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Strain Variations in the Murine Cellular Immune Response to the Phenolic Glycolipid I Antigen of *Mycobacterium leprae*

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The cellular immune response to the *Mycobacterium leprae*-specific phenolic glycolipid I was examined in inbred mice immunized with *M. leprae* by in vivo delayed cutaneous hypersensitivity and in vitro lymphocyte proliferation. Whereas all mouse strains responded to *M. leprae*-induced delayed-type hypersensitivity and lymphocyte proliferation, only BALB.K was responsive in both assays to the glycolipid. Responsiveness was determined in part by non-*H-2* genes, while the influence of *H-2* genes was not apparent. Among congenic BALB/c mice differing only at *Igh-C* allotype loci, variations in responsiveness were found in both delayed-type hypersensitivity and lymphocytes proliferation assays, indicating a possible role for *Igh-C* loci-linked genes. Unresponsiveness in the lymphocyte proliferation assay to the glycolipid was inherited as a dominant trait in one set of responder × nonresponder F_1 progeny. We conclude that after immunization with *M. leprae* organisms, the cell-mediated responses to the glycolipid, endowed with a single carbohydrate epitope, are under polygenic control, predominantly non-*H-2*-linked genes.

The wide range of clinical types in leprosy is probably determined by variations in host responses to the *Mycobacterium leprae* bacillus (23). Host response is, in part, determined by genetic predisposition. A number of familial studies and analyses of HLA associations have identified HLA gene product markers for predisposition to develop tuberculoid leprosy (25) or lepromatous leprosy (28). Variation of host response among clinical types of leprosy is represented by the lymphocyte unresponsiveness to lepromin in lepromatous leprosy (15) in the face of a vigorous specific antibody response. The link between these immunological paradigms on the one hand and genetic predisposition on the other has been obscured, however, by the complexity of immunoregulation, the lack of an animal model paralleling human leprosy, and finally the lack of immunodominant antigens.

Among a number of specific glycolipid antigens recently isolated and structurally characterized (5, 17), phenolic glycolipid I (PhenGL-I) is highly antigenic in animals and leprosy patients (6, 9, 29). PhenGL-I represents approximately 2% of the mass of the organism and is present in large quantities in infected tissues from which the bacilli have been removed (5). Since PhenGL-I may represent a significant interface between the defense mechanisms of the host and the organism itself, we undertook a series of studies to examine the genetic control of immune responses to PhenGL-I in inbred mouse strains. An earlier study examined the genetic control of antibody responses against the carbohydrate epitope of PhenGL-I (26a). This present work addresses the genetic control of cell-mediated immune responsiveness to PhenGL-I in various uninfected mouse strains when the mice are immunized with a moderate dose of nonviable *M. leprae* and PhenGL-I in liposomes.

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MATERIALS AND METHODS

Mice. The following strains of inbred female mice and F_1 hybrids were used: A/J, A.SW/SnJ, B10.BR/SgSnJ, BALB/cByJ, CBA/J, C57BL/6J, C57BL/10J, DBA/1J, DBA/2J, SJL/J, SWR/J, (C57BL/6J × A/J)F_1, (SWR/J × A/J)F_1, (SJL/J × CBA/J)F_1 (Jackson Laboratory, Bar Harbor, Maine); BALB.B, BALB.K, BALB.Ig^c (N-20), BALB.Ig^e (N-10), BALB.Ig^f (N-18), BALB.Ig^g (N-11, F-3), BALB.Ig^n (N-11) (obtained from Noel Warner and Ed Walker, University of New Mexico School of Medicine, Albuquerque). All mice were 8 to 20 weeks of age at the start of an experiment.

Antigens. *M. leprae* was purified from armadillo liver as described previously (16). PhenGL-I was finally purified to homogeneity by thin-layer chromatography (17) and diluted in 100% methanol. Control glycolipid antigens included galactocerebroside (Sigma Chemical Co., St. Louis, Mo.) and a different triglycosyl diacyl phthiocerol (mycoside A) purified from *Mycobacterium kansasii* (16). PhenGL-I was incorporated into liposomes as described previously (17). Concanavalin A was obtained from Sigma.

Immunization. Purified gamma-irradiated *M. leprae* was emulsified in incomplete Freund adjuvant (ICFA) (Marcol-Arlacel, 4:1). Primary and secondary immunizations with 0.1 ml of emulsion containing 50 μg of *M. leprae* were carried out intraperitoneally (i.p.) on day 0, day 21, and day 42. Serum was obtained by tail bleeding 2 weeks before primary immunization and on day 30 postimmunization.

Delayed hypersensitivity (DTH) testing. Ten days after the third i.p. immunization, the right hind footpad was injected with 25 μg of PhenGL-I in 0.025 ml of a liposome suspension prepared as described previously (14), and the left hind footpad was injected with liposomes devoid of antigen. Simultaneously, the right earlobe was injected with 10^6 sonicated *M. leprae* bacilli in 0.025 ml of physiological saline (0.01 M), and the left earlobe was injected with the same volume of saline. Ear or footpad thickness was measured in triplicate with a spring-loaded caliper (Dyer Co. Inc., Lancaster, Pa.) before injection and again 24, 48, and 72 h

TABLE 1. DTH response of inbred mice to *M. leprae* and PhenGL-I after immunization with *M. leprae* organisms

Mouse strain	*H-2* allele	*Ig* allotype	No.	DTH reaction (48-h corrected ear or foot swelling [mm])[a]			
				M. leprae	*P*[b]	PhenGL-I	*P*
B10.BR/SgSnJ	*k*	*b*	7	0.15 ± 0.04	<0.01	0.16 ± 0.03	<0.02
CBA/J	*k*	*a*	6	0.31 ± 0.06	<0.002	0.06 ± 0.04	NS[c]
BALB.K	*k*	*a*	5	0.33 ± 0.03	<0.001	0.15 ± 0.09	<0.001[d]
C57BL/10J	*b*	*b*	6	0.47 ± 0.09	<0.005	0.12 ± 0.03	<0.02
C57BL/6J	*b*	*b*	5	0.35 ± 0.02	<0.001	0.13 ± 0.04	<0.05
BALB.B	*b*	*a*	7	0.10 ± 0.03	<0.002	0.06 ± 0.03	NS
DBA/1J	*q*	*c*	5	0.39 ± 0.04	<0.001	0.05 ± 0.02	NS
SWR/J	*q*	*c*	5	0.36 ± 0.05	<0.001	−0.06 ± 0.02	NS
A/J	*a*	*e*	5	0.29 ± 0.05	<0.002	0.05 ± 0.04	NS
A.SW/SnJ	*s*	*e*	6	0.32 ± 0.05	<0.002	0.25 ± 0.06	<0.01
SJL/J	*s*	*c*	5	0.20 ± 0.10	<0.001	0.06 ± 0.13	NS
DBA/2J	*d*	*c*	6	0.52 ± 0.07	<0.001	0.05 ± 0.05	NS
BALB/cJ	*d*	*a*	52	ND[e]		0.12 ± 0.10	
BALB/cBy	*d*	*a*	9	0.11 ± 0.01	<0.001	0.03 ± 0.04	NS
BALB.Ig^c	*d*	*c*	10	0.10 ± 0.02	<0.001	0.21 ± 0.02	<0.001
BALB.Ig^e	*d*	*e*	9	0.46 ± 0.03	<0.001	0.13 ± 0.03	<0.01
BALB.Ig^f	*d*	*f*	9	0.10 ± 0.03	<0.001	0.10 ± 0.03	<0.02
BALB.Ig^g	*d*	*g*	10	0.08 ± 0.03	0.02	0.14 ± 0.04	<0.01
BALB.Ig^h	*d*	*h*	10	0.10 ± 0.03	<0.001	0.02 ± 0.05	NS

[a] Means ± standard error of mean.
[b] Compared with control.
[c] NS, Not significant.
[d] Define.
[e] ND, Not done.

after injection. Experiment ear swelling was obtained by subtracting the preinjection from the postinjection ear thickness. Antigen-induced swelling was obtained by subtracting the control ear from the experiment ear swelling. Statistical analysis of the difference between experimental and control ear or footpad swelling was based on the paired *t* test, whereas differences between the corrected ear or footpad swelling were analyzed by the Student *t* test.

Cell harvest and purification. Ten days after footpad injections, the peritoneal cavity was injected with 2.5 ml of aged thioglycolate broth. Four days later peritoneal exudate cells (PEC) were harvested by exsanguinating the mouse, injecting the peritoneal cavity with 5 ml of RPMI 1640 medium containing 5 U of heparin per ml, and then withdrawing the medium and cells. Popliteal lymph node cells (PLNC), mesenteric lymph node cells, and spleen cells were teased out and pressed through a fine wire mesh. Each cell population was the product of a pool from 7 to 15 donors. Cells were washed twice before purification.

T cells were prepared from PEC by the method of Schwartz et al. (24). In brief, PEC were suspended in RPMI 1640 medium containing 20% fetal calf serum at a concentration of 5×10^6 cells per ml and incubated on glass petri plates for 90 min at 37°C. Nonadherent cells were gently rinsed off the glass, washed once, and incubated on a nylon wool column for 45 min at 37°C. Eluted cells contained 95 ± 2% small mononuclear cells which were always greater than 90% viable by trypan blue exclusion. T lymphocytes in the eluted cells were analyzed by flow cytometry with a monoclonal antibody against the theta antigen. Forward light scatter revealed a bimodal distribution of cell volume. The larger small-cell peak contained 80 to 90% theta-positive cells, and the smaller large-cell peak contained 65 to 75% theta-positive cells.

Cell cultures. Cell cultures were performed in quadruplicate in 96-well round-bottom plastic microtiter plates (Nunc, Neptune, N.J.). Glycolipid antigens suspended in methanol were dried on the microtiter well by a stream of sterile air. Soluble antigens were added to the wells in 20 μl of phosphate-buffered saline. Cells were suspended at a concentration of 10^6/ml in RPMI 1640 medium with 10% nontoxic horse serum, penicillin, streptomycin, and 10 mM glutamine and added to the wells in a volume of 200 μl. The plates were incubated at 37°C for 5 days in a 10% CO_2 atmosphere. Cultures were pulsed for 1 h with [^{3}H]thymidine (60 μCi/μmol; New England Nuclear Corp., Boston, Mass.) (22), harvested on a MASH-II, and counted in a beta scintillation counter. Responsiveness was defined as significantly greater [^{3}H]thymidine incorporation in cultures containing antigen compared with controls without antigen, as determined by the Student *t* test.

RESULTS

DTH reactions to *M. leprae*. Since no significant difference was noted between the values obtained at 24, 48, and 72 h (data not shown) and since maximum reactivity was noted at 48 h in almost all mouse strains, only the results obtained at 48 h are presented. Although positive DTH reactions were noted in all mouse strains (Table 1), there were wide variations in the distribution of reactivity. Control mice immunized with ICFA demonstrated no reactivity. Among mouse strains of the *H-2* haplotypes *k*, *b*, and *d*, there were high responders (greater than 0.25 mm corrected ear swelling) and low responders, suggesting the importance of non-major histocompatibility complex (MHC) genes. However, comparison of congenic BALB/c mice with different *Igh-C* allotypes (Table 1) revealed that a single strain with the *Ig*^{e} allotype is a higher responder. This suggests that genes governing *Ig* allotypes may contribute to DTH reactivity.

To display possible interaction between MHC and non-MHC genes, the results of the study on BALB/c and C57BL/10 *H-2* congenic mice were redrawn (Fig. 1). With the C57BL/10 background, the $H\text{-}2^b$ haplotype was a high responder, and $H\text{-}2^k$ was a low responder. In contrast, with the BALB/c background, $H\text{-}2^k$ was the high responder and $H\text{-}2^b$ was the low responder.

DTH reactions to PhenGL-I. Compared with DTH reactivity to *M. leprae*, only weak reactivity to PhenGL-I was detected. Of the 13 strains that exhibited reactivity to *M. leprae*, only 6 showed a DTH reaction to PhenGL-I (Table 1), including B10.BR, C57BL/10, C57BL/6, A.SW, BALB.K, and BALB/cJ. For the latter two strains, the reactivity of 48 immune mice was maximal at 48 h, as compared with the group of nonimmunized mice of the corresponding strain (for BALB.K, 0.15 versus 0.04 mm [$P < 0.02$]; for BALB/cJ, 0.12 versus 0.04 mm [$P < 0.02$]). Reactivity to PhenGL-I did not correlate with the degree of reactivity to *M. leprae*. Among the BALB/c $H\text{-}2^d$ congenic mice with various *Igh-C* allotypes, mice bearing Ig^a and Ig^n allotypes were nonresponders, and BALB/c with Ig^c, Ig^e, Ig^f, and Ig^g were responders. Mice with Ig^c and Ig^e allotypes were responders on the BALB/c background but were nonresponders on most other backgrounds, suggesting possible interaction between genes encoding *Ig* allotypes and other non-MHC genes.

Lymphocyte proliferation (LP) to *M. leprae*. PEC and PLNC from two mouse strains were studied in detail. PEC from either BALB/cJ or BALB.K mice immunized three times i.p. with *M. leprae* responded vigorously to *M. leprae* (for BALB.K, stimulation index [SI] was 25.4, with Δcpm of 6,019; for BALB/cJ, SI was 22.5, with Δcpm of 2,796). In nine additional experiments, PLNC from BALB.K mice immunized with two to four injections of *M. leprae* in the footpads and i.p. were studied. The SIs ranged from 4 to 14, with an average SI of 8.4; and Δcpm ranged from 758 to 5,284, with an average Δcpm of 2,154.

Characteristics of LP response to PhenGL-I. Dose-response curves and optimum immunization protocols were studied in a responder strain, BALB.K. Immunization with three injections of ICFA alone or with three i.p. injections of ICFA containing *M. leprae* failed to elicit PhenGL-I-induced LP, indicating that the footpad injection of liposomes containing PhenGL-I, in addition to i.p. ICFA containing *M. leprae*, was necessary for eliciting in vitro lymphocyte reactivity. Of four cell populations assessed for PhenGL-I-induced proliferation, only PEC and PLNC were responsive; mesenteric lymph node cells and spleen cells did not show specific proliferation. PLNC responded (SI = 2.2) only at the highest concentration tested, 10 μg/ml (200 ng of PhenGL-I per well). In two separate studies PEC responded optimally (SI = 4.0 and 2.2) at 0.1 μg/ml (2 ng of PhenGL-I per well). The responses of other mouse strains to PhenGL-I are presented below only for 2 ng per well.

PhenGL-I-induced lymphocyte proliferation among murine strains. Among the 13 strains tested earlier for DTH reactivity to PhenGL-I, only 5 strains demonstrated an in vitro proliferative response to PhenGL-I (Fig. 2). Two strains showed a clear response (DBA/1 and BALB.K) with Δcpms of 750 and 940, respectively. Three other strains were weak responders (SJL, BALB.B, and B10.BR), so designated since the SI was less than 2.0. Of the 13 strains, 6, including 4 responder strains, were immunized with ICFA alone; none showed significant PhenGL-I-induced proliferative response (SIs ranged from 0.69 to 1.28). In four of five *H-2* haplotypes in which two or more mouse strains were studied, both responder and nonresponder strains were found (Fig. 2); thus, non-MHC genes are important in determining responsiveness.

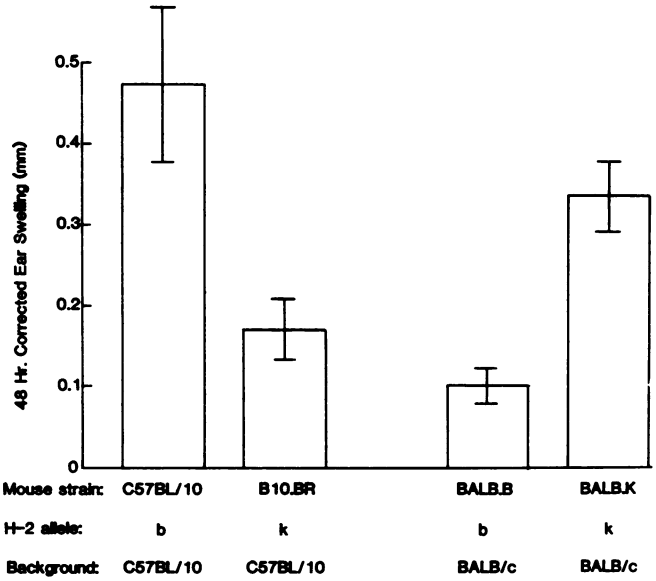

FIG. 1. DTH response of inbred mice to *M. leprae*, derived from data in Table 1, rearranged to show the interaction between *H-2* allele and strain background (non-*H-2* genes). Ear swelling is expressed as means ± standard errors.

The F_1 offspring of weak-responder (SJL) and nonresponder (CBA) parents, immunized with *M. leprae* i.p. and PhenGL-I in footpads as described above, were nonresponders (SI of 1.07). The F_1 offspring of two other nonresponder crosses (C57BL/6J × A/J and SWR/J × A/J) were both nonresponders (SI of 0.94 and 1.12, respectively).

Among four strains of BALB/c $H\text{-}2^d$ mice congenic at the *Igh-C* allotypes, three strains (Ig^c, Ig^e, Ig^n) were responders (Fig. 3). A fifth BALB/c strain (BALB/cBJ; see Fig. 2) bearing the Ig^a allotype was a nonresponder. Mouse strains other than BALB/c bearing Ig^e or Ig^c allotypes were responders (DBA/1) or nonresponders (SWR, A, A.SW, or DBA/2). Thus, the in vitro LP response to PhenGL-I appears to be influenced by genes encoding *Ig* allotypes.

Correlation of immune responses to PhenGL-I. The results of the in vitro proliferative and in vivo DTH responses to PhenGL-I were compared with anti-PhenGL-I antibody responses after immunization with *M. leprae*. Quantitative comparison showed no correlation between proliferative response and DTH among the 13 strains shown in Fig. 2 ($r = 0.01$). Of the four DTH-responsive strains, only one responded in the LP assay. Conversely, of the five strains responsive in the LP assay, only one was DTH responsive.

There was no correlation between DTH and antibody levels, and there was no correlation between the proliferative response and total levels of anti-PhenGL-I antibodies, most of which belong to the immunoglobulin M class. There was a trend to correlation ($r = 0.43$) between proliferative response and the titer of IgG class anti-PhenGL-I, as measured by a protein A-binding assay (26a), but the correlation was not statistically significant (data not shown). In addition, the mouse strain (BALB.K) with the highest proliferative response also had the highest titer of anti-PhenGL-I antibody of the IgG class.

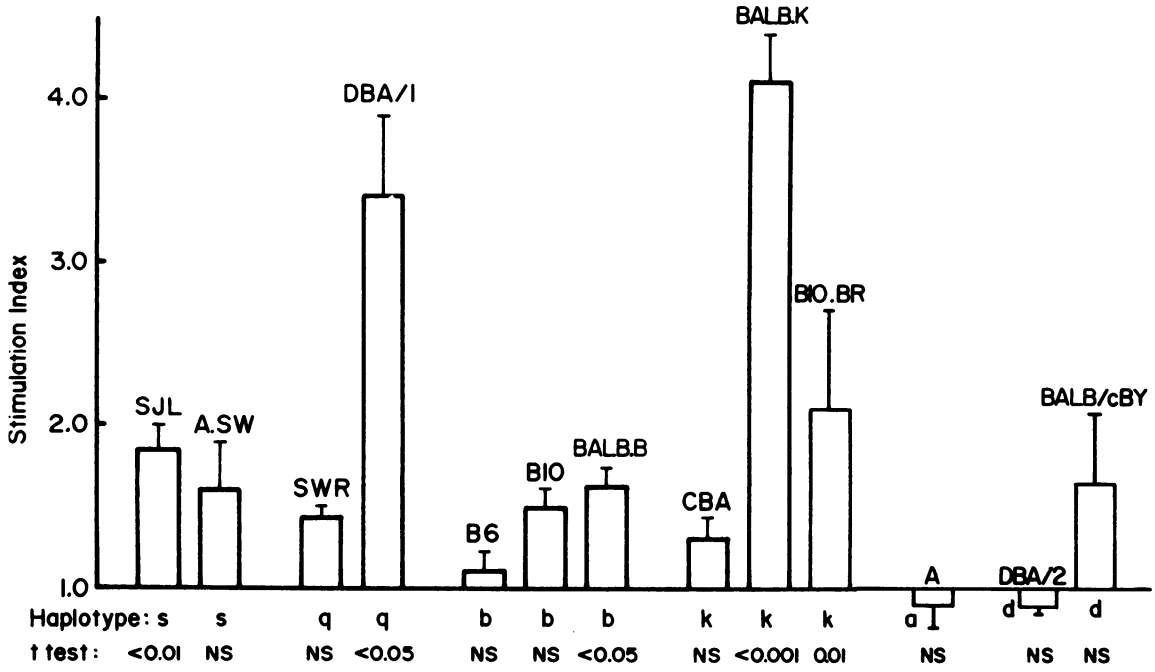

FIG. 2. LP response of inbred mice to PhenGL-I. BALB.K and DBA/1 strains are responders, while B10.BR, SJL, and BALB.B strains had marginal responses indicated only by statistical analysis. SIs are expressed as means ± standard errors. NS, Not significant.

DISCUSSION

In this study, we examined two measures of cell-mediated immunity, in vivo DTH and in vitro LP, to the specific PhenGL-I in several inbred strains of mice immunized with *M. leprae* bacilli. The present study not only addresses T-cell response to a carbohydrate antigenic determinant but also to an antigen of highly restricted heterogeneity. In a study with murine monoclonal antibodies to PhenGL-I (29) and antibodies to PhenGL-I from infected humans (14), the primary specificity for antigen-antibody interaction was shown to reside in the terminal 3,6-di-*O*-methyl-glucopyranosyl residue; there was no evidence for antigenicity on the lipid portion of the glycolipid molecule (9). Based on studies with deglycosylated fragments of PhenGL-I, it also appears that human T cells recognize this single 3,6-di-*O*-methyl-glucopyranosyl epitope, resulting in stimulation of suppressor T lymphocytes (19).

The in vitro LP response to PhenGL-I was small in magnitude and more easily demonstrated in PEC than PLNC, despite the fact that the PhenGL-I was injected into the adjacent footpad. The ability to show in vivo DTH further confirmed the existence of sensitized T cells to PhenGL-I. A recent study on a T-independent antigen, polyvinylpyrrolidone, indicates that this class of antigen can elicit helper-T-cell response (4). However, the demonstration of helper-T-cell response is masked by concomitant induction of suppressor T cells. Helper-T-cell response was noted when the animals were immunized with a low antigen dose, when they were treated with cyclophosphamide, or when the responder cell population was first depleted of Lyt-2+ T lymphocytes. Studies with bacterial capsular antigens have demonstrated that T cells respond to polysaccharide antigens both by in vitro proliferation and in vivo DTH (26). We confirmed the observation that lymphocytes of immune CBA mice fail to respond to PhenGL-I (7).

The demonstration of responder and nonresponder strains to PhenGL-I may represent presumptive evidence for polygenic control of lymphocyte responsiveness to PhenGL-I. The observation of continuous distributions of antibody response for antigens of limited heterogeneity such as the $(Glu^{60}Ala^{30}Tyr^{10})_n$ (GAT) (12) and pneumococcal polysaccharides (3) allowed a similar conclusion. The variations observed between BALB/c *Igh-C* congenic mice in their responses to PhenGL-I suggest that responsiveness to PhenGL-I may be linked to *Igh* allotype. Linkage of responsiveness to *Igh* has been similarly demonstrated in the GAT

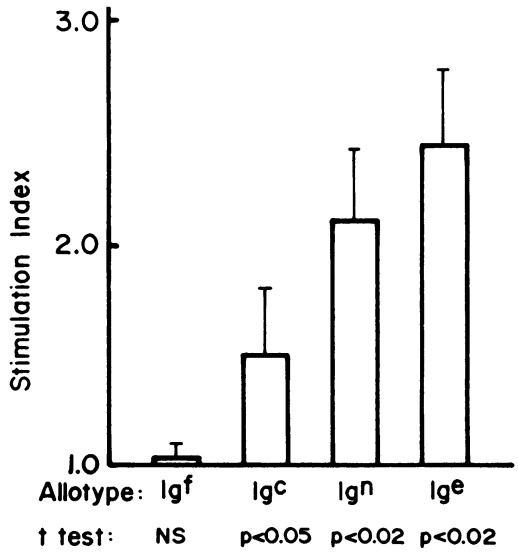

FIG. 3. LP response of BALB/c mice congenic at the *Igh* locus to PhenGL-1. Both responders and nonresponders were found. SIs are expressed as means ± standard errors. NS, Not significant.

(12) and the dextran (2) antigen systems. These findings are of considerable interest in view of the finding that genes concerned with the *Igh* allotypes are closely associated with genes that code for T-cell alloantigens on murine chromosome 12 (20).

Our study shows discordance between in vivo DTH response and in vitro LP response to PhenGL-I. Thus, three of four DTH-responsive strains did not proliferate in vitro, and conversely four of five proliferative-responder strains did not demonstrate DTH responses. The finding of discrepancy between the two assays is not unexpected for several reasons. Antigen-specific T-cell proliferation was found not to correlate with resistance to lethal infection with *Mycobacterium lepraemurium* in a small number of mouse strains tested (18). Similarly, studies based on a well-defined peptide antigen (21) have failed to show a correlation between DTH and LP response. In contrast to the proliferation assay, DTH is a complex in vivo phenomenon involving the responses of additional cell types and secretion of mediators. It is also possible that lymphocytes mediating DTH and those that proliferate in vitro are different.

In the one litter of responder × nonresponder F_1 progeny studied, nonresponsiveness to PhenGL-I in the LP assay was inherited as a dominant trait. This must be taken as preliminary data inasmuch as only one such combination has been studied and the responder parent (SJL) was only a weak responder to PhenGL-I. Nonetheless, this observation is of interest since in the study of the antibody responses to PhenGL-I, the F_1 progeny of three responder × nonresponder crosses were similarly nonresponders (26a).

If PhenGL-I is an important determinant of *M. leprae* pathogenicity (5), it is reasonable to search for a relationship between immune responses to PhenGL-I and resistance to infection. Unfortunately, the lack of a facile experimental model of *M. leprae* infection does not yet permit this analytical approach. However, since there is recent information on mouse strain variations of resistance to *M. lepraemurium* (1, 10, 11), it is tempting to compare the findings with murine cellular immune responses to PhenGL-I response. In *M. lepraemurium* infection, resistance is controlled primarily by genes linked to non-*H-2* genes (10), particularly to a gene on chromosome 1 (8), but is modified by *H-2*-linked genes (1, 11). Infectivity is also dependent on the route of infection (8, 27). Considering that *M. lepraemurium* organisms also produce substantial amounts of a glycolipid antigen (13; albeit not a phenolic glycolipid, but of the mycoside [allopeptide lipid] class [5]), we can compare the LP response to PhenGL-I established in this study with the data of Curtis et al. (10, 11) on the resistance of the same mouse strains against footpad infection of viable *M. lepraemurium*.

An inverse correlation can be discerned between the in vitro proliferative response to PhenGL-I and the in vivo resistance to footpad infection by *M. lepraemurium* (Table 2). In contrast, in vivo DTH responses to neither *M. leprae* nor PhenGL-I correlated with resistance to *M. lepraemurium*. The inverse correlation above remains when resistant *H-2*-congenic mouse strains with common background genes are compared. Thus, among mice with the BALB/c background, resistance to *M. lepraemurium* is ranked BALB/c > BALB.B10 > BALB.K (10). However, in vitro proliferation to PhenGL-I is ranked in reverse, BALB.K > BALB.B10 > BALB/c. Similarly, among two C57BL/10 congenic strains, B10 was more resistant than B10.BR (10, 26a), yet B10.BR exhibited a higher proliferative response to PhenGL-I.

TABLE 2. Comparison of cell-mediated immune responses to PhenGL-I and resistance to *M. lepraemurium* infection in six strains of inbred mice

Strains	*H-2* allele	*M. leprae* in vivo DTH[a]	PhenGL-I in vivo DTH[a]	PhenGL-I in vitro LP[b]	In vivo resistance to *M. lepraemurium*[c]
BALB.K	*k*	++	−	+	±
BALB.B10	*b*	+	−	±	+
BALB/cBy	*d*	+	−	−	++
B10.BR	*k*	+	+	+	+
C57BL/10	*b*	++	+	−	++
C57BL/6	*b*	++	+	−	+++

[a] Derived from Table 1.
[b] Derived from Fig. 1.
[c] Derived from references 1, 9, and 10.

The above inverse correlation would argue against a straightforward role for immune responses to PhenGL-I in resistance to infection. Nevertheless, the finding that PhenGL-I selectively stimulates T suppressor cells (19) suggests some influence of PhenGL-I on immunity to leprosy infection. In light of this, we speculate that a genetic predisposition to recognition and response to PhenGL-I may impair host resistance to *M. leprae*. Considerably more effort will be required to define the precise role(s) of PhenGL-I and the immune responses to PhenGL-I in the pathogenesis of leprosy.

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LITERATURE CITED

1. **Adu, H. O., J. Curtis, and J. L. Turk.** 1983. Role of the major histocompatibility complex in resistance and granuloma formation in response to *Mycobacterium lepraemurium* infection. Infect. Immun. **40**:720–725.
2. **Blomberg, B., W. R. Geckeler, and M. Weigert.** 1972. Genetics of the antibody response to dextran in mice. Science **177**: 178–180.
3. **Braley, H. C., and M. J. Freeman.** 1971. Strain differences in the antibody plaque-forming cell responses of inbred mice to pneumococcal polysaccharide. Cell. Immunol. **2**:73–81.
4. **Braley-Mullen, H.** 1985. Regulation of IgG memory responses by helper and suppressor T cells activated by the type 2 antigen, polyvinylpyrrolidone. J. Exp. Med. **161**:1357–1367.
5. **Brennan, P. J.** 1984. New-found glycolipid antigens of mycobacteria, p. 366–375. *In* L. Leive and D. Schlessinger (ed.), Microbiology—1984. American Society for Microbiology, Washington, D.C.
6. **Brett, S. J., P. Draper, S. N. Payne, and R. J. W. Rees.** 1983. Serological activity of a characteristic phenolic glycolipid from *Mycobacterium leprae* in sera from patients with leprosy and tuberculosis. Clin. Exp. Immunol. **52**:271–279.
7. **Brett, S. J., C. Lowe, S. N. Payne, and P. Draper.** 1984. Phenolic glycolipid I of *Mycobacterium leprae* causes nonspecific inflammation, but has no effect on cell-mediated responses in mice. Infect. Immun. **46**:802–808.
8. **Brown, I. N., A. A. Glynn, and J. Plant.** 1982. Inbred mouse strain resistance to *Mycobacterium leprae murium* follows the Ity/Lsh pattern. Immunology **47**:149–156.
9. **Cho, S.-N., D. L. Yanagihara, S. W. Hunter, R. H. Gelber, and**

P. J. Brennan. 1983. Serological specificity of phenolic glycolipid I from *Mycobacterium leprae* and use in serodiagnosis of leprosy. Infect. Immun. **41**:1077–1083.
10. **Curtis, J., H. Adu, and J. L. Turk.** 1982. *H-2* linkage control of resistance to subcutaneous infection with *Mycobacterium lepraemurium*. Infect. Immun. **38**:434–439.
11. **Curtis, J., H. Akuffo-Adu, and J. L. Turk.** 1984. *H-2*-linked genes which modify resistance of C57BL/10 mice to subcutaneous infection with *Mycobacterium lepraemurium*. Infect. Immun. **46**:635–638.
12. **Dorf, M. E., E. K. Dunham, J. P. Johnson, and B. Benacerraf.** 1974. Genetic control of the immune response: the effect of non-H-2 linked genes on antibody production. J. Immunol. **112**:1329–1336.
13. **Draper, P., and R. J. W. Rees.** 1970. The nature of the electron transparent zone that surrounds *Mycobacterium lepraemurium* inside host cells. J. Gen. Microbiol. **77**:79–87.
14. **Fujiwara, T., S. W. Hunter, S.-N. Cho, G. O. Aspinall, and P. J. Brennan.** 1984. Chemical synthesis and serology of disaccharides and trisaccharides of phenolic glycolipid antigens from the leprosy bacillus and preparation of a disaccharide protein conjugate for serodiagnosis of leprosy. Infect. Immun. **43**:245–252.
15. **Godal, T., B. Myklestad, D. R. Samuel, and B. Myrvang.** 1971. Characterization of the cellular defect in lepromatous leprosy: a specific lack of circulating *Mycobacterium leprae*-reactive lymphocytes. Clin. Exp. Immunol. **9**:821–831.
16. **Hunter, S. W., and P. J. Brennan.** 1981. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. J. Bacteriol. **147**:728–735.
17. **Hunter, S. W., T. Fujiwara, and P. J. Brennan.** 1982. Structure and antigenicity of the major specific glycolipid antigen of *Mycobacterium leprae*. J. Biol. Chem. **257**:15072–15078.
18. **Mathew, R. C., J. Curtis, and J. L. Turk.** 1984. T cell proliferation in *Mycobacterium leprae murium* infection. I. Lack of correlation between antigen-specific proliferation of Lyt $1+23^-$ cells and resistance in lethal infections. Immunology **51**: 185–192.
19. **Mehra, V., P. J. Brennan, E. Rada, J. Convit, and B. R. Bloom.** 1984. Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid. Nature (London) **308**:194–196.
20. **Owen, F. L.** 1983. T cell alloantigens encoded by the IgT-C region of chromosome 12 in the mouse. Adv. Immunol. **34**:1–38.
21. **Peterson, L. B., G. D. Wilner, and D. W. Thomas.** 1983. Functional differentiation in the genetic control of murine T lymphocyte responses to human fibrinopeptide B. J. Immunol. **130**:637–643.
22. **Pollack, A., C. B. Bagwell, and G. L. Irwin.** 1979. Radiation from tritiated thymidine perturbs the cell cycle progression of stimulated lymphocytes. Science **203**:1025–1027.
23. **Sansonetti, P., and P. H. LaGrange.** 1981. The immunology of leprosy: speculations on the leprosy spectrum. Rev. Infect. Dis. **3**:442–445.
24. **Schwartz, R. H., L. Jackson, and W. E. Paul.** 1975. T lymphocyte-enriched murine peritoneal exudate cells. I. A reliable assay for antigen-induced T lymphocyte proliferation. J. Immunol. **115**:1330–1338.
25. **Serjeantson, S. W.** 1983. HLA and susceptibility to leprosy. Immunol. Rev. **70**:89–112.
26. **Shapiro, M. E., A. B. Orderdonk, D. L. Kasper, and R. W. Finberg.** 1982. Cellular immunity to *Bacteroides fragilis* capsular polysaccharide. J. Exp. Med. **154**:1188–1197.

26a. **Teuscher, C., D. Yanagihara, P. J. Brennan, F. T. Koster, and K. S. K. Tung.** 1985. Antibody response to phenolic glycolipid I in inbred mice immunized with *Mycobacterium leprae*. Infect. Immun. **48**:474–479.

27. **Turcotte, R.** 1980. Influence of route of *Mycobacterium lepraemurium* infection on susceptibility to mouse leprosy and on lymphoblastic transformation. Infect. Immun. **28**:660–668.
28. **van Eden, W., N. M. Gonzalez, R. R. P. de Vries, J. Convit, and J. J. van Rood.** 1985. HLA-linked control of predisposition to lepromatous leprosy. J. Infect. Dis. **151**:9–14.
29. **Young, D. B., S. R. Khanolkar, L. L. Bourg, and T. M. Buchanan.** 1984. Generation and characterization of monoclonal antibodies to the phenolic glycolipid of *Mycobacterium leprae*. Infect. Immun. **43**:183–188.