Seven counties of Wuzhou prefecture with a population of 3 672 300 and an area of 20 113 97 kilometers in the East Guangxi Autonomous Region where leprosy was medium endemic were selected for investigation. One thousand two hundred and eight three leprosy patients have been detected in 788 of 16 756 villages. Every five years from 1956 through 1955 was considered as an endemic period for observation. The method consist in linear correlation analysis and multiple regression coefficient test using average incidence of each period (AIP) as independent variable, unknown-cause incidence (UCI)※, incidence by extra-familial infection (IEFI) and incidence by familial infection (IFI) as dependent variables. The results showed that as regards the average period incidence (API), there is a significant correlation between UCI, IEFI and IFI, a very significant dependence relation between UCI and no linear regression correlation between IEFI and IFI indicating a potential change from coexistence of incidence by irregular UCI and incidence by extra-familial and familial clustered infection to clustered incidence by extra-familial and familial infection. It also demonstrated that intimate and repeated contacts with patients still remain as a risk factor of familial incidence and of extra-familial incidence as well.

※ Occurrence of clinical leprosy by an unidentified source of infection

EP63

LEPROSY IN ASTRAKHAN REGION OF RUSSIA

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Astrakhan region located in the delta of Volga-river to the north of Caspian Sea was and remains the most active focus of leprosy in Russia. With 0,7% population living in Astrakhan region here 40 to 70% of leprosy cases detected in Russia were registered. This fact may be partly explained by ancient centuries-old close economical ties with the countries of South-East and Middle Asia and Near East (the Great Silk Way). In total during the period from 1923 (beginning of State Program of Leprosy Control in the USSR) to 1998 about 2800 leprosy cases were registered on the territory of Astrakhan region, more than 90% of them falling on 1923-1962, or by decades:

578 cases - in 1923-1932;
552 - in 1933-1942;
644 - in 1943-1952;
610 - in 1953-1962;
234 - in 1963-1972;
40 - in 1973-1982;
22 - in 1983-1992,

and only 8 new cases in 1993-1998. Regular and significant decrease in leprosy incidence, beginning since the 70th years, was achieved through active case-finding and coverage of all the patients with multi-drug therapy (MDT) implemented in the USSR in 1965-1970, regular surveys of leprosy contacts, implementation of preventive chemotherapy, special measures of financial support for leprosy patients and their families, rise in hygienic and sanitary standards. Among leprosy cases detected in the last 25 years women prevail (55%), more than 50% of leprosy cases are over 60 years old and there no children under 14 years old. Duration of incubation period in 75% of all these cases exceeds 10 years. Data Bank on ex leprosy cases is on record since 1923. A retrospective analysis of distribution and activity of leprosy foci is being carried out.

EP64

LEPROSY IN KARAKALPAKSTAN.
PROSPECTS FOR ITS ERADICATION

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For Karakalpakstan leprosy is an ancient heritage, but regular efforts towards leprosy control were initiated since 1933 after national health protection and leprosy service had been organized. To intensify antileprosy activity in 1962 Karakalpak Leprosy Control Branch of Uzbek Institute for Study of Dermatovenerology was opened and two outpatient clinics and six leprosy units were organized. Since 1957 active case-finding through regular sample and mass surveys with annual coverage of up to 100 000 inhabitants were carried out, identified foci of leprosy were put under surveillance with chemotherapy of both leprosy cases and their contacts, and just in 1960-1964 these efforts resulted in finding 490 new leprosy cases. Rates of leprosy incidence sharply contrasted between north and south parts of the country. Thus, in 1960 when the highest rate of total incidence for the country was observed (30,4 per 100 000) it was 43,7 in the north districts, approaching 185,6 in Muinak while in the southern parts it reached only 2,8 per 100 000. This difference is due to various factors, including genetic, besides living and economical conditions (Abdirov Ch.A. et al., 1973, 1977). Successful combined chemotherapy resulted in gradual decrease in leprosy incidence. By 1980 a tendency towards sporadic incidence began to be observed, ranging from 0,83 to 0,7 per 100 000 in 1987. In recent years only single cases of leprosy have annualy been registered in the north territories, and Karakalpak focus acquired features of disappearing endemicity. Meanwhile, difficult ecological and economical situation, long incubation period, absence of antileprosy vaccine and high susceptibility of a part of the population to leprosy could induce burst of leprosy incidence on Karakalpak territory.

EXPERIMENTAL

EX01

A GLOBAL STRATEGY FOR BASIC BIOMEDICAL RESEARCH ON LEPROSY ERADICATION.

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Basic biological research on *Mycobacterium leprae* and on the immunology and pathogenesis of leprosy conducted over a period of the past 20 years has contributed little to the present optimistic state of global leprosy. Indeed, proceedings from the recent historical joint workshop of ILEP and WHO/LEP (July 1997) made no mention of a role for such research in identification/confirmation of the still appreciable numbers of new leprosy cases, or in gauging the extent of sub-clinical leprosy in the community, or in the ultimate eradication of the disease. Yet, there is the need for new diagnostic and epidemiological tools, for a deeper understanding of the bacteriological and immunological basis of pathogenesis, perhaps resulting in new treatment for reactions, for probes and assays for drug resistance and, possibly, for a vaccine for ultimate eradication of the disease. Those principles are still the driving force behind small, poorly funded but enthusiastic and well coordinated research groups throughout the world. Recent watershed meetings in Bangkok (March 1996) and Addis Ababa (February 1998) have identified disease-related issues to be addressed, most promising immunological and molecular approaches to follow, and shared centralized resources required to fulfill the mission. The International Leprosy Congress provides the ideal forum for the exposition and promulgation of a shared, interdisciplinary, interdependent global research strategy.

EX02

ANOMALIES IN NEUROFILAMENT PROTEINS: SOME CLUES TO MOLECULAR MECHANISM OF PASSIVE NERVE DAMAGE IN LEPROSY.

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Mechanisms of nerve damage in leprosy remains very poorly understood. In a recent morphological study, it was noted that, a

decrease in axonal caliber precede paranodal demyelination which in turn precede nodal demyelination in human leprous nerves (Shetty and Antia,1995). As a logical extension of this observation, the biochemical basis for such a reduction in diameter of the myelinated fibres that leads to secondary demyelination was sought in the present study.

Neurofilaments (NFs) are the important constituents of the axonal cytoskeleton and C-terminal phosphorylation of NF-H is known to govern the axonal caliber (Hoffman et al 1984). Therefore the state of NFs and its phosphorylation were investigated in human and in experimental mouse leprous nerves, using immunocytochemical technique, SDS-PAGE and Western blot analysis.

Reduced SMI-31 staining, decrease in NF protein in triton insoluble fraction and a corresponding increase in total protein in triton soluble fraction were noted in minimally involved human leprous nerves as well as in sciatic nerves of mice injected in foot pads with live and heat killed M.leprae as compared to normal nerves. Protein bands corresponding to NF triplet were not seen in SDS-PAGE and immunoblotting. Results indicate alterations in the 3-D structure of NF protein as well as in NF phosphorylation. The results will be presented and discussed.

EX03

STUDY ON ANTI-ND-O-BSA IgM ANTIBODY IN SERA FROM MICE

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In order to explore the variation of the specific anti-ND-O-BSA IgM antibodies in the mice which were infected with M. leprae, the authors tested 50 serum specimens from normal mice and 131 from the M. leprae infected mice. The results indicated that the IgM level of infected sera was significantly higher than that of normal sera ($p<0.01$). And there was a significant difference between untreated and treated mice ($p<0.01$). In addition, the specific antibody levels showed distinct differences among the mice which had taken different drugs or different dosages of the same drug ($p<0.01$ or $p<0.05$). The results also suggested that IgM antibody levels of mice ran parallel with bacilli load and were related to the degree of infection and effect of chemotherapy.

EX04

THE LIMITED DIVERSITY IN RESPONSES TO LEPROMIN-A AMONG ARMADILLOS IN THE SOUTHERN UNITED STATES.

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Though sylvan leprosy is rare or absent in most locales it is highly prevalent among armadillos in the southern Mississippi river valley and contiguous coastal marshes. We examined the histological responses of 392 armadillos taken from various locations in Florida, Louisiana, Mississippi and Texas to determine if armadillos in different locales preferentially express different forms of the disease or if variations in host resistance to leprosy might influence this distribution. After adapting to captivity $1x10^7$ heat killed *M.leprae* prepared as Lepromin-A were inoculated to the abdominal skin and the site biopsied after 21 days. Many of the animals were later experimentally infected with *M. leprae*. The Mitsuda reaction was described as Multibacillary or Paucibacillary and further sub-classified by the Ridley-Jopling scale as LL, BL, BT, or TT. The percentage of animals classified as different types varied significantly ($p < 0.05$) between groups. Animals from non-enzootic areas in Florida showed the lowest Multibacillary percentage (65%) but those from another non-enzootic area in central Texas showed the highest (95%). The main variation seen was in the percentages of animals classified as either BL or TT and may have been influenced by the reaction of animals already exposed to *M. leprae* or harboring the agent. The Multibacillary rates of animals from enzootic areas and non-enzootic areas were identical (82%). The minor variations in Lepromin reactivity seen here could not underlie the major geographical disparities seen in the distribution of leprosy among wild armadillos. Results of experimental infections show that the majority of Multibacillary spectrum armadillos succumb to leprosy but 10-20% of the animals reliably resist the disease. The relationship between Mitsuda reactivity and resistance is complex and should not be confused as an indicator of susceptibility.

EX05

M. VACCAE VACCINATION OF MYCOBACTERIUM LEPRAE INFECTED MICE

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#Genesis Research and Development Corporation, Auckland, New Zealand

The study aims to determine the effect of M. vaccae immunization on the growth of Myco leprae in mice.

Preliminary results of a pilot study conducted at the LWM Laboratory indicated significant reduction of M. leprae growth in vaccinated mice compared to non-immunized controls. An expanded study using acid fast bacilli from histoid, BL relapsed and untreated LLs patients were used to infect inbred CBA/J mice. These were immunized with 1 mg of M. vaccae I.P. at day 0, 3 weeks later infected with 5,000 AFB into each hind footpads and re-immunized with M. vaccae 90 days post infection. A non-immunized group was used as control. Mice in both groups were sacrificed at 6,9, and 12 months post-infection. Hind pads from individual mice were harvested, pooled, stained and the AFB enumerated.

When compared with controls, results of harvest in the treated group inoculated with AFB from LLs patient showed a percent inhibition (P.I.) of 99.15%, 72.4%, and 62.4% at 6,9,12 months respectively. Those inoculated with AFB from the histoid patient showed a P.I. of 83.9%, 65.9% and 43.4% at 6,9,12 months. In the BL relapsed group a 95.6% and 92.8% P.I. was noted at 6 and 9 months, and a 83.8% and 68.7% P.I. was seen at 6 and 9 months in the other LLs patient.

Data will be collated, analyzed and discussed.

This investigation is funded by Genesis Research and Development Corporation, Auckland, New Zealand and Leonard Wood Memorial-American Leprosy Foundation (LWM-ALF).

EX06

AN ATTEMPT OF OPTIMIZATION OF MICE FOOT PAD TECHNIC BY SHEPARD

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Previosly we found out that intensity of phagocytic responses of granuloma macrophages of from lepromatous leprosy patients towards M.leprae is directly related to the activity their of myeloperoxidase (MPO). The main function of MPO is binding of H_2O_2, thus, interfering its intracellular accumulation. Enzyme therewith becomes inactive. Mice were introduced 0,03-0,05 ml of 0,6% H_2O_2 solution into their foot pads. Then, 1-2 hours later, the animals were inoculated with passed M.leprae at a dose of 10^4 into the same foot pads. An introduction of H_2O_2 activated multiplication of M.leprae in foot pads. By 5 month after the inoculation of M.leprae mycobacterial counts in foot pads of test-animals exceeded control values (without previous introduction of H_2O_2). At this time a few leprosy mycobacteral were discovered in lungs of test-mice. In 7 months a generalization of leprosy process was observed (M.leprae were found in large numbers in lungs and in less numbers - in spleen). By 9 month a large number of M.leprae was found out in spleen of test animals with decrease of mycobacterial counts in lungs. Electron cytochemically a decrease in MPO activity was found out in phagocytes of test-mice. The data obtained should permit a further elucidation of leprosy pathogenesis and will be used in attempts of new therapeutical approaches.

EX07

PROTECTION OF MICE AGAINST *Mycobacterium leprae* BY SKIN-TEST ANTIGENS

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BALB/c mice, bred and maintained under SPF conditions, were administered *Mycobacterium leprae* soluble antigen from which lipoarabinomannan had been extracted (MLSA-LAM), *M. leprae* cell-wall antigen (MLCwA) or MLCwA-LAM subcutaneously in the flank, the antigens had been suspended in incomplete Freund's adjuvant (IFA) or aluminum hydrogel (AlOH), an adjuvant suitable for use in humans

After one month, the mice were challenged in the right hind footpad, each with 5000 freshly harvested *M. leprae* of the "Thai 53" strain. Harvests of the organisms four months later yielded the following results:

Treatment	Median no. AFB per footpad (x 10^3)
IFA	9.50
MLSA-LAM in IFA	1.78[a]
MLCwA in IFA	4.68[a]
MLCwA-LAM in IFA	2.56[a]
AlOH	6.74
MLSA-LAM in AlOH	6.13
MLCwA in AlOH	6.30
MLCwA-LAM in AlOH	3.46

[a] The probability that the results from the group of mice indicated had been drawn from the same population as were those from the relevant control group is < 0.05

Thus, all three skin-test materials appeared to protect the mice when suspended in IFA, whereas no protection by these materials could be demonstrated when they had been suspended in AlOH.

EX08

REPORT OF AN ATTEMPT TO EXPERIMENTALLY INFECT GOATS AND PIGS WITH *M. LEPRAE*.

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Recently an association between goats and prevalence of leprosy in Indian villages was reported. It is well known that these animals live in close proximity to humans in this environment. Following this report a histopathologic study of biopsies from ears of over 300 goats and domestic pigs were studied. No evidence of leprous infection was detected. In this experiment 3 one-month-old baby goats and 2 one-month-old Yorkshire pigs were intradermally infected in the ears with saline suspensions of 10^7 *M. leprae* and the animals were followed up for 12 to 18 months. Finding of a nodule showing granulomatous inflammation at the infected site will be described and its significance discussed.

EX09

INVESTIGATION OF THE POSTANTIBIOTIC EFFECT OF RIFAMPIN AGAINST *MYCOBACTERIUM LEPRAE* USING A BACTEC SYSTEM.

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The postantibiotic effect (PAE) is defined as a measure of the suppression of bacterial growth following exposure and removal of an antibiotic. Although *M. leprae* cannot be cultured, its viability can be maintained for some time in artificial media. We have recently investigated the postantibiotic effect of rifampin against *M. leprae* by measuring $^{14}CO_2$ release with a BACTEC system and the concomitant incorporation of 1-^{14}C-palmitic acid (substrate for BACTEC system) into the *M. leprae*-specific glycolipid, phenolic glycolipid-I (PGL-I).

Previous reports in the literature have shown that 3 hours after an oral administration of 600 mg rifampin, a mean peak serum concentration of approximately 15 µg/ml was observed. *M. leprae* harvested from the footpads of experimentally-infected athymic nude mice were incubated for 3 hours at 33°C in the presence of 10 µg rifampin and 30 µg rifampin (a concentration approximating the area under serum concentration vs time curve). After incubation, the samples were centrifuged and the pellets washed 2x with medium. The final bacterial pellets were resuspended in fresh drug-free medium and transferred to BACTEC vials. Growth Index (GI) readings were taken at prescribed intervals for 14 days. The contents of the vials were then frozen and lyophilized. After total lipid extraction, PGL-I was separated by column and thin-layer chromatography and radioactivity enumerated.

Although the GI ($^{14}CO_2$ evolved) and the ^{14}C-palmitate-PGL-I incorporation values were lower in the rifampin-exposed organisms, when compared to controls, there was no discernible postantibiotic suppression observed at either concentration of the drug. Two factors may influence the postantibiotic effect of rifampin on *M. leprae*: exposure time and drug concentration. Future experiments will attempt to address these variables. However, this investigation demonstrates a radiometric procedure of determining PAE for *M. leprae* and may be helpful in considering dosage regimens for drugs to be used against *M. leprae*.

EX10

AN IN-VITRO MODEL OF HUMAN CELLS PHAGOCYTOSING M LEPRAE BE USED TO ASSAY THE EFFECT OF ANTILEPROTICS

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In order to establish a model of Schwann cells phagocytosing M. leprae in vitro, the Schwann cells taken from the human peripheral nerves were cultured and identified by electron microscope and immunohistochemistry detecting S-100 and lysosome in the cultured cell, then enabled them to phagocytose the M. leprae taken from skin lesion of lepromatous leprosy patients. For the purpose of assaying the effect of antileprotics, we used them to treat the model respectively, after 10 to 20 days, double color fluorescent stain method was utilized to demonstrate the live or death of M. leprae. The results have indicated that Rifampin and Ofloxacin had a strong effect on M. leprae, the death rates were 91.08% and 90.9% respectively. Under the electron microscope, M. leprae underwent degeneration and necrosis. However, the death rates of M. leprae in Dapsone and the control group were 29.25% and 27.8% respectively ; there was no difference between them. These results showed that Dapsone only had weaker effect on M. leprae. In conclusion, the above results have suggested that the model may be used as an assaying method for scanning and detecting sensitivity of antileprotics.

EX11

IMMUNOLOGICAL POTENTIAL OF SCHWANN CELLS AND ITS IMPLICATIONS IN REACTIONS IN LEPROSY

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The nature and origin of the signal that invites an immunological attack on the peripheral nerves is largely unknown. The documentation on localized reactions in nerves, the immuno competence of peripheral nerve glial cells and the selective entry and multiplication of M.leprae in Schwann cells favors it as an initiator of an inflammatory reaction in nerves. This was investigated in murine dissociated Schwann cell cultures (DSC) to which viable M.leprae or its subcellular antigens (r-65KD/LAM) were added in the presence of lymphoid cells. Evidence from lymphoproliferative and multiple functional assays could conclusively demonstrate the immunological potential of Schwann cells albeit there were differing requirements for accessory cells and sensitization levels of lymphoid cells. The time-Kinetics of co-culture and the presence of fibroblasts and endothelial cells revealed significant modifications in patterns of DSC induced sensitization.

The observations raise doubt about specific reaction- precipitating antigens and restrict their role to differential activation requirements, genetic restriction and tissue distribution rather than in their effector potential. The role of the blood-nerve barrier, pre-inflammatory markers in nerves and the role of CNS will also be highlighted.

EX12

MYCOBACTERIUM LEPRAE INFECTION RESULTS IN DIFFERENTIALLY TRANSCRIBED GENES IN CULTURED RAT SCHWANN CELLS

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Invasion and destruction of Schwann cells (SCs), the principle support cells in the peripheral nervous system, by *Mycobacterium leprae* is the hallmark of leprosy and the major cause of disability. However, the interaction of *M. leprae* with SCs and the events which result in nerve destruction have not been fully elucidated but may be augmented by certain inflammatory cytokines. A better understanding of the direct effect of *M. leprae* infection on SCs may provide information to identify early nerve damage and potentially serve as a rationale basis for the development of therapeutic strategies that interrupt the *M. leprae*-SC interaction. To determine the potential effects of *M. leprae* infection on SCs we have employed the use RT-PCR and differential display technologies to study differential gene expression in infected cells. A rat SC line (33B) was infected with mouse footpad-derived *M. leprae* at an MOI of 100. After 24 hr exposure total RNA was obtained from infected and non-infected SCs and mRNA was reverse-transcribed into cDNA. Optimal conditions for semiquantitative RT-PCR analysis of gene transcripts encoding the myelin proteins, peripheral myelin protein (Po) and myelin basic protein (MBP) were developed. These myelin gene transcripts were present in the sciatic nerve preparation but MBP was not transcribed in detectable levels in 33B cells and was not up regulated in 33B cells 24 post infection. The gene encoding Po was transcribed in cultured 33B cells, however, there was no apparent effect of *M. leprae* infection on transcription of this gene 24 hr post infection. Longer time periods post infection are being analyzed. The effect of *M. leprae* infection on the transcription of other genes in 33B cells was assessed using Differential Display (RNA fingerprinting). cDNA from *M. leprae*-infected and non-infected cells was amplified by PCR using arbitrary and poly(dT) primer combinations. Polyacrylamide gel electrophoresis of products demonstrated that several gene transcripts were differentially transcribed in *M. leprae*-infected cells 24 hr post infection. These transcripts have not yet been identified. In conclusion, this 33B SC/*M. leprae* infection model has limited use for the study of myelin gene regulation. Presently, a rat SC/neuron co-culture system is being established in our laboratory. Since myelin genes are expressed in this system it will be used to study the direct effect of *M. leprae* infection on myelin sheath production, maintenance, degradation and repair, as well as, the expression of other genes.

EX13

MYCOBACTERIUM LEPRAE BINDS TO A 25 KDa PHOSPHORYLATED GLYCOPROTEIN OF HUMAN PERIPHERAL NERVE.

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Mycobacterium leprae, the causative agent of leprosy, specifically invades and destroys the peripheral nerve, which results in the main clinical manifestation of the disease. Little is known about the bacteria-nerve protein interaction.

We show in the present work that when Gamma [32 P] phosphorylated rat and human peripheral nerve proteins were used in binding assays, M leprae was found to bind to a 28-30 KDa protein of rat and a 25 KDa protein of human peripheral nerve. Partial characterisation of these binding proteins revealed that they are glycoproteins whose biochemical identify is similar to the` P zero' protein.

Pre-treatment of M leprae resulting in proteolytic digestion, delipidification or carbohydrate removal resulted in decreased binding suggesting that structural integrity of M leprae is essential for binding. These M leprae - protein interaction could be important in the pathogenesis of leprosy.

EX14

REGENERATIVE POTENTIAL OF LEPROUS NERVES

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In peripheral nerve lesions, Schwann cells (SCs) actively participate in nerve repair where they are aided by macrophages. Therefore any alteration in SC functions would hamper regeneration. The aim of the present study was therefore to determine the effect of M leprae infection on the regenerative functions of SCs and their modulation by macrophages which form a major proportion of the cells that infiltrate the nerve. Observations in 2 strains of mice, Swiss White (SW) and C57BL/6 were compared in view of their divergent responses to M leprae infection.

M leprae infected SCs from SW mice showed a decrease in proliferation and expression of NgCAM and nerve growth factor receptor p^{75}. In addition, they responded to infection with an increase in laminin and collagens type I, III and IV. In contrast infected C57BL/6 SCs showed a decrease in expression of p^{75} and secretion of fibronectin.

Macrophages, modulated SC response to infection by enhancing proliferation and the expression of NgCAM and p^{75} and decreasing extracellular matrix (ECM) secretion in both strains. The decrease in ECM was a function of protein breakdown by macrophage derived protease and also active regulation by macrophage secreted cytokines.

A similar modulation on SC metabolism in leprous nerves would have major ramifications on damage and repair activities. In addition ECM proteins would also influence the composition of the infiltrating cell population in lepromatous and tuberculoid nerves. The strain variation in Schwann cell response to M leprae infection suggests diverse mechanisms of nerve damage in tuberculoid and lepromatous leprosy patients.

Financial support received from The Wellcome Trust, U.K.

EX15

DRUG RESISTANCE MONITORING IN NEPALI LEPROSY PATIENTS

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Dapsone resistance has been recognised as a problem in leprosy treatment since 1964. In Nepal, dapsone monotherapy was introduced in 1956 and Multi-drug therapy (MDT) in 1983. However MDT coverage has only recently exceeded 95%. At Anandaban Leprosy Hospital, a mouse foot pad (MFP) laboratory was established in 1981. New previously untreated multibacillary cases and relapses presenting at the hospitals clinics are screened for dapsone and rifampicin resistance by MFP culture.
In the period 1987-1993, among 157 cases, there was a 6% dapsone resistance among new cases and 47% dapsone resistance among previously treated cases. In a new series of 69 patients presenting between 1993 and 1997, the rate of primary dapsone resistance was found to be 10.5% and the secondary dapsone resistance rate was 8%. Whereas in the first cohort all primary dapsone resistant cases were resistant at low (0.0001%) dapsone, the new cohort include one high (0.01%) DDS resistant case. All secondary DDS resistant cases have been previously treated with dapsone monotherapy. No cases of rifampicin resistance were found. It is vital that surveillance of resistance to drugs used in MDT should continue at sentinel centres.

EX16

COAGULATION METHODS FOR EARLY DIAGNOSIS OF LEPROSY.

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Diagnosis of leprosy is essentially clinical based on finding one or more signs of disease and supported by observing in some cases acid fast bacilli (mycobacterium) is slit skin smear.
These two methods are liable to subjective interpretation.
Hyperimmune sera of different mammalian species including the human were mixed with dead staphylococcus aureus strain rich protein A content, the immunoglobulins attach to protein A by their Fe portion leaving the Fab region free to bind the homologous antigens
Urine was collected from patients with lepromatous leprosy and tuberculoid leprosy, concentrated and freezed at - 20 C
The work is currently in progress and the results, statistical analysis and interpretation will be presented

EX17

INHIBITION OF LIPID SYNTHESIS IN *MYCOBACTERIUM LEPRAE* BY THE ANTIBIOTIC CERULENIN.

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Cerulenin is an antibiotic isolated from the fungus, *Cephalosporium caerulens* and was found to be the first specific inhibitor of fatty acid synthesis. Fatty acid synthesis has been proposed as a possible target for antimycobacterial drugs. *M. leprae* has a lipid-rich envelope which contributes to virulence and antibiotic resistance. We have tested the effect of cerulenin on the synthesis of the *M. leprae*-specific lipids, phenolic glycolipid-I (PGL-I) and phthiocerol dimycocerosic acid (DIM).

Exogenous ^{14}C-palmitate is readily incorporated into the specific lipid fractions of *M. leprae* maintained, but not growing, in axenic medium. *M. leprae* harvested from the footpads of experimentally-infected athymic nude mice was dispensed into Bactec vials containing 1-^{14}C-palmitic acid in 7H12 medium and cerulenin (5µg/ml). Vials without cerulenin served as control. The vials were incubated at 33°C and at prescribed time intervals the $^{14}CO_2$ evolved was measured in the Bactec radiometric system and the contents of the vials lyophilized. After total lipid extraction of the lyophilized media, PGL-I and DIM fractions were separated by column and thin-layer chromatography and the radioactivity enumerated.

Cerulenin totally inhibited the incorporation of 1-^{14}C-palmitic into the PGL-I and DIM fractions of *M. leprae*. Although the presence of cerulenin resulted in a reduction of $^{14}CO_2$ evolved, the inhibition was not comparable to that seen in the lipid fractions. This indicates that the oxidation of palmitate was not adversely effected. Mycocerosic acid is an integral part of both the PGL-I and DIM structures and is formed by a condensation reaction involving long chain fatty acids and propionic acid. We can hypothesize from this data that the condensation reaction is inhibited by cerulenin. Further studies with cerulenin may provide an insight into the synthesis of these characteristic lipids found in *M. leprae* and the suitability of using this compound as an antimycobacterial agent.

EX18

THE INFLUENCE OF DAPSON ON BIOLOGICAL RHYTHMS OF BLOOD COUNT

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A chronobiological approach is increasingly used in studies of pathogenic mechanisms of different immune-mediated diseases, including infections, as well for improvement of available methods of therapy. Our preliminary data on changes in biological rhythms of some blood counts in rats given a long-time therapy with dapsone were presented at the XIV International Leprosy Congress (1993). We continued studying the influence of DDS-therapy on biorhythms of red and white blood picture in mice (circadian rhythms), in rats (season rhythms) and, retrospectively, in leprosy patients under therapy of long duration (perennial rhythms). It was found out that one-time administration of dapsone to mice resulted in changes in chronostructure of red and white blood counts. The effect was dependent on the time (morning-evening) and season of administration. In rats continuous administration of dapsone resulted in changes in perennial fluctuations in chronostructure of lymphocyte count and other blood indices, including biochemical ones. In long treated leprosy patients the most significant changes were noted in annual rhythms of lymphocyte counts. Thus, it may be concluded that if while short-term administrations of DDS cause dissimilar chronostructural changes in blood picture, these changes are abated in prolonged treatment with dapsone, probably, in the course of adaptive process. The most significant changes are noted in chronostructure of lymphocyte count that might be due to immunotropic activity of the drug.

EX19

CHROMOSOMAL ABERRATIONS IN BONE MARROW AND GERM CELLS OF MICE TREATED WITH ANTI-LEPROTIC DRUGS.

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The frequencies of chromosomal aberrations (CA) caused by antileprotic drugs (Dapsone-D, Rifampicin-R, and Clofazimine-C) used in the multidrug therapy in bone marrow(BM) and germ cells (GC) of Swiss albino mice, with or without M.Leprae inoculation, were studied. It was shown that the drugs under study caused CA both in BM and GC. In the M.Leprae inoculated animals (treated and untreated) both mitotic and meiotic cell division appeared to be inhibited as shown by low mitotic and meiotic indices. The mean frequencies (100 metaphase/animal) of major CA (chromosome rings and dicentric) in BM of M.Leprae inoculated (MLI) and non-inoculated (MLN) mice after 2 months of treatment with 2 drugs (D+ R) were in MLI - 0.18 and in MLN - 0.27 and with 3 drugs (D+R+C) in MLI - 0.15 and in MLN - 0.25. In GC of drug treated MLI mice, in addition to chromosome rings and dicentric, more numbers of fragmented chromosomes were also observed. After 2 months of drug treatment the mean frequencies of CA in GC of MLI mice were D+R - 0.27 and D+R+C - 0.14 and of MLN mice, D+R - 0.16 and D+R+C - 0.14. The prolongation of drug treatment to 10 months showed a decrease in CA both in MLI and MLN animals. The antileprotic drugs and M.Laprae caused CA types which are likely to result in cell killing of both BM and GC.

EX20

REACTIVITY OF A POPULATION OF ARMADILLO LYMPHOCYTES WITH AN ANTIBODY TO HUMAN Γ,Δ T-CELLS.

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Background. Inability to identify distinct lymphocyte populations in the nine-banded armadillo (*Dasypus novemcinctus*) has hindered immunologic studies of *M. leprae* infection in this animal model.
Design. Leukocytes from 47 armadillos were screened by flow cytometry with a panel of 16 monoclonal antibodies (mab) against human or murine leukocyte antigens.
Results. The only reactivity observed was with antibody TCRδ1, directed against an epitope on the human δ chain. Among circulating lymphocytes, this mab identified a distinct, bright population in all animals, accounting for 2.0 - 47.1% of lymphocytes (median 10.6%). Compared to blood, the percentage of γ,δ-reactive lymphocytes was smaller in lymph nodes and greater among intestinal intra-epithelial lymphocytes; the percentage was variable in the spleen, thymus, bone marrow, and in cutaneous lepromas. Armadillos with disseminated *M. leprae* infection had a significantly greater percentage of circulating γ,δ-reactive lymphocytes than did naive or resistant armadillos (P<0.05).
Conclusion. This is the first described reactivity of armadillo lymphocytes with an antibody to a lymphocyte surface antigen. The percentage andistribution of γ,δ-reactive lymphocytes is similar to that of sheep and bovines. The role (if any) of these cells with *M. leprae* infection in armadillos remains to be determined.

EX21

FATE OF M.LEPRAE INJECTED INTRANEURALLY IN A MURINE MODEL : ITS VIABILITY, FOLD INCREASE AND CLEARANCE IN THE SCIATIC NERVE OF SWISS WHITE MICE.

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In an experimental study,freshly harvested M.leprae inocula containing around 10^7 bacilli were microinjected into the sciatic nerves of normal and immunosuppressed (TR) mice. Massive but localized predominantly epitheloid and macrophage granulomas were readily obtained in normal and TR mice respectively. In order to determine the fate of M.leprae within the neural granuloma, its load, viability and clearance if any were investigated sequentially using semiquantitative measures.

The average M.leprae yield per nerve assessed at regular intervals beginning 0, 24, 72 hours and 2, 3, 4, 12 and 24 weeks showed neither a significant increase nor a decrease. Viability of M.leprae derived from the intraneural compartment were tested

using the standard mouse foot pad method. There was a marginal decrease in viability as compared to baseline growth, effective at 24 hours and remained more or less static till six months. There was no significant difference in the morphological index of M leprae. These findings were reaffirmed in 3 independent experiments and using histopathology as well as electron microscopy. One of the most important future application of this model lies in assessing the efficacy of vaccine/ immunomodulators in clearing and killing the bacteria from within the nerve.

EX22

PROTEIN PHOSPHORYLATION STUDIES IN LEPROSY

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Protein phosphorylation mediated by protein kinases play an important role in the regulation of cellular signalling mechanisms. Protein phosphorylation plays a role in uptake of pathogenic bacteria by host cells.

The study had the following objectives:

1. To study protein phosphorylation in normal human peripheral nerve and in patients affected with leprosy.

2. To study the effect of M.leprae on peripheral nerve protein phosphorylation.

The results show that :

i) The nerves of 10 out of 11 leprosy patients showed decreased 25 KDa protein phosphorylation.

ii) Purified M.leprae inhibits phosphorylation of the 28-30 KDa protein of rat/mouse peripheral nerve and the 25 KDa protein of human peripheral nerve.

The significance of these results in the pathogenesis of leprosy and nerve damage will be discussed.

EX23

PROTEIN PHOSPHORYLATION IN MYCOBACTERIUM LEPRAE

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Bacterial protein kinases are shown to regulate cell cycle, metabolite transport and sporulation. Protein phosphorylation studies in M leprae have not been reported. The present study has been taken up to observe the existence of protein phosphorylation by intrinsic protein kinases in purified mycobacterium leprae derived from human leproma skin biopsies.

Reaction mixtures in which M leprae homogenate was incubated with gamma [32 P] ATP under normal assay conditions were subjected to SDS gel electrophoresis and autoradiography. The autoradiogram showed two prominent high molecular weight proteins 200 K and 150 K phosphorylated. The other bands are 100K, 70 K and 45 K. The phosphorability of these bands reduced significantly with time after isolation.

When M leprae was used as a substrate for cyclic AMP dependent protein kinase it phorphorylated 220 K, 150 K, 100 K, and 70 K protein bands. Alkali treatment of the polyacrylamide gel to distinguish the serine, threonine and tyrosine residues suggested that protein phosphorylation is mainly on serine residues.

EX24

THE SUSCEPTIBILITY TO *MYCOBACTERIUM LEPRAE* OF NF-IL6 KO MICE AND INDUCTION OF CYTOKINES

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Transcription factor, NF-IL6 recognizes the same nucleotide sequences as C/EBP and it is predominantly expressed in macrophages. Tanaka et al. reported that NF-IL6 KO mice are highly susceptible to *Listeria monocytogenes* and *Salmonella typhimurium*, due to impairment of bacteria killing by activated macrophages. We have tried to see the susceptibility for *Mycobacterium leprae* infection in the NF-IL6 KO mice and also we examined the cytokine gene expression and induction of cytokines such as TNF-α, IL-1-α, IL-6, IL-10, IL-12 and IGIF/IL-18 in the peritoneal macrophages and then IFN-γ and IL-10 by splenocytes.

NF-IL6 KO mice was found many leprosy bacilli in the peritoneal macrophages on 30 days after inoculation while that of the wild(NF-IL +/+)mice was showing disappear. Besides TNF-α IL-1-α and IL-12 production were observed stronger in culture supernatant of peritoneal macrophages of NF-IL6 KO mice than that of the wild mice. NF-IL6 KO mice shows predominantly multiplication of *M.leprae* on the abdomino-organs, such as omentum and also scrotum on 10 months after intraperitoneal inoculation.

IMMUNOLOGY

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A STUDY ON SERUM SIL-2R AND TNF-α LEVEL IN MB LEPROSY

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In order to study the cytokine level in multibacillary (MB) leprosy patients and get more knowledge of the probable correlation between the cytokine secretion as anti-infection immunological response and that due to specific cellular immuno deficiency in leprosy patients, the authors measured the serum SIL-2R and TNF-α level in 10 active MB patients (including 7 relapses), 18 cured persons affected by MB leprosy and 19 normal local subjects in Lanxi and Tongxiang cities of Zhejiang province.

The results showed that the serum SIL-2R and TNF-α levels were markedly higher than those in normal individuals and clinically cures of MB leprosy ($p<0.01$-0.001), and there was a positive correlation betwee SIL-2R level and TNF-α level in MB patients ($t=2.536$, $r=0.667$). The SIL-2R level in active LL patients and the TNF-α level in relapses of MB were the highest as compared with those in others, but the SIL-2R and TNF-α levels in cures of MB were lower than those in normal