association was restricted to individuals without prior BCG scar. Leprosy risk was also negatively correlated with prior DTH to tuberculin in unvaccinated but not in vaccinated individuals. Age/sex/BCG scar-adjusted prevalence of DTH to the MLSA antigens was inversely related to prevalence of leprosy within different ecological areas. Naturally-acquired DTH to mycobacterial antigens may be a stronger correlate of protective immunity to leprosy than is DTH induced by BCG vaccination.

EP53

INTRAFAMILIAL TRANSMISSION OF LEPROSY IN VELLORE TOWN, INDIA

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Intrafamilial risks in leprosy reported mostly from rural areas are likely to be different in urban setting due to several socio-demographic and environmental factors. Urban sample surveys are expensive and frustrating due to problems of stability, cooperation and logistics. In this paper we describe a hospital based study done from 1968 to 1991 to determine risks and extent of intrafamilial transmission in relation to characteristics of index cases and contacts in urban areas. Families were examined annually by doctors. Person-years of followup were used for calculation of incidence rates.

Of the 120 index cases 44% were MB. 410 contacts were registered and followed up. 14 contacts developed leprosy of whom 12 were under 15 years of age. 83% were detected during the first 5 years. The incidence rate (IR) per 1000 was 5.1 with no gender bias. The IR was 7.3 and 2.8 among contacts of MB and PB leprosy ($P < 0.05$). Importance of active surveillance by hospital based survey is emphasized and may be designed to focus on persons below 15 years, with intensive followup for first 5 years. This model is feasible and can be integrated into general health service of any hospital.

EP54

DETECTION OF *MYCOBACTERIUM LEPRAE* NASAL CARRIAGE IN A LEPROSY ENDEMIC POPULATION.

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In order to better understand the role of *M.leprae* nasal carriage in the maintenance of infection reservoirs and transmission of leprosy, we applied a polymerase chain reaction (PCR) detecting a 531 bp fragment of the *pra*-gene of *M.leprae* on nasal swab specimens collected through a total population survey from individuals living in an area endemic for leprosy. False-positive reactions were controlled by the application of dUTP/UNG. False-negative reaction were monitored using a modified control. A total of 1228 nasal swabs specimens were analysed; 7.8% were found positive. No clear age-related pattern could be revealed. It was found that only 3.1% of the households was associated with 27% of all PCR-positive individuals. The results of this study further add to the already available evidence that infections occur readily throughout the endemic population. Assuming that the specific and sensitive detection of *M.leprae* DNA through PCR indeed reflects the presence of bacilli, this is to our knowledge the first time that *M.leprae* nasal carriage has been specifically detected at the population level.

EXPERIMENTAL

EX1

EARLY IMMUNOLOGICAL RESULTS OF EXPERIMENTAL M. LEPRAE CHALLENGE OF MONKEYS AFTER ATTEMPTED IMMUNIZATION WITH LIVE BCG OR BCG + HEAT-KILLED M. LEPRAE.

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Groups of 10 rhesus monkeys (RM) and 7 sooty mangabeys (SM) were immunized and boosted with either live BCG alone or BCG + low dose heat-killed M. lepraе (LD HKML) or BCG + high dose (HD) HKML. These plus an unvaccinated group were challenged with live ML and studied immunologically and clinically at intervals before and after vaccination.

Blastogenic responses of blood mononuclear cells (MNC) to lepromin (lep) and Rees soluble protein antigen (Ag) were initially baseline, but increased in BCG + HKML groups after vaccination. Lep skin tests of BCG + HKML groups of RM 2 months postvaccination were strongly positive in all 20 RM.

Changes were observed in the following blood MNC subsets by flow cytometry after monoclonal antibody (Ab) staining: CD4, CD8, CD4/4B4, CD4/2H4 and CD16.

Ab profiles to ML-specific phenolic glycolipid-I (PGL-I) Ag by ELISA showed elevated IgG and little IgM in groups receiving BCG + HKML compared to others. We previously reported that this pattern is present in leprosy-resistent monkeys.

These results together with histopathology suggest that BCG + HKML or BCG alone have a protective anti-leprosy effect. Long term follow-up is in progress to determine if this will result in protection against progressive, disseminated leprosy.

EX2

LEPROSY IN PHILIPPINE CYNOMOLGUS MONKEYS [MACACA FASICULARIS]

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Nonhuman primate models of leprosy provide valuable information on the pathogenesis of leprosy in humans.

We initiated leprosy studies in 23 Philippine cynomolgus monkeys to determine their susceptibility to the disease. The animals were infected with either human or mangabey-derived M. leprae.

Acid fast bacilli [AFB] have been detected in nasal smears of 4 of the animals 9 to 50 months postinoculation. One of the 4 animals died 14 months after inoculation from causes unrelated to leprosy and histopathologic evaluation confirmed lepromatous lesions in the nasal mucosa of the animal. In 2 of the 4 animals, an increase in anti-PGL-I antibodies [IgM] correlated well with the appearance of AFB in nasal secretions. No lesions are apparent at the cutaneous inoculation sites. The colonization of the nasal mucosa with M. leprae in the absence of other clinical manifestations implicate the nose as a primary site of infection in this species. Additional details of the experimental disease will be presented along with the results of an ongoing survey for naturally-acquired leprosy in wild-caught Philippine cynomolgus monkeys.

EX3

RECONSTITUTION OF *M. leprae* IMMUNITY IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE

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SCID mice have an autosomal recessive mutation that prevents the formation of functional B and T lymphocytes. We found that SCID mice infected with *M. leprae* developed a significantly ($P < 0.05$) more profound footpad infection than BALB/c mice. To test whether *M. leprae*-immune T cells can confer protection against infection with leprosy bacilli, groups of SCID mice were reconstituted 24 hours prior to *M. leprae* footpad infection both with a BALB/c-derived *M. leprae*-responsive T cell line, which produces gamma interferon upon stimulation with *M leprae*, as well as *M. leprae* non-immune T cells. The transfer of *M. leprae*-immune T cells resulted in a significant ($P < 0.03$) reduction in the number of *M. leprae* found in the footpads of infected SCID mice, and to levels, also, lower than that found in mice receiving *M. leprae* non-immune T cells ($P < 0.03$) and normal BALB/c mice ($P < 0.05$).

Flow cytometric analysis of spleen confirmed effective reconstitution with both CD_4+ and CD_8+ T cells. *In-vitro* lymphokine production and the proliferation of spleen cells from the reconstituted mice established that the donor cells had maintained their functional activity for the duration of the study (275 days). While spleen cells from non-reconstituted SCID mice upon stimulation with Con A failed to incorporate tritiated thymidine or produce detectable levels of cytokines, reconstituted SCID mice incorporated tritiated thymidine (stimulation index 9.6) and produced interferon gamma and IL-4 (41-72 ng and 600-800 pg per 10^6 cells, respectively).

These experiments demonstrate that *M. leprae*-immmune T cells home effectively, function, and control *M. leprae* infection in SCID mice.

EX4

ON THE POTENTIAL OF THE SCID MOUSE AS AN ANIMAL MODEL FOR LEPROSY

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The SCID (severe combined immunodeficient) mouse lacks both B and T cells and tolerates cells transferred from other species including man, the principal host of *M. leprae*. A series of experiments were carried out to determine if this animal is susceptible to infection with the leprosy bacillus. It was determined that SCID mice are susceptible to inocula of highly viable bacilli. Dissemination can be observed beyond the footpad. However, SCID mice may be able to resist inocula of less viable organisms better than conventional mice. Other investigators have shown that SCID mice have highly active natural killer (NK) lymphocytes producing amounts of interferon-γ capable of activating macrophages to destroy intracellular bacteria. Results will be presented on the effects of treatment to abrogate NK cell and macrophage function at the time of injection of *M. leprae* on the growth of the organism in both SCID and congenic normal mice possessing an intact immune system. Preliminary data should also be available on the effects of activated human cells on the growth of *M. leprae* in SCID mouse footpads.

EX5

MOUSE VACCINATION AGAINST LEPROSY WITH *M. leprae* SUBUNIT VACCINES ARE MORE EFFECTIVE THAN WITH WHOLE SONICATED *M. leprae*.

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Previously, we demonstrated that intradermal vaccination of mice with several progressively more purified and largely proteinaceous cell wall fractions of *M. leprae* diluted in Freund's incomplete adjuvant (FIA) conferred significant protection against footpad multiplication of live leprosy bacilli administered 1 month subsequently. It was noted in these studies that the most complex of the effective cell wall vaccines, the so-called cell wall insoluble fraction (CWIF), afforded protection when the amount of material utilized was as little as that derived from 10^5 *M. leprae*, while 10^7 or more killed *M. leprae* or furthert refined cell wall fractions derived from 10^7 or more *M. leprae* were required to provide protection.

In subsequent studies, we found that vaccination with a SDS-soluble fraction of CWIF, "soluble proteins", provides both unique and consistently (14 of 14 instances) prolonged mouse protection. While heat-killed *M. leprae* and progressively more refined cell wall fractions of *M. leprae* (CWP and PPC) generally protected when the interval between vaccination and challenge was 1-3 months, only soluble proteins protected when the interval between vaccination and challenge was extended to 6, 9, and 12 months.

Lastly, 10 density gradient subfractions of this material were eluted from a superose 12 column; certain of these subfractions in FIA were ineffective vaccines (fractions 11 and 12) while the others were effective (fraction 8, 9, 10, 13, 14, 15, 16, and 22), some at amounts of protein much lower than that in the whole killed *M. leprae* vaccine utilized herein (particularly fractions 8-10) and some significantly more protective (fractions 8, 13, 14, and 15). Analysis of SDS-PAGE of these soluble protein subfractions stained with $AgNO_3$ suggests that the likely critical protective *M. leprae* proteins therein are particularly the 10 kD protein and 1-3 kD proteins, and, to a lesser extent, the 65 kD protein.

EX6

BACILLARY PERSISTERS IN MURINE LEPROSY

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Studies on bacillary persisters were made in murine leprosy. Female mice were given a large inoculum of M. leproemurium in order to obtain a shorter survival time (SVT) of animals and to assure that an adequate number of bacillary persisters be included in the study. The SVT of normal animals was 535 days, and that of infected animals was 126 days. Dapsone was able to increase the SVT by 36 days, SM by 74 days, PZA by 126 days, INH by 154 days and kanamycin by 278 days. The average SVT of clofazimine (CLO)-treated animals was 519 days, approaching that of the normal mice. All animals revealed tremendous growth of murine leprosy throughout the visceral area at the time of their death except those treated with CLO, in which case there was no macroscopic growth at all. The last CLO-treated animal was sacrificed at 816 days and there were still a few organisms present in the pelvic fat. These organisms multiplied well in previously unused mice and CLO again showed

excellent suppressive activity (they were evidently not drug-resistant). Since nearly all inoculated organisms have been eliminated by CLO and drug-resistant organisms have never emerged under CLO treatment, it may be concluded that CLO is the first drug capable of killing the drug-sensitive organisms, preventing the emergence of drug-resistance and, more importantly, suppressing the growth of the persisters. In animals treated with a combination of INH and CLO for a period of 816 days the emergence of INH-resistant organisms was markedly delayed.

EX7

UV-B IRRADIATION OF MICE IMPAIRS THE PHAGOCYTIC ABILITY OF MACROPHAGES, DECREASES IMMUNITY TO MYCOBACTERIUM LEPRAEMURIUM, AND INCREASES DISEASE PATHOGENESIS.

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Ultraviolet (UV) radiation decreases immune responses to a variety of antigens introduced both locally and at distant, non-irradiated sites in experimental animals. In addition, exposure of humans to natural and artificial sources of UVB (280 - 320 nm) radiation can decrease immune function. These findings have raised concerns that increased environmental UV-B radiation, resulting from decreases in stratospheric ozone might affect the incidence or severity of infectious diseases. We are testing this hypothesis in a murine model of mycobacterial infection in which Mycobacterium lepraemurim (MLM) is injected into the hind footpad and disease progression is monitored by assessing the number of bacteria in the infected footpad and lymphoid organs. We demonstrated that exposure of BALB/c mice to a single dose of UV-B radiation, varying from 0.35 to 45 kJ/m^2, from FS-40 sunlamps suppressed the induction of a delayed type hypersensitivity (DTH) response to MLM in a dose-dependent manner. This was associated with an increase in the number of bacteria in the infected footpad and the lymphoid organs. Furthermore, UV-B radiation reduced the survival time of mice infected either in the footpad or intravenously with MLM. To determine whether the impaired clearance of bacteria seen after UV radiation was associated with altered macrophage function, we studied the uptake of MLM by macrophages collected from the peritoneal cavity of UV-B irradiated mice. Macrophages obtained from mice exposed to doses of UV-B radiation at or above $1.4kJ/m^2$ showed a significant reduction in their phagocytic ability when infected in vitro with MLM. These studies demonstrate that UVB radiation can alter the immune response to and increase the pathogenesis of a chronic mycobacterial infection in mice and suggest that impaired clearance of bacteria in vivo may result from an alteration in macrophage function.

EX8

MURINE STRAIN VARIATION IN M.LEPRAE INFECTED SCHWANN CELL FUNCTIONS AND THEIR MODULATION BY MACROPHAGES.

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Marophages (mφs) constitute the bulk of inflammatory cells in nerves of leprosy patients. Earlier studies indicate dysfunction in both ML infected human lepromatous as well as murine Swiss white (SW) mos. This dysfunction is important in processes of immunopathology, subsequent nerve damage and regeneration: this was assessed in Schwann cell tissue culture (DSC) in the 2 strains of mice (SW & C57Bl/6) that markedly differ in mφ response to ML infection. The parameters examined were a) Ability to support intracellular ML growth b) Expression of NGF & NgCAM c) Production of secretory proteins viz. fibronectin and collagen.

Constitutionally, DSC of the 2 strains responded differently to viable ML infection with respect to release of secretory proteins and NgCAM expression. However differences in mos of the 2 strains played no role in modulation of growth of ML or in their expression of NgCAM & NGF. Their role in modulating secretory proteins was at best temporary. Preliminary results of intraneural injections with supernatents of normal and patient mφs displayed diversity in the composition of intraneural granulomas that ensued.

EX9

THERAPEUTIC EFFICACY OF KRM-1648 IN COMBINATION WITH OTHER ANTIMICROBIALS AGAINST M.LEPRAE INFECTION INDUCED IN NUDE MICE

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A new benzoxazinorifamycin derivative, KRM-1648 (KANEKA Corporation), is known to have excellent in vitro and in vivo antimycobacterial activities, and is more potent than rifampicin (RMP). In this study, the therapeutic efficacy of KRM-1648, alone or in combination with DDS or clofazimine (CFZ), was evaluated against M.leprae infection induced in athymic nude mice. BALB/c nude mice infected sc with 1×10^6 of M.leprae Thai-53 strain were given test drugs by gavage, once daily six times per week, for up to 50 days from day 31 to day 80. The growth of organisms was observed in the hind footpad during the 12 months following infection, by counting the number of acid-fast bacilli in the tissue homogenate according to Shepard's method. In a dose-dependent manner, KRM-1648 markedly reduced the growth of leprosy bacilli at the site of infection (0.001~0.01 mg/mouse/day), and its therapeutic efficacy was greater than RMP. Furthermore, in vivo anti-M.leprae activity of KRM-1648 (0.001 mg/mouse) was enhanced when combined with other antimicrobial agents, such as DDS (0.2 mg/mouse) and CFZ (0.1 mg/mouse), as compared to the efficacy of either drug alone. From these findings, multi-drug regimens consisting of KRM-1648, instead of RMP, may be more efficacious for treatment of leprosy patients. Further studies on the therapeutic effect of KRM-1648 in combination with other antimicrobial drugs, such as clarithromycin, are now in progress.

EX10

COMBINATIONS OF RIFAMPICIN OR RIFABUTIN PLUS FLUOROQUINOLONES AGAINST MYCOBACTERIUM LEPRAE

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The treatment of leprosy worldwide is limited mainly to dapsone, clofazimine and rifampicin, either singly or in multiple drug therapy. Because of emergence of drug resistant M. leprae and toxicity of these drugs, there is an urgent need for new bactericidal drugs. The discovery of quinolones has given a new armamentarium in the fight against leprosy. The activities of several fluoroquinolones against several strains of armadillo-derived M. leprae were determined, both singly and in combination with rifampicin or rifabutin, in the in vitro system as well as in mouse foot pad system.

When incorporated singly into culture medium, ciprofloxacin, clinafloxacin, ofloxacin, sparfloxacin and temafloxacin were found to be most active against M. leprae, with MIC ranging from 0.75 to 1.5 μg/ml. In similar studies, it was determined that rifabutin was more active than rifampicin, both in the in vitro system and in mouse foot pad system. Excellent synergism was observed when either clinafloxacin, ofloxacin, sparfloxacin or temafloxacin was combined with rifabutin, but not with rifampicin. When ofloxacin was tested in mouse foot pad system, similar synergism was obtained with rifabutin, but not with rifampicin. Thus, it seems there are more effective candidates now available for incorporating into MDT regimens in leprosy.

EX11

STUDIES OF MACROPHAGE TRAFFIC INTO THE EXPERIMENTAL LEPROMATOUS LESION OF THE *MYCOBACTERIUM LEPRAE*-INFECTED NU/NU MOUSE FOOT PAD.

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In lepromatous leprosy (LL) the failure of the macrophage (MΦ) to cope with *M. leprae* (*ML*) is conspicuous; lepromatous granulomas consist of enormous numbers of MΦs packed with leprosy bacilli. They are not immortal cells but their half-life in the tissues is not known. Obviously there must be a continuous influx of new MΦs to support the growth of bacilli. In the athymic (nu/nu) mouse *ML* grows to enormous numbers producing a granuloma similar to that in human LL in man. In this experimental model for LL we have observed a high rate of turnover of *ML*-infected foot pad MΦs. Although newly arrived bone marrow-derived MΦ are heavily infected with *ML*, the fate of the initial granuloma MΦs and mechanisms by which they acquire their *ML* burden from the existing infected MΦs is not known. Our studies have explored the role of natural killer (NK) as well as normal and activated MΦs in the lysis of *ML*-infected MΦ target cells and the fate of the *ML*. Fresh NK cells and lymphokine (IL-2) activated killer cells (LAK) from popliteal lymph node (LN) draining the sites of foot pads inoculated with *ML* were cytotoxic for *ML*-infected target MΦs. NK and LAK activity was also detectable in LN from 14 month-old *ML*-infected nu/nu. The importance of dose and viability of *ML* in the target MΦs as well as the duration of their infection was examined. In an *in vitro* model for MΦ turnover, normal effector MΦs acquired the *ML* from target MΦs and continued to sustain their growth (oxidation of ^{14}C-palmitic acid to $^{14}CO_2$) but IFNγ-activated MΦs attacked infected target MΦs, acquired their load of bacilli and had a marked deleterious effect on these *ML*. These studies describe 2 mechanisms for maintenance of granulomas in LL that could underlie sustenance of *ML* viability in the absence of treatment. Chemotherapy and the eventual clearance of bacilli could depend heavily on acquisition of *ML* by a continuous turnover of new MΦs. Immunotherapy that activates MΦs could override the protection afforded to the bacilli by their normal host cell and lead to their subsequent destruction and clearance.

EX12

THE TRANSMISSION OF LEPROSY THROUGH THORN PRICKS IN NUDE MICE

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The mode and route of *Mycobacterium leprae* transmission is of continued interest. In our earlier studies we demonstrated the transmission of leprosy in nude mice through nasal mucosa, dekeratinized, unbroken skin, abraded skin and by subcutaneous and intravenous injection. Leprosy infection in feral nine-banded armadillos (Dasypus novemcinctus) is well documented. In an earlier study we observed thorns in the ears and nose of Louisiana armadillos infected with *M. leprae* in the wild. (Thorny bushes and briars are indigenous to the southern regions of the USA.) The study suggested that *M. leprae* may have entered the armadillo tissue by means of thorn pricks.

The present investigation was an attempt to simulate the transmission of leprosy through thorn pricks. The spines of the Bunny-Ear cactus were used to introduce *M. leprae* into the skin of nude mice. *M. leprae* (1×10^7) harvested from nude mouse footpad was placed on the dorsum of both hind feet of 10 anesthetized nude mice and allowed to partially air dry. A piece of cactus tissue was vapor sterilized with formaldehyde and the spiny portion of the cactus was immersed in suspension of *M. leprae* (1×10^9 AFB/ml). The plant tissue was then pressed against the mouse foot on the area containing *M. leprae*. The cactus spines detached from the plant and remained embedded in the skin. All of the mice developed swelling in the dorsum of the feet within six months. The infection progressed in a manner similar to that observed in nude mice experimentally infected with *M. leprae* by needle injections.

This study demonstrates that it is possible to transmit leprosy through contact with *M.leprae*-contaminated thorns. It is plausible that individuals living in endemic leprosy regions may encounter similar exposures to the leprosy bacillus, especially in those areas where going barefoot is common.

EX13

THE NEONATALLY THYMECTOMIZED LEWIS RAT AS A MODEL FOR THE ELIMINATION OF "PERSISTERS" BY CHEMOTHERAPY

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We have utilized the NTLR model to simulate chemotherapy of the "persister" state. In these studies we infected NTLR in the hind footpads with 5,000 *M. leprae*, and 1 year later, when the number of *M. leprae* per footpad was consistently $\geq 10^7$, these NTLR were treated with various regimens for 4 months; 2 or more months after discontinuing therapy, treated NTLR footpads were harvested so as to assess the presence of any surviving "persisters" by subpassage and assessment of viability in footpads both with small *M. leprae* inocula (5,000) in BALB/c mice and with larger inocula (generally 10^6 or more) in 2 NTLR. *M. leprae* from treated NTLR were judged viable if 1 year after subpassage either: (1) an increase of $\geq$ 5-fold *M. leprae* was found in any single NTLR supassage footpad, each footpad being harvested individually, or (2) the number of *M. leprae* per footpad in 4 foot mouse pools was found to be $\geq 10^5$. We found several regimens which do not regularly eliminate "persisters" (number of NTLR harboring persisters/number of NTLR treated) in this system: rifampin alone (7/11), 2 schedules of rifampin + dapsone (16/21), dapsone + ethionamide (5/11), minocycline alone (14/18), and rifampin + clofazimine (6/11). On the other hand, "persisters" were essentially entirely eliminated and the percentage of treated NTLR harboring "persisters" were statistically significantly less ($P < 0.02$) than with the previously described regimens when treatment consisted of: (1) rifampin + minocycline (0/13), (2) rifampin + ofloxacin (1/10), and (3) rifampin + ethionamide (0/14). This study of experimental chemotherapy suggests that these three combinations offer the most potential for effective short-course therapy of leprosy.

EX14

SURVEY OF MONOCLONAL ANTIBODIES AGAINST MYCOBACTERIUM LEPRAE FOR USE IN IMMUNOHISTOCHEMICAL AND IMMUNOULTRASTRUCTURAL LOCALIZATION STUDIES.

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Monoclonal antibodies (MAbs) against M. leprae components have become generally available for study through "libraries" maintained for the purpose. However, despite their specific characterization and use in a variety of immunologic studies, work on antigen localization by immunohistochemical methods or by immunoelectronmicroscopy has been sparse.

Antigenically characterized anti-M. leprae IgG MAbs were obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA, and from active Hansen's disease workers. Lymph nodes and lepromas were obtained from heavily infected armadillos. For immunohistochemical studies, frozen sections were reacted with a variety of MAbs in an avidin/biotin-peroxidase/diazobenzidine system. For immuno-electronmicroscopy, embedded sections were exposed to MAbs, then to a gold-labelled ligand. In the immunohistochemical study, the best specific staining was obtained with mouse MAbs IIC8 (65 Kd protein), mc8026 (18 Kd protein), mc6225 (30-40 Kd glycolipid), mc2924 (broad 30-40 Kd carbohydrate), and a human polyclonal anti-lepromin A serum. However, although some ultra-structural localization of specific antibodies was obtained with the human polyclonal serum, gold labelling with MAbs has generally been poor, possibly reflecting degradation of antigenic sites by EM-preparative methods.

EX15

CHARACTERIZATION OF THE SPECIFIC RECOGNITION SITES OF MONOCLONAL ANTIBODIES TO THE PHENOLIC GLYCOLIPID I OF M. LEPRAE

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By the study using synthetic sugar-constructs of phenolic glycolipid I (PGL I) of M. leprae, we have revealed that the haptenic specificity of PGL I was on the sugar part of PGL-1. To

study the roles of each sugar-constructs, various kinds of sugar analog were synthesized. They included the outer monosaccharide (NM-P), outer disaccharide (ND-P), trisaccharide (NT-P) inner monosaccharide (IM-P), inner disaccharide (ID-P) and the trisaccharides with different anomeric configurations. They were coupled to BSA, methylated BSA (MBSA) or KLH, giving synthetic antigens.

Recognition sites of the various kinds of the monoclonal antibodies (MAb's) produced by immunizing mice with these synthetic antigens were determined with the set of synthetic sugar-constructs by ELISA and microHA. MAb recognizing outer monosaccharide (mAb (1-24), mAb (1-25)), outer disaccharide (PG2B8F, ml 6A12, ml 8A2, ml 8B2), trisaccharide (SF-1), inner monosaccharide (DZ1, A8) were characterized. Among these MAb's SF-1 had extremely high specificity. Namely, SF-1 required complete structure of three sugar residues and complete anomeric configurations of three glycosidic linkages. The set of MAb's could be very useful for the development of the sensitive method of quatitation of PGL I, immunohistochemistry and so on.

EX16

DENATURED MUSCLE AUTOGRAFTS IN PERIPHERAL NERVE REPAIR IN A MODEL OF LEPROSY

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The technique of denatured muscle autografting was used to examine nerve regeneration in a model of leprosy nerve damage. Granulomas were induced in the tibial nerve of guinea pigs, by the intraneural injection of cobalt - irradiated *Mycobacterium leprae* organisms. Peak granuloma formation and nerve damage occurred at 5 weeks. At this time, the granuloma was excised and the nerve gap was repaired with a denatured muscle autograft. Nerve regeneration was followed over 20 weeks, by assessment of return of sensation in the footpad and muscle function in the foot. The conduction velocities of the fastest fibres in the tibial nerve were measured by electrophysiology, and quantitative morphometric assessment of myelinated fibres in the tibial nerve, distal to the graft was carried out, at 8, 12, 16 and 20 weeks after grafting. The results were compared with nerve regeneration after muscle grafting of a normal, non-granulomatous nerve.

Nerve regeneration occurred in the grafted granulomatous nerve, where there was fibrosis, at a slightly slower rate than in the grafted normal nerve.

EX17

SUBPLASMALEMMAL LINEAR DENSITIES IN MONONUCLEAR CELLS INDUCED BY AN ANTIGEN IN HUMAN SENSORY PERIPHERAL NERVE.

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Subplasmalemmal linear densities(SPLDs) consist of electron dense deposits lying immediately under the plasma membrane of mononuclear cells occurring in the chronic inflammatory lesions of sarcoidosis and multiple sclerosis. We describe the presence of these mononuclear cells with SPLDs in an animal model of nonlepromatous leprosy.

Rabbits and Strain 13 guinea pigs develop skin lesions similar to those of nonlepromatous leprosy when injected with human sensory peripheral nerve suspension, or a non-myelin fraction derived from human dorsal roots. SPLDs were found in mononuclear cells in the dermis of these skin lesions in 3 out of 4 rabbits, and in 3 out of 4 Strain 13 guinea pigs. SPLDs were also found in mononuclear cells at skin test sites in 6 out of 10 rabbits displaying granulomatous hypersensitivity and were readily seen when a deoxycholate extracted fraction from sural nerve in doses of 1µg was used as skin test antigen.

Although mononuclear cells with SPLDs have not been reported in human leprosy, 'plasma like' cells surrounded by basal lamina have recently been described in sural nerves and may be similar to the plasmacytoid cells occurring in sarcoidosis, which have SPLDs and which are now considered to be precursors of epitheliod cells.

EX18

OBSERVATION ON PHAGOCYTOSIS TO M.LEPRAE BY CULTURED HUMAN SCHWANN CELLS

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Schwann cells from 25 cases(fetus 17, adult 8) of human peripheral nerves have been successfully cultured by means of tissue plantation merhod. Among these the Schwann cells of 3 fetus could be subcultured up to 10th generation covering more than 100 days. It was proved that the growing cells were Schwann cells through study by immunohistochemical reaction against S-100 protein and lysozymes, and also by electron microscopy which showed many microvilli on the surface along with plenty of lysosome and Golgi's complex in the cytoplasm. Even when fragments of peripheral nerve tissue or the cell suspension were stored in liquid nitrogen for serveral months, the nerve tissue or the cells were still able to survive and proliferate after rapid thawing.

The Schwann cells and M. Leprae were co-cultured by coverslip method, and the coverslips were stained(acid-fast stain) and the phagocytosis of M. Leprae by Shwann cells was observed under light microscopy in regular intervals. About 15% of Schwann cells phagocytosed M. Leprae 10 hours after infection. Later on the number of the cells phagocytosed M. Leprae steadily increased and reached the peak (95%) of phagocytic index 72 hours after co-culture. M. Leprae globi could asto be found in Schwann cells. By electron microscopy many M. Leprae could be observed among the microvilli as well as in the cytoplasm of the infected Schwann cells. Ninety six hours after infection the Schwann cells which phagoctosed many M. Leprae underwent degeneration and necrosis, but the M. Leprae in the cells still existed with their morphology unchanged.

EX19

EARLY CLINICAL AND PATHOLOGICAL RESULTS OF M. LEPRAE CHALLENGE OF MONKEYS AFTER ATTEMPTED PROTECTIVE IMMUNIZATION WITH LIVE BCG OR BCG + HEAT KILLED M. LEPRAE.

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Seventeen monkeys (10 rhesus and 7 sooty mangabeys) per group were challenged with live Mycobacterium leprae (ML) after vaccination and boosting with either live BCG or BCG + low dose (LD) heat-killed ML or BCG + high dose (HD) HKML. An additional 17 unvaccinated monkeys were also challenged. Biopsies of leprosy lesions were removed at intervals longitudinally and were studied histopathologically and clinical results were recorded concurrently.

The following histopathological criteria were observed in the biopsies: numbers of epithelioid cells, multinucleated giant cells and lymphocytes; numbers and viability (morphologic index) of acid-fast bacilli (AFB); necrosis and average lesion size at inoculation sites.

Some differences were noted between the 2 monkey species in the types and/or proportions of cells in the infiltrates. In both species, however, there were decreasing numbers and viability of AFB in unvaccinated > BCG only > BCG + LD HKML > BCG + HD

HKML groups. These observations together with clinical data and immunologic studies strongly indicate that anti-ML immunization has been achieved. Long-term observations are needed to verify these conclusions and to determine whether protective immunization against progressive clinical leprosy has occurred.

EX20

DETECTION OF Ig A ANTI-PGL-I IN MANGABEY MONKEY INOCULATED WITH MYCOBACTERIUM LEPRAE

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3- Neurology Institut,Wien Univ.-Austria.

M. leprae affects differents parts of the body but specially the peripheral nerve, skin and mucosae, where bacilli are found in large amount in lepromatous patients By infection with microorganisms through skin and mucosae predominantely Ig A class antibody is ilicited. however, the IgA antibody developement in leprosy patients has escaped the attention. The little knowledge about the developement of the immune response in leprosy patients is caused by the difficulty to make a longitudinal study in human leprosy, because of the unknow mode of transmission and the long incubation period.

The mangabey monkey has been reported to be a good model to study the immune respense in leprosy, since the course of the infection with M. leprae in this host is similar to human.

Using sera from 8 mangabey monkeys we could demonstrate that IgA antibodies against M. leprae specific PGL-I antigen were present in sera of some monkeys. These monkey sera were obtained in the course of 100 months before and after experimental infection with M. leprae suspensions, where by two monkeys each were inoculated with different numbers of bacilli.

Ig A antibidy levels to PGL-I in monkey sera were compared with Ig G and Ig M antibody levels and clinical course of infections

EX21

A NEW PATHOGENICITY MODEL OF LEPROSY : MUTILATION OF TOES IN MICE EXPERIMENTALLY INFECTED WITH M. LEPRAE/CAN BACTERIA.

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The leprosy bacillus had been shown to multiply outside the human body, chiefly in the mouse footpads, armadillos and some non-human primates, yet no animal mutilation model, comparable to the human disease, exists. We describe here such a model in mouse. Infant mice (white, 'Swiss' strain, inbred; 6-10 days old) were inoculated in both the (mouse) footpads (MFP) with 10^7/ 10^8/ 10^9 CFU of CAN/M. leprae bacteria in 0.05 ml volume containing 40 ug of sterile collagenase (Type VII, lyophilised, Sigma Labs., USA). Each batch consisted of 20 mice. Controls consisted of uninoculated mice of the same litters as well as those inoculated with collagenase alone. The animals were observed for 6 months. Mutilation developed in several animals belonging to different test batches after 3 months or later with or without being accompanied or followed with deformities or contractures. None of the control animals living in the same environment developed mutilations/deformities. Microbiological and histopathological studies of the lesions showed significant bacillary proliferation with disintegration/dissolution of the connective tissue and their replacement by fibrous tissues in the affected areas.

EX22

EVALUATION OF SODIUM STIBOGLUCONATE AND UREASTIBAMINE ON MOUSE, EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM LEPRAE FROM HUMAN LEPROMA.

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The in vitro effects of any one of the currently used drugs in the treatment of leprosy are not well studied or understood, for which the present knowledge about the efficacy of these drugs on the in vivo or human systems is based on clinical trials or on the effects on mouse footpads. We have cultivated in vitro a large number of leprosy derived chemoautotrophic nocardioform (CAN) bacteria which appear to have a very close parallelism with the leprosy bacillus; these have been examined for in vitro susceptibility to Na-stibogluconate (a pentavalent arsenical), urea stibamine, ofloxacin, norfloxacin, rifampicin, DDS, as well as, for in vivo effects of these agents on the multiplication of the freshly harvested leprosy bacilli from human leproma and inoculated into the mouse footpads; pathological changes in the footpads as well as the internal organs were also studied.

We found that Na-stibogluconate most significantly reduced the bacterial multiplication and development of lesions in the internal organs compared with ureastibamine and all the other drugs, and also significantly with respect to the untreated but infected control mice.

EX23

UTILITY OF BEIGE MOUSE IN CHEMOTHERAPEUTIC STUDIES IN LEPROSY

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Animal models are very essential in the development of new drugs, a step between in vitro screening and clinical trials. Valid information is needed on the pharmacokinetics and toxicity of the drug and its effect on the in vivo multiplication of the organism. Animal models currently being used in leprosy — BALB/c mice, nude mice and armadillos — have their own drawbacks. The high susceptibility of Beige (C57BL/6/bg/bg) mice to M. avium complex (MAC) strains and its success to chemotherapeutic investigations for MAC infections led us to investigate its utility in leprosy.

Dissemination of M. leprae to visceral organs was seen within four months only in Beige mice, but not in BALB/c mice, following Iv or Ip inoculation. Bacilli harvested from Beige mice exhibited all the characteristics of M. leprae, including growth patterns in the foot pads of BALB/c mice. M. leprae inoculated into foot pads of Beige mice multiplied faster than those inoculated into foot pads of BALB/c mice. When Beige mice were fed ad libitum a diet containing 0.0005% dapsone, complete suppression in the multiplication of M. leprae in visceral organs as well as in foot pads was observed. Thus, Beige mouse has a potential usefulness in evaluating chemotherapeutic activities of new antileprosy drugs.

EX24

SIMULATION OF LEPROSY INFECTION IN MICE

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These investigations are an extension of the work of recent years devoted to studying

the peculiarities of mononuclear phagocyte system in leprosy. Models of leprosy infection in mice with previously affected macrophage compartment of their immunity were proposed and developed by Professor F.E.Vishnevetsky, now the deceased. Two novel approaches to leprosy simulation are presented. The first approach involves the formation of a defect of mononuclear phagocyte system by means of lavages of macrophages from peritoneal cavities of mice before their inoculation by Shepard's technique. The second way is to affect phagocytic activity of macrophages by means of introducing synthetic tetrapeptide tuftsin (Serva, Germany). Both approaches allow to shorten the experimental terms through stimulating M.leprae multiplication at the site of their inoculation. Furthermore, a generalized leprosy infection with the appearance of lepromatous granulomas in the internal organs has been observed in M.leprae-infected laboratory animals.

The data obtained suggest a value of the approaches described for leprosy simulation experiments and might be used for screening of the compounds with potential antileprosy activity and for elucidation of some aspects of leprosy pathogenesis.

EX25

PENETRATION OF DAPSONE, RIFAMPICIN AND CLOFAZIMINE INTO MACROPHAGES AND MYCOBACTERIA.

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The penetration and hence the presence of a drug inside the mycobacteria as well as the phagocytic cells macrophages is perhaps one of the key mechanism of drug mediated killing of invading organisms. We have examined the penetration of three antileprosy drugs-dapsone, rifampicin and clofazimine into M. lufu and M. smegmatis and mouse peritoneal and human macrophages.

M. lufu and M. smegmatis (10^8/ml) and cultured mouse peritoneal and human macrophages (10^6/ml) were incubated with 5-10 ug/ml of dapsone, rifampicin and clofazimine for varying periods and the levels of drugs inside the mycobacteria and macrophages were estimated by using spectrophotometry, TPLC and fluorimetry. The penetration of polylysine conjugated dapsone was compared with that of dapsone only. Other factors like temperature period of incubation and pH were also studied.

All three drugs penetrated into mycobacteria to an extent of 20-40% and the polylysine conjugation enhanced dapsone penetration by an additional 50%. Ionophores too enhanced the penetration of dapsone. The macrophage exhibited a permeability of 15-40% for the three drugs with moderate variation between the drugs. The process of drug penetration seems to be passive.

It may be possible to enhance the drug penetration through various methods of drug presentation such as conjugation of drugs to polyaminoacids, ionophores etc. Kinetic studies on drugs using macrophage as model need to be carried out.

EX26

THE ACTIVITY OF COMBINATIONS OF EFFECTIVE ANTIBIOTICS AGAINST *M. leprae*-INFECTED MICE

Robert H. Gelber, Lydia P. Murray, Mabel Tsang, and Patricia Siu.

Medical Research Institute of San Francisco, CA, USA.

Groups of female BALB/c mice were infected in both hind feet with 5,000 *M. leprae* and treated from day 60-150 afterwards with low but active schedules of the following 5 drugs singly and in all possible combinations of both 2 and 3 drug regimens: clarithromycin (C) 0.001% in diet, sparfloxacin (S) 5 mg/kg by gavage 5 times weekly, rifampin (R) 20 mg by gavage once monthly, minocycline (M) 0.004% in diet, and dapsone (D) 0.0001% in diet. At the completion of therapy and 4 & 7 months subsequently, the number of *M. leprae* in 2 mice (4 feet) was enumerated. Multiplication was considered to have occurred if the number of *M. leprae*/footpad in footpad pools of 2 mice (4 feet) was $\geq 10^5$. From the results of the first 2 harvest intervals all single agents were found to be active, but at the harvest 7 months after therapy was discontinued, *M. leprae* had multiplied in mice treated with each agent singly. At that time *M. leprae* had not multiplied in only 4 of 10 of the 2-drug regimens (S + R, S + M, S + D, R + D). The 10 3-drug combinations resulted in no *M. leprae* multiplication at all 3 harvest intervals, except for a single combination (C + M + D), which had demonstrated multiplication only at the last harvest.

These studies suggest additive or synergistic and certainly not antagonistic activity for combinations of antimicrobials effective against *M. leprae*. Furthermore, combinations of 3 active drugs were found to be generally superior to that of 2. Lastly, these studies confirm the previous work of Shepard on the activity of combinations of effective antibiotics against *M. leprae* in mice wherein more rapid bacterial killing usually resulted from the use of drug combinations. The implications of these findings to the combination therapy of leprosy will be discussed.

EX27

THE ACTIVITY OF CERTAIN NEWER QUINOLONE ANTIBIOTICS AGAINST *M. leprae*-INFECTED MICE

Robert Gelber, Ali Iranmanesh, Patricia Siu, Mabel Tsang, & Lydia Murray. Medical Research Institute, San Francisco, CA, USA.

Previously pefloxacin and ofloxacin were found to be active against *M. leprae in vitro*, in experimental animals, and in clinical trials of lepromatous leprosy patients. In this study we compared certain more recently developed fluoroquinolones (lomefloxacin, PD 124816, WIN 57273, temafloxacin, and sparfloxacin) with pefloxacin and ofloxacin in *M. leprae*-infected mice by the kinetic technique of Shepard (treatment day 60-154 after footpad infection), each by gavage at doses of 50, 150, & 300 mg/kg 5 times weekly. The number of *M. leprae* in footpads of 2 mice (4 feet) from untreated controls and all treatment groups was enumerated microscopically at the completion of therapy and at intervals of 2-3 months thereafter, generally up to 9-12 months subsequently. We judged drugs inactive (IA) if at the end of therapy the number of AFB was the same as in the untreated controls, bacteriostatic (BS) if at the end of therapy the number of AFB was less than in untreated controls but bacillary multiplication commenced immediately thereafter, partially bactericidal (PBC) if multiplication was further delayed, and fully bactericidal (FBC) if *M. leprae* did not grow for 9 or more months after therapy was completed:

	15	30	50	150	300 (all mg/kg)
pefloxacin	-	-	IA	BS	PBC
lomefloxacin	-	-	BS	BS	PBC
PD 124816	-	-	BS	BS	FBC
ofloxacin	-	-	BS	FBC	FBC
WIN 57273	-	-	PBC	FBC	FBC
temafloxacin	-	-	FBC	FBC	FBC
sparfloxacin	FBC	FBC	FBC	FBC	FBC

All 7 fluoroquinolones studied were active against *M. leprae*, temafloxacin and sparfloxacin being the most active, fully bactericidal at all 3 dosage schedules. Additionally, sparfloxacin was found fully bactericidal at 15 mg/kg and 30 mg/kg 5 times weekly. This study demonstrates that certain of the newer fluoroquinolones, particularly sparfloxacin, are more active than pefloxacin and ofloxacin against *M. leprae*-infected mice and merit clinical trial.

EX28

THE ACTIVITY OF MACROLIDE ANTIBIOTICS AGAINST *M. leprae*-INFECTED MICE

Robert H. Gelber, Lydia P. Murray, Patricia Siu, and Mabel Tsang.

Medical Research Institute of San Francisco, CA, USA.

Franzblau *et al.* first demonstrated in cell-free and macrophage culture, as well as in *M. leprae*-infected mice, that clarithromycin inhibited *M. leprae*. We tested a series of macrolide antibiotics at clinically achievable levels (0.06%-0.1% in diet) against *M. leprae*-infected mice by the kinetic method of Shepard (treatment day 60 to 150 after footpad infection) and found that while erythromycin and azithromycin were inactive, roxithromycin and clarithromycin were bactericidal, clarithromycin being found

more active than roxithromycin. Later we found by the proportional bactericidal test, wherein the actual killing of *M. leprae* is quantitated by using groups of mice infected with three serial 10-fold *M. leprae* dilutions, that azithromycin (0.1% in diet) resulted in no significant killing of *M. leprae* (11% ± 74%), while 0.1% in diet of both roxithromycin and particularly clarithromycin were found to be both bactericidal for *M. leprae* (82 ± 13% and 96% ± 2% respectively). Furthermore, we found that clarithromycin's minimal inhibitory dietary concentration for *M. leprae* in mice was exceedingly low, 0.001% (serum level < 25 mμg/ml and footpad level ≤ 0.7 μg/gm). We also found by the proportional bactericidal test that the minimal bactericidal dietary concentration was, however, somewhat higher, 0.05%. Lastly, clarithromycin (0.1% in diet) both 3 days weekly (M, W, F) and 1 day weekly entirely inhibited the growth of *M. leprae*, while administration only 1 day monthly was partially active. These studies demonstrate that clarithromycin inhibits *M. leprae*, is bactericidal, and is effective on intermittent administration, all encouraging for its application to the treatment of leprosy.

EX29

STUDIES ON THE ACTIVITY OF MINOCYCLINE AGAINST *M. LEPRAE*-INFECTED MICE.

Robert H. Gelber, Patricia Siu, Mabel Tsang, and Lydia P. Murray.

Medical Research Institute of San Francisco, CA, USA.

Previously we had demonstrated that minocycline treatment of *M. leprae*-infected mice inhibited the growth of *M. leprae* at serum levels (0.1-0.2 μg/ml) well below those achieved in man (2-4 μg/ml) by a standard daily adult dose of 100 mg, was consistently bactericidal (both by the kinetic method of Shepard and the proportional bactericidal technique), and additive in its activity when combined with other antimicrobials (dapsone, rifampin, & kanamycin).

We, also, evaluated the minimal concentrations of minocycline in the diet and in serum required to inhibit the growth of 7 *M. leprae* isolates in mice, including both a partially dapsone-resistant and fully dapsone-resistant isolate. Minocycline concentrations of 0.01% and 0.04% in the diet, which resulted in serum levels of ≤ 0.17 and 0.51 μg/ml, respectively, were consistently and completely inhibitory. Even 0.004% dietary minocycline (levels in serum, ≤ 0.08 μg/ml) partially inhibited 5 of these strains, while 0.001% minocycline was consistently inactive. We can now report that very low levels of dietary minocycline (0.01%) consistently inhibited the growth of all 18 *M. leprae* isolates studied. For 5 *M. leprae* isolates, minocycline at a concentration of 0.04% in the diet given 3 days weekly (M, W, F) and 1 day weekly completely inhibited the growth of *M. leprae*, and minocycline given even 1 day monthly was partially inhibitory for 3 of these 5 *M. leprae* isolates

Furthermore, dietary concentrations of minocycline 0.01%, 0.04%, 0.06%, & 0.1% were found bactericidal (P≤ 0.02) for *M. leprae* by the proportional bactericidal test, establishing that minocycline's minimal inhibitory concentration and minimal bactericidal concentration for *M. leprae* are similar.

EX30

LONG-TERM EFFECTS OF DAPSONE ON IMMUNE RESPONSES IN BALB/c MICE.

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Long-term exposure to Dapsone of BALB/c mice revealed a concentration dependent suppressive effect on humoral and cell-mediated immune responses with an initial enhancement at 4 weeks exposure. Adult mice were exposed to 0.01% and 0.001% dapsone concentrations in the diet for 24 weeks and the immune functions were assessed at the interval of every 4 weeks. Delayed type hypersensitivity reaction to SRBC, proliferation and IL-2 production to T-cell mitogen Con A by splenocytes was suppressed to both the concentrations of dapsone throughout the exposure period with a sharp initial increase at 4 weeks exposure. Lymphoproliferation to B-cell mitogen-LPS and PFC numbers to T-dependent (SRBC) and T-independent (LPS-SRBC) antigens were elevated at 4 weeks exposure and gradually declined to base level at 12 to 16 week exposures. Thereafter, proliferation to LPS and PFC profiles were suppressed in the subsequent exposures. Though dapsone at both concentrations showed similar effect on the above immune functions, the magnitude of the early stimulation and later suppression was higher at 0.01% dapsone concentration when compared to 0.001% concentration. This indicated a concentration-dependent response of identical nature. The implication of these results will be discussed.

EX31

STUDY ON NUDE MICE INOCULATED WITH MYCOBACTERIUM LAPRAE BY THE MULTIPLE ROUTES

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The immune-deficient nude mice were inoculated with nude mouse-derived mycobacterium leprae by multiple routes(intravenously, subcutaneously at the foot pads and ears). The results showed that these inoculated animals were capable of producing a great number of Mycobacterium leprae to the level of 10^{11-12} per gram of tissue, and much heavier lepromatous lesions were detectable histopathologically. The dessemination of the infection were particularly found in the sites with lower body temperature. The organisms are prone to proliferate in the striated muscles and peripheral nerves.

The authors suggest that the experimental leprosy in the nude mice is a very useful tool in leprosy research, especially in the countries without armadillos. Compared with the single-route inoculation reported previously, the multiple-route inoculation is of more practical value.

EX32

EXPERIMENTAL LEPROSY IN Dasypus hibridus IN ARGENTINA

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In Argentina leprosy bacilli could be obtained only from biopsy specimens of patients. To assure the continuous production of lepromin and other antigens used in serological assays, it was decided to focus the research on the armadillo. Appropriate facilities to breed and keep armadillos in captivity were built at the Sommer Hospital. In 1990 eigth animals Dasypus hibridus, which were sended by the Panamerican Zoonoses Center (PAHO/WHO), were inoculated with leprosy bacilli. A general examination was performed before the inoculation and each 60 days. The animals were weighed and the following laboratory tests were requested: blood count (red and white cells count, hemoglobin, leukocytary formula, hematocrit), sedimentation rate, prothrombin time, glycemia, uremia, alkaline phosphatasa, SGOT, SGPT, bilirrubin, serological and microbiological tests for the detection of leprosy, tuberculosis and blood parasitical diseases. The inoculum was injected with a tuberculin syringe in the external femoral vein under anaesthesia It was prepared with a human leproma obtained from an untreated patient. One mililiter of this inoculum adjusted to 10^8 b/ml was inoculated to each animal. One armadillo showed disseminated leprosy 26 month after inoculation. Abundant solid bacilli appeared in a skin ulcer (leproma), liver, spleen and lymph nodes. It was possible to purify bacilli from the infected tissues. The inoculation of bacilli into mouse foot pad and Piridin extraction were positive. The culture in diferent medium were negative.

EX33

NERVE CONDUCTION STUDY TECHNIQUE IN THE ARMADILLO

JOSÉ GARBINO, JORGE ALMEIDA, MARCOS VIRMOND

THE ARMADILLO (DASYPUS NOVEMCINCTUS) HAS BEEN ACCEPTED AS THE CHOICE ANIMAL FOR LEPROSY RESEARCH. IN ORDER TO STUDY THE POSSIBILITY OF ESTABLISHING THE ARMADILLO AS A MODEL FOR NEURAL LEPROSY INVOLVEMENT EXPERIMENTALLY, THIS REPORT DESCRIBES THE NERVE CONDUCTION STUDY TECHNIQUE IN THIS ANIMAL, PROVIDED THE LITERATURE ON THIS ISSUE IS SCARCE.

WE EXAMINED THE TIBIAL NERVE OF BOTH SIDES OF 10 ANIMALS FROM THE ARMADILLO FARM OF THE INSTITUTO LAURO SOUZA LIMA. THE TECHNIQUE PERFORMED WAS NERVE CONDUCTION STUDY, THE COMPOUND MUSCLE ACTION POTENTIAL WAS MADE FROM THE PLANTAR MUSCLES IN THE FOOT PAD OF THE LOWER LIMBS. THE STIMULATION SITES WERE DISTALLY, BELLOW THE ANKLE, AND PROXIMALLY JUST CLOSE TO THE KNEE IN THE MEDIAL ASPECT OF THE LIMB. THE DISTANCE BETWEEN THESE TWO POINTS WERE MEASURED WITH A TAPE MEASURE AND THE TEMPERATURE WAS MEASURED BY MEANS OF AN DIGITAL SKIN THERMOMETER WHICH ELECTRODES WERE PLACE HALF WAY OF THE ABOVE MENTIONED POINTS, IN BOOTH SIDES.

WE CONCLUDED THAT THE MOTOR NERVE CONDUCTION STUDY IN THE ARMADILLO IS A FEASIBLE AND EASY TECHNIQUE TO BE PERFORMED IN A STANDART LABORATORY AND COULD BE OF UTMOST IMPORTANCE TO BE USED IN EXPERIMENTAL LEPROSY NEURAL INVOLVEMENT. OUR DATA WITH STATISTICS STUDIES WILL BE PRESENTED.

EX34

THE ARMADILLO AS A MODEL FOR LEPROSY

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Leprosy is unique among human diseases in that the bacillus causing it does not grow in artificial culture media, and until 1971 would not produce disseminated disease in experimental animals. Research was at a standstill. Since then, leprosy bacilli grown in armadillos have been used to produce lepromin-A, a reagent used to predict the course of disease; and PGL-1, a reagent used for its diagnosis. Armadillo-derived vaccines for prevention of leprosy are under test on 470,000 people throughout the world. The biochemistry and genome of the leprosy bacillus, once complete mysteries, are slowly unraveling. As an animal model, the armadillo has led to a better understanding of the pathology, immunology and transmission of disease. The armadillo provides the ultimate answer to people who would like to ban use of animals in medical research. Without this model there would be no research on diagnostic reagents or vaccines. Leprosy would still linger in the shadows of medieval medicine.

EX35

LEPROSY IN WILD ARMADILLOS

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Florida Institute of Technology, Melbourne FL

Until the coming of AIDS, leprosy was the most feared of infectious diseases because the Bible linked it with corruption of both spirit and body. It was a punishment by God for transgression. Most physicians do not think that Biblical leprosy was the disease we know today, but these ancient fears lingered into modern times. In 1975, just four years after the discovery of the armadillo as an animal model, we found that some wild armadillos are naturally infected with leprosy. This was a remarkable coincidence that caused great consternation in the lay and scientific press. Since then, other workers have confirmed that leprosy occurs in many wild armadillos. A few years later, a mangabey monkey housed in our animal colonies at Gulf South Research Institute was found to have leprosy. Within a few years, leprosy was downgraded from its ancient status as a Biblical curse to just another disease common to humans and animals. This discovery opened up a vast natural laboratory for studies of transmission of leprosy in wild animal populations.

IMMUNOLOGY

IM1

THE 65 KDa PROTEIN OF MYOBACTERIUM HABANA AND ITS PUTATIVE ROLE IN IMMUNITY AGAINST M. TUBERCULOSIS.

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Mycobacterium habana (M. simiae serovar-1) an atypical mycobacterium has protective efficacy against M. tuberculosis $H_{37}Rv$ and M. leprae infections in mouse. It generates cell mediated immune responses and shares several immunodominant proteins with these mycobacteria.

The 65 KDa protein of this mycobacterium has been isolated in pure form by isotachophoresis. The isolated protein was run on SDS-PAGE gel, alongwith molecular weight marker, electro-transferred on nitrocellulose membrane and probed with two monoclonal antibodies (mab) IIC8 and IIH9. Both the mabs have identified a single band discrete protein at the same molecular mass. The yield from single dose of (1.5 mg weight = $6.27X10^8$ = 63.3 ug protein) M. habana vaccine is 3 ug. This dose has provided significant degree of protection in mice. The leucocytes/lymphocytes obtained from vaccinated animals and patients of T.B. & Leprosy had stoppage of migration and had shown strong lymphoproliferative response under antigenic influence. Strong CMI responses have been generated by this protein in animal against homo and hetrologous antigens.

IM2

A 25kDa PORTION OF 65kDa PROTEIN OF MYCOBACTERIUM LEPRAE HAS IMMUNO PROTECTIVE PROPERTIES.

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Rabbit antibodies to delipidified cell components (DCC) of M.leprae were used to screen the λgtll library of M.leprae genes and reactive colonies were picked out. One such colony had 1.6kb insert DNA and was expressing a 65kDa protein. This protein was identified in immunoblot using antibodies to DCC. This protein was not reactive with M.leprae 65kDa specific IIIE9 Mab. The DNA sequence showed that the insert started from 1.15kb portion of the classical 65kDa protein gene (Mehra et al,1986). This protein was reactive to another monoclonal antibody to DCC of M.leprae, but this monoclonal had no reactivity to 65kDa hsp of M.leprae. The DNA sequence and the antibody reactivity indicated this protein as a second 65kDa protein of M.leprae. The pUC19 lysate containing this 65kDa had good immunoreactivity listed below. However this 1.6kb insert on recloning in a modified pET vector expressed in BL21 De3 E.coli, a 25kDa protein. This was because of the restricted open reading frame available. This protein has reactivity with specific Mab IIIE9. The protein both in the crude lysate and as partially purified protein