INFECTION AND IMMUNITY, Mar. 1995, p. 1047–1054 Vol. 63, No. 3
0019-9567/95/$04.00+0

Infection of SCID Mice with *Mycobacterium leprae* and Control with Antigen-Activated "Immune" Human Peripheral Blood Mononuclear Cells

PAUL J. CONVERSE,[1]* VICKY L. HAINES,[2] ASSEFA WONDIMU,[1]† LINDEN E. CRAIG,[1] AND WAYNE M. MEYERS[3]

Department of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health,[1] and Division of Comparative Medicine, Johns Hopkins University School of Medicine,[2] Baltimore, Maryland 21205, and Armed Forces Institute of Pathology, Washington, D.C. 20306[3]

Received 1 August 1994/Returned for modification 23 August 1994/Accepted 7 December 1994

The SCID (severe combined immunodeficient) mouse lacks both B and T cells and tolerates injected mononuclear cells from humans, the principal hosts of *Mycobacterium leprae*. A SCID mouse model of leprosy could be useful to investigate potential vaccine strategies using human cells in a context in which the growth of the organism is monitored. Initial experiments determined that SCID mice are more susceptible than normal mice to infection and dissemination of *M. leprae*. Cells from humans, either BCG vaccinated or from countries where leprosy is endemic, were stimulated in vitro with a number of mycobacterial antigens—whole *M. leprae*, *M. leprae* cell walls, purified protein derivative of *M. tuberculosis*, and *Mycobacterium bovis* BCG—and tested for proliferation and production of interleukin-6, tumor necrosis factor alpha, and gamma interferon. Cell walls were the most efficient and consistent in inducing all of these activities. In vitro-activated human cells retain function better after injection into SCID mice than nonactivated cells. To test the ability of cells to affect the growth of *M. leprae* in the footpads of SCID mice, cells from a known responder to mycobacterial antigens and from a nonresponder were activated by *M. leprae* cell wall antigens. The cells were harvested and coinjected with fresh *M. leprae* into the right hind footpads of SCID mice. After 3 months, there was no growth of *M. leprae* in the footpads of mice coinjected with cells from the mycobacterial antigen responder, while growth was uninhibited in mice receiving cells from the nonresponder. Future experiments will determine requirements for antigen specificity in inhibiting *M. leprae* multiplication.

The severe combined immunodeficient (SCID) mouse lacks a gene, probably that encoding recombinase, necessary for the generation of T and B cells that can mount an effective cellular and humoral response to foreign antigens. This defect is lethal within 4 months for the mouse unless it is protected by physical barriers, i.e., under axenic conditions, by chemoprophylaxis against opportunistic pathogens such as *Pneumocystis carinii* with trimethoprim-sulfamethoxazole, or by biological rescue with transplanted immunological tissue from a syngeneic normal BALB/c mouse. With these procedures, mice can survive for 18 months or more. It is now known that these mice can also be protected by injection of human fetal tissue or even with peripheral blood cells obtained from adult humans (22, 26). The transplanted immune system in these mice protects the animals from opportunistic infections and produces human immunoglobulin and, presumably, cytokines.

The observation that human tissue can survive and function in SCID mice, designated SCID-hu after xenogeneic transplantation, has opened the possibility of using such mice to study human diseases where immunological dysfunction is a distinctive part of the disease process. Studies of SCID-hu mice in the context of human immunodeficiency virus infection and AIDS have already been undertaken, and the impact of the principal compound used in treatment, azidothymidine, has undergone an initial assessment (23). Other investigators (34) have uncovered mechanisms of the immunopathogenesis of Lyme disease caused by *Borrelia burgdorferi*. Investigations regarding susceptibility as well as intraspecies adoptive transfer protection experiments have been undertaken in this animal model with *Mycobacterium leprae* (5, 17, 18, 41).

Susceptibility of humans to leprosy is not universal. Certain populations or even individuals within families appear to be more susceptible than other communities or family members. The basis for this susceptibility is poorly understood. Linkage with the major histocompatibility complex has been shown on the basis of family studies (12). Numerous studies (reviewed in reference 8) indicate that resistance to multibacillary leprosy correlates with cell-mediated immunity as measured by the lepromin test in vivo or the lymphoproliferative responses and gamma interferon (IFN-γ) production in vitro (reviewed in reference 8) (9, 29). In addition to an absence of function by lepromatous patient mononuclear cells in the presence of *M. leprae* antigen, *M. leprae*-driven suppression and the elaboration of suppressor factors have been reported (24, 30, 33). Cytokine production measurements instead of lymphoproliferation alone may relate these diverse findings. For example, interleukin-6 (IL-6) induces polyclonal B-cell activation and the production of immunoglobulin, a phenomenon also observed in lepromatous leprosy. Distinctive cytokine production patterns even in unexposed individuals (2) may provide useful criteria in choosing whose cells are appropriate for injection into SCID mice.

Our strategy was to determine which *M. leprae* antigens were most efficient at promoting putative protective immune responses. We evaluated lymphoproliferative responses and cy-

* Corresponding author. Mailing address: Department of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205. Phone: (410) 955-6845. Fax: (410) 955-0105.

† Present address: Armauer Hansen Research Institute, Addis Ababa, Ethiopia.

tokine production in two broad classes of individuals. We measured responses in subjects with possible exposure to *M. leprae* antigens due to prolonged residence in a country where leprosy was endemic and/or to the cross-reacting antigens of *Mycobacterium bovis* BCG. At the same time, we carried out these tests on subjects with no history of BCG vaccination and, in all likelihood, no exposure to leprosy patients. We wanted to use this information to examine the possibility that in vitro-activated human cells could affect the multiplication of *M. leprae* in the SCID mouse. Therefore, we also determined that such mice were indeed susceptible to infection with this organism and investigated the ability of a cytokine, transforming growth factor β, (TGF-β), known to abrogate natural killer (NK) cell activity (38), which is, in turn, the principal known defense mechanism of SCID mice, to modify its susceptibility. After evaluating lymphoproliferation and cytokine production in the presence of different mycobacterial antigens, two individuals were selected as donors of cells to be activated by *M. leprae* cell walls and injected into SCID mice in the presence of viable nude mouse-derived *M. leprae*.

MATERIALS AND METHODS

Mice. SCID mice were obtained from the breeding colony at Johns Hopkins University for most of the experiments or from Taconic Farms (Germantown, N.Y.). BALB/c mice or those of the congenic strain, C.B-17, were obtained from the same sources. Mice were housed in microisolator cages and offered autoclaved rodent chow and water without antibiotics ad libitum. The husbandry of the mice was carried out in a facility conforming to the standards of the American Association for Accreditation of Laboratory Animal Care.

Lymphoproliferation and cytokine production. Peripheral blood mononuclear cells were obtained from donors at Johns Hopkins University School of Hygiene. The likelihood of exposure to the leprosy bacillus or to BCG vaccine was noted for each donor, and informed consent was obtained. Donor mononuclear cells (1.5×10^5 per well) isolated by Ficoll density centrifugation (9) were incubated in flat-bottom microtiter plates with an optimal concentration of antigen or mitogen. After overnight incubation and after 66 to 72 hours of incubation, supernatants from lymphocyte cultures were harvested by well-standardized procedures in our lab and stored frozen at −70°C until they could be tested in an enzyme-linked immunosorbent assay (ELISA) or in a bioassay for cytokines. After 6 days, cells were pulsed for 18 h with [^{3}H]thymidine to test for lymphoproliferation. Cells were harvested with a Packard Filtermate 196 cell harvester and counted with a Matrix 96 counter.

Antigens and mitogens. *M. leprae*, cell walls of *M. leprae*, soluble *M. leprae* antigens and lipoarabinomannan (all kindly provided by Patrick Brennan, Fort Collins), *M. bovis* BCG, and purified protein derivative of *Mycobacterium tuberculosis* (PPD; obtained from Statens Serum Institute, Copenhagen, Denmark) were used for mononuclear cell stimulation. Nonspecific and mitogen controls for maximal cytokine production included monoclonal anti-CD3 (filtered supernatant of cell line; generously furnished by the laboratory of Hans Wigzell, Karolinska Institute, Stockholm, Sweden) and phytohemagglutinin (GIBCO, Gaithersburg, Md.).

M. leprae. *M. leprae* was obtained from one of the following sources in these experiments. Armadillo-derived organisms were supplied courtesy of Eleanor Storrs (Florida Institute of Technology, Melbourne), Patrick Brennan (Colorado State Univ., Fort Collins), and the Armed Forces Institute of Pathology. Nude mouse-derived bacilli were kindly provided by Scott Franzblau (Hansen's Disease Center, Baton Rouge, La.) for the co-inoculation experiment with activated human mononuclear cells.

Cytokine detection. ELISAs for IFN-γ were performed with minor modifications by the technique of Andersson et al. (3) with monoclonal antibody pairs (Chromogenix, Mölndal, Sweden), 1-D1K for coating and biotinylated 7-B6-1 for detection. The international standard Gg-23-901-530 provided by Craig Reynolds of the NIAID Biological Resources Branch was used in all of the assays. IL-6 determinations were made with a commercially available ELISA (Biosource, Camarillo, Calif.) or by the B9 bioassay (1). Tumor necrosis factor activity was measured by serially diluting supernatants in a WEHI cytotoxicity assay, with cell viability being determined by the ability to transform MTT (Sigma, St. Louis, Mo.) to formazan (27).

Histopathology. Necropsies were performed on each of the 69 SCID, 30 C.B-17, and 8 BALB/c mice, and sections of lung, liver, spleen, and footpad were fixed in 10% buffered formalin. Rear limbs and heads were also fixed and decalcified. The tissues were then embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin and by the Fite-Faraco method for detection of acid-fast bacilli (AFB). The footpad sections and rear limbs were then examined for histopathologic changes and AFB. The remaining tissue sections were examined for dissemination of bacilli as were the ear pinna, testicles, and nasal turbinates in mice positive for AFB on initial screening. Tissue sections positive for AFB were graded on a semiquantitative scale: 0+, no AFB observed; 1+, <10 AFB per high-powered field; 2+, 10 to 100 AFB per high-powered field, 3+, >100 AFB per high-powered field; 4+, too numerous to count.

Enumeration of AFB. Each infected footpad was split in half. One half was kept for histological evaluation as described above. The other half was minced and homogenized before placing a known volume of supernatant on a 7-mm-diameter glass slide (Cel-Line Associates, Newfield, N.J.) for Ziehl-Nielsen staining and counting of organisms (37). All readers were blinded for the evaluation of histopathologic changes and enumeration of AFB as well as to the treatment the mice had received.

RESULTS

In vitro responses of human subjects to mycobacterial antigens. Subjects were assigned to categories of exposed and nonexposed. Exposure included a history of BCG vaccination and/or growing up in a country where leprosy is endemic in most parts of it. Nonexposed individuals had no history of BCG vaccination and no long-term residence in an environment where leprosy is endemic. Four of the mycobacterial antigens tested—*M. leprae*, *M. leprae* cell walls, BCG, and PPD—were found to be capable of inducing lymphoproliferation and cytokine production in exposed individuals. Lipoarabinomannan was capable of inducing tumor necrosis factor alpha (TNF-α) and IL-6 production but not lymphoproliferation or IFN-γ in both exposed and nonexposed individuals (data not shown). Soluble *M. leprae* failed to induce any responses in our hands. Figure 1 displays the results for the antigens capable of inducing all four responses in exposed individuals and the results measured in both the exposed and the nonexposed groups. Table 1 indicates the numbers of individuals tested in the different categories and the statistical significance of differences between the two groups. Responses were consistently higher in the exposed than in the nonexposed individuals, although one nonexposed individual responded vigorously to BCG and PPD but not well to whole *M. leprae*. This individual may have been exposed to tuberculosis, but this could not be evaluated. Both *M. leprae* cell walls and BCG induced some degree of proliferation and cytokine production in most of the exposed individuals and even in some of the nonexposed ones.

Tests for significance were carried out by the Mann-Whitney U method. Significant differences in proliferative responses between the two groups were found for PPD and *M. leprae* ($P < 0.01$) and BCG ($P < 0.05$) but only a trend towards a significant difference for *M. leprae* cell walls ($P = 0.07$). A significant difference in IFN-γ production was found for PPD ($P < 0.02$) and a marginally significant one for BCG ($P = 0.05$). In response to *M. leprae*, 5 of 10 exposed individuals and 1 of 4 nonexposed individuals produced detectable IFN-γ. Two of the nonproducing exposed individuals were BCG vaccinated but were from countries where leprosy is not endemic; one of the two had a vigorous proliferative response to *M. leprae* (stimulation index = 250), while the other had a marginal proliferative response (stimulation index = 4). BCG vaccination alone does not appear to necessarily induce a full range of responses to *M. leprae*. No statistically significant differences were found between the two groups in the production of either TNF-α or IL-6, although four of seven exposed individuals and no nonexposed individuals made detectable levels of IL-6 (ranging from 873 to 4,598 pg/ml of supernatant) in response to *M. leprae*. TNF production was induced by all the stimuli in both groups. There appeared to be a good correlation with exposure status and IFN-γ production in response to either whole *M. leprae* or PPD. Overall, *M. leprae* cell walls appeared to be a more potent stimulus than whole *M. leprae*. In the exposed group, significantly greater production of IFN-γ and

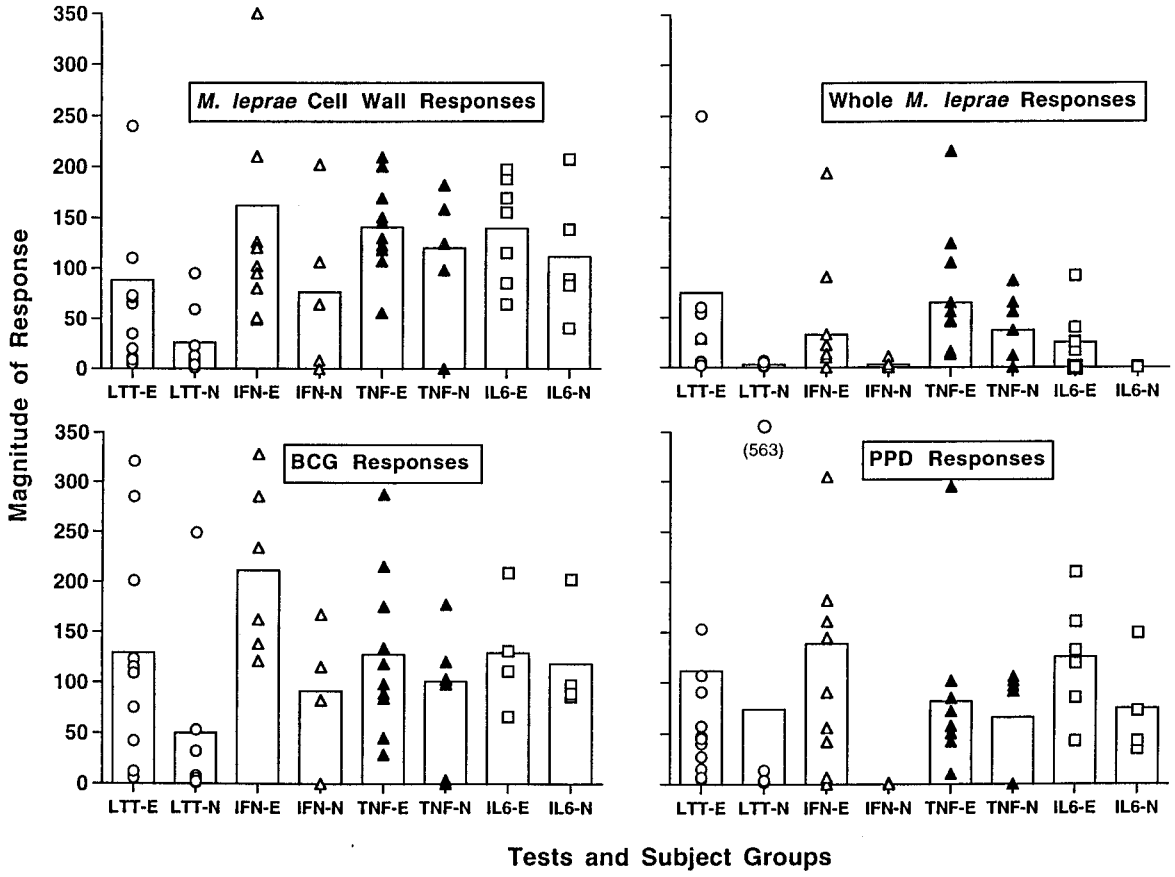

FIG. 1. Responses to four mycobacterial antigens (*M. leprae*, cell walls of *M. leprae*, BCG, and PPD of *M. tuberculosis*) in healthy subjects from countries where leprosy is endemic and/or where BCG vaccination is practiced (-E) and countries where leprosy is not endemic and where BCG vaccination is not practiced (-N). Responses measured were lymphoproliferation (LTT; stimulation index, i.e., counts of [^{3}H]thymidine in response to antigen divided by counts of [^{3}H]thymidine in response to medium alone) (○), IFN-γ production [(units per milliliter) divided by 2] (△), TNF production [(picograms per milliliter) divided by 200] (▲), and IL-6 production [(picograms per milliliter) divided by 50] (□). Each bar indicates the mean response of the group. Points, some of which are superimposed, represent the responses of the individual subjects. Note that real magnitudes vary by the constants indicated above.

IL-6 ($P < 0.01$) and TNF-α ($P < 0.025$) was observed in response to the cell wall preparation compared with that to whole *M. leprae*. Proliferative responses also appeared stronger, but the difference was not statistically significant ($P = 0.055$). These observations prompted us to choose the cell wall preparation as the activator for human cells that would be transferred to the mouse footpads.

TABLE 1. Numbers and exposure status of individuals tested for immune responses to mycobacterial antigens[a]

Response measured	No. of individuals with response to:							
	M. leprae		Cell walls		BCG		PPD	
	E[b]	N[b]	E	N	E	N	E	N
LTT	13	8[c]	14	8[d]	10	7[e]	14	8[c]
IFN-γ	10	4	10	5	6	4[e]	10	4[f]
TNF-α	10	6	10	6	10	6	10	6
IL-6	4	4	7	5	4	4	7	4

[a] Responses measured included lymphoproliferation (LTT), IFN-γ production, TNF-α production, and IL-6 production. Antigens tested included *M. leprae*, cell walls of *M. leprae*, BCG, and PPD.

[b] E, exposed individuals, i.e., those from a region where leprosy is endemic or where BCG vaccination is practiced; N, nonexposed individuals, i.e., those from a country or regions of a country where leprosy is not endemic and where BCG vaccination is not practiced.

[c] Compared with values for exposed individuals, $P < 0.01$.

[d] Compared with values for exposed individuals, $P > 0.05 < 0.1$.

[e] Compared with values for exposed individuals, $P \leq 0.05$.

[f] Compared with values for exposed individuals, $P < 0.025$.

Susceptibility of SCID mice to infection with *M. leprae*. Our initial experiments (not shown) failed to provide evidence on the susceptibility of either SCID or normal mice to *M. leprae* infection since the inoculum had been frozen and thawed. In all subsequent experiments, organisms freshly harvested from armadillos or nude mice were brought to the lab on ice at 4°C and injected within 1 week. Figure 2 shows the results of an experiment with armadillo-derived organisms inoculated at three different concentrations into the SCID mouse footpads. Multiplication of organisms was observed in homogenates of footpads in mice infected with 5×10^3, 5×10^4, and 5×10^5 *M. leprae* organisms per footpad. There is a suggestion of a plateau in growth after the first 3 months in mice that had received the smaller inocula, although the growth did continue. However, in this small pilot experiment, individual variation in the mice may have been a factor.

Histologically, the SCID mice of the 9-month harvest group had the largest numbers of AFB (4+) and the most significant lesions (Fig. 3). Infection starts in the dermis of the footpad, does not involve the epidermis, but does disseminate into the subcutaneous tissue and beyond over time. Perineural and perivascular histiocytic and epithelioid cell infiltration and rather well organized granulomas were the most prevalent

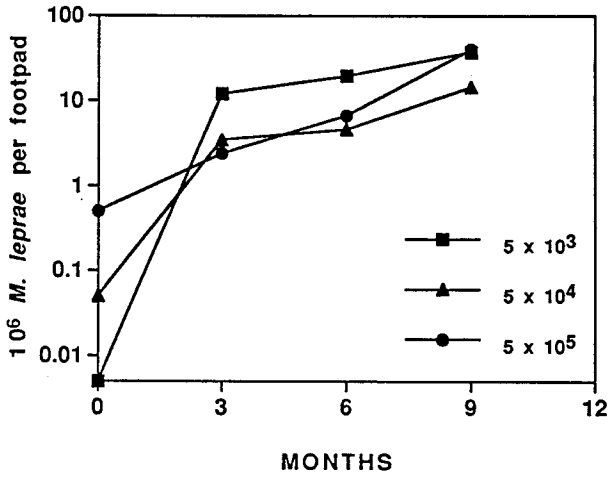

FIG. 2. Growth of *M. leprae* in SCID mice inoculated with different numbers (5×10^3, 5×10^4, or 5×10^5) of bacilli. Each point represents a single mouse.

lesions noted. One mouse demonstrated dissemination to the submucosa of the nasal turbinates (Fig. 3C) and submandibular lymph node. Another mouse had organisms that had disseminated to the spleen (Fig. 3D). Infiltration of neutrophils was noted in several of the lesions of mice sacrificed at 9 months. Two of the mice harvested at this time had extensions of histiocytic infiltrate to the joint surfaces (tarsometatarsal, in one case, and tibiotarsal, in the other). Dissemination to the bone marrow was noted in all of the mice sacrificed at 9 months, and one of these mice was also found to have AFB in the popliteal lymph node of the contralateral leg.

In contrast, mice sacrificed at 3 months generally demonstrated lesser, more localized lesions, with young macrophages and histiocytes predominating, without epithelioid cell granuloma formation or with very early epithelioid cell granulomas. These lesions tended to focus around sweat glands and small vessels of the footpad and subcutis as compared with lesions of mice sacrificed at 6 or 9 months. The latter lesions developed later, which were concentrated around and extended upward along the plantar nerve, its branches, and associated vasculature.

Mice in the 6-month category generally had higher numbers of AFB in histologic sections than did the 3-month group. Their lesions reflected characteristics of both the 3- and 9-month harvest groups.

None of the histologic sections from any of the three time points revealed any invasion of the basal cell layer of the epidermis. In most cases, a distinct clear zone was noted between the inflammatory infiltrate and the basal layer (Fig. 3A and B). Many club forms of AFB, as described, for example, by Wise (40), were noted on Fite-Faraco-stained sections, particularly in the lesions of the 9-month group.

Attempts to abrogate innate resistance in the SCID mouse. Data (not shown) from the initial large experiment with frozen and thawed organisms suggested that BALB/c mice might be more susceptible than SCID mice to infection with *M. leprae*. Innate resistance to *Listeria* infection mediated by NK cell-derived IFN-γ activation of SCID macrophages has been demonstrated by Bancroft et al. (6). Local inoculation of cytokines in the footpad has also been shown to drastically alter responses in experimental leishmaniasis in a murine model (35). These lines of evidence and the observation that TGF-β, a structurally highly conserved cytokine between species, could abrogate NK cell function in mice (38) led us to attempt coinoculation of the cytokine with *M. leprae* in SCID and normal mice. Figure 4 presents the results of this experiment. SCID mice that received no TGF-β showed linear growth of the organisms, as measured at the time points of 3, 6, and 9 months. C.B-17 mice, congenic to the SCID mice but having functional immune systems, showed a growth curve typical of normal mice, with a peak at 6 months and a slight decline by 9 months. TGF-β treatment resulted in no enhancement of growth in the SCID mice. In fact, there appeared to be a reduction in the number of organisms at the later time points. A slight enhancement of growth was observed in the conventional mice. Therefore, TGF-β treatment under conditions that should have blocked NK cell function in the early phase of infection was not able to enhance growth of the organisms in SCID mice nor to any significant degree in immunologically intact mice.

Histological evaluation showed that lesions progressed over the course of the experiment, from mild infiltrates composed of young macrophages and histiocytes in the 3-month group to discrete granulomas in the 9-month SCID group. By semiquantitative analysis, SCID mice at 9 months consistently had a 4+ AFB score while the scores of the C.B-17 mice at this time point in the untreated group were never greater than 2+. With the exception of one mouse, fewer AFB were also seen in the C.B-17 mice treated with TGF-β at 6 months. Lesions in mice that had received TGF-β tended to be minimal, with lower numbers of AFB in both SCID and C.B-17 mice. The only TGF-β-treated C.B-17 mouse harvested at 9 months did have high numbers (4+) of bacilli. This animal was in a cage in which all other mice died early in the experiment from causes unrelated to the experimental protocol.

Transfer of in vitro-activated human cells plus *M. leprae* to SCID mice. On the basis of the experiments with various mycobacterial antigens and cellular responses in humans, *M. leprae* cell wall antigens were selected as activators of cells in vitro before transfer to mouse footpads with viable *M. leprae*. In vitro activation has been reported to enhance human lymphocyte function and possibly homing mechanisms in SCID mice (4, 14). Two donors whose cells had been tested on multiple occasions for lymphoproliferation and cytokine message expression and production were chosen. One had a history of BCG vaccination and had lived more than 25 years in a country where leprosy was endemic. The other donor had not had BCG vaccination but had lived for more than 4 years in a country where leprosy was endemic without developing any in vitro lymphocyte reactivity to any mycobacterial antigens tested. The first donor was designated immune, and the second was designated nonimmune. IFN-γ mRNA could be detected by reverse PCR in response to PPD in the immune donor but not in the nonimmune donor (data not shown). The only cytokine that could be detected in the nonimmune donor was for IL-10 mRNA, a molecule that inhibits the effects of IFN-γ and macrophage activation. After overnight incubation with cell wall antigen at a concentration of 5 μg/ml, mononuclear cells were harvested. Freshly obtained nude mouse-derived *M. leprae* organisms (7.5×10^3) were mixed with either immune or nonimmune activated cells (2×10^6) and injected in a volume of 30 μl into each SCID mouse footpad.

Tissues were harvested at 3 months after infection, and coded samples were counted by an independent observer. When the code was broken, the results (Fig. 5) indicated that in three of four mice that had received cells from the immune donor, no growth could be observed at this time point. Also, in three of four mice that had received cells from the nonimmune donor, growth of *M. leprae* (3.7×10^4 to 5.2×10^4 bacilli)

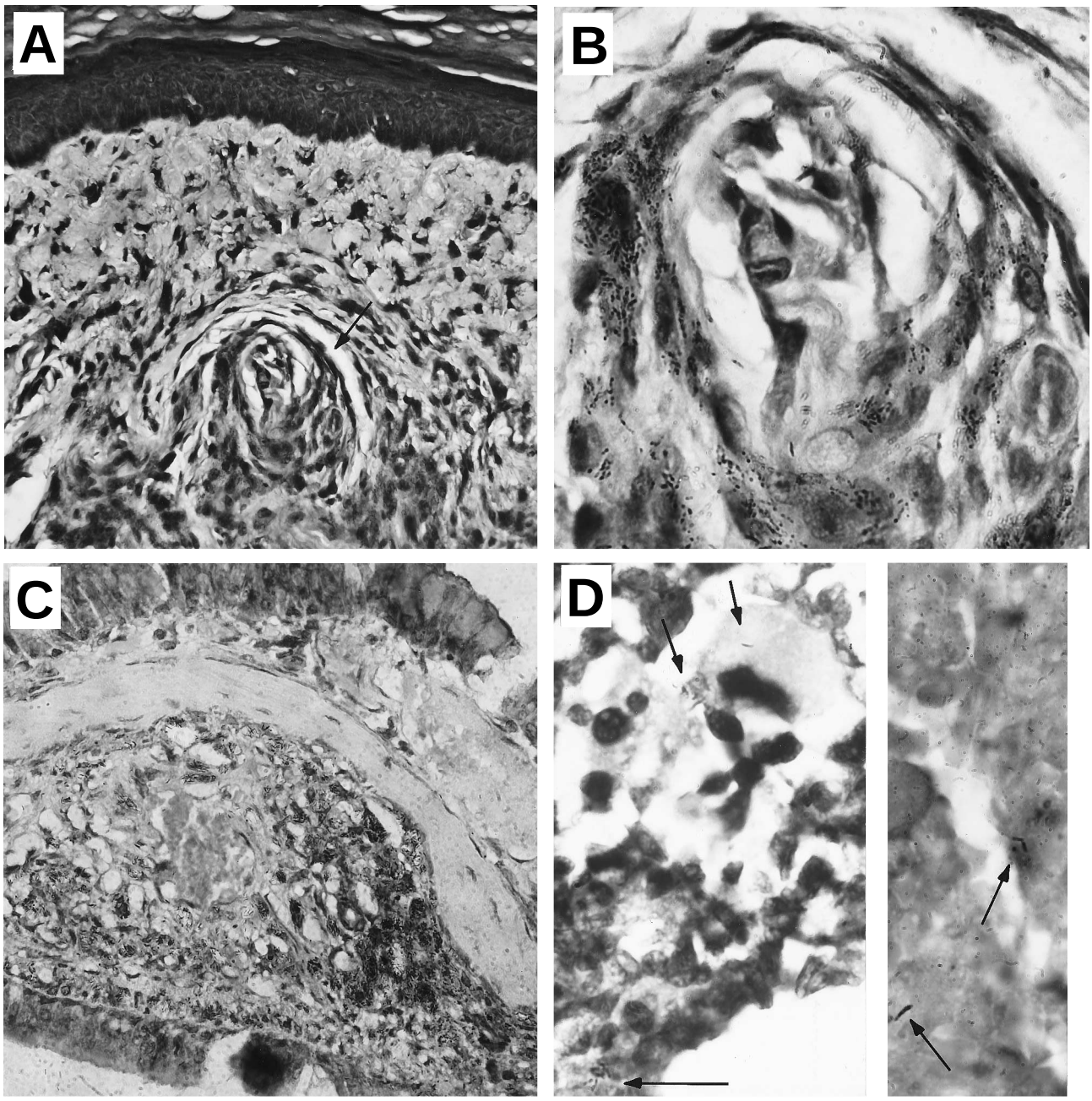

FIG. 3. Histopathologic changes in Fite-Faraco-stained sections of tissues from SCID mice 9 months postinfection with *M. leprae*. (A) Footpad showing subepidermal clear zone, histiocytic infiltrate in dermis, and perineural thickening (arrow). Magnification, ×250). (B) High-power magnification of dermal nerve shown in panel A. Note AFB within nerve and surrounding histiocytes. Magnification, ×1,000. (C) Dissemination of *M. leprae* in histiocytic infiltrate in submucosal area of nasal turbinate. Magnification, ×400. (D) Dissemination of *M. leprae* to lymphoid system. Left, moderate numbers of AFB (arrows) in cytoplasm of cells in bone marrow of tibia contralateral to injected footpad (magnification, ×1,000); right, scarce bacilli in spleen (magnification, ×1,560).

similar to that in SCID mice that had not received donor cells was seen. In one mouse from each group, intermediate growth to 2.6×10^4 bacilli was detected. The difference between the two groups was statistically significant ($P < 0.01$) by the Student *t* test.

DISCUSSION

Currently, there are a number of animal models for leprosy, and all have strengths and weaknesses (25). We have assessed the value of a new model, the SCID mouse, which has the advantages of being relatively inexpensive to obtain and maintain. In addition, it has the scientific virtue of potentially serving as a medium in which the activities of cells from human immune systems may be evaluated for their ability to control *M. leprae* growth.

M. leprae cannot yet be cultivated on artificial media in vitro and grows only to limited numbers in the footpad of BALB/c and other mouse strains (36). Larger numbers can be obtained in nude mice (21) and in armadillos (20), but both of these

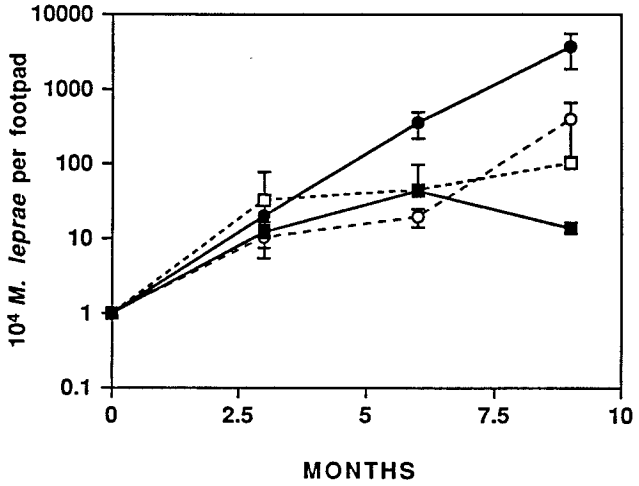

FIG. 4. Coinjection of *M. leprae* with TGF-β. Mice (groups of four) receiving TGF-β are indicated with open symbols and dashed lines. Solid symbols and lines represent mice injected with *M. leprae* in diluent. SCID mice are represented by circles, and immunologically intact C.B-17 mice are represented by squares. Standard deviations of mouse group means are indicated by the bars.

animals are difficult or expensive to maintain and breed and neither is ideal for the study of the pathogenesis of leprosy as it is known in humans. Further progress on the recent observations of *M. leprae* infections in monkeys susceptible to disseminated leprosy is complicated by the scarcity of the appropriate primate species (7) and relatively long gestation periods. SCID-hu mice offer the potential advantages of being easier to handle than monkeys, rapid breeders, and a model for studies of the human immune system both in normal function and in response to infectious agents.

Successful growth of *M. leprae* in SCID and SCID-hu mice may open a number of potential avenues of research. At present, any vaccine against *M. leprae* can be tested only in conventional mice in which there are only minimal and self-healing lesions. Vaccine trials in humans such as those currently under way in Venezuela, Malawi, and India (10, 31, 39) have been possible only after acquiring considerable baseline data on the population. While early observations are inconclusive, the ultimate efficacy of this vaccine will require years of follow-up in these populations. At the end of the trials, we will know whether the combination of BCG and *M. leprae* is effective. If it is not effective, it will be difficult to marshal the enthusiasm and resources for the trial of another essentially untested candidate vaccine. However, the organization and evaluation of the current vaccine trials could be reutilized if supported by trials of an improved, well-defined vaccine in a small animal species having an immune system essentially similar or identical to that of the normal host. Such vaccines could be tested in individual mice by use of immune system cells from a range of genetically distinct human sources analogous to what would exist in a mixed human population.

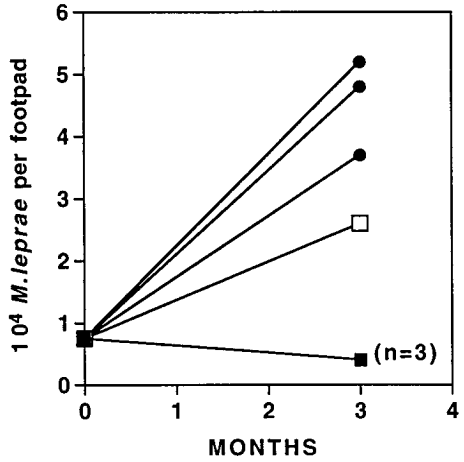

FIG. 5. Growth of *M. leprae* in SCID mice that were coinoculated with in vitro-activated mononuclear cells. *M. leprae* cell wall antigens (5 μg/ml) were incubated overnight with cells from either an individual responsive to mycobacterial antigens (■) or from an individual who was nonresponsive to mycobacterial antigens (●). Each mouse received 2×10^6 cells from either the immune donor (■) or from the nonimmune donor (●). One mouse from each group had intermediate growth detected; these two mice are represented by an open square.

The data from the BCG vaccine trials (for examples, see references 10 and 31) have all, with one exception, indicated an efficacy of ≥50% protection against the development of leprosy. In our experiments in vitro, we found very similar patterns of lymphoproliferation and cytokine secretion in BCG-vaccinated subjects from countries where leprosy is endemic and from those where it is not and therefore did not separate them for purposes of analysis (Fig. 1). It would appear that such vaccination imparts at least some reactivity to antigens of *M. leprae*.

Recently, the growth of *M. leprae* in normal mice and that in SCID mice were compared (41). Like the nude mouse, these immunodeficient mice have no barrier to the multiplication and dissemination of *M. leprae* bacilli and can be infected with either high or low numbers of bacteria. Our experiments confirmed the susceptibility of SCID mice to high numbers of organisms, as did findings by Guebre-Xabier et al. (17). Unlike the latter authors, we observed dissemination to tissues other than the footpad, although not to the extent found by Yogi et al. (41). In our hands, good growth was observed at the lower concentrations of bacilli and was detectable at 3 months. A concentration of 5,000 to 10,000 bacilli per footpad was chosen for subsequent experiments.

Although SCID mice are immunodeficient in terms of T- and B-cell function, they are not defenseless. This phenomenon has been best studied by Bancroft et al. (6) using a *Listeria* model. In those studies, it was confirmed that normal T-cell-bearing mice are capable of resolving the infection within a 2-week period. In SCID mice, there is no resolution, but the numbers of organisms reach a plateau that is never as high as the peak of infection in T-cell-replete mice. In addition, the plateau ($<10^6$ bacteria) is reached only at 2 weeks instead of at 4 days in normal mice. At 4 days, there are 2-logs-fewer *Listeria* organisms in the SCID mice. The SCID macrophages are activated Ia-positive cells. For their activation, NK cells producing IFN-γ are required. On the basis of these observations and unreported data from preliminary experiments, we sought to abrogate NK cell function to enhance growth of *M. leprae* in the SCID mice. Since our focus has also been on cytokine function, we selected TGF-β, which has the capacity to inhibit NK cell activity (38) as well as the virtue (unlike, e.g., IL-4) of being highly homologous and functional between species. On the basis of the experience of others (35) using the *Leishmania* model, we chose to concentrate the effects locally and temporally by injecting the molecule in the footpad together with the *M. leprae*. However, from the results, it would appear that TGF-β had little or no effect on the multiplication of bacilli under these conditions. In future experiments, we would suggest intraperitoneal injections before and after injecting *M. leprae* in the footpad together with TGF-β.

The significance of neutrophils in the SCID mouse lesions at 9 months is unknown. A hallmark of erythema nodosum leprosum in humans, however, is the neutrophilic infiltrate. Such an infiltrate was noted in several of the SCID mice in this series, suggesting that SCID mice may have the potential to develop erythema nodosum leprosum.

The major rationale for using SCID mice rather than nude mice in these studies was that SCID mice, if susceptible to *M. leprae* infection, would also permit the inoculation of human cells that might influence the course of infection. The results do pose a number of questions. First, what is the antigen-presenting cell in this system? If *M. leprae* organisms are taken up by the donor's monocytes and antigens of the bacilli are then presented to the donor's T cells, the results reported here are easily explained. In mice injected with cells from the immune donor, bacilli are largely eliminated, but bacilli survive and increase in the presence of cells from a donor without cellular immunity to mycobacterial antigens. However, the SCID mouse is not without defenses. Leakiness can occur but was not found, in spite of repeated attempts to find immunoglobulin, in any of the animals in the present study on the basis of a sandwich ELISA for murine immunoglobulin G, A, and M with a sensitivity of less than 50 μg/ml (data not shown). Both NK cells and macrophages are present. If the mouse macrophages take up the bacilli and the presence of foreign tissue activates the NK cells to produce IFN-γ, thereby activating macrophages of the mouse, bacilli would also be eliminated. However, given the absence of growth inhibition in the mice receiving cells from the nonimmune donor, one would have to postulate that somehow the cells of the immune donor were recognized as more foreign than the cells of the nonimmune donor and were better able to evoke a xenogeneic response. This explanation is not likely. It cannot be ruled out that both donor and mouse monocyte/macrophages took up bacilli and that components of both systems were involved in elimination of bacilli. Immune donor cells may have already been making IL-2. IL-2 of human origin is routinely assayed with a murine IL-2-dependent cell line. NK cells of the mice could have had an enhanced response (28) in the presence of immune donor cells secreting IL-2. The mouse NK cells then could have secreted IFN-γ and activated host macrophages, and the result would reflect a composite immune system (13).

On the basis of the findings shown in Fig. 1, the cells of both individuals were probably producing TNF-α in response to *M. leprae* cell walls. Inflammatory infiltrates were observed histologically in these mice. TNF-α is important in granuloma formation (19). However, granulomas at the times assayed were observed inconsistently and were poorly organized (data not shown). If macrophages at the site of injection were highly activated as a consequence of the injection of the cells of the immune donor, elimination may have been so rapid as to have aborted granuloma formation, as can happen in lesions of indeterminate leprosy cases that do not evolve toward a polar form of the disease. In fact, in the normal BALB/c mice at 9 months after infection, when *M. leprae* numbers were declining, the granulomas contained fewer lymphocytes and neutrophils than in the mice sacrificed at 6 months while the macrophages were showing fatty change. These changes could be consistent with signs of regressing granulomas (32). Only the mice receiving cells from the individual capable of making the mycobacterium-specific response showed an effective inhibition of the growth of *M. leprae*. These results suggest that TNF-α, probably produced in the two donors at levels comparable to one another, was less important than other responses in controlling bacillary multiplication in this system.

Likewise, IL-6 is unlikely to have played a role since cell walls of *M. leprae* induced production of this cytokine by all individuals (Fig. 1). Reported findings on the role of this cytokine in mycobacterial infections of macrophages are controversial (11, 16). Production of IFN-γ in response to cell walls tended to be higher in the BCG-vaccinated subjects from countries where leprosy was endemic than in nonvaccinated subjects from countries where leprosy was not endemic, but the difference was not statistically significant.

Future experiments using *M. leprae*-specific T-cell clones from donors of known HLA type are planned. Mice will be treated before and after injection with anti-asialo GM1 antisera to ablate NK cell function. Such abrogation has permitted the establishment of human T-cell leukemia type 1-positive cell lines (15) in SCID mice that otherwise reject those lines. In the *M. leprae* system, a possible role of SCID mouse defenses should be considerably reduced by anti-NK treatment.

ACKNOWLEDGMENTS

This work was supported by grants from the Damien-Dutton Society for Leprosy Aid, the Heiser Program for Research in Leprosy, the National Foundation for Infectious Diseases, and by a Faculty Development Award from Johns Hopkins University. V.L.H. was supported by NIH grants RR0130 and RR07002.

The contributions of Richard Markham, Patricia Wilcox, and Meng Wu to the completion of this study are gratefully acknowledged.

REFERENCES

1. **Aarden, L. A., E. R. De Groot, O. L. Schaap, and P. M. Lansdorp.** 1987. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. **17:**1411–1416.
2. **Akuffo, H. O., and S. F. Britton.** 1992. Contribution of non-Leishmania-specific immunity to resistance to *Leishmania* infection in humans. Clin. Exp. Immunol. **87:**58–64.
3. **Andersson, G., H.-P. T. Ekre, G. Alm, and P. Perlman.** 1989. Monoclonal antibody two-site ELISA for human IFN-γ. Adaptation for determinations in human serum or plasma. J. Immunol. Methods **125:**89–96.
4. **Armstrong, N., F. Cigel, W. Borcherding, R. Hong, and V. Malkovska.** 1992. In vitro preactivated human T cells engraft in SCID mice and migrate to murine lymphoid tissues. Clin. Exp. Immunol. **90:**476–482.
5. **Azouaou, N., R. H. Gelber, K. Abel, D. T. Sasaki, L. P. Murray, R. M. Locksley, and N. Mohagheghpour.** 1993. Reconstitution of *Mycobacterium leprae* immunity in severe combined immunodeficient mice using a T-cell line. Int. J. Lepr. **61:**398–405.
6. **Bancroft, G. J., R. D. Schreiber, and E. R. Unanue.** 1991. Natural immunity: a T-cell-independent pathway of macrophage activation, defined in the SCID mouse. Immunol. Rev. **124:**5–24.
7. **Baskin, G. B., B. J. Gormus, L. N. Martin, R. H. Wolf, J. L. Blanchard, R. Malaty, G. P. Walsh, W. M. Meyers, and C. H. Binford.** 1987. Experimental leprosy in African green monkeys (*Cercopithecus aethiops*): a model for polyneuritic leprosy. Am. J. Trop. Med. Hyg. **37:**385–391.
8. **Bloom, B. R., and T. Godal.** 1983. Selective primary health care: strategies for control of disease in the developing world. V. Leprosy. Rev. Infect. Dis. **5:**765–780.
9. **Converse, P. J., T. H. Ottenhoff, N. Gebre, J. P. Ehrenberg, and R. Kiessling.** 1988. Cellular, humoral, and gamma interferon responses to *Mycobacterium leprae* and BCG antigens in healthy individuals exposed to leprosy. Scand. J. Immunol. **27:**515–525.
10. **Convit, J., C. Z. Sampson, M. Zuniga, P. G. Smith, J. Plata, J. Silva, J. Molina, M. E. Pinardi, B. R. Bloom, and A. Salgado.** 1992. Immunoprophylactic trial with combined *Mycobacterium leprae*/BCG vaccine against leprosy: preliminary results. Lancet **339:**446–450.
11. **Denis, M., and E. O. Gregg.** 1991. Recombinant interleukin-6 increases the intracellular and extracellular growth of Mycobacterium avium. Can. J. Microbiol. **37:**479–483.
12. **De Vries, R. R. P., R. F. M. Lai A Fat, L. E. Nijenhuis, and J. J. van Rood.** 1976. HLA-linked genetic control of host response to *Mycobacterium leprae*. Lancet **ii:**1328–1330.
13. **Donjon, C. M., H. Morkowski, and R. K. Cheung.** 1993. Reversion of the SCID phenotype by human T-cell grafts—development of cross-species immunocompetence. J. Immunol. **151:**5948–5954.
14. **Duchosal, M. A., S. A. Eming, P. Fischer, D. Leturcq, C. F. Barbas III, P. J. McConahey, R. H. Caothien, G. B. Thornton, F. J. Dixon, and D. R. Burton.** 1992. Immunization of hu-PBL-SCID mice and the rescue of human monoclonal Fab fragments through combinatorial libraries. Nature (London) **355:** 258–262.
15. **Feuer, G., J. A. Zack, W. J. Harrington, Jr., R. Valderama, J. D. Rosenblatt,**

W. Wachsman, S. M. Baird, and I. S. Y. Chen. 1993. Establishment of human T-cell leukemia virus type I T-cell lymphomas in severe combined immunodeficient mice. Blood **82:**722–731.
16. **Flesch, I. E. A., and S. H. E. Kaufmann.** 1990. Stimulation of antibacterial macrophage activities by B-cell stimulatory factor 2 (interleukin-6). Infect. Immun. **58:**269–271.
17. **Guebre-Xabier, M., R. C. Howe, and D. Frommel.** 1992. Infection and elimination of *Mycobacterium leprae* in SCID C.B-17 mice. C. R. Acad. Sci. Ser. III Sci. Vie **314:**99–103.
18. **Ishaque, M.** 1992. Inoculation of foot pads of severe combined immunodeficient mice with *M. leprae*. Int. J. Lepr. **60:**661.
19. **Kindler, V., A. P. Sappino, G. E. Grau, P. F. Piguet, and P. Vassalli.** 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56:**731–740.
20. **Kircheimer, W. F., and E. E. Storrs.** 1971. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. Int. J. Lepr. **39:**693–702.
21. **Lancaster, R. D., G. R. F. Holson, A. C. McDougall, and M. J. Colston.** 1983. *Mycobacterium leprae* infection in nude mice: bacteriological and histological responses to primary infection and large inocula. Infect. Immun. **39:**865–872.
22. **McCune, J. M., R. Namikawa, H. Kaneshima, L. D. Shultz, M. Lieberman, and I. L. Weissman.** 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science **241:**1632–1639.
23. **McCune, J. M., R. Namikawa, C.-C. Shih, L. Rabin, and H. Kaneshima.** 1990. Suppression of HIV-infection in AZT-treated SCID-hu mice. Science **247:**564–566.
24. **Mehra, V., L. H. Mason, J. P. Fields, and B. R. Bloom.** 1979. Lepromin-induced suppressor cells in patients with leprosy. J. Immunol. **123:**1813–1817.
25. **Meyers, W. M., B. J. Gormus, and G. P. Walsh.** 1994. Experimental leprosy, p. 385–408. *In* R. C. Hastings (ed.), Leprosy. Churchill-Livingstone, London.
26. **Mosier, D. E., R. J. Gulizia, S. M. Baird, and D. B. Wilson.** 1988. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature (London) **335:**256–259.
27. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65:**55–63.
28. **Nandi, D., J. A. Gross, and J. P. Allison.** 1994. CD28-mediated costimulation is necessary for optimal proliferation of murine NK cells. J. Immunol. **152:**3361–3369.
29. **Ottenhoff, T. H., P. J. Converse, N. Gebre, A. Wondimu, J. P. Ehrenberg, and R. Kiessling.** 1989. T cell responses to fractionated *Mycobacterium leprae* antigens in leprosy. The lepromatous nonresponder defect can be overcome in vitro by stimulation with high molecular weight *M. leprae* components. Eur. J. Immunol. **19:**707–713.
30. **Ottenhoff, T. H. M., D. G. Elferink, P. R. Klatser, and R. R. P. de Vries.** 1986. Cloned suppressor T cells from a lepromatous leprosy patient suppress *Mycobacterium leprae* reactive helper T cells. Nature (London) **322:**462–464.
31. **Pönnighaus, J. M., P. E. Fine, J. A. Sterne, R. J. Wilson, E. Msosa, P. J. Gruer, P. A. Jenkins, S. B. Lucas, N. G. Liomba, and L. Bliss.** 1992. Efficacy of BCG vaccine against leprosy and tuberculosis in northern Malawi. Lancet **339:**636–639.
32. **Ridley, D. S.** 1990. Skin biopsy in leprosy, 3rd ed. Ciba-Geigy, Basel.
33. **Salgame, P., R. Modlin, and B. R. Bloom.** 1989. On the mechanism of human T cell suppression. Int. Immunol. **1:**121–129.
34. **Schaible, U. E., M. D. Kramer, K. Eichmann, M. Modolell, C. Museteanu, and M. M. Simon.** 1990. Monoclonal antibodies specific for the outer surface protein A (OspA) of *Borrelia burgdorferi* prevent Lyme borreliosis in severe combined immunodeficiency mice (SCID) mice. Proc. Natl. Acad. Sci. USA **87:**3768–3772.
35. **Scott, P.** 1991. IFN-γ modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. J. Immunol. **147:**3149–3155.
36. **Shepard, C. C.** 1960. The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. J. Exp. Med. **112:**445–454.
37. **Shepard, C. C., and D. H. McRae.** 1968. A method for counting acid-fast bacteria. Int. J. Lepr. **36:**78–82.
38. **Su, H. C., R. Ishikawa, and C. A. Biron.** 1993. Transforming growth factor-beta expression and natural killer cell responses during virus infection of normal, nude, and SCID mice. J. Immunol. **151:**4874–4890.
39. **Walia, R., K. G. Sarathchandra, R. M. Pandey, S. K. Parida, S. A. Zaheer, H. K. Kar, A. Mukherjee, R. Mukherjee, and G. P. Talwar.** 1993. Field trials on the use of *Mycobacterium w* vaccine in conjunction with multidrug therapy in leprosy patients for immunotherapeutic and immunoprophylactic purposes. Lepr. Rev. **64:**302–311.
40. **Wise, M. J.** 1963. Club-forms of *Mycobacterium leprae*. Lepr. Rev. **34:**68–72.
41. **Yogi, Y., K. Nakamura, T. Inoue, K. Kawatsu, Y. Kashiwabara, Y. Sakamoto, S. Izumi, M. Saito, K. Hioki, and T. Nomura.** 1991. Susceptibility of severe combined immunodeficient (SCID) mice to *Mycobacterium leprae*: multiplication of the bacillus and dissemination of the infection at early stage. Jpn. J. Lepr. **60:**139–145.