Nadu to select the Focal Areas as per criteria and conduct focal surveys.

As per local needs, based on criteria, all over Tamil Nadu focal areas were identified and Focal Surveys were carried out in Jan-April 2001.

The paper would highlight the outcome of the Focal Surveys:

• Coverage of population and leprosy cases found and follow through action right from patient education, treatment and release of patients.

• Effectiveness of the approach, conclusions, suggestions and recommendations

OHE 23

THE PROMOTION OF BEHAVIORS OF LEPROSY PATIENTS IN COMMUNITY HOSPITALS THROUGH GROUP PROCESS WITH SOCIAL SUPPORT

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The objective of this quasi -experimental research is to identify group process with social support in order to promote behaviors of leprosy patient. The subjects was recruited from multibacillary leprosy patients in community hospitals There were 61 subjects who passed eligible criteria and then divided purposively into experimental group (31 subjects) and control group (30 subjects). Data was collected by interview before and after the intervention was implemented. The results were analyzed by Percentage, Mean, Standard Deviation, Student t Test, Paired Sample, T-test, Z- test, Pearson Product Mòment Correlation and Chi-square Test.

The results show that there are increasing of the perception in term of susceptibility, severity, positively benefit among experimental group. In addition, the behavior of leprosy patients is improved significantly. The proportion of contact cases among experimental group is increased significantly. More over, perception of positive benefit and services satisfaction are correlation significantly with behavior of leprosy patients.

The researcher recommended that group process with social support should be implemented in community hospitals. The community hospital staff who are responsible for leprosy should be trained to give good quality of services.

OHE 24

TREATMENT DEFAULT AMONG PATIENTS DISCOVERED DURING LEPROSY ELIMINATION CAMPAIGN (LEC): EXPERIENCE OF KANO STATE NIGERIA.

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Leprosy Elimination Campaign is an initiative adopted and recommended by the World Health Organization with the main objectives of creating community awareness on leprosy activities, capacity building (most especially among the lower cadre of health providers), and to enhance case finding and case holding. A Statewide LEC was conducted in Kano State, Nigeria, in the first half of the year 1999. The exercise was conducted with the set target of achieving the above objectives. The campaign was mainly sponsored by the World Health Organization, with support of the Netherlands Leprosy Relief and the Federal Ministry of Health. The campaign was to a large extent, a huge success taking into consideration its objectives. All villages in the State were visited, and over 1000 general health workers were trained on Leprosy. More than 68% of patients registered during the year were found during the campaign. However the State Tuberculosis and Leprosy Control Program observed a high default among the patients discovered during the exercise, and conducted a defaulter retrieval activity and also made an attempt to find outwhy these particular patients defaulted. Statistics on all the patients treated, defaulted, retrieved and lost are collated and analyzed.

IMMUNOLOGY

OI 1

A NON-INVASIVE METHOD FOR DIAGNOSIS OF LEPROSY BASED ON DETECTION OF SPECIFIC ANTI-MYCOBACTERIAL ANTIBODIES IN SALIVA

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The aim of the present work was to improve methods of diagnosis of leprosy through the development of immune test system for anti-*M. leprae* antibodies (Ab) in saliva. Samples of saliva and blood sera from 116 leprosy patients at different stages of their disease and 23 healthy donors (control group) were

studied with using indirect enzyme labeled immune assay. As test-antigen for detection of anti-*M. leprae* Abs, preparations of M. lufu, a value of which for leprosy serodiagnosis was proved by us earlier, were used. Rabbit Abs against human peroxidase-labeled immunoglobulins (IgG, IgA, IgM) were used as conjugate. The investigations showed that Ab titers in saliva and blood sera were comparable both in patient and control groups. Correlation analysis indicated interrelations between Ab titers in saliva and serum samples. Dynamic investigations showed high titers of Abs both in saliva and blood sera in patients with active leprosy. Inactive leprosy patients giving seronegative results for 1-2 and more years, showed no specific anti-mycobacterial Abs in their samples of saliva. In the periods of activation of the disease (relapses, exacerbations of leprosy neuritis) Ab levels against *M. leprae* in saliva were increasing in parallel with increasing anti-*M. leprae* Abs in blood sera. Thus, a test-system was developed, high diagnostic value and reliability of which was achieved owing to using a new antigen from M. lufu and conjugate of peroxidase-labeled rabbit Abs against human immunoglobulins of IgG, IgA, IgM classes. Detection of anti-*M. leprae* Abs in saliva opens possibilities for early diagnosis of leprosy infection in leprosy contacts and general population of leprosy endemic areas. Monitoring of Ab levels in saliva of leprosy patients under treatment allows estimating effectiveness of antileprosy therapy.

OI 2

ANTIGENIC SPECIFICITY OF THE *Mycobacterium leprae* HOMOLOGUES OF ESAT-6 AND CFP-10.

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The recent completion of the sequencing of the genomes of *M. tuberculosis* and *M. leprae* provides the opportunity to identify leprosy-specific antigens. An analogous approach applied to *M. bovis* BCG allowed the identification of deleted genes and the development of antigens that can distinguish between *M. tuberculosis* infection and vaccination with BCG. Among those antigens which have shown promise are two low-molecular weight *M. tuberculosis* culture filtrate proteins, ESAT-6 (*esat-6*) and CFP10 (*lhp*), both encoded by genes in the RD1 region, a genetic segment that has been deleted from all strains of BCG. Because the *M. leprae* ESAT-6 (ML0049) and CFP-10 (ML0050) proteins have only 36% and 40% identity, respectively, to their homologues in *M. tuberculosis* (Rv3875 and Rv3874), we decided to analyze the immunologic cross-reactivity of these proteins in mice by characterizing the B and T cell epitopes recognized. We had previously reported this analysis of the ESAT-6 homologues, and found that the dominant B and T cell epitopes recognized in H-2^d haplotype (BALB/c) strain mice for the *M. tuberculosis* and *M. leprae* proteins were in different regions. In addition, polyclonal antisera against the two forms of ESAT-6 did not cross-react at the level of the whole protein or with any of the heterologous peptides. We have since performed a similar immunological analysis of cross-reactivity with the CFP-10 homologues, and found that polyclonal antiserum raised against ML0050 did not cross-react with the *M. tuberculosis* homologue, and vice versa. We are currently in the process of analyzing antibody and T cell immune responses against members of the ESAT-6 family of proteins and other unique proteins discovered in the analysis of the *M. leprae* genome.

OI 3

CELL MEDIATED IMMUNITY IN LEPROSY PATIENTS WITH ERYTHEMA NODOSUM LEPROSUM (ENL)

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The dramatic resolution of erythema nodosum leprosum (ENL) following therapy with thalidomide suggests that immunologic changes associated with this treatment may afford insights into the pathogenesis of ENL. It has been reported recently that thalidomide may promote Th-1 immunity. However, it is unknown if thalidomide acts in this way in patients with ENL.

Aim: To study cell-mediated immune responses in Nepali leprosy patients with ENL undergoing thalidomide treatment, and to compare their response to those of lepromatous patients without ENL.

Methods: Venous blood was obtained from appropriate (LL) patients: 20 with and 20 without ENL. Plasma levels of interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and interleukin 12 (IL-12), and of soluble IL-2 receptor (sIL-2R) were measured using a standard immunoassay. Leprosy-specific and mitogen-induced IFN-γ producing cells in the peripheral blood were measured by ELISPOT and flow cytometry, respectively on days 0, 7 and 21 of a 21 day course of thalidomide.

Results: Thalidomide-induced activation of Th-1 immunity was suggested by an increase in numbers of T cells induced ex-vivo to produce interferon IFN-γ as assessed by both ELISPOT and flow cytometric assays ($p > 0.01$). This activation was transient, however, observed on day 7 of thalidomide treatment. Although

it is difficult to discern obvious trends in the plasma cytokine levels, there is some correlation between the patterns in TNF-α levels and those for IL-12.

Conclusions: Our results suggest transient T cell activation following thalidomide treatment, and may give some clues to the pathological processes underlying ENL as well as to new treatment strategies.

OI 4

CYTOKINE LEVELS IN TYPE 1 REACTIONS: RELATION TO NERVE DAMAGE AND THE RECURRENCE OF REACTION.

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Aim: To elucidate the role of cytokines during and after steroid treatment of Type I Reaction (T1R), and their relation to recurrent reaction episodes

Methods: We enrolled 192 borderline (BT, BB, BL) leprosy patients, 96 with T1R and 96 without, in this study. Blood was collected from T1R patients at various timepoints before, during and after prednisone treatment, and used in a standard 24 hour whole blood assay.

Results: Leprosy specific antigen-induced levels of IFN-γ, TNF-α and IL-10 were measured in a 24-hour whole blood assay in T1R patients. Cytokine levels were significantly increased when compared with appropriately matched borderline leprosy patients without T1R. Steroid treatment lowered levels of IFN-γ, but levels of TNF-α increased as the doses of steroids were lowered. IL-10 levels increased during steroid therapy. High TNF-α levels in untreated patients (higher than 75th percentile) was associated with a 5 times greater risk of reactivation of symptoms during treatment phase. High levels of TNF-α after treatment with 30mg of steroids was associated with a 3-5 times greater risk of nerve function impairment or failure to improve nerve function. The relationship between cytokine levels and subsequent reactions was investigated by follow up for up to three years after initial observations.

Conclusion: This study seeks to link cytokine levels with recurrent T1R reactions and nerve function impairment and offers a means to identify patients failing to respond adequately to steroid therapy.

OI 5

DENDRITIC CELL-MEDIATED PRODUCTION OF IL-12 AND IFN-γ BY *Mycobacterium leprae*-DERIVED CELL MEMBRANE

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The development of reliable vaccination agents toward leprosy is currently desired. In order to identify molecules capable of inducing effective cellular immunity against *Mycobacterium leprae*, the antigenicity of *M. leprae*-derived cell membrane fraction was examined using human dendritic cells (DCs). Immature DCs internalized and processed the cell membrane components, and expressed Ags, which reacted to lipoarabinomannan mAb or to leprosy patient's sera. The expression of MHC class II, CD86 and CD83 Ags on surface DCs was up-regulated indicating that the DCs were potently stimulated by the membrane Ags. Moreover these stimulated DCs induced significantly higher proliferation of autologous $CD4^+$ and $CD8^+$ T cells and higher IFN-γ production by the T cells than those pulsed with equivalent doses of *M. leprae*-derived cytosol fraction or whole live *M. leprae*. The involvement of CD40 ligand signaling on membrane pulsed DCs enhanced the IFN-γ production. $CD4^+$ and $CD8^+$ T cells from tuberculoid leprosy patients produced marked and significantly higher IFN-γ than those from healthy donors, when they were stimulated by autologous cell membrane pulsed DCs. The $CD8^+$ T cells stimulated for 10 days by DCs pulsed with the membrane and CD40L, produced intracellular perforin in the Ag dose or CD40L dependent manner, in 50% of lymphocytes donors. Furthermore, the *M. leprae* cell membrane was more efficient in the CD40L-associated IL-12 p70 production from DCs than the cytosol fraction, but was less efficient than cell membrane from *M. smegmatis*. Both hydrophobic and hydrophilic fractions of *M. leprae* cell membrane induced IL-12 p70. These results suggest that *M. leprae* cell membrane has pleural antigenic molecules that might be useful as the vaccinating agents against leprosy

OI 6

EFFICACY OF SHORT TERM MULTIDRUG THERAPY ON THE CONTROL MULTIBACILLARY LEPROSY

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The purpose of this study is verify whether the short term multidrugtherapy (MDT) on leprosy treatment can be efficient on the immune response and the consequent control of the evolution of the disease. To evaluate this hypothesis 67 multibacillaries leprosy patients (MB) (lepromatous leprosy-LL and border-

line leprosy-BL) were randomized to receive 12 or 24 doses of MDT and 9 healthy controls were evaluated. All the patients were classified by Ridley-Jopling criteria and the bacilloscopic index (BI) serum antibody anti PGL-1 (APGL-1), inflammatory cytokines and the co-stimulatory and adhesion molecules serum levels were measured before and after MDT. The APGL-1, IFN-γ, IL10, IL6 and TNF alpha serum levels were determined by ELISA assay. The results showed that MDT 12 and 24 doses can reduce the APGL-1 levels in a similar range. The decrease of BI and APGL-1 levels is followed by the augment of IFN-γ serum levels associated with enhancement of LAF/CD4+ molecules. The quantification of the LAF/CD8+ molecules is higher in MB patients before the treatment and after 24 doses of MDT the values are similar to the normal controls. Since IFN-γ is a cytokine able to induce an enhanced cellular immunity this results can suggest that 12 doses of MDT might be efficient on the control of MB leprosy specially in those patients with moderate bacillary index (below 3,0)

OI 7

ENUMERATION OF IFN-γ-PRODUCING CD4+ T CELLS AS A TOOL FOR SELECTING HIGH-LEVEL IFN-γ-INDUCING *Mycobacterium leprae* ANTIGENS

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The sequencing of the genomes of *M. leprae* and other mycobacteria has afforded new opportunities for the development of pathogen-specific diagnostic tests for mycobacterial infections, critical in the context of leprosy eradication. Recently, antigen-specific IFN-γ production was used with success for the diagnosis of latent tuberculosis. We evaluated the IFN-γ inducing capacities of various fractions derived from *M. leprae* itself (*e.g.* ammonium sulfate or ethanol precipitated cytosolic proteins; cytosolic proteins fractionated by anion exchange chromatography or isoelectric focusing) and various recombinant proteins dictated by analysis of the genome (*e.g.* ESAT-6, CFP-10, 10kDa, Ag85B, Hlp, EfTu, MMP-I, MMP-II) and genetically fused versions of some of these recombinant proteins (*e.g.* CFP10-ESAT-6; ESAT-6-Ag85B). The initial screening was done with blood samples from two untreated newly diagnosed leprosy patients (TT and BT; from Leprosy Lab Outpatient Unit, Rio de Janeiro). The presence of IFN-γ-producing CD4+ T cells (IFN-T) was detected by intracellular cytokine assay, using flow cytometry, in response to several of these antigens and, when observed, was associated with high-level IFN-γ in the culture supernatants as determined by ELISA. In particular, some of the native *M. leprae* fractions, as well as MMP-I, had high frequencies of IFN-T and induced high IFN-γ supernatant levels, comparable for instance to that of *S. aureus* enterotoxin-B. (Research was supported by NIAID, NIH and FAPERJ).

OI 8

EXPRESSION OF CHEMOKINES AND THEIR RECEPTORS IN LEPROSY SKIN LESIONS

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Chemokines are small peptides that are potent activators and chemoattractants for leukocyte subpopulations and some nonhaemopoietic cells. Chemokines and their receptors have been associated with infectious diseases.

Study: We have investigated the expression of chemokines and their receptors in leprosy skin lesions using immunohistochemistry. Skin biopsies from 25 leprosy patients across the leprosy spectrum, 11 patients undergoing Type I reversal reactions and 4 normal donors were immunostained by ABC peroxidase method using antibodies against CC and CXC chemokines and their receptors. We have also investigated mRNA production for MCP-1, RANTES and IL-8 chemokines using an *in situ* hybridisation technique.

Results: Chemokine and receptor expression was detected in all leprosy skin biopsies. Expression of CC chemokines MCP-1 ($p < 0.05$), RANTES ($p < 0.005$) and CXC chemokine, IL-8 ($p < 0.005$) were significantly elevated in borderline tuberculoid leprosy in reversal reaction compared to borderline tuberculoid leprosy. However, the expression of CC (CCR2 and CCR5) and the CXC (CXCR2) chemokine receptors did not differ across the leprosy spectrum. Similarly, there was no significant difference in the expression of MCP-1 and IL-8 mRNA. Nevertheless, a small but significant elevation in RANTES mRNA ($p < 0.05$) was detectable in borderline lepromatous leprosy in reversal reaction compared to borderline lepromatous leprosy.

Conclusion: Surprisingly, we did not find any difference in the expression of chemokine receptors across leprosy spectrum. In addition, RANTES expresion was slightly elevated in borderline tuberculoid leprosy in reaction. The presence of a neutrophil chemoattractant IL-8 in leprosy lesions, which do not contain neutrophils, here strongly suggests a role of IL-8 as a monocyte and lymphocyte recruiter in leprosy lesions.

OI 9

HUMAN T CELL RECOGNITION OF FRACTIONATED ANTIGENS FROM *Mycobacterium leprae*: POTENTIAL AS DIAGNOSTIC REAGENTS.

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One approach to the development of species-specific mycobacterial antigens is to progressively fractionate the antigens in whole bacteria until functional specificity is achieved. Equivalent preparations of *Mycobacterium leprae* and *Mycobacterium tuberculosis* cell wall and cytosolic antigens were used to test lymphocyte proliferation and IFN production in 6 day assays in leprosy and tuberculosis patients in Pakistan. The *M. leprae* antigenic preparations were less potent for T cells from tuberculoid leprosy patients than the *M. tuberculosis* antigens, and also induced T cell responses in tuberculosis patients. Further fractionation of *M. leprae* cytosolic antigens (MLSA) produced components of potentially greater specificity. The presence of *M. leprae*-specific antigens within MLSA was confirmed in healthy, non-BCG-vaccinated young adults in Malawi, where IFN-γ production to MLSA in diluted whole blood assays was more strongly associated with skin test indurations to Rees MLSA than to *M. tuberculosis* PPD. Thus fractionation of the cell wall or cytosolic proteins of *M. leprae* may yield specific diagnostic reagents for leprosy.

OI 10

IFN-γ DETECTION AND ABSENCE OF IL4 *IN SITU* UNDER NON STIMULATED CONDITIONS IN PAUCIBACILLARY SINGLE SKIN LESION LEPROSY

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Objectives: Define in early leprosy lesions the Cell Mediated Immunity by cytokine profiling: IFNγ, IL12, IL10, IL 4, TNFα and MIP1 and assess *M. leprae* DNA.

Methods: 259 SSL-PB leprosy patients were enrolled (1997-98) for ROM therapy and were clinically monitored. Six cytokines- IFN-γ, IL10, IL12, IL4, TNFα and MIP1α had mRNA assessed by RT-PCR using Real Time PCR (ABI Prism 7700 Perkin Elmer) in skin biopsies from 39 patients with different clinical outcomes. *M. leprae* DNA-PCR was performed in skin biopsies using primers for the specific 18KDa protein gene.

Results:: Highest values of IFN-γ were among the TT group (median=1.77) with well-formed granulomas, followed by BT (1.08) and I group (0.02). IL10 values were similar for TT (0.79) and BT (0.72) groups. MIP1γ detection was higher in TT lesions followed by BT and I groups. IL4 values were zero for all specimens tested. Statistically significant correlation was observed between IL12 and IFN-γ ($r=0.4$, $p=0.02$) and between IFN-γ and IL10 ($r=0.67$, $p<0.05$), possibly reflecting regulatory measures related to macrophage activation. Also IL10 and IL12 correlation was observed ($r=0.6$,$p<0.01$) suggesting *in situ* relationship between induction and control mechanisms in early leprosy lesions. 48.6% *M. leprae* DNA-PCR positivity was observed.

Conclusions: Our results support the concept that SSL-PB leprosy patients are tuberculoid-like with reasonable strong CMI contributing to the good prognosis after early treatment with ROM. *TDR/WHO grant 98100*

OI 11

IMMUNOPHENOTYPIC STUDY IN PAUCIBACILLARY SINGLE SKIN LESION LEPROSY

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Objectives: Assess the microanatomy of single skin lesion paucibacillary leprosy (SSL-PB) characterizing the phenotypes of different cell populations present in the cellular infiltrates.

Methods: 36 SSL-PB leprosy patients from Central Brazil, treated with ROM therapy were evaluated by immunostaining in skin biopsies collected before drug intake. Immunophenotypic study was performed in deparaffinized skin biopsies using monoclonal antibodies and immunoperoxidase methods, after microwave antigen retrieval. The distribution, location and estimated proportion of seven cell populations were evaluated: T lymphocytes ($CD3^+$), B lymphocytes ($CD20^+$), T lymphocyte subpopulations ($CD4^+$, $CD8^+$) and NK cells, CD 68^+ macrophages and mast cells. Samples were previously coded and all laboratory tests performed in independently, by different experts. Data were analyzed taking into account conventional histopathology and *M. leprae* DNA- PCR findings.

Results: 50% of SSL-PB were classified as BT, 27.8% TT and 22.2% I. *M. leprae* DNA was detected in 14/36 (45%). Cell phenotypes immunohistochemistry markers were observed in all preparations regardless of the morphological classification. Presence of neural aggression observed in histopathology was associated with positivity for *M. leprae* DNA by PCR ($p<0.05$). Detection of different cell phenotypes in early leprosy lesions, many of them with confirmed *M. leprae* DNA detection, provides indepth evaluation of the *in vivo* immune/inflammatory response in early paucibacillary leprosy. TDR/WHO grant 98100

OI 12

IMPROVING SUBUNIT DNA VACCINES AGAINST MYCOBACTERIAL INFECTIONS.

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Immunisation with *Mycobacterium bovis* (BCG) confers significant protection against leprosy and has contributed to the control of the disease. We have demonstrated that immunisation with a DNA vaccine expressing the immunodominant 35 kDa antigen of *M. leprae* causes equivalent protection to BCG in the mouse footpad model of *M. leprae* infection (1). We have investigated two ways of improving the efficacy of this approach, using DNA expressing the *M. avium* homologue of the 35 kDa protein (DNA-35), which shows 95% aa identity to the *M. leprae* protein, and infection with virulent *M. avium*. First, we co-immunised mice with DNA-35 and a plasmid producing both chains of IL-12 as a self-cleaving protein. This resulted in increased frequency of antigen-specific IFN-γ secreting T cells, and a reduction in specific IgG antibody responses. Moreover, following IVI infection with *M. avium,*, these mice showed significantly reduced bacterial loads compared to mice immunised with DNA-35 alone or BCG. This increased protection was associated with a significantly stronger IFN-γ response to both the 35 kDa protein and *M. avium* sonicate after challenge. Second, we examined whether targeting the 35 kDa protein to the B7 molecules on antigen presenting cells increased the vaccine efficacy. The 35 kDa gene was fused to the gene for CTLA-4-Ig within the DNA vaccine. Mice immunised with this construct showed an increase in both specific IFN-γ T cell and IgG responses. However, this was not associated with increased protection against *M. avium* infection. Therefore plasmid IL-12 is an effective adjuvant to increase the protective effect of DNA vaccines against *M. avium*. We are testing whether this increases protection against *M. leprae* infection. Future subunit vaccines against tuberculosis should also include dominant *M. leprae* antigens to ensure they provide cross-protection against leprosy.

1- Martin E, et al (2001) DNA encoding a single mycobacterial antigen protects against leprosy infection. Vaccine 19;1391-6

OI 13

IN SITU EXPRESSION PATTERN OF IFN-γ, IL-4 AND *M. leprae* ANTIGENS ACROSS SPECTRUM OF LEPROSY REFLECT DISEASE ACTIVITY RELATED TO REACTIONS

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Recent studies on lesional skin derived T cell clones (TCC) from leprosy patients experiencing reversal reaction (RR) showed a polarised shift of *M. leprae* responsive TCC to Type-1 like phenotypes with predominant production of IFN-γ/TNF-α and low production of IL-4/IL-5/IL-13. With this background knowledge, we assessed the presence of IFN-γ and IL-4 (both protein and mRNA) in lesional skin biopsies of untreated leprosy patients during RR and erythema nodosum leprosum (ENL) reactions. The *in situ* cytokines were identified on frozen biopsies by both immunohistochemical staining and *in situ* hybridisation and RT-PCR methods. On the other hand *in situ* presence of *m.leprae* antigens was identified in both frozen and parafin embeded biopsies by specific monoclonal antibodies to phenolic glycolipid-1 (PGL-1) lipoarabinomanan (LAM) by immunohistochemical single and double stainings. We found that both IFN-γ and IL-4 protein and mRNA were opresent in varying amounts in the lesions of untreated paucibacillary (PB) and multibacillary (MB) patients irrespective of their spectral status. No significant differences were seen regarding the in toto presence of

these cytokines in individual lesions although their presence varied indifferent granulomas within one lesion. However in lesions with RR and ENL higher levels of IFN-γ and IL-4 were seen although in ENL IL-4 was relatively higher although not significant. These data of *in situ* expression of T cell cytokines appear to indicate the ongoing disease activity as is the case in patients with reactions. The *in situ* presence of PGL-1 and LAM with the macrophages in lesions of MB patients decreased dramatically with the treatment. However, in some patients, presence of these antigens persisted in lesions of MB as well as in PB patients, but with differing staining pattern. Such dynamics in the expression pattern of PGL-1 and LAM, in leprosy lesions appeared to he associated with reactions. Our studies suggest that the evaluation of *in situ* expression pattern of IFN-γ, IL-4 and *M. leprae* antigens can be regarded as important differential diagnostic criterium for recognising leprosy lesions and may have predictive value for recognising reactions during the evalution of the disease

OI 14

INTERLEUKIN-10 PROMOTER SINGLE NUCLEOTIDE POLYMORPHISMS: MARKERS FOR DISEASE SUSCEPTIBILITY AND DISEASE SEVERITY IN BRAZILIAN PATIENTS

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Single nucleotide polymorphisms at positions –3575, -2849, and -2763 on the promoter region of the interleukin-10 gene are arranged to form haplotypes that affect levels of IL-10 production. In this study we have determined IL-10 genotype and haplotype frequencies in leprosy patients compared to controls, and analyzed their occurrence with particular forms of leprosy (multi- vs. paucibacillary as severe and mild forms, respectively). No significant differences was observed in genotypes comparing patients to controls, but in -2849 SNPs genotypes were different in multibacillary (MB) when compared to paucibacillary (PB) patients (P=0.04). The observation of haplotypes suggested that –3575T/-2849A/-2763C is associated with the occurrence of the disease (P=0.038) irrespective of the further clinical outcome. Besides, AGC haplotype was diminished in patients group as compared to controls (P=0.001). The comparison among patient groups demonstrated that the rarely found IL-10 haplotype AAA was strongly associated to the development of the severe (P=0.003) form of leprosy and TGA haplotype was more frequent in paucibacillary group. The data suggest that distal IL-10 promoter haplotypes could be used as genetic markers that predict susceptibility as well as disease severity in leprosy.

OI 15

LATERAL FLOW ASSAY FOR CLASSIFICATION OF LEPROSY PATIENTS AND IDENTIFICATION OF HIGH-RISK CONTACTS

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The interruption of leprosy transmission is one of the main challenges for leprosy control programs since no consistent evidence exists that it has been significantly reduced after introduction of MDT. Sources of infection are particularly patients with high loads of bacteria and infected persons in which the clinical signs have not yet become apparent. Detection of antibodies to PGL-I of *M. leprae* to identify those cases may be a tool for the interruption of transmission. An operational applicability of serology within the leprosy control services requires a simple test system.

We have developed a lateral flow assay for the detection of antibodies to PGL-I which takes only 10 minutes to perform. We have compared its performance with that of ELISA. An agreement of 89.7% was observed between ELISA and the lateral flow assay when tested on 620 serum samples; the agreement beyond chance (Kappa value) was 0.76. No significant difference was found between the lateral flow assay and ELISA when seropositivity rates obtained in groups of leprosy patients, household contacts and controls were compared. Storage of the only reagents required, the lateral flow test and the running buffer, for up to a month at high temperatures, does not influence the results of the assay.

The lateral flow assay is a fast and easy-to-perform method for the detection of IgM antibodies to PGL-I of *M. leprae*; it does not require any special equipment and the highly stable reagents make the test robust and suitable for use in tropical countries.

OI 16

LEPROSY TRANSMISSION AND MUCOSAL IMMUNE RESPONSE

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Decrease in the prevalence rate of leprosy has not reflected in the incidence of new cases. Very little is known about the transmission of leprosy. Though household contacts of multi-bacillary cases are at high risk of developing disease, majority of the new cases have no history of household contact. A study was undertaken to look at the transmission and the development of mucosal immunity. Subjects (3035) from three villages were examined and followed twice at six monthly intervals. Polymerase Chain Reaction (PCR) was used to detect presence of *M. leprae* DNA on the nasal mucosa and mucosal immunity was tested by measuring the salivary *M. leprae* reactive IgA antibodies (sML-IgA) using ELISA. More than 60% of the subjects from all the three villages showed sML-IgA. This response was seen in all the age groups. The response between BCG vaccinated and non-vaccinated individuals did not show any difference. Overall PCR positivity (presence of *M. leprae* on nasal mucosal) was found to be 1.65% (42 out of 2552), 4.5% (56 out of 1252) and 1.9% (25 out of 1308) in the initial screening, 1st follow-up and 2nd follow-up respectively. Most of the positive subjects in the follow-up were negative in either the previous or subsequent follow-up suggesting transient nature of the PCR positivity. Presence of *M. leprae* reactive antibodies in the majority of population suggested a possible widespread exposure to *M. leprae*. This mucosal immune response could be of protective importance as most of the subjects showing presence of nasal *M. leprae* had these antibodies. Shorter intervals between the follow-ups may shed more light on role of mucosal immunity and fate of *M. leprae* in the nasal passage.

OI 17

M. leprae INDUCES NF-B NUCLEAR TRANSLOCATION IN PBMC FROM LEPROSY PATIENTS

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NF-B is a transcription factor involved in the regulation of many inflammatory genes, including TNF and IL-1. It has also been suggested to be involved in the regulation of apoptosis. *M. leprae* is known to induce TNF production by PBMC from leprosy patients *in vitro*. Recent data from our laboratory showed apoptosis to occur in a dose-dependent manner and that TNF seems to be a mediator of this process. In order to investigate whether NF-B is activated in response to *M. leprae*, nuclear proteins from stimulated PBMC were analyzed by EMSA. Initial results demonstrate that *M. leprae*, when added at 1 and 10g/ml, induces nuclear translocation of this transcription factor. To identify NF-B subunits activated by *M. leprae*, samples were assayed by super-shift. Subunits p65 and p50 were detected, while p52, c-rel and Rel-B were not. These results indicate that NF-B is activated in PBMC by *M. leprae*. Moreover, its role in the induction of TNF synthesis and apoptosis of TNF synthesis and apoptosis is under further investigation.

OI 18

M. leprae-SPECIFIC, HLA CLASS II-RESTRICTED KILLING OF HUMAN SCHWANN CELLS BY CD4+ TH1 CELLS: A NOVEL IMMUNOPATHOGENIC MECHANISM OF NERVE DAMAGE IN LEPROSY.

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Peripheral nerve damage is a major complication of reversal (or type-1) reactions in leprosy. The pathogenesis of nerve damage remains largely unresolved, but detailed in situ analyses suggest that type-1 T cells play an important role. *Mycobacterium leprae* is known to have a remarkable tropism for Schwann cells of the peripheral nerve. Reversal reactions in leprosy are often accompanied by severe and irreversible nerve destruction and are associated with increased cellular immune reactivity against *M. leprae*. Thus, a likely immunopathogenic mechanism of Schwann cell and nerve damage in leprosy is that infected Schwann cells process and present Ags of *M. leprae* to Ag-specific, inflammatory type-1 T cells and that these T cells subsequently damage and lyse infected Schwann cells. Thus far it has been difficult to study this directly because of the inability to grow large numbers of human Schwann cells. We now have established long-term human Schwann cell cultures from sural nerves and show that human Schwann cells express MHC class I and II, ICAM-1, and CD80 surface molecules involved in Ag presentation. Human Schwann cells process and present *M. leprae*, as well as recombinant proteins and peptides to MHC class II-restricted CD4(+) T cells, and are efficiently killed by these activated T cells. These findings elucidate a novel mechanism that is likely involved in the immunopathogenesis of nerve damage in leprosy.

OI 19

MATRIX METALLOPROTEINASE (MMP) mRNA EXPRESSION IN THE SKIN LESION OF LEPROSY PATIENTS

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Introduction: MMPs are a zinc-dependent proteases family that collectively are able to degrade most extracellular matrix components. In general, MMPs are not constitutively expressed by the cell in vivo, but their expression can be modulated by cytokines such as TNF-á.

Objective: To determine the pattern of expression of MMPs in reactions in leprosy patients who showed enhanced TNF-á production.

Methods: Skin biopsies of 20 leprosy patients and 2 controls were collected and total RNA was extracted. RT-PCR to MMP-2, MMP-9 and TNF-á was performed, and the amplified products analyzed through electrophoresis in agarose gel.

Results: The dermis of all (n= 15) reactional patients (RR and ENL) were positive to TNF-á and MMP-9 mRNA, and 84% (n= 12) to MMP-2 mRNA. In the dermis of the 5 unreactional patients, 60% (n= 3) were positive to MMP-2, MMP-9 and TNF-á mRNA. The 2 health controls are positive to TNF-á mRNA, but negative to MMP mRNA. In 50% of the patients MMP mRNA expression decreased during the treatment. In the epidermis of leprosy patients the TNFá mRNA was detected in all patients and MMP-2 and MMP-9 in 50% (n=3) and 16% (n= 1), respectively. TNFá and MMP mRNA was not observed in the epidermis of the unreactional patients. Preliminary experiments with Real Time PCR confirm the above data.

Conclusion: The MMP mRNA expression was detected only together with TNF-á expression both in the dermis and epidermis. It is likely that these enzymes play a role in inflammatory reaction in leprosy.

OI 20

MHC AND IMMUNE REPONSE ACROSS THE LEPROSY SPECTRUM.

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We earlier showed that HLA Class II genes modulate immune response following infection with *M. leprae* with preferential interaction among mycobacterial epitopes and particular Class II motif characterized by 'Arg' rich pocket 4 residues of the DRB1 molecule. It has been observed that cytokine polarized clinical states of leprosy correlate with the representation of phenotypable fine T cell subsets. We found that the functionally polarized subsets of CD4 memory T cells are identifiable on the basis of expression of CD11a, CD45RA and CD62L, viz those that primarily produce IL- 4 (MT_2, CD45 RA- CD62 L + $CD11a^{dim}$) or primarily γ-Ifn (MT1, CD45 RA- CD62 L - $CD11a^{bright}$). The frequency and representation of phenotypically definable memory T cell subsets (MT_1 and MT_2) was defined in all patients. We observed an overrepresentation of MT1 cells among BT subjects (Median, 53) compared to those with BL leprosy (Median, 15). Conversely MT_2 cells were over represented in BL patients (Median, 23) as opposed to BT subjects (Median, 3). Although median value of MT_1 representation among BL subjects was significantly lower than BT subjects, certain BL/LL patients had comparable higher frequency of MT_1 cells as observed in BT cohorts. These results suggest a possible heterogeneity of immune response against *M. leprae* in these patients. Careful analysis of the data with regards to the bacillary load of BL patients revealed that MT_1 cells were increased in only those BL/LL patients who had become bacillary negative following therapy. All BL/LL patients with a bacillary index of 2 or 4 showed lower frequency of MT_1 cells. Our data indicates immune dynamics in leprosy and provides evidence that functional immune response as measured by cytokine producing memory T cells is dictated by the antigen load and presentation of relevant peptides of *M. leprae* by the host MHC.

OI 21

NEURAL PATHOLOGY DURING TREATMENT AND RFT

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Histological findings of 24 nerves, 15 developing pain and tenderness during treatment and 9 after RFT are presented in this study. Eight cases showed macrophage type and 15 had epithelioid cell type of granulomas. All cases were active except 3 macrophage granulomas where regressive changes were evident. One case had fibrosis with lymphocytic infiltration. No morphological difference could be observed between nerves biopsied during treatment and

during RFT. Histology of epitheliod cell granulomas had interesting and diverse manifestations such as severe type I reaction, caseation necrosis with lequefaction and calcification. Three macrophage granulomas showed regressive changes, two were in ENL, while one case showed histoid changes. All the cases studied showed similar granulomatous response irrespective of whether the pain and swelling appeared during treatment or during surveillance. Nerves are thought to be immunologically more protected structures but once the raction is triggered the special nature of neural tissue seems to make the reactional episodes more explosive. Ascertaing relapse in nerves is more ambiguous due to smouldering nature of neural pathology.

OI 22

QUANTITATIVE ESTIMATION OF SALIVARY IgA ANTIBODIES IN LEPROSY BY ELISA USING INDIGENOUS POOLED SALIVA AS STANDARD

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Background: *M. leprae* being the first organism to be discovered, yet a specific method for cultivation of *M. leprae* is still lacking. Leprosy is a unique disease; the disease is difficult to define due to the lack of gold standard for diagnosis. It is known that the primary lesions may be in the nasal mucosa. All mucosal sites are linked by lymphocyte re-circulation. The mucosal immune system is of importance in a putative protective response to infection.

Aim: To investigate *M. leprae* reactive antibodies in saliva as a marker of anti-*M. leprae* immunity using indigenous *pooled saliva* as *standard.*

Materials and Methods: Saliva samples were collected from subjects in leprosy endemic areas. ELISA studies were performed on all the saliva samples using whole ã-irradiated *M. leprae*. Saliva showing high IgA concentration was pooled in appropriate quantities and a two-fold dilution of the pooled saliva was done to construct the standard curve. HRP conjugated antihuman IgA was added and the reaction was developed using o-phenylenediamine containing 0.05% hydrogen peroxide.

Results: Standard curves prepared using pooled saliva samples were used to determine the concentration of IgA in terms of arbitrary units (AU) and used to compare the antibody levels in different individuals. For most of the saliva samples tested, 1:04 dilution of saliva appeared to be the right dilution. The optimum concentration of *M. leprae* cells used for coating was $1x10^7$ cells/ml and the secondary antibody was diluted 1: 40,000.

Conclusion: The pooled saliva used as a standard contributes to the uniformity in ELISA results. It was seen that this kind of quantitation was sufficiently a robust technique to give reproducible results.

OI 23

REAÇÃO DE MITSUDA EM PACIENTES PORTADORES DE HANSENÍASE NA FORMA TUBERCULÓIDE E DIMORFA DURANTE E APÓS O SURTO REACIONAL.

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A hanseníase, de acordo com o sistema de classificação adotado pelo VI Congresso Internacional de Leprologia, 1953, apresenta duas formas polares, clínica e imunologicamente distintas: o tipo virchoviano e o tuberculóide. Apresenta ainda dois grupos instáveis, o indeterminado e o dimorfo. Em todas elas, exceto na indeterminada, a evolução crônica pode ser interrompida por surtos agudos, denominados de tuberculóide reacional (TR), dimorfo reacional (DR) e, quando na virchoviana, de eritema nodoso. A resistência à Hanseníase pode ser avaliada através da Reação de Mitsuda e, neste sentido, diferentes autores relatam que esta pode estar aumentada, diminuída ou inalterada em pacientes Tuberculóides e Dimorfos durante os surtos reacionais, sem contudo realizarem trabalhos específicos sobre o tema. Com esse objetivo, nesta apresentação, avaliou-se o comportamento clínico da reação de Mitsuda em 43 pacientes das formas Tuberculóide e Dimorfo, durante e após o surto reacional, e observou-se que há predominância da manutenção dos resultados da Reação de Mitsuda - tanto quando a avaliação foi feita separadamente, nos grupos TR e DR, quanto quando conjuntamente. Ademais, as alterações, quando ocorreram, foram predominantemente no sentido de aumento após o surto, ou seja, é possível que, na realidade, durante o surto reacional, alguns indivíduos possam apresentar uma diminuição de sua resistência específica ao *M. leprae*

OI 24

RT-PCR ANALYSIS OF PATHOGEN AND HOST EXPRESSION IN LEPROSY PATIENTS

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Molecular analysis of nucleic acids in Paraffin-embedded infected tissues (PET) using RT-PCR is an effective approach to analyse gene expression of both host and pathogen genes. Punch biopsies obtained from leprosy patients across the leprosy spectrum (n=46) were fixed in formaldehyde and embedded in wax. RNA extraction conditions were optimized and first strand cDNA synthesis was carried using oligo dT primers. Semi-quantitative PCR was carried out using human cytokine specific primers as well as M. *leprae* specific primers.

Analysis of the expression of regulatory cytokines, TGF- and IL-10 in relation to Th1/Th2 cytokines in different disease states confirm and extend the earlier observations that the LL condition is a mixed type of response having both Th1/ Th2 cytokine production. Also, TGF- mRNA was found to be up- regulated at the LL end of the spectrum and could be responsible for the absence of any effect of TNF- and IFN-γ in borderline and lepromatous conditions. Finally the reactional conditions, reversal and ENL show similar cytokine profiles.

Till date there is no documentation of the gene expression of *M. leprae* in infected host tissue. The same RNA extracted for cytokine analysis was also used for analysis of *M. leprae* specific genes 18kDa heat shock protein, 35kDa Major Membrane Protein I and two genes belonging to the *Mce* operon (*M*ammalian *c*ell *e*ntry) namely *mceIA* and *lprK*. Amplification of these genes by RT-PCR from leprosy biopsies confirmed the expression of these genes *in vivo*. All amplified products have been cloned and sequenced to confirm the reliability of the system.

OI 25

SCREENING NEW LEPROSY ANTIGENS FOR POTENTIAL AS LEPROSY SKIN TESTS

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There remains a requirement for leprosy-specific tests to detect leprosy exposure in communities with high levels of tuberculosis. We have previously demonstrated that levels of the cytokine interferon-gamma (IFN-γ produced in a simple overnight whole blood culture with leprosy antigens are increased in healthy contacts of leprosy patients.

Aim: To investigate the efficacy of three *M. leprae* antigens (35kD antigen, 45kD antigen, and the *M. leprae* homologue of ESAT-6 (ESAT-6 ML)) as potential new skin test antigens.

Methods: Whole blood was collected from almost 300 Nepali leprosy patients, TB patients, leprosy household contacts and unexposed subjects and used in overnight whole blood assays. Cells were stimulated with the above named antigens, and IFN-γ was measured in supernatants. The resultant cytokine levels in these stimulated short-term cultures were compared with longer (5-day) culture and with T-cell proliferation.

Results: Very high IFN-γ response levels were observed in leprosy health workers compared with healthy control subjects. Both 24 hour and 5-day cultures gave similar results. In both cases cytokine levels observed were highest in response to *M. leprae* 35kD antigen, followed by ESAT-6 and 45kD.

Conclusion: These data indicate the potential of these three relatively leprosy-specific antigens for use as leprosy skin tests in the future.

OI 26

SCREENING OF NEW *Mycobacterium leprae* ANTIGENS AS CANDIDATES FOR THE DEVELOPMENT OF TESTS FOR THE EARLY DIAGNOSIS OF LEPROSY

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The greatest needs from leprosy research are definitive diagnostic antigens to help understand transmission and allow early detection of disease. In order to further investigate the *in vitro* potential of new *M. leprae* antigens for *in vivo* skin tests or related *in vitro* tests, PBMC from leprosy patients, contacts of leprosy multibacillary patients, operational contacts, TB patients, and exposed or non-exposed healthy controls from leprosy endemic and non-endemic areas were stimulated with antigens from a collection of 26 *M. leprae* antigens, including crude and fractionated subcellular fractions of *M. leprae* and recombinant antigens. Cell-mediated responses were measured through IFN-γ secretion using ELISA, and some T-cell activation parameters (such as CLA and

CD69 expression) were estimated by flow cytometry. Initial results obtained with these different groups of subjects indicate that some fractions/antigens are good inducers of IFN-γ production, and of CLA and CD69 expression, in leprosy patients, but not in TB patients. Responses were lower in lepromatous leprosy patients. These preliminary results suggested that some of our *M. leprae* antigens hold promise as specific diagnostic tools for leprosy. (Research supported by NIAID, NIH).

OI 27

STUDY OF EXPERIMENTAL LEPROSY IN INTERLEUKIN-12 DEFICIENT MICE

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IL-12, a key regulatory cytokine of the immune system, induces the production of IFN-γ by T cells and NK cells and promotes the development of a Th1 type cell mediated immune response. To study its role in experimental leprosy, *Mycobacterium leprae* infection was evaluated in IL-12 knockout (KO) mice. Wild type control mice (C57Bl/6) and IL-12 KO mice were infected in both hind foot pads with 6×10^3 viable *M. leprae* and bacterial growth, cell profiles, histology, and gene expression were monitored for over twelve months. In wild type mice, growth of the bacilli in the foot pads peaked on the order of 10^5 at six months post infection. In contrast, growth of *M. leprae* was enhanced in IL-12 KO mice, reaching 10^5 by three months post infection ($P<0.01$) and continuing to multiply to reach 10^6 by 12 months post infection ($P<0.01$). Histopathologically, control mice exhibited mild lymphocytic and histiocytic infiltrates at 12 months post infection. IL-12 KO mice also developed a mild inflammation with equal numbers of lymphocytes, macrophages and epithelioid cells. Lymph node cells from the draining popliteal lymph nodes were examined throughout infection for lymphocyte differentiation and activation surface markers. Cells bearing the $CD44^{high}$, $CD45RB^{low}$ markers (activation/memory phenotype) constituted only $15.57 \pm 3.93\%$ of the $CD4^+$ cells in the lymph nodes of wild type mice at three months post infection. This cell population increased to $29.14 \pm 4.17\%$ by six months and to $36.26 \pm 10.49\%$ by twelve months. A similar profile was observed in the lymphocytes of IL-12 KO mice. In summary, IL-12 KO mice exhibited a decreased ability to control *M. leprae* KO mice, yet did so without the massive granulomatous infiltration observed in those mice.

OI 28

T CELL RESPONSES TO PEPTIDES FROM *M. leprae* 10 KDA PROTEIN IN THAI LEPROSY PATIENTS, HEALTHY CONTACTS AND NON-CONTACTS

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Detection of *M. leprae* infected individuals using a T cell based assay is hampered by the lack of suitably specific test reagents. Thus the objective of this study was to identify *M. leprae*-specific immunogenic peptides from *M. leprae* 10 kDa protein. Although this protein has a homologue in *M. tuberculosis*, it was previously found to contain *M. leprae*-specific epitopes located within amino acid residues 24-39 as tested by murine T cell hybridoma and T cell clone. In this study, we analyzed the proliferative responses of peripheral blood mononuclear cells (PBMCs) to three synthetic peptides from 10 kDa protein of *M. leprae* among 73 paucibacillary (PB) and 124 multibacillary (MB) leprosy patients, 57 healthy household contacts and 20 non-contacts. These 18-mer peptides were located in 3 areas between residues 11-28, 22-39 and 55-72 containing 3-4 residues distinct between *M. leprae* and *M. tuberculosis*. Surprisingly, the result showed that frequencies of responders to all peptides were similar among the PB and MB patient groups. The most immunogenic peptide was p55-72, recognized by 34% and 48 % of PB and MB leprosy patients, respectively. *M. leprae*-specific P22-39 was recognized by 23-27% of patients but only 7% of healthy contacts which might be useful for discriminating between disease and sub-clinical infection. All peptides were recognized by non-contacts with significantly lower frequencies than the patient groups, suggesting that they were likely to be *M. leprae*-specific. Determination of the exact species-specificity would require further evaluation using T cell lines or clones. Combination of these peptides may increase the sensitivity of a prospective diagnostic test reagent above that, observed for the individual peptides.

OI 29

T CELL SUBSETS EXPRESSING NEURAL CELL ADHESION MOLECULE: ASSOCIATION WITH ANTIGEN INDEPENDENT, MHC UNRESTRICTED T CELL CYTOTOXICITY IN LEPROSY PATHOLOGY

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We have investigated the role of Neural Cell Adhesion Molecule (NCAM or CD56) in the killing of Schwann cells and other NCAM positive targets by an NCAM expressing human T cell subset, isolated from leprosy patients. Involvement of NCAM expressing T cells in leprosy pathology was suggested by the observations that NCAM expressing T cells could be isolated from inflamed neural tissue. Furthermore, antigenic stimulation of these cells with *Mycobacterium leprae* increased both the number of $NCAM^+$ T cells and their cytolytic activity against $NCAM^+$ target cells. The cytolytic activity of $NCAM^+$ T cells was antigen independent and could be attributed to the $CD8^+$ T cell subpopulations. NCAM expression was not a stable but rather seemed an acquired characteristic, since it could be modulated *in vitro* on sorted, $NCAM^+$ cell populations.

In addition, a longitudinal analysis of leprosy patients undergoing active erythema nodosum leprosum (ENL or type 2 leprosy reactions) showed that *M. leprae* stimulation increased NCAM expression on $CD8^+$ peripheral T lymphocytes only at the time of active ENL. In line with these observations, stimulation with *M. leprae* increased antigen independent lysis of NCAM positive target cells in close association with the period of active ENL. At the same time, $CD8^+$ $NCAM^+$ T cells could be visualized in ENL skin lesions.

These results reveal a novel mechanism of antigen independent, T cell mediated tissue damage, which is likely to play a role in leprosy and possibly other peripheral neuropathies

OI 30

THE APPLICATION OF SEROLOGICAL TOOLS IN PATIENT MANAGEMENT AND LEPROSY CONTROL

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The detection of human antibodies to the *Mycobacterium leprae* cell wall component phenolic glycolipid I (PGL-I) and its semi-synthetic derivatives can be performed using ELISA, agglutination tests, dipsticks or lateral flow tests. The results from these tests used to answer a number of important questions that are of direct relevance to patient management and leprosy control.

Specifically, serological testing can be used to assist with the classification of leprosy patients into multibacillary (MB) and paucibacillary (PB) after clinical diagnosis, to detect patients who have an increased risk of relapsing after treatment and to identify contacts of leprosy patients that are in danger of developing leprosy in future.

This presentation will give a critical overview of the various techniques in use, their relative advantages and limitations and the way in which these tools can be used to assist in patient management and leprosy control.

OI 31

THE ROLE OF MANNOSE BINDING LECTIN AND INFLAMMATORY RESPONSE IN LEPROSY

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Mannose binding lectin (MBL) is a serum protein component of the innate immune system. MBL is able to enhance the phagocytosis of the pathogens by binding to the sugars on the microbial surface through an opsonin mechanism resulting in complement system activation. The deficiency of MBL is generally associated to susceptibility to infections. Leprosy is a chronic inflammatory and infective disease caused by the intracellular parasite Mycobacterium *leprae* which strongly bind to MBL. To evaluate the involvement of MBL on the evolution of the disease were selected 58 untreated leprosy patients, classified by Ridley & Jopling criteria (lepromatous leprosy-LL = 14, borderline lepromatous-BL = 8, borderline tuberculoid-BT = 11 and erythema nodosum leprosum-ENL = 25) and 10 healthy controls. Sera samples from the patients and controls were analyzed for determination of MBL, inflammatory cytokines (TNFα. IL6, IFN-γ), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and anti PGL1 antibody (APGL 1). It was observed that all ENL patients presented significantly higher levels of MBL (M = 2335,3 ng/ml) than LL (M = 301,3 ng/ml), BL (M = 428,2 ng/ml), BT (M = 486,1 ng/ml), or the normal control (M = 508,7 ng/ml). Additionally significantly elevated values of all the inflammatory parameters were found in ENL, when compared with the other forms of the disease and to controls. ALthough the bacilloscopic index (BI) of ENL and LL patients were similar (4.5 ± 1.1 and 5.0 ± 1.5 respectively) the APGL1 levels (ENL 7.5; LL = 3.1), inflammatory cytokines (TNAFα, IL6, IFN-γ, ESR and CRP levels were significantly higher in ENL than in LL, indicating that the reactional episode, type 2 reaction, could stimulate the liver cell to produce more MBL and induce the phagocitosis of the parasite, increasing the intracellular destruction. Thus, the data suggests that the protein MBL may act protecting the leprosy patients against the dissemination of the infection.

OI 32

THE ROLE OF TH1 AND TH2 CYTOKINES IN ACUTE LEPROSY NEURITIS

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Study: To assess cytokine production in nerves from patients with acute neuritis (defined as tenderness and/or loss of function within the last six months). The clinical samples skin and nerve biopsies from 57 patients with acute neuritis (BT = 30, BL = 18 and LL = 9) were collected.

Immunohistochemistry was done on skin and nerve sections to detect the cytokine proteins IFN-γ, IL-6, IL-10, IL-12, IL-13, TNF-α, TGF-β and iNOS.

Results: Morphology: Granulomas were better defined and organised in nerve lesions. Cellular infiltration also more prominent in nerve. Th1 type cytokines (IFN-γ and IL-12) were present at high levels in skin and nerve. Nerves from LL patients had both low levels of IFN-γ and IL-12 and moderate levels of IL-6 and TGF-(3. Th2 type cytokines IL-6, IL-10 and IL-13 were present across the spectrum.

Comments: Nerve damage may occur through two mechanisms, a Th1 dependent mechanism in BT and BL patients and a Th2 dependent mechanism in LL patients.

OI 33

TNF PROMOTER GENOTYPE INFLUENCE TNF PRODUCTION IN LPS- BUT NOT *Mycobacterium leprae*- STIMULATED WHOLE BLOOD CELLS IN VITRO.

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Single nucleotide polymorphisms (SNP) on TNF promoter are associated with the risk and progression of infectious and inflammatory diseases. Mutations at position -308 in TNFα (TNF2) promoter gene might affect levels of the cytokine production that are central in the outcome and the natural course of leprosy. The study was set out to investigate the contribution of TNF2 SNP in the cytokine mRNA expression and protein secretion in vitro. Paucibacillary leprosy patients were genotyped by PCR-RFLP for the presence of TNF2 allele (carriers = 13, non-carriers = 21). Whole blood cells from these patients were stimulated with LPS (1ng/ml) and *M. leprae* (1μg/ml). To mRNA expression analysis, semi-quantitative RT-PCR was performed after 3h stimulation. TNFα mRNA did not show any differences among the patients analyzed regardless the stimulus or the genotype of the patients. Nevertheless, LPS induced an increased in TNFα secretion in TNF2 carriers as compared to non-TNF2 carriers at 6h only ($p<0,05$). In *M. leprae*-stimulated cultures no significant differences were achieved. TNF2 allele influence the increased production of LPS-stimulated TNFα production was time dependent and restricted at the protein levels suggesting a post transcriptional regulatory role associated to the promoter polymorphism.

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OM&BM 1

ACCUMULATION OF NORFLOXACIN AND DAPSONE IN *M. smegmatis*

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Quinolones are being increasingly used as second-line agents in the treatment of tuberculosis caused by multidrug-resistant strains. Dapsone is the main component of the MDT regimen for leprosy. At this juncture adequate knowledge of the transport of these chemotherapeutic agents will be of help in the development of new agents. A preliminary study has been conducted at this Institute on the accumulation of norfloxacin and dapsone using modified fluorescence methods. By employing exogenous norfloxacin concentration of 10 ug/ml, a steady state concentration (SSC) of 100 ng of norfloxacin/mg cells, by dry weight was obtained for *M. smegmatis*. Adequate care was taken to nullify the errors due to drug adsorption to the cell surface and to maximise the desorption using several standardised washings in the buffer. The addition of either dinitrophenol (2.0 mM) or CCCP (150 uM) 10 minutes before or