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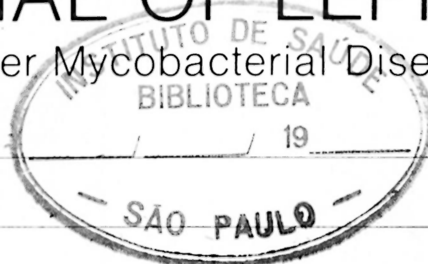
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# Immunity to Leprosy. IV. Murine T-Cell Proliferative Responses to Mycobacteria[1]

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To investigate how thymus-derived (T) lymphocytes respond to the antigens of *Mycobacterium leprae,* we have described an experimental system which measures the proliferation of murine T lymphocytes in response to immunization with *M. leprae* ([3]). Mice are injected at the base of the tail with irradiated *M. leprae* emulsified in Freund's incomplete adjuvant (FIA), and 7–14 days later the draining lymph nodes (inguinal and para-aortic) are removed for cell culture with different concentrations of *M. leprae* antigen. The subsequent proliferation of T cells is readily quantitated and has been shown to be specific for *M. leprae.* Our objective is to use this assay to identify antigens that elicit strong T-cell responses and to consider whether these have potential use in the development of a protective vaccine for leprosy.

In previous publications, we have reported two findings. First, in a comparison of T-cell responses to *M. leprae* using 18 different inbred strains of mice, it was found that congenic strains of C57BL/10J mice can be divided into high- or low-responder strains ([4]). $F_1$ hybrid mice resulting from crosses between high- and low-responder strains were also low responders to *M. leprae* antigens ([4]). Second, *M. leprae* and 13 related strains of mycobacteria have been tested for their stimulatory effects in T-cell proliferation assays using spleen, thymus, and lymph node cultures ([5]). A number of strains of mycobacteria acted as direct polyclonal B-cell mitogens in lymphocyte cultures, exhibiting proliferative responses far greater than those using purified protein derivative (PPD) tuberculin, a known mycobacterial mitogen ([5]).

The objective of the experiments presented here was to analyze antigens which may be shared between *M. leprae* and other mycobacteria using murine T-cell proliferation as the response assay. The species of mycobacteria which exhibit polyclonal B-cell mitogenic properties have been a particular problem. By immunizing mice with mitogenic strains of mycobacteria, and then preparing lymph node cultures from these mice for challenge *in vitro* with *M. leprae,* a non-mitogenic strain of mycobacteria, antigens common to different species could be assessed. T-cell proliferative responses to a number of strains of mycobacteria have been

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examined in high- and low-responder congenic strains of mice, as well as $F_1$ hybrid progeny. Of particular interest was that while six mycobacteria elicited normal T-cell proliferative responses in these high- and low-responder strains of mice, as well as the $F_1$ hybrid progeny, *M. marinum* yielded a response pattern in congenic and $F_1$ mice which followed that observed using *M. leprae* as antigen.

## MATERIALS AND METHODS

**Animals.** C57BL/10J and congenic strains B10.M, B10.Q, BALB/cJ, and DBA/2J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, U.S.A., and maintained in the breeding facility of the Department of Immunobiology in the Auckland University School of Medicine, Auckland, New Zealand. $F_1$ hybrid mice, (B10.M × C57BL/10J), (B10.Q × C57BL/10J), and (BALB/cJ × DBA/2J), were bred from inbred parental stocks obtained from the Jackson Laboratory. Mice were used at 6–10 weeks of age for all experiments.

*M. leprae* purified from armadillo tissue was kindly provided after irradiation and lyophilization by Dr. P. Brennan, Colorado State University, Fort Collins, Colorado, U.S.A. ([1,7]). All other mycobacteria were prepared by culturing on Löwenstein-Jensen medium at the Tuberculosis Reference Laboratory, Greenlane Hospital, Auckland. Each mycobacterial species was separately harvested from the medium, suspended in sterile saline, washed twice in RPMI 1640 tissue culture medium, washed once in sterile saline, distilled water and in 0.1 M ammonium acetate. The mycobacteria were lyophilized and then sterilized by irradiation (60,000 Rad) at the ICI Tasman Vaccine Laboratory, Wellington, New Zealand. All mycobacteria were then stored at 4°C under sterile conditions until use. For use in lymphocyte cultures, mycobacteria were suspended in sterile saline.

**Immunization procedure.** Each strain of mycobacteria was suspended in saline, emulsified in Freund's incomplete adjuvant (FIA) and injected subcutaneously into mice at the base of the tail. An antigen dose of 20 μg was injected in 50 μl volumes. This immunizing dose was found to be optimal, as detailed elsewhere ([3, 4]). Control animals received 50 μl of sterile saline emulsified in FIA. In each experiment, groups of three mice were injected for each dose of antigen used, and their lymph nodes pooled at the time of harvest for microculture assay.

**T-cell proliferation assay.** Mice were sacrificed 7–10 days after immunization with mycobacteria and the para-aortic (3 nodes) and inguinal (2 nodes) lymph nodes removed aseptically under a dissecting microscope. Lymph node cells were suspended in medium containing RPMI 1640 medium with 5% fetal calf serum (FCS), $5 \times 10^{-5}$ M 2-mercaptoethanol, 50 units/ml of penicillin, and 50 μg/ml streptomycin sulfate. Single cell suspensions were prepared by repeatedly drawing cells into a Pasteur pipette. The cells were washed once with medium, counted, and diluted to a final density of $4 \times 10^6$ cells/ml. Each microculture contained $8 \times 10^5$ cells in a volume of 200 μl of medium containing *M. leprae* antigen or other mycobacterial antigens as indicated in the text at concentrations of 6, 12.5, 25, 50, and 100 μg/ml. Control microcultures contained cells but no antigen. Cultures were incubated for 3 days at 37°C in an atmosphere of 6% $CO_2$ in humidified air. Cultures were then radiolabeled with 0.5 μCi of [$^3$H]-thymidine (2 μCi/mMole; New England Nuclear Corporation, Boston, Massachusetts, U.S.A.) for 6 hr. Cells were harvested on glass-fiber filter paper, and the radioactivity was measured in a liquid scintillation counter. Results were expressed as means of triplicate cultures, and standard deviations were calculated.

**Data analysis.** To compare antigens shared between different species of mycobacteria in experiments where lymph node cells from mice immunized with *Mycobacterium X* and challenged *in vitro* with either *Mycobacterium X* or *M. leprae* antigen, the data have been presented in several ways. First, the response to *Mycobacterium X* was arbitrarily taken as 100%, and the response to *M. leprae* antigen at the same concentration expressed as a percentage of this figure. Second, the response to *M. leprae* was taken as 100%, and the response to *Mycobacterium X* at the same antigen concentration expressed as a percentage of this figure. Five antigen concentrations were used in each *in vitro* challenge, leading to five percentage

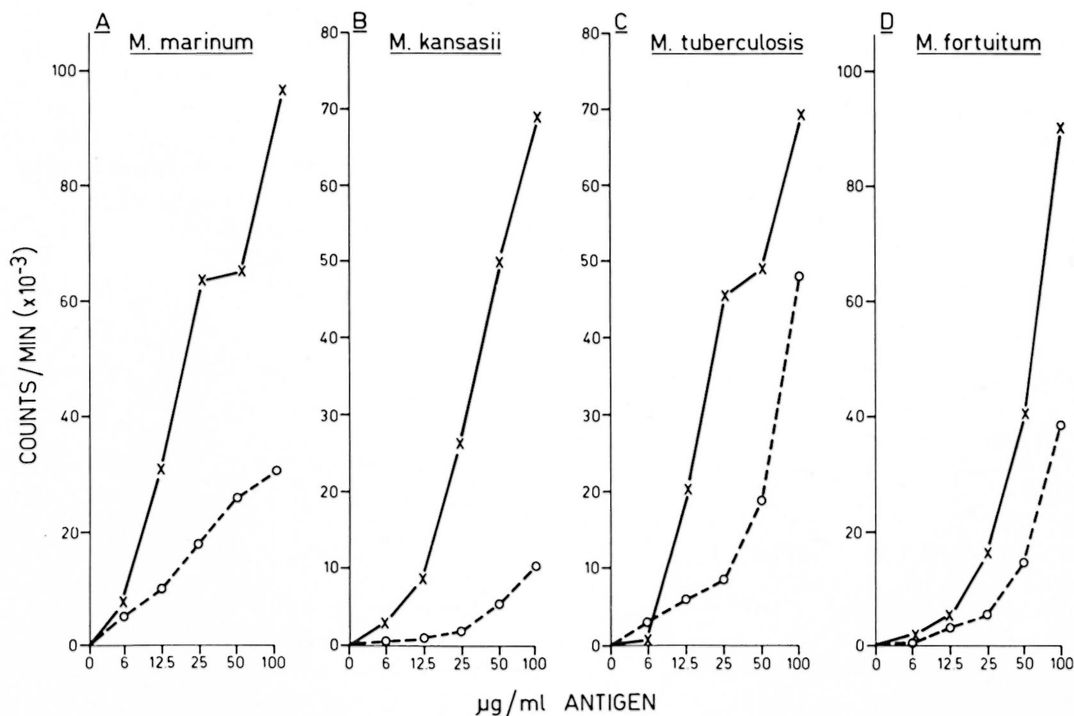


FIG. 1. T-cell proliferative responses to mycobacteria. Groups of $CDF_1$ mice were immunized with 20 µg *M. marinum* (A), *M. kansasii* (B), *M. tuberculosis* (C), and *M. fortuitum* (D). Lymph node cultures were prepared one week later and challenged with the immunizing antigen (×——×) or with *M. leprae* (○- - -○).

values. These five values were averages from each experiment and standard deviations were calculated. The data shown in Figures 2–4 have been derived in this way.

## RESULTS

In a previous study, mycobacteria have been arbitrarily divided into three groups based on the ability to elicit polyclonal B-cell mitogenic responses: low, intermediate, and high ([5]). Mycobacteria which showed little or no mitogenicity included *M. leprae, M. marinum, M. kansasii, M. tuberculosis, M. fortuitum, M. smegmatis, M. avium,* and *M. chelonei.* Mycobacteria which elicited strong mitogenic responses included *M. gordonae, M. scrofulaceum,* and *M. avium-intracellulare.* Mycobacteria which stimulated mitogenic responses intermediate between the other two groups included *M. bovis, M. vaccae,* and *M. phlei.* These groupings have influenced the design of the following experiments aimed at examining patterns of antigen crossreactivities using murine T cells.

**Immunization with *Mycobacterium X* and challenge with *M. leprae.*** The aim of these experiments was to immunize mice with each available strain of mycobacteria using standard conditions and to compare T-cell responses when node cultures prepared from immunized mice were challenged with different concentrations of either the immunizing antigen or *M. leprae* as antigen. The data presented in Figure 1 show the results obtained by immunizing separate groups of $CDF_1$ mice with 20 µg *M. marinum* (Fig. 1A), *M. kansasii* (Fig. 1B), *M. tuberculosis* (Fig. 1C), and *M. fortuitum* (Fig. 1D), and subsequently challenging lymph node cultures, either with the immunizing antigen or with *M. leprae.* The results reveal that mice immunized with *Mycobacterium X* and challenged *in vitro* with either *Mycobacterium X* or *M. leprae* yielded strong T-cell proliferative responses to the immunizing antigen in all cases, but varying and lesser responses to *M. leprae* (Fig. 1).

We have used this experimental design to systematically compare 13 mycobacterial

species, and have presented the data in two ways. First, the response obtained from each group of mice immunized *in vivo* and challenged in culture with *Mycobacterium X* was taken as 100%, and the responses resulting from *in vitro* challenge of the same cells with *M. leprae* is expressed as a percentage of this value. This compares how T cells from mice immunized with *Mycobacterium X* responded to *M. leprae* in comparison to their own antigens. These data are presented in Figure 2, together with the standard devia-

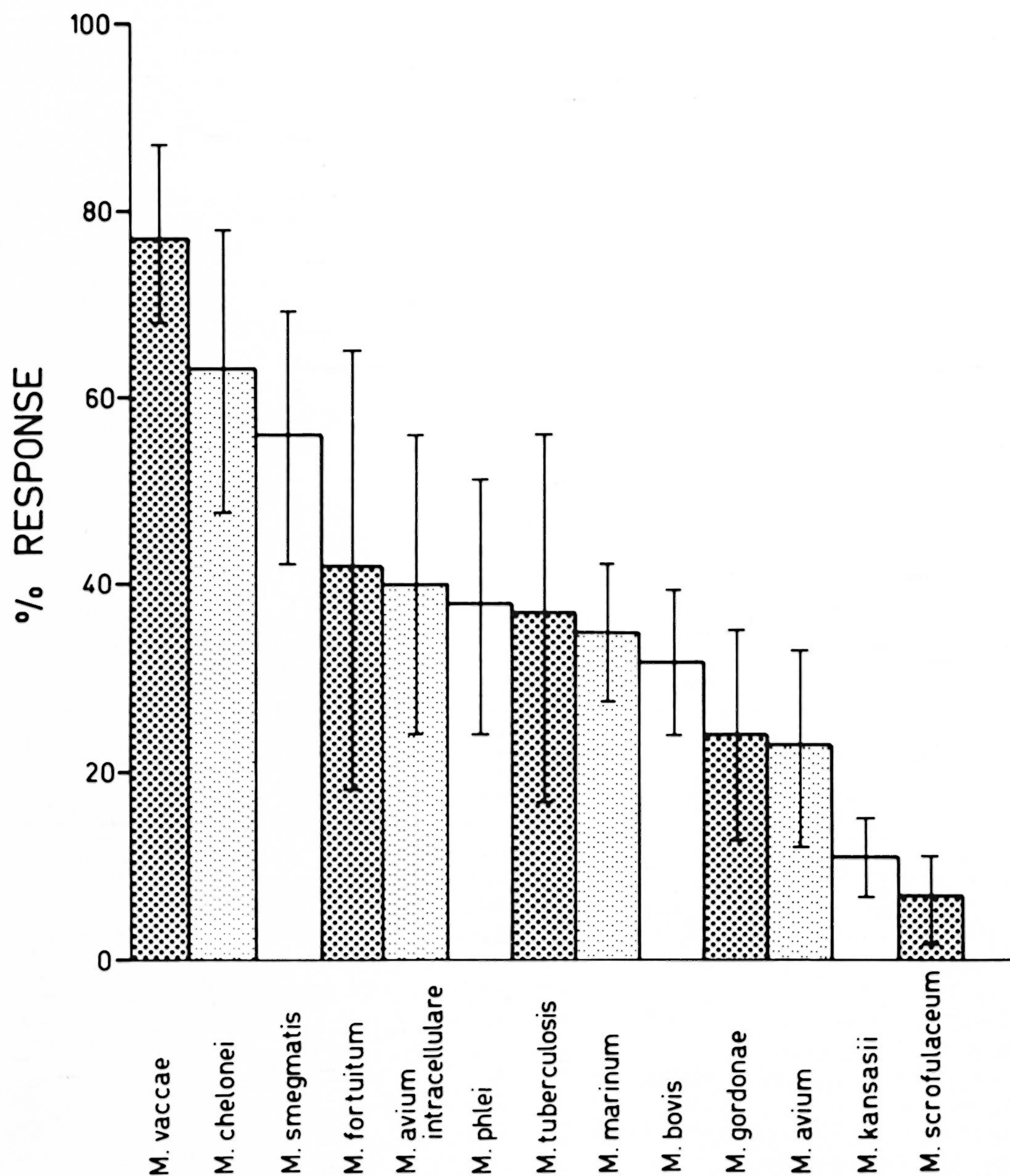


FIG. 2. A comparison of how mice immunized with *Mycobacterium X* responded to *M. leprae* in T-cell proliferative responses. Groups of $CDF_1$ mice were immunized with 20 μg of each mycobacterial species shown, lymph node cultures were prepared and challenged with the immunizing antigen or *M. leprae*. The response obtained from each group of mice immunized *in vivo* and challenged in culture with *Mycobacterium X* was taken as 100%, and the response resulting from *in vitro* challenge of the same cells with *M. leprae* is expressed as a percentage of this value.

tions. The results show that the resulting T-cell proliferative responses varied considerably. Mice immunized with *M. vaccae, M. chelonei,* and *M. smegmatis* and challenged with *M. leprae* yielded significant T-cell responses to *M. leprae*; whereas mice immunized with *M. gordonae, M. avium, M. kansasii,* and *M. scrofulaceum* supported only small T-cell responses to *M. leprae.* Other mycobacteria tested gave intermediate responses. These results imply that the various immunizing species of mycobacteria shared a range of antigens recognized by T cells with those found on *M. leprae.*

Another method of presenting the data in a comparative form was to use mice immunized with *M. leprae* and challenged with *M. leprae* as the 100% value. This means that all responses from mice immunized with *Mycobacterium X* and challenged with *M. leprae* are calibrated to the response of mice immunized and subsequently challenged in culture with *M. leprae.* Any mycobacteria which immunize mice better than *M. leprae* for responses to *M. leprae* readily emerge (Fig. 3). Mice immunized with *M. tuberculosis, M. scrofulaceum, M. bovis,* and *M. marinum,* and challenged *in vitro* with *M. leprae,* gave a higher response to *M. leprae* than comparable mice immunized and challenged with *M. leprae.* It must be stressed that the responses of mice immunized with *M. tuberculosis, M. scrofulaceum, M. bovis,* and *M. marinum* were much higher to their own antigens than to *M. leprae* (Fig. 2). *M. vaccae, M. phlei, M. gordonae,* and *M. smegmatis* were also effective compared with *M. leprae* in immunizing mice for subsequent responses to *M. leprae* (Fig. 3), while the remaining mycobacterial species tested provided a lesser degree of immunization in mice (Fig. 3). This variation in response to the *in vitro* challenge with *M. leprae* presumably reflects the degree to which antigens are shared between the mycobacterial species used for immunization and *M. leprae.*

**Immunization with *M. leprae* and challenge with *Mycobacterium X.*** Groups of $CDF_1$ mice were immunized with 20 $\mu$g of *M. leprae,* and after 7 days lymph node cultures were prepared and challenged separately with different concentrations of *M. leprae,* as well as several different mycobacterial strains. The data expressed in Figure 4 show that when the mice were immunized with *M. leprae* and challenged *in vitro* with *M. leprae* and *M. marinum* (Fig. 4A), *M. kansasii* (Fig. 4B), *M. tuberculosis* (Fig. 4C), and *M. fortuitum* (Fig. 4D) strong proliferative responses to *M. leprae* were obtained, but only small responses to the other mycobacterial species were seen. These experiments have been repeated a number of times using lymph node cells from mice immunized with *M. leprae* and challenged with either *M. marinum, M. kansasii, M. tuberculosis, M. fortuitum, M. smegmatis, M. avium,* or *M. chelonei.* The data have been compared in a histogram (Fig. 5), showing the response of T cells immunized to *M. leprae* and challenged separately with each of the mycobacteria expressed as a percentage value of the proliferative response observed in identical control lymph node cultures challenged with *M. leprae.* Cultures prepared from mice immunized with *M. leprae* responded well in culture to challenge with *M. leprae* and also *M. smegmatis,* with smaller and decreasing responses to the other mycobacteria (Fig. 5). The implication of these experiments is that the T cells are responding to antigens unique to *M. leprae,* many of which may be shared by *M. smegmatis,* but other mycobacterial species have varying and fewer common antigens. Thus, while *M. marinum,* for example, can be used to immunize mice for strong proliferative responses to *M. leprae* (Fig. 3), *M. leprae* does not immunize mice for strong proliferative responses to *M. marinum* (Fig. 4).

**Genetic control of T-cell proliferative responses to mycobacteria.** We have previously reported that C57BL/10J mice immunized with *M. leprae* exhibit low T-cell proliferative responses to *M. leprae* upon *in vitro* challenge, while the congenics B10.M and B10.Q are high-responder strains. The hybrid progeny of high- and low-responder strains (C57BL/10J × B10.M)$F_1$ and (C57BL/10J × B10.Q) $F_1$ hybrid mice are also low responders to *M. leprae* ([4]). We have examined T-cell proliferative responses to a range of mycobacterial species using C57BL/10J, B10.M, B10.Q, and $F_1$ hybrid mice. The data presented in Figure 6 compare the T-cell proliferative responses of C57BL/10J, B10.Q, and (C57BL/10J × B10.Q)$F_1$ hybrid mice to *M. leprae* (Fig. 6A), *M. marinum* (B), *M. kansasii* (C), *M.*

*tuberculosis* (D), *M. fortuitum* (E), *M. bovis* (F), *M. chelonei* (G), and *M. phlei* (H). Of major interest is that while six of these mycobacterial strains elicited high T-cell responses in all these strains of mice, *M. marinum* showed a response pattern similar to that observed for *M. leprae* (Fig. 6 A and B). While B10.Q mice were high responders

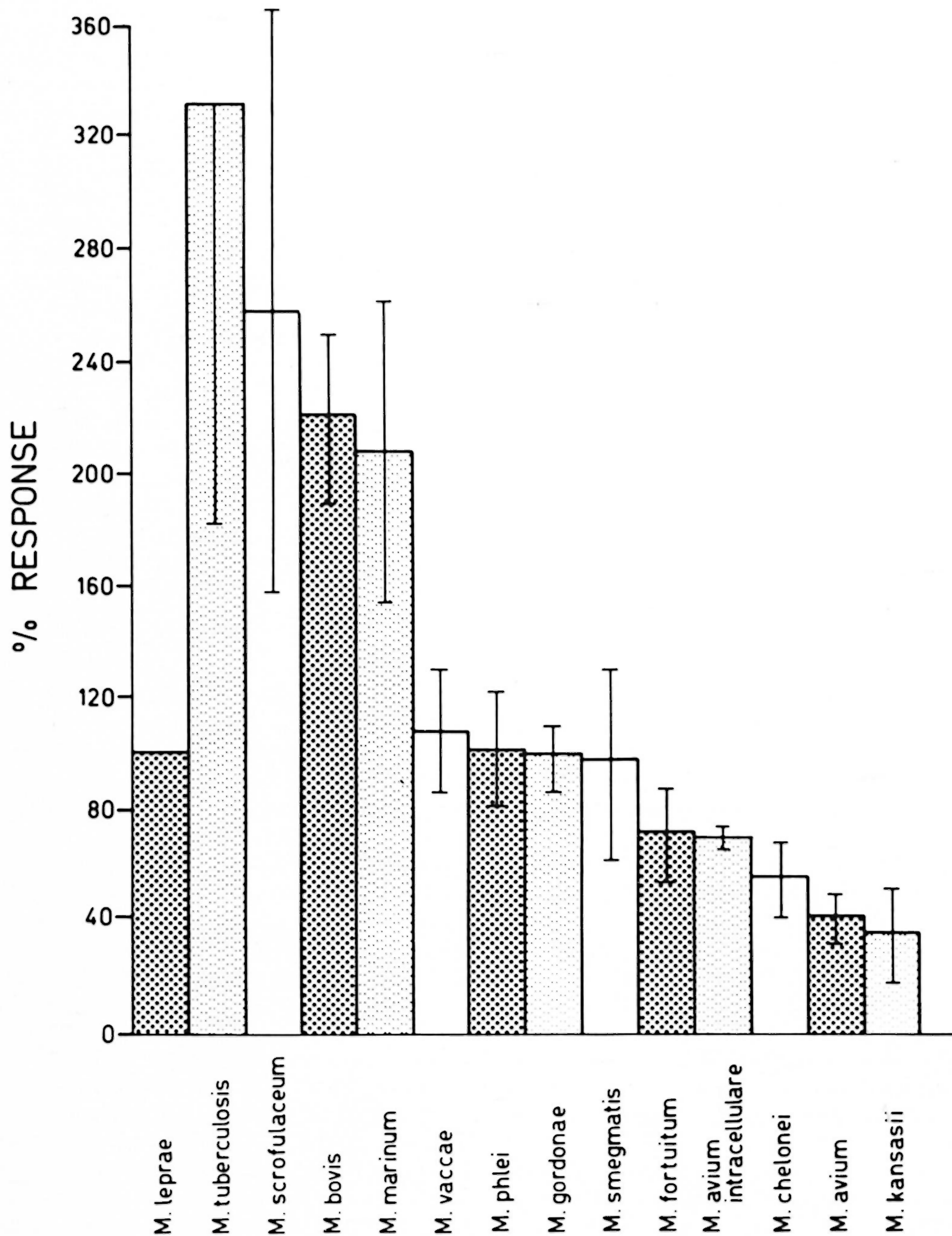


FIG. 3. A comparison of how different mycobacteria immunize T cells for responses to *M. leprae*. These data are the results from the experiments of Figure 2 presented in a different form. Groups of mice immunized with *M. leprae* and challenged *in vitro* with *M. leprae* were taken as the 100% value. Responses from each group of mice immunized with *Mycobacterium X* and challenged with *M. leprae* were calibrated to the response of mice immunized and challenged with *M. leprae*. Thus, mycobacteria which immunized mice better than *M. leprae* for responses to *M. leprae* readily emerge.

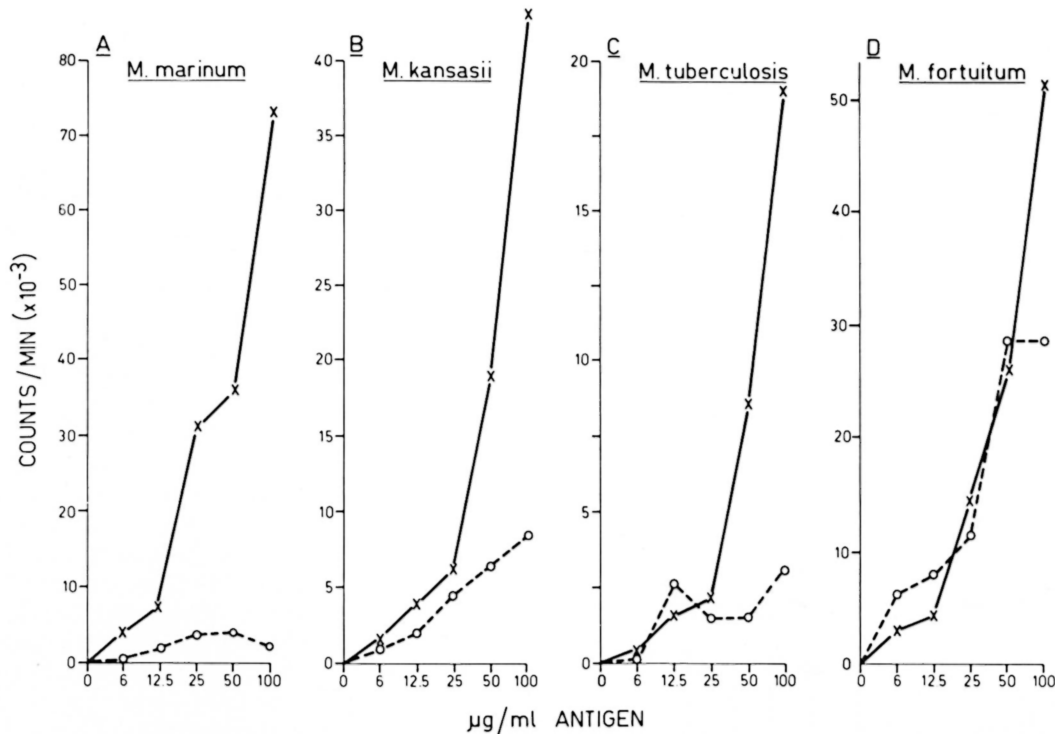


FIG. 4. T-cell proliferative responses to mycobacteria. Groups of $CDF_1$ mice were immunized with 20 µg *M. leprae*, lymph node cultures prepared one week later and challenged with *M. leprae* (×——×) or with (A) *M. marinum* (O- - -O); (B) *M. kansasii* (O- - -O); (C) *M. tuberculosis* (O- - -O); (D) *M. fortuitum* (O- - -O).

to *M. marinum*, C57BL/10J and $F_1$ hybrid mice were low responders. The T-cell proliferative responses using C57BL/10J, B10.M, and (C57BL/10J × B10.M) $F_1$ mice to *M. leprae, M. marinum*, and *M. kansasii* have been compared. The results presented in Figure 7 show that B10.M mice are high responders to each of these mycobacteria, while C57BL/10J and $F_1$ progeny mice are low responders to *M. leprae* and *M. marinum*, but respond well to *M. kansasii*.

## DISCUSSION

It is well established that T-cell-mediated immunity rather than humoral immunity is important for the *in vivo* control of multiplication of the leprosy bacillus in mice ([2, 9]). Problems with cost, patient compliance, and the emergence of *M. leprae* strains resistant to chemotherapeutic agents have added impetus to efforts to develop a safe, cheap, and effective vaccine against *M. leprae*. There have been two main approaches to the problem. The first involves the use of inactivated *M. leprae* derived from armadillos either alone or in combination with BCG ([10, 11, 15]) which has been shown to be immunogenic ([13, 14]). The second approach has been to search among the cultivable mycobacteria for organisms which share crossreactive antigenic determinants with *M. leprae* and confer protection from challenge with live *M. leprae* ([6, 8, 12–14, 16]).

Methods for examining T-cell-mediated responses to mycobacterial antigens have been indirect. Skin reactions to lepromin and tuberculin or other mycobacterial antigens can indicate delayed-type hypersensitivity (DTH) responses but are difficult to quantify and, in humans, are difficult to interpret because of the possible contributions from previous exposure to environmental mycobacteria ([6, 8]). The murine T-cell proliferative response we have used ([3, 4]) allows a simple quantitative method of measuring reactivity and crossreactivity to mycobacterial antigens. This paper describes the use of this assay to examine the crossreactivity of antigens from cultivable mycobacteria with *M. leprae*. It has been previously reported that the assay measures primarily the proliferative response of murine Lyt-1+ ([3])

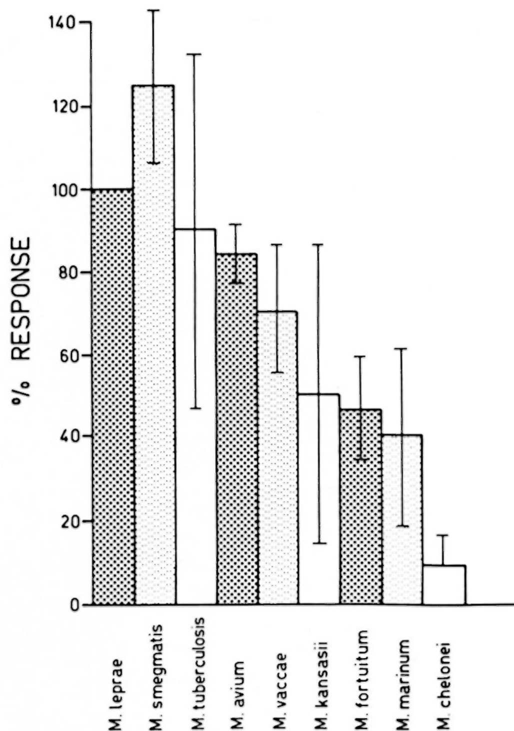


FIG. 5. Response of T cells immunized with *M. leprae* to other mycobacterial species. Groups of $CDF_1$ mice were immunized with 20 μg *M. leprae*. Lymph node cultures were prepared one week later and challenged separately with each of the mycobacteria shown. Data have been presented as a percentage value of the proliferative response observed in identical cultures challenged with *M. leprae*.

cells. Current unpublished work indicates that Ly-2+ cells in mice do not respond to immunization with mycobacteria (M. Skinner, A. G. Douglas-Jones and J. D. Watson, unpublished data).

The aim of the experiments was to examine the effect of immunizing mice with different mycobacterial species and analyzing subsequent T-cell responsiveness to *M. leprae*. The 13 mycobacteria used showed varying abilities to immunize T cells for subsequent response to *M. leprae*. It was of particular interest that mice immunized with *M. tuberculosis*, *M. scrofulaceum*, *M. bovis*, and *M. marinum* and then challenged in a T-cell proliferative assay with *M. leprae* gave a high response to *M. leprae* (Fig. 3). These responses were as high as those observed in comparable groups of mice immunized and challenged with *M. leprae*. The interpretation of this data is that these four species share antigenic determinants with *M. leprae* that are recognized by T cells (Fig. 3). However, each of these mycobacterial species have antigens not shared by *M. leprae*, since T-cell responses to their own antigens are much higher than their T-cell responses to *M. leprae* antigens (Fig. 2).

A different pattern was observed when the reverse immunization procedures were used, in which mice were immunized with *M. leprae* and subsequently lymph node cultures were challenged with a range of nonmitogenic mycobacteria. Mice immunized with *M. leprae* responded well to challenge with *M. smegmatis* and *M. tuberculosis*, but not to *M. marinum* or to the other species tested (Fig. 5). This implies that although mycobacteria may share common antigenic structures, they may vary considerably in their ability to elicit T-cell responses. This may be a result of nonimmunogenic substances in the cell wall masking antigens in different species. Clearly, these experiments are also limited by the use of whole bacilli which are complex mixtures of antigens. While other species, *M. tuberculosis*, *M. marinum*, *M. scrofulaceum*, and *M. bovis*, all immunize mice for responsiveness to *M. leprae*, the use of *M. leprae* as antigen is not as effective in immunizing mice for challenge with these species of mycobacteria.

There is a large body of literature describing the antigens of *M. leprae* and their cross-reactivity with other mycobacteria ([6, 8, 17]). Such work has largely used serological techniques which examine specificities recognized by B cells. It is not known whether those determinants recognized by B cells are also recognized by T cells. Mycobacterial

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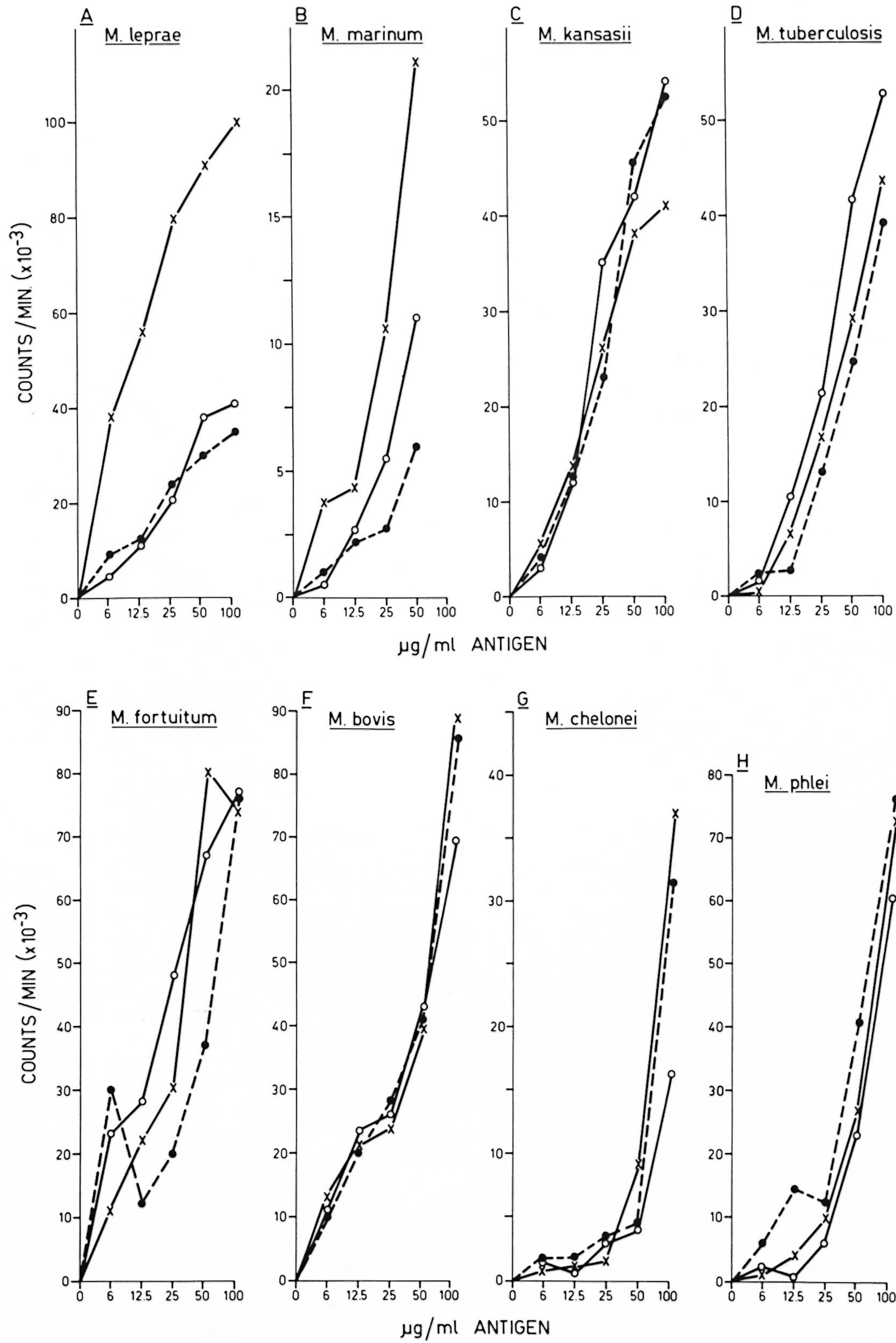
FIG. 6. Genetic control of T-cell proliferative responses to mycobacteria. Groups of C57BL/10J (●——●), B10.Q (×——×), and $F_1$ (C57BL/10J × B10.Q) hybrid mice (○——○) were immunized with 20 μg of antigen. One week later lymph node cultures were prepared and challenged with the immunizing antigen. Mycobacteria tested were: (A) *M. leprae*; (B) *M. marinum*; (C) *M. kansasii*; (D) *M. tuberculosis*; (E) *M. fortuitum*; (F) *M. bovis*; (G) *M. chelonei*; (H) *M. phlei*.


A
M. leprae
B
M. marinum
C
M. kansasii
D
M. tuberculosis
COUNTS / MIN (x10$^{-3}$)
µg/ml ANTIGEN
E
M. fortuitum
F
M. bovis
G
M. chelonei
H
M. phlei
COUNTS / MIN (x10$^{-3}$)
µg/ml ANTIGEN

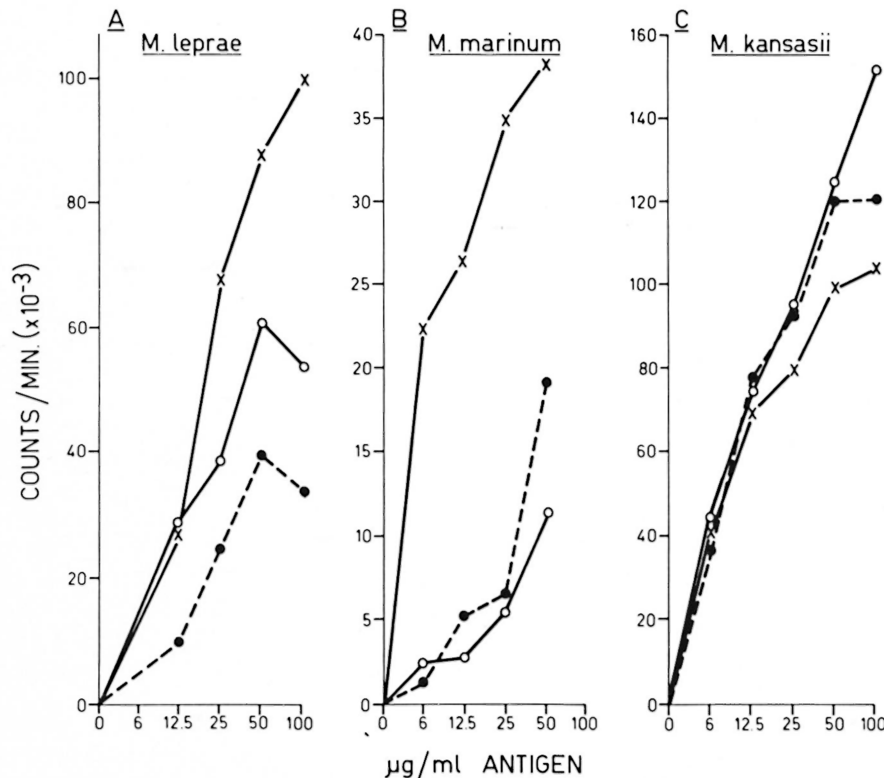



FIG. 7. Genetic control of T-cell proliferative responses to mycobacteria. Groups of C57BL/10J (●——●), B10.M (×——×), and $F_1$ (C57BL/10J × B10.M) hybrid mice (○——○) were immunized with 20 µg *M. leprae*. One week later lymph node cultures were prepared and challenged with the immunizing antigen. Mycobacteria tested were (A) *M. leprae*; (B) *M. marinum*; (C) *M. kansasii*.

antigens have been divided into four groups based on serological analysis ([3]): Group 1—shared by all mycobacteria; Group 2—shared by slow-growing species; Group 3—shared by fast-growing species; and Group 4—those limited to individual species.

*M. leprae* and *M. vaccae* alone among the mycobacteria lack Group 2 and Group 3 antigens, and it has been suggested that this might point to antigenic similarities between *M. leprae* and *M. vaccae* ([2]). In addition, lymphocyte transformation tests performed on lymph node cells of BALB/c mice after injection of *M. leprae* antigen into the foot pad showed increased responsiveness to *M. leprae* and *M. vaccae* only after 6 months. The data presented in this paper show that when T cells are primed with *M. vaccae* they respond 80% as well to *M. leprae* antigen as they do to their own antigen (Fig. 2). T cells primed with the other mycobacteria tested gave lesser responses to *M. leprae* antigen. Conversely, T cells primed with *M. leprae* also gave good responses to *M. vaccae* when compared to the responses of *M. leprae*-primed cells with *M. leprae* antigen (Fig. 5). This does suggest that *M. leprae* and *M. vaccae* share common antigenic determinants recognized by T cells.

It is interesting that in the experiments described here, *M. tuberculosis*, *M. marinum*, *M. scrofulaceum*, and *M. bovis* all immunized the mice for good T-cell proliferative responses to *M. leprae* (Fig. 3). Early *in vivo* experiments described elsewhere ([11]) indicated that immunization of mice with heat-killed *M. tuberculosis*, *M. marinum*, BCG, *M. lepraemurium*, and *M. ulcerans* showed some evidence of immunity against *M. leprae* infections. In a later search among mycobacteria for candidates for use in an-

tileprosy vaccines, mice were immunized with 17 different mycobacterial strains. Protection against infectious challenge with *M. leprae* injected into the foot pads, as assessed by a reduction in the rate of multiplication of *M. leprae*, was observed only with *M. bovis* and *M. leprae* ([15]).

The data from experiments on the genetic control of murine T-cell responses have added further clues as to antigenic relationships between non-mitogenic mycobacterial species. The proliferating T cells in this assay carry predominantly the helper phenotype ([3, 4]). Previous data have shown that within C57BL/10J congenic strains of mice there are high- and low-responder strains to *M. leprae* antigens ([4]). $F_1$ hybrids between high- and low-responder strains show low T-cell proliferative responses to *M. leprae* antigens. Genetic control of the T-cell responses involved gene complementation between genes in the H-2I region of the major histocompatability complex. Also, a marked effect of the H-2D region genes on the T-cell proliferation responses was seen ([4]). We currently believe that helper T cells from low-responder $F_1$ hybrid mice may be defective in their ability to recognize "key" antigens of *M. leprae* involved in the initiation of T-cell responses ([4]). The T-cell responses of B10.Q, C57BL/10J, and $F_1$ (B10.Q × C57BL/10J) mice to different species of mycobacteria revealed that these strains of mice responded equally well to *M. kansasii, M. tuberculosis, M. fortuitum, M. bovis, M. chelonei,* and *M. phlei* (Fig. 6). The surprising finding was that C57BL/10J and the $F_1$ hybrid mice used were low responders to *M. leprae* and *M. marinum* (Fig. 6). Similarly, B10.M mice were higher responders and (B10.M × C57BL/10J) mice were low responders to both *M. leprae* and *M. marinum* (Fig. 7). Mice immunized with *M. marinum* and challenged *in vitro* with *M. leprae* (Fig. 3) revealed that there are antigens shared between these two mycobacterial species. Similarities in the genetic control of T-cell proliferative responses to *M. marinum* and *M. leprae* were unexpected, particularly when genetic differences were not seen using six other mycobacterial species as antigens. It is a reasonable hypothesis that the basis of low responsiveness involves a closely related group of antigenic determinants. If so, these determinants might be expected to play a critical role in the induction of T-cell responses to *M. marinum* and *M. leprae* during *in vivo* immunization with whole bacilli. The T-cell immune response to a large number of antigens found in *M. leprae* or *M. marinum* may be initiated by the response to a smaller, but common set of closely related antigens present in both mycobacteria.

These studies also illustrate the need to learn more of the T-cell response to *M. leprae*. First, while the T-cell proliferative response appears to reflect the response of helper T cells to *M. leprae* antigens, there is no way to determine which of these is important in protective immunity. Second, while the T-cell proliferative responses seen after immunization of mice with different mycobacteria and *in vitro* challenge with *M. leprae* reflect the presence of shared antigens, it remains impossible to determine whether any of these have potential in protective immunity to leprosy.

Our current research that has emerged from these studies has now focused on two directions. First, by electrophoretically separating sonicates of bacilli, different molecular species from *M. leprae* are being examined for antigenic properties in the T-cell proliferative response assay. Second, the cellular basis of high and low responsiveness to *M. leprae* and *M. marinum* in C57BL/10J and congenic strains of mice is being examined. It is hoped that a combination of genetic and molecular approaches to the basis of T-cell responses to *M. leprae* may lead to a rationalization of how to develop an effective protective vaccine for leprosy.

## SUMMARY

The nature of antigens shared between *Mycobacterium leprae* and other species of mycobacteria has been examined using a murine T-cell proliferation assay. Mice were immunized with different mycobacteria, and lymph node cultures were prepared one week later and challenged with *M. leprae* antigen. The 13 species of mycobacteria tested as antigens in this assay revealed that several species shared antigens in common with *M. leprae* as recognized by T-cell responses.

C57BL/10J mice and congenic strains exhibit differences in T-cell responsiveness to *M. leprae*. B10.M and B10.Q mice are high responders and C57BL/10J are low responders, while $F_1$ (C57BL/10J × B10.M) and (C57BL/10J × B10.Q) hybrid progeny are also low responders. These genetic differences were not observed when six other mycobacterial species were used as T-cell antigens. An unexpected finding was that the genetic pattern of T-cell responsiveness to *M. marinum* was identical to that observed for *M. leprae* using these strains of mice. Helper T cells may recognize antigenic determinants shared by *M. leprae* and *M. marinum*. These antigens may initiate the induction of T-cell responses to these two species of mycobacteria.

## RESUMEN

Usando un ensayo de proliferación de células T murinas, se examinó la naturaleza de los antígenos compartidos entre el *Mycobacterium leprae* y otras especies microbacterianas. Se inmunizaron ratones con diferentes micobacterias y una semana después se prepararon cultivos de células de gánglios linfáticos los cuales se estimularon con el *M. leprae*. El ensayo reveló que varias cepas de las 13 especies micobacterianas usadas como antígeno comparten antígenos comunes con el *M. leprae*. Los ratones C57BL/10J y sus cepas congénicas exhiben diferencias en sus respuestas T hacia el *M. leprae*. Los ratones B10.M y B10.Q son respondedores altos, los C57BL/10J son respondedores bajos y los híbridos $F_1$ C57BL/10J × B10.M y C57BL/10J × B10.Q, son también respondedores bajos. Estas diferencias genéticas no se observaron con antígenos otras 6 especies microbacterianas en el ensayo con células T. Un hallazgo inesperado fue que el patrón genético de la respuesta celular T al *M. marinum* fue idéntico al observado con *M. leprae* en las cepas de ratón usadas. Las células T "cooperadoras" pueden reconocer determinantes antigénicos compartidos por el *M. leprae* y el *M. marinum*. Estos antígenos pueden iniciar la inducción de respuestas dependientes de T contra estas dos especies micobacterianas.

## RÉSUMÉ

La nature des antigènes communs à *Mycobacterium leprae* et à d'autres espèces de mycobactéries, a été étudiée par une épreuve de prolifération des lymphocytes T de la souris. Des souris ont été immunisées à différentes mycobactéries, et des cultures de ganglions lymphatiques ont été préparées une semaine plus tard et mises au contact de l'antigène de *M. leprae*. Les 13 espèces de mycobactéries utilisées comme antigènes dans cette étude partageaient des antigènes communs avec *M. leprae*, ainsi qu'on a pu le mettre en évidence par les réponses des lymphocytes T. Des souris C57BL/10J de même que des souches apparentées, ont montré des différences dans la capacité de réponse des cellules lymphatiques à *M. leprae*. Les souris B10.M et B10.Q ont présenté des réponses prononcées, alors que la réponse était faible chez les souris C57BL/10J. La réponse était également faible chez les hybrides descendant de croisements entre C57BL/10J et B10.M, et C57BL/10J et B10.Q. Ces différences génétiques n'ont pas été observées avec six autres espèces mycobactériennes utilisées comme source de cellules lymphatiques antigéniques. On a également été surpris de constater que le profil génétique de la capacité de réponse des lymphocytes-T à l'égard de *M. marinum* était, avec ces souches de souris, identique à celui observé pour *M. leprae*. Les lymphocytes-T adjuvants peuvent reconnaître des déterminants antigéniques communs à *M. leprae* et à *M. marinum*. Ces antigènes peuvent déclencher une réponse des lymphocytes-T à ces deux espèces de mycobactéries.

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