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

IM01

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

controls. The authors suggested that there were abnormal cytokine secretion of SIL-2R or TNF-α and inhibited immuno function in MB patients. High TNF-α level was not protective to the patients. Serum SIL-2R and TNF-α levels were closely associated with the severity of the disease of MB patients and with relapse of the disease as well. It told us that determination of SIL-2R and TNF-α levels may be of some value to predict the patient's immuunological status and prognosis of their disease.

IM02

DEVELOPMENT OF A NEW PEPTIDE-BASED SKIN TEST FOR LEPROSY

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Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The mode of transmission of *M. leprae* is not well understood and thus improved skin test reagents for the detection of infection with *M. leprae* would be of considerable benefit for leprosy control programs.

The available skin test reagents for leprosy, like lepromin, contain cross-reactive mycobacterial antigens and it is therefore not possible to know if skin test positivity to lepromin is due to exposure to or infection with *M. leprae*, vaccination with *M. bovis* BCG, or exposure to other mycobacteria.

For the study, 19 synthetic peptide pools each containing 10 or 11 peptides were provided by WHO GPV/VRD & TDR. The peptides were then tested for their ability to induce T-cell proliferation and interferon-gamma secretion *in vitro* using blood samples from tuberculoid leprosy patients and healthy leprosy contacts.

Our results showed that many of the peptide pools induced good T-cell responses, but that no single peptide pool was immunodominant. Four synthetic peptide pools containing 40 to 43 peptides were selected for further testing. The results of the present and the future studies with the selected peptides will be discussed.

IM03

WHO SYNTHETIC PEPTIDE SKIN TEST INITIATIVE

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The overall objective of the WHO IMMYC Synthetic Peptide Skin Test Initiative is to develop a new, synthetic peptide-based skin test reagent which would allow the identification of individuals infected with, or exposed to, *Mycobacterium leprae*. A total of 193 synthetic peptides were selected for testing, based on 5/15 amino acid mismatches between the *M. leprae* and *M. tuberculosis* sequences, or sequences from the *M. leprae* genome predicted to contain DR binding motifs. Initial screening of 19 peptide pools, each containing 10-11 peptides, was performed in 4 centres. Lymphocyte proliferation and IFNγ production using PBMC from tuberculoid leprosy patients or healthy staff contacts revealed broad T cell recognition of the peptide pools. In the second round, individual peptides from 8 pools will be screened. We hope this approach will identify leprosy-specific peptides which could be pooled for use as a new skin test reagent.

IM04

EVALUATION OF A RAPID ML DIPSTICK ASSAY FOR DETECTION OF IgM ANTIBODY IN LEPROSY

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Although the ELISA has been well established for leprosy, there is no rapid and simple serologic test to measure antibody to *M. leprae* in developing countries where the disease is endemic. The ML Dipstick Assay was recently developed at the Royal Tropical Institute to meet this need by detecting binding of human IgM to a semi-synthetic analog of *M. leprae* phenolic glycolipid - I (PG-I), a well known ELISA antigen. The result of this phenomena, is a dichotomy where the technology dependent assays like ELISA are most effective in developed countries or central laboratories far from the point of transmission and occurrence of leprosy. The recent push to eliminate leprosy as a public health problem has intensified the focus on the endemic countries, bringing control efforts to local levels often located in remote regions distant from central laboratories where the ELISA might be applied. Detection of antibody to PG-I can enhance local control programs by serving as screening test for the ELISA, and assisting in recognition of potential multibacillary (MB) cases among household contacts. Since this test has important potential in the " end game" of control at the local level, we evaluated the assay against a variety of 140 known sera from leprosy patients and individuals free of leprosy living in endemic and non-endemic environments. The sera were grouped according to ELISA results. ELISA positive sera were from 69 MB leprosy patients with range 0.16 to >0.90 OD units. The ELISA values of the 19 sera were from treated patients, 21 sera from endemic negative controls from Cebu, and 31 sera from non-endemic population in Chicago, were less then 0.15 OD units. Both of the endemic and non-endemic negative control sera were obtained from individuals known to be free of leprosy. There was no significant difference between the negative control sera from the endemic and non-endemic populations($p > 0.68$). Also, the 96% of the leprosy cases were detected. In addition, the assay correctly interpreted 96% of the negative control sera, showing that the specificity is good. The treated cases did not give any conclusive results, since 53% of the treated cases showed a low positive result in the dipstick assay. In conclusion, the dipstick assay is a simple alternative to the ELISA assay, which can be used remote areas. It is quick, specific, and low in cost and effort, not requiring extensive training. It can be used in the field to identify household contacts with the high potential of incubating and developing MB disease.

IM05

THE APPLICATION *MYCOBACTERIUM LEPRAE* PARTICLE AGGLUTINATION (MLPA) TEST AND ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) ON DISTRIBUTION AND PERSISTENCE OF IGM ANTIBODIES AMONG INDIVIDUALS IN LEPROSY ENDEMIC AREA, SOUTH SULAWESI, INDONESIA.

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In this study, we applied a *Mycobacterium leprae* particle agglutination (MLPA) test and ELISA that detected a IgM against Phenolic Glycolipid-I (PGL-I) of *M. leprae* on sera collected through a total population survey and longitudinal study from individuals in leprosy endemic area, South Sulawesi Indonesia.

Among the total tested population of 1496 people, 40.4 % (604/1496) were found to be seropositive using MLPA test in the first survey (survey I). Seropositivity was shown to be randomly distributed among the population and the seropositivity rates in female were significantly higher than in males.

To evaluate the persistence of seropositivity, we followed up some persons every 6 months during one year (three times examination). These results suggested that there was no significantly different results in seropositivity rates in three times examination among individuals (survey I and survey II and III or survey I and III).

Also, we compared a MLPA test with the conventional ELISA to detect IgM PGL-I in the same person. Percentage of concordance between MLPA and ELISA for positive or negative results was satisfying and there was no significant difference in positivity rates between MLPA test and ELISA.

IM06

A SIMPLE DIPSTICK ASSAY FOR THE DETECTION OF ANTIBODIES TO PHENOLIC GLYCOLIPID - I OF *M.LEPRAE*.

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Among the many reported applications of the detection of antibodies to phenolic glycolipid I of *M.leprae*, in particular the use of seroprevalence as an indicator of the magnitude of the leprosy problem may turn out to be very useful to leprosy control programmes. An operational function of serology within the leprosy control services requires a simple test system. We have developed a simple dipstick assay for the detection of antibodies to PGL-I and we compared its performance with that of ELISA. A high degree of agreement (97.2%) was observed between ELISA and the dipstick assay when tested on 435 sera; the agreement beyond chance (Kappa value) was 0.92. No significant difference was found between the dipstick assay and ELISA when seropositivity rates obtained in groups of leprosy patients, household contacts and controls were compared. The interpretation of the dipstick results as positive or negative was unequivocal as illustrated by the high agreement between different persons reading the test (kappa values >0.88). An agreement of 94.9% (kappa=0.87) was found when dipstick results obtained with whole blood were compared with ELISA results obtained with the paired serum samples. Storage of the only reagents required, the dipsticks and the stabilized detection reagent, up to 3 months under "tropical" conditions of high temperatures, high humidity and exposure to light did not influence the results of the assay. The dipstick assay described here is an easy-to-perform method for the detection of IgM antibodies to PGL-I of M.leprae; it does not require any equipment and the highly stable reagents make the test robust and suitable for use in tropical countries. An internal control validates the performance of the assay. This dipstick assay may be the method of choice for epidemiological mapping of leprosy.

IM07

SIMPLE BLOOD TESTS TO DETECT EXPOSURE TO LEPROSY

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New tools to detect leprosy exposure in the community will be necessary for the eradication of leprosy. A simplified blood test has been applied to determine whether exposure to leprosy can be detected by the production of the cytokine, interferon γ after stimulation of whole blood cultures with leprosy proteins.
First we investigated whether the production of interferon γ after 24 hours was associated with lymphocyte proliferation and interferon γ production after 5 days, and which of the leprosy protein antigens available were the most potent. A recombinant 35kD protein and preparations of native proteins such as sonicates of armadillo derived *M. leprae* were found to be potent in inducing the production of interferon γ at 24 hours and this was significantly associated with high levels of cytokine at 6 days and with lymphocyte proliferation.
24-hour cultures of whole, undiluted blood from leprosy patients and contacts and healthy subjects without exposure were incubated with r35kd, MLS, MLS-LAM, and MLCwA antigens. A IFNγ response was detected in contacts to MLS-LAM antigen. This response was significantly higher in leprosy health workers than in healthy non-exposed subjects. These results indicate the utility of measuring leprosy exposure by a new simple overnight blood test.

IM08

NEW SKIN-TEST ANTIGENS FOR THE DIAGNOSIS OF LEPROSY.

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A major challenge of current research is to provide new tools of sufficient sensitivity and specificity to identify subclinical infection and facilitate epidemiological monitoring of the disease. Serological and gene amplification approaches have not met the needs. A remaining hope lies in new skin test antigens. Past products--the lepromins, the Rees MLSA and Convit SDA--have their place in leprosy control but fail as universal diagnostic/epidemiological tools. We have produced under prescribed pilot-plant conditions two new skin test reagents, *M. leprae*-soluble antigens devoid of LAM and other immunosuppressive carbohydrates (MLSA-LAM) and *M. leprae* cell wall antigens (MLCwA). Those have been submitted to a full gamut of biochemical and immunological analyses to identify the inherent immunogens; *in vitro* assays to confirm the absence of endotoxin; *in vivo* safety tests in mice; assays for potency, sensitivity and specificity in *M. leprae*-sensitized guinea pigs; and further *in vitro* assays for potency in stimulating T-cell proliferation and the evocation of γ-IFN and other cytokines in blood from appropriate patient groups. We are now awaiting approval by regulatory authorities for Phase I human trials in the U.S. before formulation of Phase II trials towards application of these products in leprosy endemic areas in order to test in field settings their actual potency, specificity and suitability for wide-scale application. Separately, we will report on studies of MLSA-LAM and MLCwA as subunit vaccines. Supported by NIAID/DMID Contract NO1 AI 55262.

IM09

EMERGENCE OF TH1 LIKE RESPONSES DURING LEPROSY REACTIONS

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Leprosy patients suffer from clinical episodes associated with tissue damage which are designated as Type 1 (reversal reaction) when localised to the lesions and Type 2 (erythema nodosum leprosum, ENL) when accompanied by systemic involvement. We had reported earlier that stable, non reaction lepromatous leprosy subjects show Th2 and Th0 but not Th1 like responses in the peripheral blood. To further understand the development of Th like responses during disease, 32 lepromatous patients undergoing reactions were studied using cytokine specific RT-PCR and ELISA in peripheral blood and some skin biopsies. Of interest was the emergence of Th1 like response with presence of interferon-gamma (IFN-gamma) and absence of interleukin-4 (IL-4) mRNA in the PBMC of 85% and 64% of Type 1 and 2 reaction patients respectively and in all reaction sites. Whereas Th0 was seen in some, Th2 like response was absent. IL-12p40 mRNA was seen in 21/25 ENL and all Type 1 reaction subjects irrespective of the Th phenotype. IL-12p40 and IFN-gamma were detectable in unstimulated PBMC suggesting an in vivo priming during reactions. IL-10 was mainly associated with adherent cells and showed a differential expression in the two reactions. It was present in the PBMC of ENL but not in reversal reaction patients. Moreover, it was not detectable in the skin lesions of either type of reactions. Th1 like cytokine profile was associated with immunopathology and persisted up to 6-7 months after the onset of reactions.

IM10

IMMUNOHISTOCHEMICAL ANALYSIS OF MYCOBACTERIAL ANTIGENS IN SKIN LESIONS OF LEPROSY PATIENTS RELATED TO REACTIONS

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At present identification of antigenic recognition by T cells derived from leprosy patients across the clinical spectrum, including the reactions, are still lacking. The strategy of the present study is to demonstrate in situ presence of the immunodominant mycobacterial antigens (both **m.leprae** specific and cross reactive) in relation with the local cell mediated immune status in the lesional skin specimens and systemic humoural immunity of patients. Towards this goal, we applied

immunohistochemical (IHC) methods using mAbs to **m.leprae** specific phenolic glycolipid-1 (PGL-1) and to cross reacting mycobacterial antigens (CRMA) of 30KD, 36KD, 65KD and lipoarabinomanan (LAM). In addition, we also studied the association of the *in situ* presence of antigens with the presence of viable bacilli by NASBA as well as with the circulating **m.leprae** specific antibody levels in serum.
Results: The staining patterns with mAbs to all the protein CRMA were heterogeneous that could also be seen in the lesions of other skin diseases. However, *in situ* staining of PGL-1 and LAM could only be seen in leprosy. These antigens are abundantly present in infiltrating macrophages in the lesions of untreated multibacillary (MB) patients whereas only PGL-1 was occassionally seen in scattered macrophages in some untreated paucobacillary (PB) patients. During treatment clearance of PGL-1 from granulomas in MB lesions was seen before that of LAM, although the former persisted in scattered macrophages in some treated patients. The persistance of PGL-1 in the lesions paralleled high serum anti PGL-1 antibody titres and was not indicative for the presence of viable bacilli in the lesions. Interestingly, we also observed that the dynamics in the expressions of PGL-1 and LAM in the lesions of patients with the presence of acid fast bacilli during the course of the disease are associated with the reactional states of the patients.
The implications of the present study indicate that the IHC study for *in situ* presence of mycobacterial antigens in conjunction with the estimation of anti PGL-1 antibody titres in leprosy patients will be valuable in the future Leprosy containment protocol.

IM11

THE ROLE OF CYTOKINES IN THE PATHOGENESIS OF ENL

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Erythema nodusum leprosum (ENL) is an acute inflammatory state of lepromatous leprosy characterized by painful cutaneous lesions, severe systemic symptoms and accelerated, irreversible nerve damage. We and others have reported that ENL is associated with high levels of plasma TNF-α which fall precipitously following treatment with thalidomide. The drug has been shown to inhibit the production of TNF-α but not IL-1β, IL-6 or GM-CSF. We have recently shown that thalidomide stimulates T cells *in vitro*, particularly the $CD8^+$ T cell subset. The drug increases T cell proliferation and also the production of TH1 cytokines IL-2 and IFN-γ. The effect of thalidomide on T cells *in vivo* is currently being evaluated in patients with ENL. We are also examining the biologic effects of a number of newly synthesized analogues of thalidomide which have increased TNF-α inhibitory activity compared to the parent drug. If found to be more efficient and less toxic than thalidomide, it is hoped that the drugs will provide alternative therapies for ENL.

IM12

A DISTINCT Th1/Th2 PATTERN OF CYTOKINE mRNA EXPRESSION IN LEPROSY PATIENTS UNDERGOING REACTIONAL EPISODES. Moraes MO, Sarno EN, Almeida AS, Saraiva BCC, Nery JAC, Sampaio EP. Leprosy Laboratory, Oswaldo Cruz Foundation, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, Brazil.

Leprosy reactions are still a major health problem. Patients during the natural course of
the disease may undergo reactional states that can be characterized by immunoinflammatory episodes. Our previous studies have indicated that TNFα play a key role in the inflammatory process mediating tissue damage during erythema nodosum leprosum. Since the role of other cytokines in the regulation of the inflammatory response in leprosy is not fully understood, we decided to evaluate the cytokine profile of blood and tissue samples obtained from patients among the clinical spectrum of leprosy, by using RT-PCR. We analyzed 20 blood samples (BL/LL = 5; BT/TT = 5; ENL = 5; RR = 5) and 14 skin biopsies (BL = 4; ENL = 7; RR = 3). For a qualitative approach, we analyzed the presence of a PCR product for each cytokine tested in agarose gel electrophoresis. We observed that there is an increase of mRNA synthesis of IFNγ, GM-CSF, p55 and perforin in patients during the reactional episodes in blood samples. On the other hand, in biopsy specimens, we were able to detect both IFNγ and IL-10, in LL or ENL and RR lesions. Moreover, IL-6 mRNA is observed only in the reactional biopsies. The sensitivity of the approach did not allow a distinction among patients' group for most cytokines tested. Then, we performed a semi-quantitative RT-PCR, and verified that there is an up-regulation of TNFα, IFNγ and IL-6 mRNA during the inflammatory episodes. The data reported so far suggest a distinct Th1/Th2 pattern observed in, leprosy reactions. Besides, a more complex mRNA cytokine profile showing IFNγ, IL-10, TNF,IL-4 and IL-6 is detected. This pattern results probably from an immunological reactivation that might lead to the acute inflammatory response in these patients.

Supported by CNPq and WHO/TDR grants

IM13

A POSSIBLE ROLE FOR IFNγ AND IL-12 IN THE PATHOGENESIS OF ERITHEMA NODOSUM LEPROSUM. Sampaio EP, Moraes MO, Saraiva BCC, Almeida AS, Teles RMB, Sarno EN. Leprosy Laboratory, Oswaldo Cruz Foundation, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, Brazil.

Acute inflammatory episodes can occur during the natural course of leprosy. A classification of these reactional states is obtained according to the clinical status of the patients. The type I or reversal reaction (RR) is described as an activation of the immune response that is triggered into a previous unresponsive patient. The mechanisms that are involved in the development of erythema nosodum leprosum (ENL) or type II reaction is not fully understood, although a pivotal role for TNFα has already been demonstrated. It has been previously described high TNFα levels in ENL patients' sera, as well as an increase of TNFα mRNA and protein expression in the tissue during ENL. In order to understand the immunological reactions that can lead to the acute inflammatory response, semi-quantitative RT-PCR for IFNγ and IL-12 was employed in the skin biopsies of leprosy patients undergoing or not reactional episodes. Samples of the same patient were collected before and during ENL (n=2) or after thalidomide (n=3) and pentoxifylline (n=4) treatment. As a control, tissue samples from patients undergoing RR and after being treated with prednisone (n=3) were also included. IFNγ mRNA was up-regulated during the reactional episodes and it was observed that after anti-inflammatory treatment, IFNγ mRNA was down-regulated. Besides, IL-12 mRNA was intensely expressed in the skin biopsies during the reaction, and was negatively regulated after thalidomide and prednisone administration. These results together suggest that IFNγ and IL-12 could be participating in the establishment and development of ENL reactions.

Supported by CNPq and WHO/TDR grants

IM14

LYMPHOCYTE-MACROPHAGE CONTACT PROVIDES A POTENT STIMULI AMPLIFYING TNFα PRODUCTION IN LEPROSY. *Sampaio EP., *Oliveira RB., **Warwick-Davies J., **Griffin GE., *Hernandez MO., * Sarno EN, **Shattock RJ.
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During the natural course of disease, leprosy patients may undergo reactional states which represent immunoinflammatory episodes in response to the mycobacterium. Our previous studies have indicated that TNFα plays a key role in this process mediating local and systemic symptoms mainly in type II reaction (ENL). Increased interest has developed to define the cells and or secreted mediators that are involved in the amplification of TNFα production in leprosy. Previous reports have demonstrated that engagement of integrin receptors regulated both mRNA expression and production of inflammatory mediators. In the present study, we investigated whether T cell-macrophage contact had any role in amplification of TNFα secretion in leprosy.
Co-cultures of activated (PMA 5 ng/ml and PHA 1 μg/ml) paraformaldehyde fixed T-cells (HUT-78 T-cell line) and human monocytes prepared by centrifugal elutriation were established in vitro in the presence or absence of *M. leprae* (0.3 - 10 μg ml), and supernatants harvested after 18-20 hours for determination of TNFα levels (Elisa, R&D Systems).
Interaction of the T-cell line with monocytes up-regulated *M. leprae*-induced TNFα secretion by monocytes. Cell contact between T-cells and monocytes was required since physical separation of these cells abrogated this response. Pre-activation of human monocytes with IFNγ also lead to enhanced TNFα secretion induced by *M. leprae*. In this context, we have observed that PBMC obtained from ENL patients released increased TNFα compared to purified monocytes obtained from the same individuals. Moreover, if purified monocytes were reconstituted in vitro with lymphocytes, TNFα release was similar to that released in the original PBMC cultures from the same individuals. In summary, direct contact between T-cells and monocytes amplifies production of TNFα by monocytes in response to *M. leprae*. This finding is relevant to the observation of increased expression of TNFα in the reactional leprosy lesion where lymphocytes and macrophages are in close proximity, a phenomena not seen in the unreactional tissue.

Supported by a Wellcome Trust grant

IM15

ROLE OF INTERLEUKIN-12 (IL-12) AND IL-12 RECEPTOR SIGNALLING IN PATIENTS WITH DISSEMINATING INFECTIONS WITH M. LEPRAE AND OTHER MYCOBACTERIA

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IL-12 plays a crucial role in immunity to mycobacterial pathogens by promoting T helper-1 differentiation, IFN-γ production and natural killer cell function. Lepromatous leprosy patients' specific T cell nonresponsiveness to M. leprae could be overcome effectively by the synergistic action of IL-12 and IL-2 in vitro. In addition, patients with disseminated, nonleprous mycobacterial infections appeared to produce strongly reduced levels of IFN-γ. This was due to genetic mutations in the IL-12R complex, which led to lack of IL-12R expression and function. These findings document a novel immunodeficiency and highlight the crucial role of IL-12 in immunity to mycobacterial pathogens in man.

IM16

LEPROSY PATIENTS WITH LEPROMATOUS DISEASE RECOGNISE CROSS REACTIVE T CELL EPITOPES IN THE M. LEPRAE 10KDA ANTIGEN

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T cell responses play a critical role in determining protective responses to leprosy. Patients with self limiting tuberculoid leprosy show high T cell reactivity while patients with disseminated lepromatous form of the disease show absent to low levels of T cell reactivity. Since the T cell reactivity of lepromatous patients to PPD, a highly cross reactive antigen, is similar to that of tuberculoid patients we queried if lepromatous patients could recognise cross reactive epitopes in M. leprae antigens as well. T cell responses were analysed to a recombinant antigen 10kDa (a heat shock cognate protein) which is available from both M. tuberculosis (MT) and M. leprae (ML) and displays 90% identity in its amino acid sequence. Lymphoproliferative responses were assessed to ML and MT 10kDa in newly diagnosed leprosy patients (lepromatous; N=23; tuberculoid; N=65;). Lepromatous patients showed similar lymphoproliferative responses to ML and MT 10kDa while tuberculoid patients showed much higher responses to ML 10kDa compared to MT 10kDa suggesting that the tuberculoid patients may be recognising both species specific and cross reactive epitopes in ML 10kDa while lepromatous patients may be recognising only cross reactive epitopes. This was further supported by linear regression analysis. Lepromatous patients showed a high concordance in T cell responses between ML and MT 10kDa using (r= 0.658; p<0. 0006) not observed in tuberculoid patients (r=.203; p>0.1). Identification of cross reactive T cell epitopes in M. leprae which could induce protective responses should prove valuable in designing second generation peptide-based vaccines.

IM17

T CELL AND CYTOKINE REACTIVITY TO THE *Mycobacterium leprae* 45 kDa ANTIGEN BY LEPROSY PATIENTS, CONTACTS, AND ENDEMIC CONTROLS

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Peripheral blood mononuclear cell (PBMC) proliferation and interferon γ (IFN) production by leprosy patients, household contacts, and endemic controls to the *M.leprae* 45 kDa antigen and other mycobacterial proteins were compared. A total of 48 leprosy patients (34 lepromatous and 14 paucibacillary) were diagnosed and recruited in Mexico according to the Ridley & Jopling criteria. The contact group consisted of 17 individuals living in the same house as the leprosy patients, and 20 healthy subjects were studied as leprosy endemic controls. T-cell proliferation assays and ELISA for the detection of IFN in culture supernatants were performed following standard protocols in our laboratory. A Mann-Whitney U test was used for the statistical analysis. All patients, household contacts, and endemic controls gave strong proliferative responses and IFN production in response to the mitogen PHA. The majority of TT/BT cases responded to the *M.leprae* 45, and 30/31 kDa antigens, whereas the lepromatous, household, and endemic control groups showed significantly lower responses to these proteins. A similar pattern was observed with the IFN production. Our results demonstrate that the *M.leprae* 45 kDa protein is a potent T-cell antigen. The responses to the *M.leprae* 45 kDa were on average higher than those obtained with the 65, 30/31, and 10 kDa leprosy antigens. The 45 kDa antigen was recognised by a high proportion of leprosy cases and household contacts but not by healthy endemic controls or patients with other mycobacterial disease. This antigen may have potential as a reagent for skin testing.

IM18

HEAT SHOCK PROTEIN AND APOPTOSIS OF PERIPHERAL BLOOD MONOCYTES IN LEPROSY PATIENTS AND HEALTHY INDIVIDUALS

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Factors determine the outcome of exposure of human immune system to Mycobacterium leprae antigen are poorly understood. Estimation of heat shock protein (Hsp) 65kd and 70 kd which may represent one of the major targets of cell mediated immune response were evaluated by Western blot in skin biopsies of 46 untreated leprosy patients, 15 reactional leprosy patients and 25 age and sex matched healthy control . Results showed that 65 kd Hsp was significantly higher in reactional state groups, multibacillary leprosy and baucibacillary. Seventy kd Hsp was significantly lower in control group than other groups. Reversal up grading reaction (type I) showed highly significant expression of 65 kd and 70 kd Hsp.

The possible involvement of apoptosis or programmed cell death (PCD) of peripheral blood mononuclear cells (PBMC) was evaluated in leprosy patients. The peripheral blood mononuclear cells were prepared and incubated for 24, 48 and 72 hours in tissue culture medium. The percentage of apoptotic PBMC at 0 time, 24, 48 and 72 hours of each culture media was assessed by both morphology and DNA fragmentation. There was significant increase of percentage of apoptotic cells with aging in culture medium from normal control subjects, paucibacillary, multibacillary and reactional groups (typeI, II).

There was no significant difference in the percentage of apoptotic PBMC between paucibacillary and healthy control subjects. On the other hand there was higher significant difference in multibacillary than other groups. Interestingly patients with upgrading reversal reaction (type I) showed inhibition of PBMC apoptosis in comparison to other groups. These results suggest that prompt death of PBMC in multibacillary leprosy may be due to decrease cell mediated immune response also could be considered as protective mechanism against highly replicated bacilli. It could be concluded that the increased expression of 70 Kd and CD5 kd Hsp increase resistance to apoptosis in leprosy.

IM19

DICHOTOMY AND PARADIGM OF TH1 AND TH2 SUBSETS IN RELATION TO THEIR CYTOKINE PROFILE IN LEPROSY PATHOLOGY.

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Changeable immunostatus of borderline leprosy patients during the course of the disease represent an unique model to investigate the paradigm of Th1 and Th2 in humans. During the course of the disease, some of these patients show excerbated immune activities in the form of reversal reaction (RR) and erythema nodosum leprosum (ENL). In order to explore the role of T cell subsets in relation to their cytokine profile, we generated T cell clones (TCC) from the lesional skin of 7 untreated patients and again from 3 patients undergoing RR during treatment. Both ml responsive (mlR) and non-responsive (mlNR) TCC were generated with minimum selection. TCC consisted of all phenotypes ($CD4^+$, $CD8^+$, $TCR\gamma\delta^+$) that had similar distribution pattern of in vivo situation. In respect to the ratio INF-γ/IL-4 secretion profile, TCC were Th1, Th0 and Th2 irrespective of the clinical status of the patients. Interestingly, in 3/7 patients who (re)experienced RR a polarised shift to Th 1 among mlR TCC were seen whereas this shift did not occur among mlR TCC. Further analysis of a broad range of cytokines, showed a positive correlation in the co-production of INF-γ/TNF-α (xr = 0.81) and in that of IL-4/IL-5 (r = 0.83), IL-4/IL-13 (r = 0.80) by the mlR TCC. Furthermore, Th1 and Th2 mlR TCC can be characterised by the co-production of INF-γ/TNF-α and IL-4/IL-5/IL-13 respectively. Such clear cut correlated co-production of these cytokines was not seen among mlNR TCC of distinct isotypes of mlR Th1 and Th2. Interestingly, isotypic forms of both mlR Th1 and Th2 TCC can be recognised further by differential expression of these cytokines including IL-10 and IL-6. The present results suggest that distinct isotypes of mlR Th1 and Th2 characterised by a panel of cytokine profile reflect a pathogen associated characteristic of T cell subset. Furthermore, in the context of future leprosy containment programme in the next millennium, we hypothesise that monitoring the in vitro INF-γ/IL-4 section of primary isolates of lesional T cell lines could be useful for the diagnosis of reactions versus reactivation of the disease.

IM20

GRANULOMA FORMATION AND GROWTH OF *MYCOBACTERIUM LEPRAE* IN INOS DEFICIENT MICE.

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Production of nitric oxide (NO), a free radical generated from L-arginine by cytokine inducible nitric oxide synthase (iNOS), is a major anti-microbial mechanism of activated macrophages (ACT MΦ). To study the effects of NO on *Mycobacterium leprae* infection in mice, C57Bl/6 (B6) control mice, B6 mice given drinking water supplemented with 2.5% aminoguanidine (AG), an inhibitor of iNOS, and iNOS gene knockout (KO) mice were infected via two routes with freshly harvested, viable, nu/nu mouse-derived *M. leprae*. iNOS deficient mice inoculated in the foot pads exhibited a marked enlargement of the foot pads. Immunostaining for CD4 and CD8 differentiation antigens showed an enhanced infiltration of CD4+ cells into the foot pads of iNOS KO mice compared to controls. In mice infected intravenously, liver tissue from control mice showed slow development of granulomas, with moderate sized, non-organized inflammatory infiltrates surrounding the bacilli. However, AG mice and iNOS KO mice exhibited large, focally organized granulomas, many of which contained multinucleated giant cells. iNOS mRNA was detected in granulomatous liver tissue from control mice, but none was found in iNOS KO mice. Interestingly, high levels of iNOS mRNA were detected in AG mice. Immunostaining revealed a pattern similar to that seen in human tuberculoid granulomas, with many CD4+ cells dispersed throughout the granuloma and fewer CD8+ cells located at the periphery. In vitro, ACT MΦ from iNOS KO mice, as well as B6 ACT MΦ treated with AG, produced negligible nitrite and were incapable of killing *M. leprae*. Like ACT B6 MΦ, though, ACT iNOS KO MΦ generated elevated levels of TNF-α. Intracellular mycobacteriocidal events may thus be suppressed in vivo in the absence of NO, and perhaps in compensation, granuloma formation is greatly enhanced. These data suggest that NO, generated by iNOS, is an important regulator of cell mediated immunity to *M. leprae*.

IM21

THE ROLE OF TRANSFORMING GROWTH FACTOR - β (TGFβ) AND INDUCIBLE NITRIC OXIDE SYNTHASE IN LEPROSY REVERSAL REACTIONS: AN IMMUNOHISTOLOGICAL STUDY

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Inducible nitric oxide synthase (iNOS) synthesises reactive nitrogen radicals which have a role in mycobacterial killing. Expression of iNOS is induced during macrophage activation. Transforming growth factor β (TGF-β) antagonises Th1, mediated macrophage activation and promotes fibroblast activity.

Using immunocytochemical techniques iNOS and TGFβ were localised in paraffin embedded skin biopsies from lesions in 33 patients across the leprosy spectrum and a further in 15 patients with reversal reactions.

iNOS expression was highest at the turberculoid pole of the spectrum and increased during reversal reaction. TGFβ was detected throughout the leprosy spectrum but was highest at the lepromatous pole. Levels of TGFβ decreased during reversal reaction.

We propose that reduced levels of TGFβ may contribute to unregulated inflammatory responses during reactional episodes.

IM22

A STUDY ON SERUM TNF-α AND SIL-2R IN RELAPSES OF LEPROSY

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In order to study the immunofunction and cytokine's role in clinically cured persons affected by leprosy and get more knowledge of immunophathogenesis of leprosy, serum TNF-α level and SIL-2R level were measured in 62 cures of leprosy, 28 their lineal relatives and 28 randomly selected local normal persons in Lanxi City, Zhejiang Province. The results showed that the TNF-α level in all cures of leprosy was marked lower ($p<0.001$) and the SIL-2R level in cures of MB leprosy was lower than those in controls ($p<0.002$). The TNF-α and SIL-2R levels in cures of LL leprosy were the lowest ($p<0.001$, $p<0.005$). This suggested that there were still abnormal cytokine secretion and macrophages function disturbance in cured persons affected by leprosy. It also suggested that there were some disturbances in T cell's activation function in cures of MB leprosy. The persistence of lower TNF-α level and SIL-2R level may be correlated with the sensitivity of leprosy patients to M. leprae and may play an important role in immunopathogenesis of leprosy.

IM23

BCG PROPHYLAXIS FOR LEPROSY IN SOUTH INDIA

Leprosy Prevention Trial, Madras

Indian Council of Medical Research

Indian Council of Medical Research conducted a large scale BCG trial in Chingleput district in South India to study efficacy of BCG in preventing tuberculosis from 1968 to 1985. Leprosy component was added in 1978 and apart from the baseline survey, which covered about 200,000 available individuals from the "vaccinated" cohort, four follow-up surveys were conducted at 2.5 years' intervals. This study was a placebo controlled double blind study, involving 2 different strains (French and Danish) of BCG and 2 different doses (0.1 mg and 0.01 mg) of BCG in a factorial design. Initial screening of the population was done by paramedical workers and confirmation of diagnosis was done by medical officers. Analysis of this study has been recently completed.

French and Danish strains provided similar levels of protection. Usual dose of BCG (0.1 mg) consistently gave higher protection (24.4%), compared to the lower dose of 0.01 mg (17.4%) over 5-15 years of follow-up. Females were somewhat better protected (26.7%) than males (22.8%), but the difference was not statistically significant. Children in the age-group 0-4 years were protected best. However, the protective efficacy waned from 58% to 18% from 5 to 15 years after BCG vaccination. BCG provided protection against all clinical forms of leprosy. However, smear positive leprosy was not prevented. A total of 121 new smear positive cases were distributed uniformly over the three arms. Protective effect was also observed during the first 5 years based on the results of the baseline survey conducted 5 years after vaccination.

IM24

VACCINATION AGAINST LEPROSY USING 'NAKED DNA' VACCINES

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Two plasmid constructs containing the gene for the 35kD leprosy protein were constructed incorporating a cytomegalovirus (CMV) promoter and with or without a tissue plasminogen activator (TPA) leader sequence to give a membrane expressed or secreted 35kD protein.
Swiss albino mice 6-8 weeks were immunised with 3 doses of 100μg of DNA intramuscularly, at intervals of three weeks. Control mice received either 10^7 heat-killed *M. leprae* or 10^6 live BCG intradermally or three doses of PBS or control plasmid without the gene insert intramuscularly. One month after the last immunisation, all mice were infected in the hind foot pads with 10^4 live *M. leprae*. The effect of the immunisation was measured by the growth of *M. leprae* six months after infection.

IM25

MYCOBACTERIUM LEPRAE- AIDS VIRUS INTERACTIONS IN MONKEYS

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Seven of eight rhesus monkeys (RM) coinfected with *Mycobacterium leprae* (ML) and simian immunodeficiency virus (SIV, 8-10 months pre-ML) harbored acid-fast bacilli (AFB) at sites of dermal inoculation and/or at disseminated sites (nasal mucosa) at times of humane sacrifice (up to 270 days post-ML inoculation) due to SIV-induced debilitation or, in one long term survivor's case, to date over 3 years post-ML inoculation. Detectable AFB were cleared in biopsies of inoculation sites of RM inoculated with ML alone after 63 days postinoculation; these sites have, so far, remained AFB-negative, thereafter.

Compared to animals infected with ML alone, RM coinfected with SIV plus ML showed: 1) completely suppressed serum antibody responses to ML-specific phenolic-glycolipid-I and to mycobacteria-common lipoarabinomannan antigens, but strong anti-SIV Gp120 antibody responses; 2) impaired sensitization of blood mononuclear cells (MNC) to *in vitro* recognition of ML-specific antigens in blastogenic stimulation assays; 3) impaired *in vitro* responses of blood MNC to nonspecific (ConA) blastogenic stimuli and 4) early post-ML inoculation, there was a significant incremental diminution of percentages of blood CD4+CD29+ T-cells in addition to the existing SIV-induced diminished percentages of the CD4+CD29+ subpopulation of T-cells.

A follow-up study in groups of 6 RM showed that SIV given at the same time as ML is significantly more lethal than SIV inoculated 2 weeks prior to ML; both of these inoculation procedures are effective in inducing increased susceptibility to leprosy. Differences in relative timing of inoculation with the 2 agents result in differing degrees of modification in immune responses to ML antigens.

The results indicate that humoral and cellular immune responses to ML antigens are compromised in ML-inoculated RM that are coinfected with SIV and provide an immunologic basis for the demonstration of enhanced ML persistence or leprosy susceptibility in SIV-ML coinfected RM.

IM26

SIMIAN IMMUNODEFICIENCY VIRUS (SIV)-ASSOCIATED DELAY OF CD4 CELL ACCUMULATION AT *M. leprae* SITES *IN VIVO* IS ACCOMPANIED BY A DELAY IN LYMPHOCYTE RESPONSES *IN VITRO*.

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Background. The mechanisms by which immunity to mycobacteria is deranged in HIV infected individuals (and SIV-infected rhesus monkeys) is not known. HIV is reported to have little or no effect on leprosy.
Objectives: To assess T-cell subsets and cytokines at *M. leprae* inoculation sites in normal (SIV-)and SIV+ monkeys.
Methods. Biopsies were taken at cutaneous *M. leprae* inoculation sites in 4 SIV[-] rhesus monkeys and 8 SIV[+] rhesus monkeys (*Macaca mulatta*). CD4+ and CD8+ cells were counted in immunostained frozen sections, or in blood by flow cytometry. Lymphocyte transformation (LTT) was measured by ^{3}H-thymidine uptake in 6 day cultures with *M. leprae* sonicate antigen (MLS). mRNA for IL-2 and TNFα were evaluated by RT-PCR using primers & probes developed for rhesus monkeys.
Results. The percentage of CD4 lymphocytes in skin peaked at 5 days in healthy, SIV[-] monkeys, but was delayed until 27 days in the SIV[+] animals. In the SIV[-] group, IL-2 mRNA was detected in all animals by day 5 and the percent reacting in LTT increased steadily from 3 weeks onward. In SIV[+] animals, the percent of animals positive for IL-2 mRNA increased slowly until day 63; LTT reactivity was not observed until 5 weeks and only 4/5 were responsive at 15 weeks. TNFα expression was observed at inoculation sites in all animals, similar in both groups.
Conclusions. (1) CD4+ T-cell participation in situ in SIV[+] animals is delayed. (2) Robust mechanisms of recruitment appear to remain in SIV[+] animals: the percentage of CD4+ cells at inoculation sites ultimately reaches levels similar to the maximum in SIV[-] animals. (3) The delay in CD4 recruitment appears to be associated with a delay in the initiation of specific immunity to *M. leprae* in SIV[+] animals, as indicated by a delay in expression of IL-2 mRNA and in LTT. (4) *M. leprae* infection in rhesus monkeys may be a useful model in which to study the early immunologic aberrations induced by SIV, as a model for abnormal responses to mycobacteria in HIV-infected patients.

IM27

MODULATION OF LEPROMIN RESPONSE IN MULTIBACILLARY LEPROSY FOLLOWING IMMUNOCHEMOTHERAPY (M.D.T.+Mw vaccine) AND CHEMOTHERAPY (M.D.T.+PLACEBO):- A comparitive assesment.

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In clinical trials with *Mycobacterium w* (Mw) anti-leprosy vaccine, (materials & methods discussed breifly in the abstract of immunotherapy paper) the data pertaining to 300 cases (155 vaccine & 145 placebo group) is available which have been followed up for 8 years with respect to thier lepromin status. Better response has been observed in vaccine group as compared in the two groups taking into consideration the 3 parameters viz. (a) percentage of conversion from lepromin negativity to positivity, (b) actual measurement of lepromin response in mm and (c) the duration for which the lepromin positivity was sustained. The average duration of positivity observed in LL, BL & BB types was 2 years, 1.9 year and 3.75 years in vaccine group. The corresponding figures for placebo group were 0.3, 1.0 and 2.5 years respectively. Overall, 16 out of 162 (9.8%) cases in vaccine group failed to convert to lepromin positivity and remained lepromin negative through out, as compared to 92 of 148 (62.1%) cases in the placebo group. The average lepromin response was 3.39 mm at the end of 1 year in LL type as compared to 1.1 mm in placebo group, the difference being highly statistically significant ($p<0.001$). Similar difference was observed up to 4 years from commencement of therapy in LL leprosy, and up to 3 years in BL type.

IM28

IMMUNOTHERAPY WITH *Mycobacterium w* VACCINE IN MULTIBACILLARY LEPROSY ACCELERATES B.I. DECLINE, REDUCES THERAPY DURATION FOR SMEAR NEGATIVITY: A 7 years experience.

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The clinical trials for evaluation of immunotherapeutic effects of Mycobacterium w (Mw) anti-leprosy vaccine have been in progress since 1986, at Urban Leprosy Centres of two hospitals of Delhi. Out of 422 patients of multibacillary leprosy inducted, 315 have persued a regular course of treatment. Initially all cases were untreated, lepromin negative and bacteriologically positive and divided randomly into vaccine and placebo groups. Both groups received MDT as per NLEP regimen for a minimum period of 24 pulses and continued thereafter till the skin smear negativity. The vaccine group received, in addition, Mw vaccine given intradermally, at 3 monthly interval, till a maximum of 8 doses. The rate of bacteriological fall in the two groups showed statistically significant difference in LL and BL types during first 3 years of therapy (2 years immunochemotherapy followed by chemotherapy alone). Among 300 (155 vaccine & 145 placebo) cases, the number of patients released from therapy (RFT) after 24-29 months of treatment in LL & BL types were 84 out of 133 (60.3%) in vaccine group, and 30 out 120 (25.0%) in the placebo group, the difference being statistically significant ($p<0.01$). No statistically significant difference was observed in the two groups with respect to incidence of type-1 and type-2 reactions, episodes of neuritis. The addition of vaccine to MDT did not lead to any rise in the incidence of disabilities of all grades over & above those encountered with MDT given alone. In post-RFT follow up for varying durations (from 1-8 years) in different patients, there has not been any case of bacteriological or clinical relapse in both the groups.

IM29

RESPONSES OF ACTIVATED ARMADILLO MACROPHAGES TO CHALLENGE WITH *MYCOBACTERIUM LEPRAE*.

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Upon inoculation with *Mycobacterium leprae*, the nine-banded armadillo (*Dasypus novemcinctus*) often develops lepromatous leprosy, and extensive histopathological studies have been done. However, due to a lack of reagents, little work has been done to evaluate immune

responses in vitro. In this study, macrophage activating factor (MAF) was prepared by incubating armadillo spleen cells in medium containing Concanavalin A (ConA) for 48 hr. Control MAF (cMAF) was prepared by incubating the spleen cells in medium and adding an equivalent amount of ConA at the end of the culture period. Peripheral blood was collected from armadillos and the monocytes were cultured for 6 days. These monocyte-derived MΦ were stimulated for 24 hr with cMAF or MAF, infected with *M. leprae*, and reincubated with cMAF or MAF. The infected MΦ were then lysed and the viability of the released *M. leprae* was evaluated in a ^{14}C- palmitic acid oxidation assay. As a control for anti-microbial activity, similarly treated MΦ were evaluated for the ability to kill the intracellular protozoan, *Toxoplasma gondii*. MAF-treated armadillo MΦ exhibited increased spreading compared to cMAF-treated cells, and were capable of killing *T. gondii*. In contrast, MAF-treated armadillo MΦ did not consistently inhibit the metabolic activity of *M. leprae*. In addition, no nitrite, an end-product of the L-arginine-dependent production of reactive nitrogen intermediates (RNI), was detected in the supernatants of MAF-treated MΦ. This profile of RNI-independent killing of *T. gondii* and an inability to inhibit mycobacteria is similar to that seen with IFN-γ-treated human monocyte-derived MΦ.

IM30

STUDIES ON LYMPHOCYTE PHYSIOLOGY - STUDY OF MARKER ENZYMES OF METABOLIC PATHWAYS

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Infection with M.Leprae, diagnosis and the reasons behind occurrence of the reactions and relapse all the things remain scientifically obscure although we are nearing the goal of global elimination of leprosy. Although the abnormality may be the result of defect in CMI and variations in number, quantity and functions of T-Lymphocytes and certain cytokines. The changes that occur in the physiology of lymphocyte might be one of the physiology of lymphocyte might be one of the reasons for their depressed functions, specially in the effector limb. We have studied a number of enzymes to elucidate different metabolic pathways like Arginase (an enzyme associated with one cytokine which is basically a LPS in nature). Iso-enzymes of LDH and Aldolase, SOD and Peroxidase, Adenosine deaminase and rate of translation by labeled aminoacids. Lymphocyte Arginase showed an increase of activity in LL (5.48 units/mg), BB and BT (2.54 units/mg), TT (1.81 units/mg) in comparison to healthy controls (0.87 units/mg). ADA shows a reversal of this trend as in LL (265 units/mg), TT (375 units/mg) which is common in immunoproliferative disorders. Activities of all other enzymes also varied throughout the spectrum of the disease, which is well correlated with LTT and LMIT. Enzyme induction studies have been carried out to rectify the defects in lymphocytes which showed varying responses to different antigens including M.Leprae.

IM31

HLA-DR-B1 AND DQ ALLELES IN LEPROSY PATIENTS WITH UVEITIS

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This study was planned to investigate the role of HLA-DRB1 and DQB1 alleles in Turkish patients with uveitis.

The subjects were 27 Turkish leprosy patients (15 uveitis and 13 non uveitis). Controls were 27 healthy subjects.

We have performed DR and DQ "low resolution" typing by the PCR-SSP technique. We used 32 primer mixes for per sample. Twenty one PCR reactions were performed for identifying DR1-DR18, in addition, 3 PCR reactions were performed for the DR51, 52, 53 superspecifities, 8 PCR reactions were performed for identifying DQ1-DQ9.

There was no significance difference between leprosy patients with and without uveitis and healthy controls with any of HLA-DRB1 and DQB1 alleles except DR16 ($p<0.05$).

This study has been continued.

IM32

STUDY ON SEROLOGICAL ACTIVITY OF THREE RECOMBINANT OF ANTIGENS-85B IN LEPROSY

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The authors compared the natural trisaccharide of PGL-1 conjugated BSA (NT-P-BSA) with the three recombinant antigens (TRA)-M. bovis BCG 85B (BCG85B), M. kansasii 85B (KAS85B) and M. leprae 85B (ML85B) to observe the distribution of the antibody titers in leprosy patients, leprosy household contacts, tuberculosis patients, pregnant women and controls and evaluate the value of the TRA in the serodiagnosis of leprosy.

The results indicated that: 1) in the 95% of specificity, the sensitivity of the TRA was lower than that of NT-P-BSA, but the sero-positive rates of the antibodies against the TRA in leprosy patients was relatively high reaching 82.3%-87.5% in lepromatous patients in particular and significantly higher than that of non-leprosy patients and controls; 2) among 138 leprosy household contacts with anti-NT-P-BSA antibody positive, only 0.0%-4.3% of them had positive anti-TRA antibody levels, but among 57 leprosy household contacts with high titer of anti-NT-P-BSA antibody, 4.3% had the antibody against the TRA.

The authors believed that the TRA should and could be considered as an alternative to NT-P-BSA in the serodiagnosis of leprosy, but could not replace it entirely. It appeared not be useful for detection of subclinical infection and high risk group in leprosy.

IM33

STUDY OF AFFINITY OF ANTI-PGL-I ANTIBODIES IN THE LL PATIENTS WITH TYPE I AND TYPE II REACTIONS

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This communication describes the differential response of anti-PGL-I antibodies in the sera of leprosy patients suffering from type I and type II lepra reactions. Both IgG and IgM classes of antibodies in the serum samples obtained from the lepromatous patients and the patients during acute onset of both reactions and following clinical remission of the same after steroid therapy were quantified and their binding constants (K) determined. It was observed that the mean K of IgM antibodies in both types of reactional patients got markedly reduced in comparison with LL patients without reactions. However, the affinity of IgG class (K) of anti-PGL-I antibody in the sera of patients during episodes of both type I and type II reactions increased drastically with respect to control LL patients. The decreased K of IgM antibodies during lepra reactions is likely to suggest switching of IgM synthesis to IgG variety. Immunological implications of the present findings will be discussed.

IM34

IMMUNOASSAYS WITH 29/33KD DOUBLET AND 65KD ANTIGENS OF *MYCOBACTERIUM TUBERCULOSIS* FOR SERODIAGNOSIS OF BORDERLINE LEPROSY PATIENTS

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Immunologically unstable borderline leprosy patients, classified as borderline tuberculoid (BT), mid borderline (BB) and borderline lepromatous (BL) patients, often undergo reactions such as reversal reaction (RR) and erythema nodosum leprosum (ENL) in the course of the disease. It is often difficult to discriminate between BT and BL patients because of previous treatment. Moreover, differential diagnosis of these patients is essential in order to monitor the risk for developing distinct types of reactions. Presently known all serologic assays are only applicable in discriminating multibacillary (MB) and paucibacillary (PB) patients and there are no immunoassays for distinguishing between BL and BT patients.

In the present retrospective study we examined a total of 121 serum samples (40 TT, 22 BT, 26 BL and 33 LL) of leprosy patients for the antibodies to mycobacterial antigens using immunoblotting and enzyme linked immunosorbent assays (ImBA and ELISA, respectively). The ImBA showed seroreactivities to 29/33 KD doublet bands of *m tuberculosis* (RIVM 7611 strain) in 99% and 77% of LL and BL patients respectively, whereas none of TT, BT and control patients did not show such specific antibodies. However, most of the TT and BT patients' sera (94% and 63% respectively) reacted to 65KD antigen(s) but not in disease specific manner when compared to controls. Interestingly, by ELISA mean, serum IgG antibody levels to either gel purified 29/33 KD doublet and 65 KD antigens were found significantly higher in lepromatous (LL/BL) and tuberculoid (TT/BT) leprosy patients respectively as compared to control sera consisting of tuberculosis, Crohn, sarcoidosis patients and patients with different skin diseases. After establishing a cut off point, 97% and 88% sera of LL and BL patients were positive with the 29/33 KD doublet as compared to the positivities of 41% and 20% of BT and TT patients respectively. In contrast 86% TT and 82% BT patients' sera showed high positivity for high antibody levels to 65 KD antigens as compared to 50% BL and 14% LL patients' sera. The present study showed that the measurement of antibody activities to 29/33 KD and 65 KD mycobacterial antigens by ImBA and ELISA could clearly discriminate BL and BT patients from the respective polar forms. We recommend that the presently described immunoassays should be used in a field investigation as an additional supportive tool for clinical classification of leprosy spectrum.

IM35

IN SITU EXPRESSION OF IFN-γ AND IL-4 IN THE LESIONAL SKIN ACROSS THE IMMUNOPATHOLOGICAL SPECTRUM OF LEPROSY: REFLECT DISEASE ACTIVITY

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The dynamics of immune responses in leprosy patients display a clinical and histopathological spectrum from the paucibacillary (PB) type of polar tuberculoid leprosy (TT) to the multibacillary form (MB) of polar lepromatous leprosy (LL). Between the two polar forms of leprosy, the majority of the patients are the immunologically unstable borderline patients. A considerable number of these patients (20-30%) show changing clinical status in the course of the disease, known as reactions, resulting in clinical and pathological alterations accompanied by tissue damage. The reversal reaction (RR) is accompanied by augmented *m leprae* specific CMI-DTH responses that often parallels the destruction of peripheral nerves. In the erythema nodosum leprosum (ENL) reaction, tissue damage appears to be mediated by the local deposition of immune complexes and complement fixation. Type 1- and type 2-like *m leprae* responsive T cells, characterised by a predominant production of either IFN-γ or IL-4, are recognized *in vitro* to parallel CMI-DTH or humoral immune responses within the immunopathological spectrum of leprosy. In the present study we assessed the *in situ* presence of IFN-γ and IL-4 both at protein and mRNA levels in lesional skin biopsies of untreated leprosy patients, and in patients during a RR or an ENL, as well as after treatment. We observed that both IFN-γ and IL-4 were present in varying amounts in the lesions of untreated paucibacillary (PB) and multibacillary (MB) leprosy patients. Moreover, no significant differences were seen in regard to the protein level of both of these cytokines in one individual lesion. However, high levels of *in situ* protein of IFN-γ and IL-4 were seen in all lesions with RR, and in ENL lesions the IL-4 protein was relatively higher than IFN-γ. Interestingly, *in situ* protein expression of IFN-γ and IL-4 was found significantly lower in the lesions of PB and MB patients who were released from treatment. Similarly, both cytokines decreased in the lesions of patients with RR or ENL with treatment. Using a RT-PCR method, the *in situ* expression of IFN-γ and IL-4 mRNA, relative to the same amount of T cell mRNA also did not differ between untreated PB patients, untreated MB patients, and those patients experiencing an ENL. However, similar to the immunohistochemical findings, the lesions of patients experiencing RR showed high level of both IFN-γ and IL-4 mRNA as compared to the other groups of patients. These data implicate that although *in vivo* expression of IFN-γ and IL-4 in leprosy lesions may not necessarily reflect the role of discrete type 1- or type2-like *m leprae* responsive T cells in the pathology of different types of leprosy. The monitoring of lesional cytokines will be useful for the evaluation of disease activity (particularly in regards to the development of reactions) of patients in a leprosy control programme.

IM36

ROLE OF MACROPHAGES IN THE PATHOGENESIS OF MYCROBARCTERIUM TUBERCULOSIS.

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The macrophages were collected from two sources:

Alveolar macrophages (AM) obtained from the bronchoalveolar lavage and adjusted to 10^6 cell/ml, mononuclear cells (PMN) were prepared and adjusted to 10^7 cell /ml. Both AM and PMN were infected with Mycobacterium tuberculosis (MTB) adjusted to 10^7 cfu/ml. AM and PMN were stimulated with soluble mycobacterial preparation PDD and lipopolysaccharide (LPS) and serve as control.

The concentration of chemokines IL-8, Rantes, MCP and MIP Alpha were assayed in the supernatant by ELISA. The migratory activity for MN and Lymphocytes in supernatant of MTB infected PMN and AM containing the chemokines was measured. Northern blot analysis of RNA extracted from AM and PMN was performed.

The results and statistical analysis will be presented.

IM37

CELL MEDIATED IMMUNE RESPONSE IN HEALTHY EXPOSED INDIVIDUALS SETTLED IN NON- ENDEMIC AREA - A PILOT STUDY

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Introduction: It is believed that upon infection with M.leprae the nature of the T-cell immune response in the exposed individual determines whether leprosy will develop or whether protection will be induced.

Aims and Objectives: To investigate the lymphoproliferative responses to leprosin A in the exposed individuals.

Experimental Procedure: Venous blood samples were collected from 10 subjects (8 with previous exposure and 2 with no known contact with leprosy). Lymphocytes were isolated and 3-4 day culture experiments were performed stimulating the cells with PHA,PPD and Leprosin A. The degree of lymphocyte growth was determined initially by ATP assay and later by an ELISA for gamma-interferon.

Graphical representation of ATP counts: Encouraging results with good lymphocyte growth and gamma-interferon is seen in subjects with previous leprosy exposure. Controls for the assay show no such response.

Conclusion and Discussion: The frequency of the reactive T-cells in the peripheral circulation at the time of sampling may influence the immune status of the host toward the antigens.

IM38

A POSSIBLE LINK BETWEEN TIMING OF DEVELOPMENT OF MUCOSAL AND SYSTEMIC IMMUNITY - APPARENT PROTECTION??

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The transmission of leprosy is poorly understood but infection from subclinical sources is more important than infection from active clinically apparent cases. Since the primary lesion of leprosy is thought to be in the nose, it is possible that mucosal immunity plays a major role in protecting exposed individuals from developing the disease. Little known about the early lesion of leprosy there is some evidence that they may heal by the induction of a CMI with concomittant induction of an IgA response.

Lymphocytes were isolated from venous blood samples collected during a period of time from subjects belonging to different groups including a) family contacts, b) individuals from non-endemic countries & c) fresh hospital contacts.The mononuclear cells were stimulated with a mitogen, PPD and Leprosin A. The degree of the cell growth was determined by an ELISA for gamma-interferon. Tuberculosis patients served as positive control to assess the response against PPD.

Good lymphocyte growth and production of gamma-interferon showed a strong T-cell response to *M.leprae* in some exposed individuals. Mucosal IgA responses at different time intervals alongside will help to determine the possible link between humoral and CMI and its putative role in protection.

IM39

DETECTION OF NITRIC OXIDE (NO) IN DERMAL LESION OF PATIENTS WITH LEPROSY

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The aim of this study is to determine the presence of Nitric Oxid and TGF-β1 in the skin lesions of patients with leprosy, in an attempt to correlate the presence of both with the clinical forms of the disease that progress with a specific immunosuppresion to *Mycobacterium leprae*. The presence of NO

and TGF-β1 were perfomed in biopsies from patients with lepromatous (LL, N=8) and tuberculoid (TT, N=8) leprosy. Normal skin samples (N=8) were used as control. The sections were submitted to NADPH-diaphorase activity a surrogate marker for NO-synthase and to Immunohistochemical studies using an anti- TGF-β1 and anti NOSi polyclonal antibodies and the avidin-biotin-peroxidase (ABC complex method). For the NADPH-diaphorase activity and to NOSi immunoreactivity, seven LL cases presented mild (+/++) staining and one case did not present reactivity in the dermal lesions. All TT cases were positive, five with moderate intensity (+++) and three cases presented intense (++++) staining. Immunoreactivity to TGF-β1 protein was present in the dermal lesion of all LL cases. In contrast, it was absent in all TT cases. The immunoreactivity to TGF-β1 and to NOSi were usually observed in the cytoplasm of mononuclear cells with macrophage/histiocyte morphology (CD68+). Conclusion: In the lepromatous form *M. leprae* multiplies in the cytoplasm of macrophages inducing the production of TGF-β1. Its immunosuppressive activity inhibit the differentiation and activation of these cells perpetuating the infection. In the tuberculoid forms TGF-β1 absence cause intense differentiation of macrophage cells and NADPH-diaphorase activity which eliminates most of the bacilli.

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IM40

PRODUCTION OF TGF-β1 BY BLOOD MONOCYTES FROM PATIENTS WITH DIFFERENT CLINICAL FORMS OF LEPROSY

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It has been demonstrated that there is a clear correlation among the clinical forms of leprosy and the state of mononuclear phagocyte activation in the lesions which are also associated with the presence of cytokines such as IL-2, IFN-γ, TNF-α and IL-12 in tuberculóide form and IL-4, IL-5 and IL-10 in lepromatous form. The cytokine TGF-β1 (transforming growth factor-beta 1) has been described as a macrophage-suppressing factor in diseases caused by intracellular parasites and in dermal lesions of patients with BL and LL forms of leprosy.
In the present investigation, we determined the concentration of TGF-β1 secreted in the adherent cell supernatants from human monocytes stimulated *in vitro* with PGL-1, LPS or media only. The cells were obtained from untreated patients with different clinical forms of leprosy and healthy individuals, as controls. The peripheral blood mononuclear cells were isolated by Ficoll-Hypaque and platelets were removed from monocytes by washing in Versene buffer and differential centrifugation. Monocytes were cultured in RPMI 1640 in presence of 2.5% heat-inativated human AB serum at 37° C for 1 h. The adherent monolayers were then cultured in presence of RPMI 1640 only. TGF-β1 concentrations were determined by ELISA in cell free supernatants harvested after culture of 48 h. Our results demonstrated that the adherent cells exhibited spontaneous release of TGF-β1 in all clinical forms of leprosy and in healthy individuals. However, adherent cells from patients with the BL and LL forms with erythema nodosum leprosum (ENL) displayed significantly higher concentrations of TGF-β1 when stimulated with PGL-1 ($p < 0.0124$), or LPS ($p < 0.0445$) or media only ($p < 0.0362$) than patients with other clinical forms of leprosy or healthy individuals. In addition, when stimulated with PGL-1, the cells from ENL patients exhibited high concentrations of TGF-β1 as compared with LL ($p<0.0069$), BT ($p<0.0173$) or healthy individuals ($p<0.0124$).
Comparing these observations with our previous data studying the TGF-β1 in dermal lesions, it appears that this molecule probably play different roles in leprosy, presenting a supressive action locally and features of pro-inflamatory cytokine when secreted systemically by monocytes.

IM41

CYTOKINES SERUM PROFILE AND LEPROSY SPECTRUM.

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Considering that the different clinical manifestations visualized in the spectrum of leprosy can be related with the immunological response, we have investigated serum cytokines levels in 35 leprosy patients and 10 normal individuals(controls).The patients were subdivided into LL(7), BL + BB(8), BT(7), TT(6) and ENL(7). All patients were classified by clinical, bacteriological and histological criteria. It was include only untreated patients. The serum levels of IL1, IL4, IL6 and TNFα were measured by ELISA(R&D Systems). The results showed significantly higher IL4 levels in multibacillary leprosy-MB(62.0pg/ml in LL,BL,BB), when compared with paucibacillary-PB(1.3pg/ml in HBT,TT) patients and control group(0.0pg/ml). In contrast, the IL1 and IL6 levels were significantly lower in MB patients(1.6 and 5.6pg/ml) when compared with the levels of PB patients(33.7 and 12.7pg/ml). In patients with ENL the TNFα levels(101.5pg/ml) were significantly higher than in controls(30.5pg/ml) and were associated with decreased concentrations of IL4(59.9pg/ml). In conclusion, we suggest that the capacity of immune response in leprosy is related to the ability of cytokines IL1,IL6 and TNFα, to induce the activation and modulation of the response of phagocytic cells(macrophage) and effector cells(Tlymphocites) and the immunosuppression observed in LL could be maintained by IL4 activity, through the supression of macrophage activation.

IM42

CYTOKINE Th1 AND Th2 PROFILE, TGFβ1 AND IMMUNOSSUPRESSION OF LEPROMATOUS LEPROSY.

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The aim of this study is to verify whether the correlation between peripheral cytokines levels, "in vitro" production, and inflammatory cells pattern in cutaneous biopsies from patients is related with the evolution of the polar forms of leprosy. Cultures of PMNBC from 21 leprosy patients (11 lepromatous leprosy-LL, and 10 tuberculoid leprosy-TT) were treated during 24-72 hrs with LPS (10 mg/ml), PHA (20 mg/ml) and lepromin (2×10^7 bac/ml). The supernatant and serum citokines (IL1, IL2, IL4, IL6 and TNF), were measured by ELISA (R & D Systems). In the cutaneous lesions the presence of T cell (PANT-T4, T8), macrophages (CD68) and TGF-β1, were determined by immunohystochemical assay (ABC-peroxidase), using frozen skin biopsies. IL1, IL6 and a TNFα levels in the serum and in the supernatant were higher in TT (37,5; 13,5 and 84,5 pg/ml; 63,1; 22,0 and 166,5 pg/ml; $p < 0,05$) than those levels of LL patients (0,0; 3,4; 0,0 and 15,7; 5,8; 14,4 pg/ml). IL2 was detected only in the lymphoproliferative supernatants of TT patients showing elevated levels (129,3 pg/ml) when compared to LL patients (10,4 pg/ml). IL4 was observed only in LL (89,2 e 51,2 pg/ml, serum and supernatant). It was observed predominance of macrophage (CD68) in the cutaneous inflammatory infiltrate from both leprosy patients groups. TGF-β1 was found in 85% of CD68 cells from LL patients and it was absent in TT infiltrate. 80% of T cells of TT infiltrate showed CD4+ pattern, whereas only in 10% of LL patients CD4+ pattern was observed. These results may suggest that IL4 and TGF-β1 are correlated with the immunossuppressive
mechanisms observed in LL. Since both cytokines can be induced by *M. leprae* and/or correlated antigens, we can suppose that this mechanism should explain, in part, the maintenance of the supressive response.

IM43

REACTIONAL EPISODES, CYTOKINES PATTERN AND MICROBIAL MECHANISMS IN LEPROSY

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Reactional states in leprosy can be defined as the clinical manifestations of alterations in the immunological balance between host and infecting organism. There are two types of them: *Type 1 reaction* or *reversal reaction-RR*, frequent in paucibacillary leprosy is associated with an increased cellular immune response. *Type 2 reaction or erythema nodosum leprosum-ENL*, occurs in lepromatous leprosy patients and characterize a systemic inflammatory reaction. Forzen skin lesions biopsies from 15 patients (9 in ENL and 6 in RR), were immunostained to evaluate the inflammatory cells phenotypes, expression of IFN-γ, TNFα, TGFβ1 and the were negative for TGFβ1. Memory lymphocytes, CD3+CD45RO+expressed IFN-γ in their cytoplasm. The presence of iNOS was detected in the cytoplasm of pratically all CD68+cells. In ENL cases the CD68+macrophages positively immunostained for TGFβ1 and TNFα and were negative for iNOS. However, presence of iNOS was detected in the cytoplasm of neutrophils cells (CD15+). This TNFα detected in lesion site was correlated with increased concentrations in serum of ENL patients (105.6±9.8 pg/ml), while in RR patients the TNFα levels were lower (57.3±5.0 pg/ml). The presence of IFN-γ, TNFα and iNOS in skin lesions of reactional states in leprosy may induce local production on nitric oxide leading to mycobacterium killing.

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IM44

ANTIGEN RECOGNITION MECHANISM OF ANTI-PGL-I ANTIBODY DZ 1

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To study the mechanism in which antibodies recognize antigens, anti-PGL-I monoclonal antibody DZ 1 was employed, because of that DZ 1 had unique characteristics which recognized inner part of the sugar chain of PGL-I. The activity of purified DZ 1 was tested by ELISA for synthetic sugars related to PGL-I. DZ 1 was active to the inner disaccharide 3-OMe-2-(2',3'-di-OMe-α-L-Rha)-α-L-Rha (ID). DZ 1 had no activity to the inner monosaccharide. DZ 1 had greatly reduced activity to 4-OMe, 3-OH, 3'-OH or 2',3'-di-OH derivatives of ID. But, DZ 1 was activity to 2'-OH derivative of ID. DZ 1 recognized two anomeric configurations in ID. These results showed that DZ 1 recognized the inner disaccharide, 4-OH, 3-OMe and 3'-OMe of ID.

Computer modeling and structural analysis by NMR of the trisaccharide suggested that two types of the cluster (Conformation A and B from lower energy level) were present in about 46.2% and 50.3% within 10Kcal/mol. They were in very similar conformation on the inner disaccharide which were recognized by DZ 1. NMR spectroscopy (NOE HETLOCK and pHMBC spectra) supported conformation B strongly. NMR study showed also that conformation B was dominat in solution. In confomation B the trisaccharide chain vented between Rha-Rha linkage, forming the surface open to the outside. 4-OH, 3-OMe And 3'-OMe, which were recognized by DZ 1, were present on this surface, and 2'-OMe and non-reducing-end glucose residue were oriented to 90 degree directions. Thus, it was concluded that DZ 1 bound to the trisaccharide from this direction. This is why DZ 1 recognized the inner part of the sugar chain.

IM45

LEPROMIN REACTION IN HIV POSITIVE INDIVIDUALS

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There have been several reports about AIDS patients easily acquiring tuberculous infection saying that it is one of the common causes of death. Particularly in USA the prevalence of TB since the advent of AIDS has considerably increased. However M. leprae which belongs to the same species as M. tuberculosis does not seem to affect the HIV patients with the same virulence as evidenced by the available reports. In reports where leprosy and HIV infection are associated, most of the patients belong to borderline variety rather than lepromatous type. The prevalence of HIV infection among leprosy patients is not higher than any general population. Lepromin test is one of the important tests which even now we use to measure the CMI to M. leprae in HD patients and in the general population. This study is undertaken to find out the lepromin reaction of HIV positives in 3 HIV positive individuals and discuss its significance.

IM46

AN EXPERIENCE OF USING LEUCINFERON FOR TREATMENT OF LEPROMATOUS LEPROSY PATIENTS

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Not infrequently leprosy patients, after some period of prolonged improvement against the background of antileprosy therapy, show stabilization of the process: clinical, morphological picture and immunological profile do not improve further. In such cases addition to the standard therapy of immunomodulators might be rather effective. We present our experience of using leucinferon - a combination of alpha-interferon and other cytokines ("Intecor", Russia) for the treatment of LL patients with no signs of further improvement of their disease. The results were estimated by the dynamic of clinico-morphological and immunological indices. Histological investigations were based on morphometry with using 100-point ocular system (Avtandilov,G., 1991). Antibodies to Dis-BSA and protein antigens of M.leprae were determined in ELISA. After a course of leucinferon therpy there was a marked improvement of clinical manifestations. Morphological pictuare of infiltrates changed as follows: macrophages decreased by 31,3%, fibroblasts and tissue basophils increased by 9,2% and 9,6%, correspondingly. Solid forms of M.leprae disappeared. Initial high levels of anti-M.leprae antibodies fell to normal values during 2 months of the therapy. Thus, using of leucinferon might be useful in treatment of leprosy patients inresponsive to standard therapy.

IM47

DEFINITIVE ROLE OF LEPROMIN POSITIVITY IN BACTERIOLOGICAL CLEARANCE IN MULTIBACILLARY LEPROSY.

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In clinical trials with *Mycobacterium w* (Mw) anti-leprosy vaccine, the role of lepromin status on B.I. decline was studied. From LL, BL and BB leprosy types there were 83, 48 and 24 patients respectively who received immunochemotherapy (MDT+Mw vaccine), and 81, 41 and 23 patients respectively, who received chemotherpy (MDT+placebo). All patients, in both groups, were lepromin negative initially. In LL and BL types, the rate of BI decline in the vaccine and placebo groups showed a statistically significant difference from 6 months therapy onwards. Analysed with respect to lepomin status, by segregating the patients in vaccine and placebo groups into lepromin positives and negatives, this statistically significant difference was observed only in patients converting to positive lepromin status. The difference was non-significant among cases remaining lepromin negative throughout, although slightly higher rate of BI decline was observed in vaccine group.

Apart from the well recognised role of lepromin positive status in imparting protection against contracting leprosy (immunoprophylaxis), its role in bacteriological clearance also seems definitive (immunotherapy).

IM48

AN IN VITRO MODEL OF MACROPHAGE TURNOVER IN EXPERIMENTAL LEPROSY.

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Multibacillary lepromatous leprosy is characterized by enormous numbers of *Mycobacterium leprae* in the lesions and a potent antibody response, but little cell mediated immunity. *M. leprae* engorged granuloma macrophages (MΦ), harvested from the foot pads of experimentally infected athymic nu/nu mice, as well as MΦ infected in vitro with high numbers of bacilli, are refractory to activation by interferon-gamma (IFN-γ). This suggests that any killing of *M. leprae* in infected tissues would likely be due to newly-arrived, competent MΦ activated before or shortly after their traffic into the leproma. Therefore, we have developed an in vitro model to simulate the turnover of MΦ in a lepromatous lesion. Murine target MΦ, infected with varying doses of viable *M. leprae* freshly harvested from the foot pads of nu/nu mice, were challenged with normal or activated (ACT) effector MΦ. At various times, the bacilli were recovered and, since *M. leprae* cannot be cultured in vitro, assayed for ^{14}C-palmitic acid oxidation to assess viability. *M. leprae* recovered from target MΦ possessed high metabolic activity. Bacilli from target MΦ challenged with normal effector MΦ displayed metabolism which was often greater than that of bacilli from unchallenged

targets, suggesting that turnover may actually sustain *M. leprae* viability. Bacilli recovered from infected target MΦ challenged with ACT MΦ, however, exhibited a markedly decreased metabolic activity, implying that *M. leprae* residing in normal target MΦ are not protected from the microbicidal effects of ACT MΦ. Thus, the state of the MΦ infiltrating a granuloma may markedly affect the viability of *M. leprae* residing in MΦ in the lepromatous lesion.

IM49

COMPARISON BETWEEN ANTI-PGL-I SEROLOGY AND MITSUDA'S REACTION (CLINICAL READING, MICROSCOPIC FINDINGS AND IMMUNOHISTOCHEMICAL ANALYSIS)

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A quantitative relationship between anti-PGL-I IgM levels (ELISA) and Mitsuda's reaction was studied in 44 leprosy patients (10 TT, 11 BT, 5 BB, 15 BL and 3 LL). We have performed the clinical reading and histological analysis of Mitsuda's reaction. Histological examination of lepromin reaction was classified into 6 categories. A correlation was found between clinical reading and microscopic findings. The antibody titers against PGL-I antigen increased from tuberculoid to lepromatous pole, although individual variations were found in the spectrum. When multibacillary patients presented high antibody levels, the clinical reading of Mitsuda's reaction were low (< 3,0mm) and the histological findings were an incomplete tuberculoid granuloma or absence of granuloma. Tuberculoid patients showed low anti- PGL-I titers. The Mitsuda's reaction in these patients was > 6,0 mm and the histological analysis presented a tuberculoid granuloma development. CD4+ lymphocytes were found throughout epithelioid cells and CD8+ cells were predominantly in the mantle surrounding the compact granuloma as it had been demonstrated in other tuberculoid granulomas as sarcoidosis and rhinoscleroma (MODLIN et al., 1983).

IM50

LYMPHOCYTE AND CYTOKINE RESPONSES TO SUBFRACTIONS OF THE LEPROSY BACILLI IN PATIENTS WITH TYPE I REACTIONS

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The biological basis of Type 1 reactions (T1R) is poorly understood. To investigate the immunological responses in T1R, whole blood cultures were stimulated with leprosy antigens (MLS, WML, MLS-LAM, MLCwA) for 24 hours and levels of cytokines in the supernatants were measured by ELISA. Patients with untreated T1R had significantly higher levels of interferon-gamma (IFNγ) and tumor necrosis factor alpha (TNFα) but not interleukin 10 (IL-10) in response to leprosy antigens when compared with non-T1R controls. The levels of IFNγ and TNFα showed a marked decline within one week of commencing steroid therapy. The antigenic specificity of the lymphocyte proliferation in patients with neural and cutaneous forms of T1R was also investigated.

IM51

ANALYSIS OF CITOKINE mRNA PROFILE IN LEPROSY LESIONS DURING ACUTE INFLAMMATORY EPISODES: IMPACT OF PENTOXIFYLLINE, THALIDOMIDE AND PREDNISONE TREATMENT. Moraes MO, Sarno EN, Almeida AS, Saraiva, BCC, Nery JAC, Sampaio EP. Leprosy Laboratory, Oswaldo Cruz Foundation, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, Brazil.

Cytokines play a crucial role in the establishment and development of immune response against intracellular infections. In this regard, a cytokine profile resembling a Th1 pattern is related to the resistance, while Th2 responses may lead to susceptibility to the pathogen. The clinical features of leprosy comprise a spectrum of immunological response including the tuberculoid (TT-responsive) and lepromatous (LL-unresponsive) poles. Besides, during the natural course of the disease, patients may undergo reactional episodes that are characterized by an acute immunoinflammatory response to *Mycobacterium leprae*, that can be correlated to tissue and nerve injury. They are clinically classified as type I (reversal reaction-RR) and type II (erythema nodosum leprosum-ENL) reactions. Despite of the amount of knowledge accumulated in the past few years, very little is known about the molecular mechanisms that lead to reactions in leprosy. Moreover, the understanding of cytokine balance in the development of leprosy reactions and its modulation by anti-inflammatory drug therapy is poorly understood. Our previous studies suggest that TNFα is involved in mediating the local and systemic pathophysiological events during those episodes. Furthermore, the inhibitory effects of thalidomide (THAL) and pentoxifylline (PTX) on TNFα mRNA expression and protein secretion in the sera from ENL patients have been demonstrated. Recent data from our group and others suggest that these drugs are acting in a broader range of cytokine regulatory pathways. We decided to employ semi-quantitative RT-PCR to determine the cytokine profile at the site of the lesion of leprosy patients undergoing or not reactional episodes. The effects of drug treatment were also evaluated. Skin biopsy specimens were collected from 6 unreactional (5-LL and 1-TT) and 13 reactional (10-LL undergoing ENL and 3-LL undergoing RR) patients. Biopsies were collected at the time of leprosy diagnosis, at the onset of the reactional episode, and 3-30 days after drug administration. ENL patients underwent pentoxifylline (n=8) or thalidomide (n=2), while RR patients were treated with prednisone (n=3). We observed that patients undergoing reactions present a more prominent expression of TNFα, IFNγ and IL-6 mRNA in situ. In addition, message for IL-2, IL-10 and IL-4 was also noticed. So, leprosy patients during reactions seems to present a Th0 response. Whenever the impact of the anti-inflammatory drugs was evaluated, we verified a drastically inhibition of TNFα, IFNγ, and IL-6 mRNA expression. However, the impact on IL-10 and IL-4 gene expression was dependent of the immune status during the reaction.

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IM52

SERUM IL-1ra IS ELEVATED IN LEPROMATOUS LEPROSY PATIENTS

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The pattern of specific cytokines produced has been used to define subsets of T lymphocytes in diseases caused by intracellular pathogens. This subsets of T lymphocytes (TH1, TH2) has been associated to the different poles of leprosy (lepromatous LL and tuberculoid LT).

Previous reports indicate that LL patients can be classified in responders (R) and non responders (NR), according to their activity under mitogenic stimulation. Our previous reports showed that lymphocytes from NR patients release threshlod IL-2 as compared with R patients and healthy subjects. On the other hand there is an ongoing controversy concerning to the correlation of cytokines production, and the general status in leprosy patients.

In this work we attempted to elucidate the serum levels of IL-1β, IL-1ra and TNFα in LL-, LT- patients and healthy subjects. Furthermore, we analyzed the production of IL-4, IL-6 and IL-10 in culture of T lymphocytes from LL- patients.

Our results showed no differences in the pattern of TH1 and TH2 cytokines released by lymphocytes from LL-patients and healthy subjects. This was the same for cytokines serum levels. Nonetheless, IL-1ra serum levels were dramatically higher in LL- as compared to LT-patients and healthy subjects. This could explain the lack of microbicidal activity displayed by macrophages in LL- patients.

IM53

DO γ/δ T-CELLS PLAY A ROLE IN LEPROMIN SKIN REACTIONS IN HANSEN'S DISEASE-NAIVE VOLUNTEERS?

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Studies have indicated that mycobacterial antigens, including those of *Mycobacterium leprae*, evoke γ/δ T-cells: they were found in increased numbers in lepromin skin-test sites, and comprised a significant proportion of the T-cell population in Mitsuda reactions among patients with various forms of Hansen's disease. The purpose

of this study was to evaluate the role of γ/δ T-cells in both Fernandez and Mitsuda responses in healthy, tuberculin PPD-negative volunteers who had never lived in leprosy endemic areas.

Volunteers were injected intradermally with standard lepromin A, tuberculin PPD, and lepromin diluent as a control. Biopsies were obtained at the time of injection (control), and after 48 h and 21-28 days. Suction blisters were produced over control and test sites at the same times, and blister contents were aspirated 24 h later for evaluation of cellular content. Blood was also obtained before lepromin administration, then after 48 h and 21-28 days, for fluorescence-activated cell-sorter (FACS) analysis.

As determined by cell analyses of biopsy specimens, monocytic infiltrates observed in Fernandez and Mitsuda reactions contained insignificant numbers of γ/δ T-cells, and the majority of lymphocytes were of CD4+ and CD8+ phenotype. The same results were obtained when cellular contents of supra-lesional suction blisters were examined. At 23 days, incubation of peripheral blood mononuclear cells with *M. leprae* or tuberculin PPD resulted in a significant increase in incorporation of ^{3}H-TdR by cells exposed to the former, but not to PPD ($p = 0.04$).

In conclusion, the results of our study indicate that, in contrast to published observations in patients infected with *M. leprae*, γ/δ T-cells do not appear to play an important role in the early development of responses to *M. leprae* antigens evidenced by Fernandez or Mitsuda lepromin reactions in Hansen's disease-naive individuals.

IM54

IMMUNOLOGICAL RESPONSE OF A TT LEPROSY PATIENT POST BCG VACCINATION DURING DISEASE AND AFTER HEALING OF THE LEPROSY LESION. Mônica C. B. S. Lima[1,2], Jorge L. Salgado[1], Maria C. V. Pessolani[1], Geraldo M. B. Pereira[2], Franklin D. Rumjanek[3], Nadia Duppre[1], José A. C. Nery[1], Luis C. M. S. Pôrto[4], Luciane F. S. Pontes[4], Euzenir N. Sarno[1] & Elizabeth P. Sampaio[1]
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It has been shown that BCG is an effective vaccine against leprosy, and the aplication of multiple doses to leprosy contacts has been adopted as a regular protocol by the National Control Program in Brazil. In the present study, a clinical and immunological evaluation of a household contact of a multibacillary patient who developed a self-healing TT leprosy following BCG vaccination is presented. JSS, a female aged of 55, received 1 dose of BCG vaccine and 4 months later a single leprosy lesion was observed on her face. At this point, a lepromin skin test was performed and a reaction of 22 mm was detected. HLA-typing for this individual was A31-B35-Cw4-DR16, DR51-DQ7/A2-B51. PBMC from the patient was analyzed with regard to *in vitro* proliferation and cytokine production following stimulation with *M. leprae* and BCG at 4 different periods of the follow-up (at diagnosis, and after 7, 16 and 19 months). A positive T cell response and IFN-γ production was observed along the follow-up. When PBMC from JSS were depleted of the CD4 lymphocytes, the proliferation and IFN-γ production to *M. leprae* stimulation were decreased by 44 and 34%, respectively, suggesting a major participation of this population in this type of response. In addition, mononuclear cells from this patient were able to proliferate and produce IFN-γ in response to four major purified proteins of the leprosy bacillus (10 kDa/GroES homolog, 22 kDa/bacterioferritin, 35 kDa/MMPI and the secreted 85 complex). Finally, *M. leprae* and BCG were also able to induce IL-2, TNF-α, IL-10 and IL-5 production. In this study we then demonstrated an immunological type of response which was found to be associated with a self-healing case in leprosy.

Financial support: TDR/WHO

IM55

EFFECT OF PHENOLIC GLYCOLIPID-I ON TNF-α PRODUCTION AND T CELL ACTIVATION. Pessolani MVC[1], Charlab R[2], Alvim IMP[1], Nery JAC[1], Esquenazi D[1,3], Sarno EN[1], & Pereira GMB[1,3].
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PGL-I has been associated with the selective anergy observed in lepromatous leprosy patients. Experimental findings indicated that PGL-I interferes with the cellular immune response at the level of both macrophage function (measured by the release of inflammatory cytokines and by generation of oxygen radicals) and lymphocyte proliferation. However, little is known of the molecular events triggered by PGL-I in human cells, and that are responsible for the alterations seen in their physiology and metabolism. We have studied the effect of PGL-I on the production of TNF-α by human monocytic cells. We found that PGL-I alone was unable to induce TNF-α secretion, but acted as a co-signal for the production of this cytokine when associated with *M. leprae* or PMA. PGL-I was also able to inhibit the IL-2 dependent pathway of T cell activation. In PBMC stimulated with anti-CD3 or ConA, PGL-I down modulated the expression on T cells of the α chain of the IL-2 receptor and the secretion of IL-2 itself. Moreover, PGL-I also affected the expression of CD69, suggesting that interferes at very early steps in the signaling pathways leading to T cell activation. Currently, we are working on the hypothesis that PGL-I exerts its modulatory effects by inserting its very large hydrophobic moiety into the membrane lipid bilayer, disturbing in this way, signaling pathways such as those dependent on protein kinase C.

Supported by PADCT/CNPq

IM56

IMMUNOHISTOCHEMICAL STUDY OF PATIENTS OF MITSUDA POSITIVE LEPROMATOSE LEPROSY.

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A study was carried out on a group of cured lepromatose leprosy patients who turned spontaneously to Mitsuda's test, and it was compared histologically to the granulome in Mitsuda's reaction from another group of also cured tuberculoid leprosy patients.

As parameters for the study, H-E tincture, the proteines S-100, CD-68, MAC-387, Muramidase HLA-DR, CD-3, CD-4, CD-8, CD-19 and CD-20 were used.

The results obtained are compatible with the reaction of granulomatose type caused by Mitsuda's reaction in tuberculoid leprosy.

These patients of lepromatose leprosy show an evident histological change, coming close to the tuberculoid pole and suggesting the possibility of immunological change.

IM57

STUDY OF H MORAL AND CELLULAR IMMUNOLOGY IN PATIENTS OF MITSUDA POSITIVE LEPROMATOSE LEPROSY.

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A research was carried out on a group of 43 cured leprosy patients: 32 of them belonged to the lepromatose leprosy type, 16 of whom showed spontaneous positivity to Mitsuda's reaction; the other 16, who remained negative, were used as a control group. The rest (11) was a group of patients of tuberculoid leprosy used as a control.

A group of 9 healthy, Mitsuda positive people who lived with the patients was also taken as a control.

In cellular immunity, the parameters CD3, CD4, and CD19 were studied; as for humoral immunity, the immunoglobulines IgA, IgM, IgG and the subtypes IgG1, IgG2, IgG3 and IgG4, as well as the interleukines IL-2, IL-4 and IL-10 and the Gamma-interferon were considered.

Only slight significant differences were found among the groups.

IM58

AN IMMUNOGENETIC STUDY OF DIFFERENTIAL MANIFESTATIONS OF LEPROSY IN NORTH INDIA

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In spite of the causative agent being the same i.e., *M. leprae*, different individuals manifest leprosy in different

forms ranging from paucibacillary tuberculoid pole to multibacillary lepromatous leprosy. To study the genetic factors which determine the immune response to an antigen or infectious agent, HLA-DRB1, DQA1 and DQB1 were studied in tuberculoid (TT), borderline lepromatous (BL) and lepromatous leprosy (LL) patients and compared with normal controls. While DRB1*1501 was significantly increased in patients of all types of leprosy, other alleles at DRB1, DQA1 and DQB1 loci seem to play a significant role in determining the type of leprosy one develops after infection. Since peptides presented by different MHC molecules have allele specific motifs, different peptides of *M.leprae* get presented by different HLA molecules, resulting in differential immune response to the causative agent and hence differential manifestations of the disease. The detailed analysis of hetero-dimers and trans-mers will be presented.

IM59

RAPID AND SIMPLE MEASUREMENTS OF ANTI-LEPROSY ANTIBODIES

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A rapid immunochromatographic tests was devised for the qualitative detection of human antibodies to *Mycobacterium leprae*. The test consists of a folding cardboard device containing a nitrocellulose strip and anti-human IgG attached to colloidal gold particles. The 35kDa *M. leprae* protein expressed in recombinant *M. smegmatis* (Triccas *et al* 1996, Infection & Immunity 64:1571) was immobilised across the nitrocellulose strip. When 30 µl of serum or plasma is added to a pad it diffuses across the 35kDa protein and specific IgG antibodies bind. Upon closure of the test card, the anti-human IgG attached to colloidal gold particles binds to the bound IgG producing a distinct pink line within 15 minutes.
When tested with leprosy patients serum, 23% of PB and 100% of MB patients gave positive responses. When compared with a conventional ELISA using the 35kD antigen and a cut-off determined by the mean plus 3 SD of a pooled normal serum, the test cards had a 74% sensitivity and 100% specificity.
This rapid and specific antibody test may be useful in countries with low endemicity of leprosy where delays in diagnosis are common.

IM60

NEUTROPHILS ISOLATED FROM LEPROSY PATIENTS EXHIBIT ACCELERATED APOPTOSIS IN VITRO AND RELEASE TNFα AND IL-8

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This study demonstrated that polymorphonuclear neutrophils (PMN) participate in the acute inflammatory response in some way as effector cells. Reactional lepromatous patients (Type II reaction or ENL) showed in the lesion intense infiltrate of neutrophils. Polymorphonuclear cells of nonreactional patients, health donors, and reactional patients were purified and analysed. Human neutrophils were purified from the peripheral blood by Ficoll-Hypaque density centrifugation and dextran sedimentation. The cells were suspended in RPMI 1640 (Gibco BRL) supplemented with 10% fetal bovine serum. According to Trypan blue dye exclusion tests, upon purification around 100% of PMN was viable. The first phase of the study confirmed the short lifespan of these cells in culture in addition to progressive changes characteristic of apoptosis. Apoptosis was greatly accelerated in ENL patients as shown by morphologic analysis, which was later confirmed by DNA fragmentation and quantitative analysis. Analysis of TNFα production in culture supernatants was performed by specific Elisa after 18-20h of stimulation. It was observed that neutrophils stimulated with LPS synthesize and secrete TNFα and IL-8 in significant quantities. Thalidomide a drug known to inhibit TNFα synthesis on monocytes also exerted an inhibitory effect on LPS-induced TNFα secretion in neutrophils. It was also demonstrated that neutrophils stimulated with *M. leprae* e LAM-ML secreted IL-8. TNFα gene expression was analyzed by Northern blot hybridization. The capacity of neutrophils to synthesize and secrete cytokines after stimulation by mycobacterial products suggest that these cells participate in the regulation of the immune response. In addition, the ability of reactional these cells to release TNFα suggested that they could be involved in the amplication of TNFα production at the site of leprosy lesions (ENL).

IM61

CLOFAZIMINE INTERFERES WITH T-LYMPHOCYTE ACTIVATION IN VITRO. Martins MVBS, Sarno EN, Sampaio EP. Leprosy Laboratory, Oswaldo Cruz Foundation, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, Brazil

Recent studies in our laboratory have demonstrated that clofazimine, an anti-microbicidal and anti-inflammatory drug, has a multiple effect on the regulation of the immune response. Firstly, clofazimine, but not thalidomide, showed in vitro an inhibitory effect on the in vitro proliferation of T cells, an effect which appeared to be irreversible. It was demonstrated that IFNγ production in these cultures was inhibited as well. The goal of the present study was to determine if clofazimine in any way interfered in the expression of activating molecules (CD25, CD69, HLA-DR, and B7) in PBMCs activated with PHA. It was shown that clofazimine was only able to inhibit the expression of CD25 (IL-2 receptor). In addition, it was decided to investigate whether either lymphocytes or macrophages were the target cell responsible for or that interfered with the action of this drug. As a first step, lymphocytes and macrophages were incubated separately for 1-3 hours with different drug concentrations (5, 25 and 40 µg/ml). After the incubation period, the cells were washed reconstituted with lymphocytes and macrophages (without clofazimine) respectively and stimulated with 1% PHA (in the absence of the drug). The results showed that only the cultures which had the lymphocytes that were pre-incubated with clofazimine showed inhibited proliferation.
In the second part of the study, the effect of clofazimine on the secretion of inflammatory cytokines was investigated. The kinetic of TNFα production in culture supernatants of monocytes and PBMC stimulated in vitro with LPS 1µg/ml, in the absence or presence of clofazimine was determined. Inhibition of TNFα protein synthesis was noted within the first three hours of culture. Interestingly this effect was bypassed during longer periods and, at 20 hours, no effect of the drug on TNFα production was seen.

IM62

SERUM ANTIBODY TO A SYNTHETIC PEPTIDE ANTIGEN OF THE 85B *MYCOBACTERIUM LEPRAE* PROTEIN COMPLEX IN NEWLY DIAGNOSED LEPROSY PATIENTS

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An ELISA for the quantitative determination of serum antibody to *Mycobacterium leprae* was developed using a synthetic peptide as antigen corresponding to the amino acid sequence 256 -280 of the 85B protein complex, a major and well conserved *M.leprae* antigen.

This test was applied to the sera of newly diagnosed leprosy patients, i.e. 14 multibacillary (MB) and 13 paucibacillary (PB) cases as well as to 11 healthy household contacts (HHC) and 11 endemic controls (EC). Results were compared to those of the PGL-I ELISA using phenolic-glycolipid disaccharide serum albumin (D-BSA) as antigen. Sensitivity of the peptide ELISA was 43% for MB leprosy and 46% for PB leprosy as compared to 100% and 23% respectively of the PGL-I ELISA. Using PGL-I ELISA the reactivity was 36% for HHC and 18% for EC, resulting in a specificity of 82% (EC only) and 59% if both HHC and EC are considered. The respective specificity of the peptide ELISA was 64% for EC and 59% for HHC and EC combined.

It is concluded that the detection of serum antibody directed against the 85B peptide is not useful in the diagnosis of leprosy because of the low sensitivity and specificity of the test.

IM63

EARLY LOCAL & REGIONAL EVENTS IN THE 2° RESPONSE OF RHESUS MONKEYS TO *M. LEPRAE.*

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Background: Previous studies have indicated differences between simian immunodeficiency virus-infected (SIV+) and SIV- rhesus monkeys, with regard to T-cell recruitment and function during the first month after their initial exposure to *M. leprae*.
Objectives: The 2° immune response was studied in 9 animals inoculated 18 months earlier with *M. leprae*; 3 were also SIV+. The percentage of CD45RO+ 'memory' T- cells was determined at new *M. leprae* inoculation sites, in lymph nodes and in blood.
Design: Fresh *M. leprae* (10^7/site) were injected intradermally at 12 sites (4 per limb). Biopsies were taken at 2, 5, 8, 12, 26 and 62 days post-inoculation; biopsies of lymph nodes and samples of blood were taken at days 0, 5, 26, and 62. Tissues were minced and filtered, and suspensions were analyzed by 3-color flow cytometry for CD45RO+/CD4+ and CD45RO+/CD8+ cells.
Results: A weaker granulomatous response was seen in the SIV+ group. In both SIV- and SIV+ animals, a small increase in CD45RO+ cells occurred in the skin at day 5 in both CD4+ & CD8+ subsets. A larger increase in CD45RO+ cells was observed in both subsets, in skin and lymph node, at day 62.
Conclusions: SIV+ animals are less capable of producing a granulomatous secondary 2° response to *M. leprae* and have lower CD4+ cell counts at inoculation sites, in draining lymph nodes, and in blood. Nevertheless, they are able to recruit CD45RO+ 'memory' T-cells to skin and lymph nodes in the 2° response to *M. leprae*, in a pattern comparable to that of healthy rhesus monkeys.

IM64

SEROLOGICAL DIAGNOSIS OF LEPROSY USING FULL THICKNESS SKIN CULTURES IN VITRO: A PRELIMINARY STUDY.

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Although specific serological assays using PGL-1 and 35kD antigens of M.leprae have been developed both of these specific assays fail to detect a significant level of antibody against M.leprae in clinically established cases of leprosy. It was noted that in early stages of infection (TT/BT leprosy) only 40 to 60% of patients show positivity to these assays. When the antibody levels against whole M.leprae was measured in such patients it was further noted that 40% of TT/BT leprosy do not show a significant level of antibody. This finding led us to look for antibody measurement locally in skin lesions. Methods were established to measure antigen and antibodies in skin smear suspensions taken from skin lesions of patients. It was noted that 100% of TT/BT patients were positive for antibody. To know the mechansim of local antibody secretion a full thickness skin culture has been established and all types of leprosy lesions were found to liberate antibodies in vitro in the tissue culture. The kinetic of antibody response in vitro from TT/BT and BL/LL skin lesions have been noted and the mechanism is being understood immunohistologically using B cell markers. These studies would further provide an insight into the mechanism of local immunity and in establishment of a diagnostic assay for early cases of leprosy.

IM65

IN VITRO THALIDOMIDE IS AGONISTIC TO THE SYNTHESIS OF IL-2 AND FREQUENTLY ANTAGONISTIC TO THE SYNTHESIS OF TNF-A IN ENL AND HIV SEROPOSITIVE PATIENTS.

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Recently, there has been a resurgence of interest in thalidomide (thal). Contributing factors to this renewed interest are the numerous reports of thal's successful use in the treatment of conditions other than ENL such as aphthous ulcers in patients with AIDS. The mechanism by which thal attenuates these inflammatory conditions is unknown, but modulation of inflammatory cytokines like IL-2 and TNF-α possibly plays a key role.

Prior to treatment with thal, peripheral blood mononuclear cells (PBMC's) and plasma were obtained from 4 patients with ENL and 6 HIV seropositive patients. After the patients had ingested a cumulative dose of 800 mg of thal (48h) a second sample of blood and PBMC's were taken. The PBMC's were exposed for 2 h to 4.0 μg/ml of thal and stimulated with 50 ng/ml of staphylococcal enterotoxin A (SEA). After 16 h the culture supernatant was assayed for TNF-α and IL-2.

In the four patients with ENL, prior to ingestion of thal, comparing the concentration of the cytokines in the SEA-stimulated cell cultures to those treated with thal and SEA, the thal and SEA cultures had an average increase of 163 pg/ml of IL-2 (range 397-9 pg/ml). TNF-α was decreased by an average of 95 pg/ml (range 154 to 71 pg/ml) in 3 patients and increased by 550 pg/ml in one patient. The concentration of IL-2 in the plasma prior to ingestion of thal was 10 ± 11 pg/ml and 15 ±12 pg/ml at 48 h. The concentration of TNF-α was 60 ± 54 pg/ml prior to ingestion of thal and 67 ± 49 pg/ml after 48 h. In a similar protocol in six HIV seropositive patients, the thal and SEA-treated cultures had an average increase of 182 pg/ml of IL-2 (range 392 to 58 pg/ml). TNF-α was decreased by an average of 78 pg/ml (range 215 to 62) in 5 patients and increased by 84 pg/ml in one patient. The concentration of IL-2 in the plasma prior to ingestion of thalidomide was 29 ± 15 pg/ml and 19 ± 15 pg/ml after 48 h. The concentration of TNF-α in the plasma prior to ingestion of thal was 9 ± 5 pg/ml, and 4 ± 3 pg/ml after 48 h. After 48 h, when unhydrolyzed thal achieved an average concentration of 2 ± 0.5 μg/ml in the plasma, the results from the *in vitro* studies on the cytokines from the cultured cells was highly variable.

Thal, in vitro enhanced the ability of SEA-stimulated cells to produce IL-2, and it frequently suppressed the ability of the same SEA-stimulated cells to produce TNF-α.

IM66

THALIDOMIDE CAN MODULATE THE ABILITY OF MONONUCLEAR CELLS TO INCORPORATE [^{3}H]-THYMIDINE WHEN STIMULATED WITH *M. LEPRAE* ANTIGENS.

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Thalidomide is capable of modulating the immune response. The exact mechanism by which it modulates and suppresses erythema nodosum leprosum (ENL) is unknown. In this study, *M. leprae* was incubated with mononuclear cells from a donor naive to *M. leprae*, a lepromin-positive, healthy individual and a patient with ENL. Four μg/ml of thalidomide was added into the cultures at the initiation of the incubation period and was replaced daily to maintain an estimated concentration of ≥0.5 μg/ml of unhydrolyzed thalidomide. After 6 days [^{3}H]-thymidine was added into the cultures and the cells harvested the next day. When cells cultured in the absence of *M. leprae* and thalidomide were compared to those cultured in the presence of *M. leprae*, the *M. leprae*-exposed cells from the lepromin-positive individual and the ENL patient were stimulated in their ability to incorporate [^{3}H]-thymidine. When cells cultured in the presence of *M. leprae* were compared to cells cultured in the presence of thalidomide and *M. leprae*, incorporation of [^{3}H]-thymidine was dramatically inhibited in the cells from the lepromin-positive individual and significantly enhanced in the ENL patient. The presence of *M. leprae* or thalidomide had no effect on the ability of the cells from the naive individual to incorporate [^{3}H]-thymidine.

IM67

THE POSSIBLE ROLE OF INTERLEUKIN (IL) 12 AND INTERFERON-γ-INDUCING FACTOR/IL-18 IN PROTECTION AGAINST EXPERIMENTAL *MYCOBACTERIUM LEPRAE* INFECTION IN MICE

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Cell-mediated immunity participates in host defense against mycobacterial infection. Both interleukin 12 (IL-12) and interferon-γ-inducing factor (IGIF/IL-18), produced mainly by macrophages, play a critical role in expression of cell-mediated immunity. To investigate the role of IL-12 and IGIF/IL-18 in vivo, we examined cytokine profile, bacterial growth and the potential benefit of cytokine therapy in genetically susceptible and resistant mice infected with *M. leprae*. The early expression of IL-12 p40 and IGIF/IL-18 at the site of inoculation was found in resistant mice 3-72 hr after the infection, but not in susceptible mice. Both strains of mice did not show expression of IFN-γ and IL-4. IL-12 administration resulted in a significant reduction of bacterial loads in mice with *M. leprae* infection. The results imply that susceptible mice exhibit decreased expression of type 1 helper T (Th1) response without reciprocal increased Th2 response and show responsiveness to exogenous IL-12. IL-12 therapy may be a possible rationale for treatment of *M. leprae* infection.

IM68

ANTIBODY RESPONSES WITH RECOMBINANT SELECTIVE SEQUENCE BASED PEPTIDES IN HUMAN LEPROSY PATIENTS

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Lepromatus leprosy patients(LL) and border line (BL) patients develop Type-2 and Type-1 reactions approximately 10-15% respectively. ENL (erythema nodosum leprosum) type 2 reactions are episodic, and are responsible for significant morbidity in lepromatus leprosy patients. These reactions are a prime source of morbidity and occurs before or during treatment with anti leprosy drugs. At present there is no method by which patients prone to reactions can be identified prior to onset of reactions. Such a distinction is mandatory for appropriate therapy. The aim of the present study is to evaluate MLS antigen and other sequence specific recombinant peptide as a predictor /marker of reactional states in leprosy using conventional ELISA.

To determine the immunological specificity between the different types of leprosy including ENL, we investigated the antibody responses to MLS (*M. leprae* derived sonicated antigen), recombinant peptide 2 and peptide 3 These peptides are 10-15 mer overlapping peptides synthesised on the basis of LSR amino acid sequence. We have observed that there is a selective recognition of B cell epitopes with ENL as compared to control groups. Of significance was more than 90% patients of ENL sera were recognised by recombinant peptides 2 and peptides 3. The amino acid sequences of recombinant peptide 2 and peptide 3 were GVTYEIDLTNKNAA and IDLTNKNAAKLRGD respectively. MLS antigen recognised all most all of the patients sera. The core sequences IDLTNKNAA are common in both the peptide and this may be a major target of antibody responses in ENL and lepromatus leprosy patients in Enzyme-linked immunoabsorbent assay(ELISA) These recombinant sequence specific peptides may be useful as a markers in diagnosis of early ENL.

IM69

ASSESSMENT OF ANTI-PGL I AS PROGNOSTIC MARKER OF LEPROSY REACTION

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Anti-PGL I assay is currently applied for leprosy conceived as an early marker of asymptomatic infection, early disease diagnosis and cure monitoring. Its use as prognostic marker of reaction is still a matter of controversy. We conducted a case-control study to investigate whether IgM and IgG anti-PGL I antibodies could discriminate patients at increased risk of developing reactions. Eligible cases were untreated leprosy patients at the onset of type-1 and type-2 reactions recruited from among 600 concurrent newly detected untreated leprosy patients attending outpatient clinic in Central Brazil. For each patient with reaction approximately the same number of leprosy cases without reaction matched to bacilloscopic index (BI), age and gender was randomly selected. Individuals without clinical leprosy were evaluated as healthy controls. Sera from type-1 reaction patients (N=43) and type-2 reaction (N=26) were tested by ELISA using PGL I synthetic disaccharide - BSA antigen and 1:300 sera dilution (cutoff point S0.2 OD). Antibody profiles were evaluated by exploratory data analysis and reverse cumulative distribution curves. IgG anti-PGL I response did not have a defined pattern being detected in low levels. Our results indicate that leprosy patients, independently of their reactional status, produce high levels IgM anti-PGL I demonstrating a strong correlation between the magnitude of antibody response and bacilloscopic index (BI). Patients with higher BI were at least 3.4 times more prone to produce antibody response compared to healthy controls.

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IM70

FREQUENCY OF IFNγ SECRETING CELLS IN THE PERIPHERAL BLOOD OF LEPROSY PATIENTS DEPENDS ON THE CLINICAL FORM OF THE DISEASE AND SHOW A DOMINANT CD4+ PHENOTYPE.

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It is well known that IFNγ is a critical cytokine involved in controlling resistance to many intracellular pathogens. In the present study, IFNγ production by peripheral blood mononuclear cells (PBMC) of leprosy patients in response to *M. leprae* antigens or mitogens, were investigated using the ELISPOT and ELISA methods. In vitro depletion studies using monoclonal antibodies (MACs method) were employed to determine the role of $CD4^+$ and $CD8^+$ cells in IFNγ production. In addition, cytokine production was compared to the lymphoproliferative response of the PBMC studied. Our results show that: (i) frequencies of IFNγ producing cells in response to *M. leprae* antigens were high in PBMC of TT, BB and in reactive BL patients (fl/1000); (ii) increased numbers of IFNγ - SFC (ELISPOT) are correlated with the detection of IFNγ in the supernatants (ELISA) and, with on exception, with the *in vitro* proliferative capacity of the PBMC, (iii) in all individuals tested, numbers of IFNγ SFC were increased after in vitro stimulation with PHA; (iv) PBMC of LL patients failed to produce IFNγ in response to *M. leprae* antigens, in comparison to reactive BL patient; (v) *in vitro* treatment with anti-CD4 Mab but not anti-CD8 Mab reduced (2-fold) antigen specific IFNγ production in 4/4 reactive BL patients. Our results underline the important role of CD4 cells and the marginal contribution of CD8 cells to IFNγ production in leprosy patients; and suggest that *M. leprae* induced IFNγ production is selectivelly decreased in multibacilary LL patients, but is enhanced during reaction.

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IM71

DENDRITIC CELL ENHANCEMENT OF INTERFERON-GAMMA IN LEPROMATOUS LEPROSY

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The role of antigen presenting cells was investigated in Th like responses in lepromatous leprosy patients. T cells, dendritic cells (DC) and monocytes (Mo) from PBMCs were separated on antibody coated magnetic bead columns using both positive and negative selection methods. Subsequently T cells were combined with 1 or 10 % DC or Mo, cultured for 24 hours in the presence of *M.leprae* antigen and assayed for mRNA expression of IFN-gamma IL-4, IL-10 and IL-12p40 using RT-PCR to reverse transcibe for cDNA and amplify by specific primers using polymerase chain reaction. Concurrently, quantitation by means of 32 P labelled probes was undertaken for IFN-gamma and IL-4. The results indicate that dendritic cells promoted expression of IFN-gamma even at 1 % concentration. IL-4 mRNA was not detectable in the majority of cultures. Conversely it was not possible to detect IFN-gamma signal in parallel T + Mo cultures from the same subject.

IM72

CUTANEOUS DELAYED-TYPE HYPERSENSITIVITY RESPONSIVENESS IN LEPROMATOUS LEPROSY AS DETERMINED BY MULTITEST CMI

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MULTITEST CMI is a disposable, plastic applicator with 8 sterile tines pre-loaded with 7 recall antigens and a glycerin control. To assess cellular immune function in lepromatous leprosy (LL), 37 patients were examined with the MULTITEST CMI to evaluate cutaneous delayed-type hypersensitivity (CDTH) responsiveness before or during therapy. Of 26 newly diagnosed patients who had a MULITEST CMI applied before therapy (Group 1), 11 were responsive (≥ 2 antigens positive) and 15 were hypo-responsive (≤ 1 antigen positive). Nine of the 15 were re-tested after therapy began and 6 eventually converted to a responsive state. Eight of 11 patients already on therapy (2 months to 1 year; Group 2) at first MULTITEST CMI application were responsive; 2 others eventually converted. Eleven of 12 community or household contacts without clinical evidence of leprosy were responsive.

The MULTITEST CMI applicator provided an efficient, safe, reproducible, and relatively inexpensive method to determine CDTH responsiveness in LL and control subjects. Our findings indicated that most LL patients are able to generate a CDTH response to standard recall antigens, some becoming responsive after therapy initiation. This suggests that CDTH abnormalities are neither predisposing causes nor necessary accompaniments of LL, but probably represent remote sequelae of the disease.

IM73

THE INVOLVEMENT OF HLA IN LEPROSY IN SOUTHERN CHINA

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According to the WHO-MDT we have carried out this study to know the involvement of HLA in leprosy. Seventy-two Southern Chinese leprosy patients and 120 health subjects as the control were participated in this study. HLA-A, -B and -C were serologically typed by using a standard microcytotoxicity test, and HLA-DR2 subtypes and MHC class I chain-realted A (MICA) allels were typed at the DNA level by using the PCR-SSCP method.

The frequencies of HLA-B57 antigen were significantly increassed, and those of HLA-B46 antigen were significantly decreased, in the leprosy patients as compared with the control group ($P<0.01$). The frequencises of HLA-DR2 antigen and its subtypes in the patients showed a higher tendency than those in the controls, but there were no significant differences. In addition, the frequencise of MICA alleles were not significantly different. However, the patients with B57-MICA5.1 haplotype were significantly increassed, and those with B46-MICA5 haplotype were decreassed, when compared with the control group ($P<0.01$).

This study shows that the leprosy in this district is significantly associated with particular HLA-B/MICA haplotypes (positive association with B57-MICA5.1 and negative association with B46-MICA5). These results suggest that an HLA-linked disease-control gen for the leprosy in Southern China may be locatednear the HLA-B/MICA region and not in the HLA-DR locus.

IM74

ANALYSIS OF *IN VITRO* T-CELL RESPONSES TO CANDIDATE *M.LEPRAE* SKIN TEST ANTIGENS IN LEPROSY PATIENTS AND CONTROL SUBJECTS

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A sensitive and specific method for detection of subclinical infection in leprosy remains a priority for leprosy control. Development of a tuberculin-like skin test to identify *M.lepraе*-exposed subjects would be the most practical approach for use in leprosy endemic areas. This may be achieved by isolation of *M.leprae* specific peptides, or by defined fractionation of the bacillus. A new approach to fractionation of *M. leprae* has yielded two fractions depleted of lipoarabinomannan (LAM). These fractions, derived from *M.leprae* cell cytosol (MLSA-LAM) or cell wall (MLCwA-LAM) were tested in a whole blood assay, to assess *in vitro* T-cell responses in Nepali leprosy patients, and in endemic and non-endemic (UK) control subjects. The fractions proved to be potent T-cell antigens, inducing high proliferation and IFNγ responses in paucibacillary leprosy patients and leprosy contacts. However, lepromatous leprosy patients did not respond. Responses in control subjects indicated that exposure to cross-reactive antigens in other mycobacteria may compromise the *M.leprae* specificity of these fractions. An alternative approach, to analyse differential responses to equivalent fractions derived from both *M.leprae* and *M.tuberculosis* was also explored. These field studies have demonstrated the utility of the whole blood assay in epidemiological studies as an indicator of T-cell responses, prior to *in vivo* skin test trials.

IM75

A STUDY ON THE METHODS FOR EARLY SEROLOGICAL DIAGNOSIS OF LEPROSY AND THEIR POTENTIAL USE

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The authors have established 11 methods for early serological diagnosis of leprosy, including FLA-ABS test, ELISAs with artificial products (ND-O-, ND-p-, NT-O-, NT-p-BSA, PGL-I, whole M. leprae and M. smegmatis), monoclonal antibody specific binding assay (McAB/SBA), latex agglutination test (LAT) and MLPA. These methods were compared each other on a large scale in leprosy patients in the field.

The results indicated that: 1)the excellent results were obtained when ELISAs were conducted with skim milk or egg albumin as blocking agent and by using blood from ear lobes instead of those from venipuncture; 2) according to 4 "S" standard (sensitivity, specificity, simplicity and speed), ND-O-BSA-ELISA (ND-ELISA) was proved the best and MLPA more suitable for use in the field due to its simple procedure and quick reaction; 3) in ND-ELISA, the increase or decrease of OD value was positively correlated with BI, and the order of positive rates (PR) was LL>BL>BB>BT>TT and household contact > random population > normal control of endemic area > normal control of nonendemic area; 4) in population of subclinical infection with M. leprae, the highest risk group was 15 - 25 years age group with an increase or a persistance of high OD values prior to the onset of disease, 5) OD values were gradually decreased as the treatment goes on and these declines run parallel with the declines of BI, 6) in cases cured with DDS monotherapy, an increase or a persistance of high OD values in ND-ELISA occurred prior to the onset of leprosy relapse.

In conclusion, the authors have developed 11 immuno-assays and have proved ND-ELISA as the most practical one in investigating early detection of disease, in monitoring antimicrobial therapy and even in predicting relapse of leprosy.

IM76

ANTI-LEPROSY PROTECTIVE VACCINATION OF MONKEYS WITH BCG AND BCG PLUS HEAT-KILLED *MYCOBACTERIUM LEPRAE*: IMMUNOLOGIC OBSERVATIONS

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Groups of rhesus and sooty mangabey monkeys (RM & SMM) were vaccinated and boosted with BCG or BCG + low-dose (LD) or high-dose (HD) heat- killed *Mycobacterium leprae* (ML) or were unvaccinated. Prior to and following vaccination-boosting and subsequent ML challenge, these and unvaccinated-unchallenged monkeys were observed longitudinally for several years. Paucibacillary (PB)-prone RM were significantly protected clinically by BCG or BCG + HKML; multibacillary (MB)-prone SMM were partially protected by BCG alone, but were render-

ed clinically more susceptible by BCG + HKML. Clinical interpretations were supported by phenolic glycolipid-I (PGL-I) antigen levels in plasma. Highly significant blastogenic responses (to lepromin, Rees antigen, ML 10Kd protein & tuberculin) were seen in all 3 vaccinated RM groups post-vaccination (PV), post-boosting (PB), immediately post-inoculation (PI) with ML and 2 years PI. Similar responses to ML antigens occurred mostly in BCG + HKML groups of SMM PV and PB; suppression of some responses occurred in SMM approximately 2 years PI. Blood lymphocyte subsets with the following phenotypes significantly changed in vaccinated vs unvaccinated ML-challenged SMM and RM: CD2, CD4, CD8, CD16, CD20, CD4CD29 and CD4CD45. Patterns of changes in the subsets differed between the 2 species. Serum IgG vs IgM anti-PGL-I responses supported our prior observations that serum IgG anti-PGL-I responses favor protection while IgM responses correlate with susceptibility to leprosy. Lepromin skin testing confirmed successful long-term immunization of RM and failure to induce significant responsiveness in SMM by these immunizations. SMM immunized with BCG + LDHKML had significantly suppressed lepromin skin-test responses 33 months post-ML challenge. The immune response to vaccination with HKML and/or BCG is complex, multifaceted and dose-dependent. BCG protects individuals who are susceptible to either PB or MB leprosy; BCG + HKML protects PB-prone and heightens susceptibility in MB-prone individuals.

MICROBIOLOGY

MI01

IDENTIFICATION OF SPECIES-SPECIFIC ANTIGENIC DETERMINANTS OF M.LEPRAE IN CULTIVABLE MYCOBACTERIA

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At the present stage of leprology in some countries great efforts are aimed at searches for cultivable mycobacteria having close antigenic relationship with M.leprae so to use them as vaccine and diagnostic preparations in leprosy. With using immunoblotting we studied antigens of mycobacteria isolated from tissues of lepromatous leprosy patients (M.01, M.011) and cultivated in vitro on Shkolnikova's nutrient medium modified with perfluorine decaline as well as antigens of M.lufu, known as highly susceptible to dapsone. For identification following six clones of MAbs to M.leprae antigens obtained from WHO Bank were used: anti-12KDa mc8908-MLO6-A, anti-18 kDa mc8026-L5, anti-36 kDa mc5828-F47-9-36, and mc9215-IIIE9, mc5205-IIH9 and mc2404-IVD8 against three epitopes of 65kDa-protein. For reference, a sonicate of M.leprae isolated from nine-banded armadillos (WHO Bank) was used. The obtained results were following. In antigenic reference-preparation proteins of molecular weights 12, 18, 36 and 65 kDa (IIIE9) were identified. 36 kDA-protein was detected in M.01 and M.lufu, 12 kDa - in M.011 and M.lufu. MAb mc2404-IVD8 actively interacted with 65 kDa in M.01 and M.011. Presence of antigenic determinants specific for M.leprae in mycobacterial strains under study might suggest their antigenic relationship with M.leprae, hence, necessitating further investigation of their biological properties.

MI02

DETECTION OF MYCOBACTERIUM LEPRAE IN NASAL SECRETIONS OF HOUSEHOLD CONTACTS OF MULTIBACILLARY AND PAUCIBACILLARY PATIENTS

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It is generally accepted that one mode of transmission of *M. leprae* is via aerosols originating from infected individuals shedding *M. leprae* through the nose. Workers have reported either acid-fast bacilli (AFB) or PCR-positive nasal secretions from MB and PB patients. To better understand the risk of exposure to *M. leprae* from index cases we evaluated nasal carriage rates among household contacts (HHC) of MB and PB cases in Cebu, PI. Two hundred and forty-two HHC of both treated and untreated MB and PB index cases were enrolled in the study. Evidence of *M. leprae* in secretions by PCR was found in 8 of the 242 HHC (3.3%). Two of 8 of the contacts were positive from both nasal swabs and their serum tested positive for PGL-I antibody. All PCR-positive HHC were at least 18 years old and were contacts of either LL or BL index cases. PCR-positive contacts lived with their index cases between 2-21 years. Whereas 2 index cases of the positive HHC were untreated LL's, the remaining index cases had either finished WHO-MDT or were currently under treatment with the same or a combination of rifampin and ofloxacin. All contacts of PB disease tested negative by PCR. Further serial testing of nasal secretions from contacts is warranted in an attempt to distinguish a true carrier state from transient contamination.

MI03

USE OF PCR MEDIATED AMPLIFICATION OF MYCOBACTERIUM LEPRAE IN DIFFERENT TYPES OF CLINICAL SAMPLES AND SEROLOGY IN LEPROSY PATIENTS AND CONTACTS.

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The purpose of this study is to determine the usefulness of different clinical samples obtained from skin lesions with slit-skin smears before and after biopsy, ear lobes and nasal swabs together with serum samples in different stages of the disease and compare the results of each group with the less sensitive conventional methods.

Samples were obtained from 54 leprosy patientos (42 multibacillary and 12 paucibacillary) and 37 contacts (12 household contacts and 25 leprosarium staff members). The smear and histopathological stainings were done by the classical methods. The PCR primers used were S-13 and S-62 (Hartskeerl et al.1989) that amplify specifically the 530 bp fragment of the proline-rich (pra) gene of M.leprae. The serological assays include detection of antibodies to phenolic glycolipid I (PGL-I), LAM-B and protein antigens by enzyme-linked immunosorbent assays.

The results reveal that PCR was more sensitive in detecting M.leprae in biopsy and slit-skin smears specimens with no or low bacterial loads than the conventional microscopic examination and that the serological assys also correlated well with decrease of the bacterial index (BI) suggesting their usefulness for following leprosy patients responses to therapy.

MI04

A COMPARATIVE STUDY OF THE DETECTION OF Mycobacterium leprae BY PCR-BASED METHODS

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