis (8, 41, 42); a low-responder strain would be the result of self-antigens cross-reacting with an epitope(s) on PGL-I, whereas a high-responder strain is one with minimal cross-reactivity between self-antigens and PGL-I. In this case, the continuous spectrum of magnitude simply reflects varying degrees of cross-reactivity between self-antigens and PGL-I. A similar model for molecular mimicry or antigenic cross-reactivity has been hypothesized to account for the mechanism by which HLA-
DR2 serves as a marker for susceptibility to tuberculous leprosy (38). An extrapolation of the cross-tolerance hypothesis would predict that varying degrees of cross-reactivity between HLA and M. leprae antigens dictate whether the host fails to eliminate the infectious agent or proceeds to abnormal immune reactivity (37). However, available information on the immunochemistry of the antigenic determinant of PGL-I would argue against the cross-tolerance mechanism.

Studies with pooled sera from lepromatous leprosy patients and hyperimmune anti-M. leprae rabbit antiserum in a solid-phase enzyme-linked immunosorbent assay have led to the elucidation of the primary epitope of PGL-I (4). Antigenic activity was associated only with the moieties containing the trisaccharide entity. Since the essential epitope of PGL-I required for antigen antibody interaction was found to reside in the terminal, 3,6-di-O-Me-glucopyranosyl residue (9), and since this dimethyl glycoside has not previously been recognized in nature, it would seem unlikely that cross-reactivity between self-antigens and PGL-I could be responsible for the continuous distribution of anti-PGL-I antibody responses. Multigene control as an explanation for the spectrum of anti-PGL-I responses is therefore more plausible.

Since the animals in this study were immunized with the whole M. leprae organisms, M. leprae antigens other than PGL-I can function as carrier antigenic determinants and influence the anti-PGL-I antibody response. Thus, the strain variations observed in this study could reflect different responses to the carrier determinants in M. leprae rather than to PGL-I per se. Although this is expected to lead to more complex results, the experimental design more closely mimics a study on the control of immune responses to PGL-I in leprosy patients. In fact, similar studies have been reported for immune response to streptococcal group A carbohydrate antigens after injection of whole organisms (3).

Strain distribution studies suggest an H-2-linked influence on the level of anti-PGL-I antibody response. In general, mice possessing the H-2A, H-2K, and H-2D haplotypes were high to intermediate responders, whereas mice possessing the H-2B and the H-2S haplotypes were consistently low responders. The use of H-2 congenic resistant strains also support the role of H-2-linked genes in controlling the antibody response to PGL-I. However, a discrepancy in the antibody response detectable by SaMk between the BALB/c and the C57BL/10 congenic mice indicate that gene complementation between H-2-linked genes and non-H-2-linked genes may be operative.

Control of antibody response has also been shown to involve Igh allotype complex linked genes (3, 7, 34). BALB/c allotype congenic mice were therefore examined more closely for the possible role of Igh-linked genes in controlling the level of the anti-PGL-I antibody response. This was done in spite of the lack of apparent correlation between Igh allotypes and anti-PGL-I responses in the strain distribution studies. The use of BALB/c Igh allotype congenic mice clearly demonstrate significantly higher levels of anti-PGL-I antibodies in mice with some immunoglobulin allotypes (IgG, IgM, and IgE). In addition, gene complementation between immunoglobulin allotype-linked Ir genes and other genes may play a role in governing the level of anti-PGL-I antibody response. This is exemplified by the fact that mice possessing the IgG allotype are higher responders on the BALB/c background than they are on the A/J or A.SW background (compare the BALB/c IgG response in Fig. 3 with the A/J and A.SW responses in Fig. 1). Genes linked to the IgG allotype complex have also been shown to influence the antibody responses to group A streptococcal carbohydrates (3), p-aminobenzoic acid and sulphamic acid coupled to bovine gamma globulin (29), and to sheep erythrocytes (19).

The apparent dominant inheritance of low antibody response to PGL-I in F1 hybrid progeny of high- and low-responder parental phenotypes has been demonstrated in mice with other antigen systems. Examples include the antibody response to trinitrophenylated-mouse serum albumin (39), mouse IgG2a (15), Ea-1 erythrocyte antigens (10), the random l-glutamic acid l-tyrosine polymer (6), and the autoimmune response to the liver antigen F (33). When dominant nonresponsiveness or low responsiveness is observed, the explanation has frequently been that the nonresponding strain, as well as the resultant F1 hybrids, possess cross-reactive epitopes with the test antigen. In the case of PGL-I this seems unlikely for the reasons previously discussed.

There are several alternative explanations for dominant inheritance of low responsiveness, and they are not mutually exclusive. The interaction of molecules involved in antigen presentation between high and low responders in the F1 progeny could result in the expression of a low-responder phenotype. High- and low-responder interactions occurring after antigen processing at the genetic or the cellular level, or both, could also play a role. Gene products in the F1 hybrids may be defective at the cellular level, leading to inefficient cellular cooperation, which is involved in the anti-PGL-I antibody response. Finally, preferential stimulation of suppressor activity of the immune response is characteristic of low-responder phenotypes. Thus, in F1 hybrids, as well as low-responder parental strains, the low-responder phenotype could be the expression of genetically controlled suppression of anti-PGL-I immune responses. The fact that in two out of three F1 progeny (SJL/J × CBA/J) and (SWR/J × A/J) the response was significantly below that of the low-responder parental phenotype (P < 0.05) is suggestive of an active suppressor effect by genes of the low-responder parent. Future studies in the mouse system, including the determination of the number of genes involved in dominant low responsiveness by backcross analysis, may help to elucidate the potential role of genetic influences on the complex immunoregulatory mechanisms involved in leprosy.

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LITERATURE CITED

4. Cho, S. N., D. L. Yanagihara, S. W. Hunter, R. H. Gelber, and...
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