

continuada para atuar conjuntamente com os diferentes profissionais nos serviços de saúde, podendo assim desenvolver novas habilidades, tanto no trabalho em equipe, na informação da assistência ao usuário do SUS e no agir comunicativo, possibilitando maior segurança e autonomia no desempenho de suas funções junto a população, que passa a ter nova compreensão do saber/conhecer sobre saúde, portanto mais instrumentalizado, adquirindo maior capacidade de intervenção sobre a realidade, passando a exercitar o controle social de modo mais representativo e efetivo fortalecendo a organização dos conselhos de saúde garantindo mobilização e autonomia para suas lutas. Configu-

rando-se um processo real de transformação, tanto na educação popular na saúde, quanto no processo profissional, significa provocar e enfrentar conflitos, exercitar a paciência e perseverança. O trabalhador deve ser tornado sujeito da aprendizagem, criador de condições para que se possa adquirir liberdade com responsabilidade, neste processo de construção de práticas em educação popular em saúde, o objetivo é a transformação social do sujeito. Conclui-se que o processo de mudança é imperativo, a coragem de ousar e a determinação são fatores que determina o avançar, ou limitam a realização de mudanças neste processo de educação popular em saúde continuada.

IMMUNOLOGY

PI 1

A NOVEL 33 KD LIPOPROTEIN ANTIGEN FROM *Mycobacterium leprae*

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A novel *Mycobacterium leprae* lipoprotein LpK was identified from the genomic database. The 1116 base pair open reading frame (ORF) encodes a 371 amino acid precursor protein with a N-terminal signal sequence and a consensus motif for lipid conjugation. BLAST search of the gene bank database revealed an 80% homologous gene in *M. tuberculosis* but having no N-terminal consensus lipid modification sequence.

The ORF of the lipoprotein was expressed in *Escherichia coli* under the *lac* promoter and with a histidine tag at the C-terminus of the protein. Expression of the LpK protein in *Escherichia coli*, and detection with anti-His antibody, revealed a 33 kD protein. Metabolic labeling experiments with [¹⁴C] glycerol and treatment with peptidase II inhibitor, globomycin, proved that the protein was lipidated. Furthermore, to search for the native protein in *M. leprae*, polyclonal antibodies against the lipoprotein was raised in rabbit. Western blot data with different fractions of *M. leprae*, revealed that this lipoprotein was present in the membrane fraction of *M. leprae*.

Since IL-12 is one of the cytokines induced by mycobacteria and its products and has a function of biasing CD4⁺ T cells towards Th1 differentiation which is closely associated with host defense, we have mea-

sured the cytokine level induced by LpK in human blood peripheral monocytes. The purified lipoprotein was found to induce significant production of IL-12p40. The studies imply that *M. leprae* LpK is involved in protective immunity against leprosy and may be a candidate for vaccine design.

PI 2

A PHASE II FIELD TRIAL OF NEW LEPROSY SKIN TEST ANTIGENS

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Aim: To undertake a Phase II field trial of two new leprosy skin tests in Nepal, a population endemic for both leprosy and tuberculosis.

Methods: Two new skin test reagents, MLSA-LAM (*M. leprae* soluble antigen depleted of LAM) and MLCwA (*M. leprae* cell wall antigen), are protein fractions of *Mycobacterium leprae*, and are expected to give an *M. leprae* specific response in test subjects. This study is designed to assess the safety and immunogenicity of these reagents and measure the sensitivity and specificity of the reagents relative to PPD in detecting exposure to leprosy in a population endemic for both leprosy and tuberculosis.

Subjects will be tested by intradermal injection with two concentrations (1 and 0.1 g/ml) of each test antigen, and induration measured at 48 and 72 hours and at 28 days following injection. Subjects will be recruited from the following groups: healthy non-con-

tacts, active leprosy patients, leprosy household contacts and tuberculosis patients. Volunteers will also be tested with tuberculin PPD.

Results: This trial is scheduled to begin in March/April 2002, and is designed to proceed in three parts. In part A, 10 healthy non-leprosy contacts will be tested with only one antigen each. Following the establishment of the safety of these antigens in this group, a further 90 non-contacts will be tested. In the third part, larger numbers of volunteers from each group will be tested with both antigens.

Conclusion: Details of the issues relating to the implementation of such a study will be presented. Some preliminary observations will be discussed.

PI 3

A POLYMORPHISM IN THE TOLL-LIKE RECEPTOR-2 GENE AND ITS ASSOCIATION WITH LEPROMATOUS LEPROSY

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TLR2 is critical in the immune response to mycobacterial infections and the mutations in the TLR2 have been shown to confer the susceptibility to severe infection with mycobacteria. To define this, we screened the intracellular domain of TLR2 in 131 subjects. Ten subjects among lepromatous leprosy (LL) patients had a band variant detected by SSCP (Single-Strand Conformational Polymorphism). DNA sequencing detected a C to T substitution at nucleotide 2029 from the start codon of the TLR2. The mutation would substitute Arg to Trp at amino acid residue 677, one of the conserved regions of TLR2. The mutation was involved in only lepromatous leprosy, not tuberculoid leprosy and control. We also performed the functional study on TLR2 by measurement of IL-12 production in serum and monocytes from leprosy patients with TLR2 mutation (Arg677Trp). The monocytes obtained from patients with the TLR2 mutation, in comparison to the wild-type TLR2, is significantly less responsive to MLL. It was also confirmed that patients with TLR2 mutation showed significantly lower serum levels of IL-12, in comparing with wild-type TLR2.

Our results provide the first genetic evidence that mutation in TLR2 is associated with leprosy. Thus, we suggest that the mutation (Arg677Trp) in the intracellular domain of TLR2 has a role in susceptibility to lepromatous leprosy.

PI 4

A PRELIMINARY STUDY ON LEPROSY SUBCLINICAL INFECTION AND ITS IMMUNO-EPIDEMOLOGY

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In this article, We reported the results of study on leprosy subclinical infection and its immuno-epidemiology by using PGL-1-ELISA. The blood samples were collected from 116 leprosy patients (LL30, BL30, BB16, BT20, TT20), 130 normal subjects from a non-endemic area of leprosy. 291 household contacts (HC) and 1023 random contacts (RC). In leprosy patients, blood samples were from veins; in HC and RC, blood samples were from ear lobes and absorbed onto a filter paper strip (FPS), 0.025ml or 0.05 ml for each spot. The results indicate that PGL-1-ELISA is highly sensitive and specific for detecting antibody against PGL-1 specific for *M. leprae*. Its Youden's Index (YI) is greater than 90%, and the positive and negative predicative values are more than 90%. The detected results agreed with immuno-epidemiological studies: 1. The positive rate using PGL-1-ELISA increased gradually from TT to LL leprosy patients (in HC, The positive rate of PGL-1-ELISA was much higher in contacts of multibacillary patients than those in contacts of paucibacillary patients); 2. Among RC, the positive rate detected by PGL-1-ELISA were similar in each district and in concordance with the general prevalence rates. The significance of the PGL-1-ELISA for detecting leprosy subclinical infection and studying on immuno-epidemiology for leprosy, including use of FPS, was discussed in detail.

PI 5

A SYNTHETICAL STUDY ON POSSIBILITY OF PREDICTING EARLY RELAPSE IN LEPROSY USING A ND-O-BSA BASED ELISA

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In order to investigate whether the immuno-serological method for detecting infection with *M. leprae* may be applied to predict relapse in leprosy or not, it was used that an indirect enzyme-linked immunosorbent assay (ELISA) with natural disaccharide octyl bovine serum albumin (ND-O-BSA) as antigen (i.e. ND-IgM-ELISA or ND-ELISA) to detect antibody against ND in sera from normal controls, active cases of leprosy, cases cured and relapsed for determining the relations of antibody level to types of leprosy, the relation of Bacteriological Index (BI) and changes of antibody level in sera from leprosy patients cured to relapse of leprosy. The results evaluating ND-ELISA for screening infection with *M. leprae* indicated that, in ND-ELISA, the sensitivity, specificity, PPR and NPR were all 0.96, YI was 0.92, FPR and FNR were all 0.04, LR+ was 24.0, LR- was 0.041; the titers of antibody against ND in sera of leprosy patients showed a gradual increase from the TT to the LL end of leprosy spectrum, and decrease in leprosy cured year by year, and the correlation between BI and MOD in ND-ELISA was also demonstrated by Spearman's method; when a total of 666 sera from leprosy patients cured with DDS monotherapy, was periodically determined the IgM-AbL by using above ND-ELISA. The results showed that, ? In P-MB of post-DDS, of whom 95 were Ab+, 12 of them were diagnosed as relapse in leprosy; additional 335 cases were Ab-, only 1 of them was diagnosed as relapse in leprosy; ? In PB of post-DDS, of whom 44 were Ab+ and 192 cases Ab-; one case of relapse of leprosy in both to be found; ? The risk of relapse was higher 6.7 times in MB of post-DDS than that in PB of post-DDS; ? In group of Ab+, its CRR was 13.68%, in group of Ab-, the CRR was 0.35%, RR=36.7 (RR>1), AR=13.33%; ? Even though the samples were from PB of post-DDS, the Ab would be positive at that time of relapse in the majority of them, usually the relapse did not develop until consistence positivity of IgM-AbL or gradual increase, and appearance of relapse 1-2 years (2 years in the majority) after Ab+. The period of time for relapse was 12-33 years, and change of type might be developed, although it was rare; ? IgM-AbL were gradually decreased in all of relapsed leprosy after effective treatment except one case whose IgM-AbL was consistent. The above results indicated that the ND-ELISA was useful in screening for early infection with *M. leprae* and in predicting and monitoring relapse in leprosy, especially in multibacillary leprosy.

PI 6

ALLELE FREQUENCIES FOR AN IFN- γ MICROSATELLITE IN A POPULATION OF BRAZILIAN LEPROSY PATIENT

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Leprosy is a chronic infectious disease affecting the skin and nerves. The clinical manifestations and the severity of its symptoms may vary widely in those who develop the disease. The immune response to the causative bacterium *Mycobacterium leprae* may be predominantly cellular giving rise to the tuberculoid form of the disease or it may be biased to a more humoral reaction that leads to the development of lepromatous leprosy. Borderline leprosy falls into the middle of the spectrum but often it develops towards either one of these two dichotomous forms eventually. Interferon- γ , a T_H1 cytokine with a critical role in cell-mediated immune responses, seems to be vital in the control of mycobacterial infection. We genotyped a group of Brazilian control samples and leprosy patients for a CA-repeat microsatellite polymorphism within the IFN- γ gene, which has previously been shown to influence IFN- γ production. The tuberculoid patient subgroup, in which the disease is known to be controlled by a Th1 response, had a significantly different allele distribution when compared to the control group (p=0.013). These results indicate that IFN- γ gene polymorphism may contribute to the course of leprosy post infection.

PI 7

AN ASSOCIATION STUDY BETWEEN BORDERLINE LEPROSY AND THE HLA SYSTEM ANTIGENS – PRELIMINARY RESULTS

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Leprosy is an infectious disease with chronic evolution whose etiological agent is the *Mycobacterium leprae*. The clinical forms classification was done according to the VI Hansen's Disease Congress in Madri (1953), where two clinical and immunologically distinct polar types were described: the tuberculoid (T), the lepromatous (V), and two intermediate groups that are the borderline (D) and the intermediate (I).

Because of its immunological involvement and the few studies related specifically to the immunogenetics of the borderline group, our objective was to investigate the possible association between HLA antigens in the borderline leprosy patients and compare it with normal individuals of the same ethnic group.

Our study showed a decreased frequency of the HLA-DR5 ($P=0.03$), which suggests protection against the disease. ABC antigen frequencies were not significantly altered in the patients. These findings further support the involvement of the HLA system in the pathogenesis of this clinical form of leprosy.

PI 8

ANALYSIS OF THE IN VITRO IMMUNE RESPONSE TO *Mycobacterium tuberculosis* ESAT-6 RECOMBINANT ANTIGEN IN BRAZILIAN TB PATIENTS

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Tuberculosis (TB) remains an important health problem worldwide. ESAT-6, a secreted highly specific antigen of Mtb, is a major target for potent IFN- γ secreting CD4+ T cells in humans. However, few Mtb antigens (Ag) that induce CD8+ T cells are recently recognized, whereas those for CD4+ T cells have been partly defined. Since ESAT-6 usage as a diagnostic tool for TB has been proposed, the immune response to rESAT-6 and its peptide mixture (PeptMix) was assessed in Brazilian TB patients and controls from an endemic area for leprosy and TB.

PBMC were stimulated with rESAT-6 and PPD, and IFN- γ was detected by ELISA and RT-PCR. High IFN- γ levels were observed in pulmonary (mean 1576pg/ml) and also in pleural (mean 1279pg/ml) TB patients ($p < 0.05$) when compared to control individuals (mean 491pg/ml). In addition, the rESAT-6 (untreated = 646 196pg/ml; treated = 2342 728pg/ml) and PeptMix (untreated = 1464 \pm 361pg/ml; treated = 1177 352pg/ml) PBMC stimulated obtained from 39 (20 untreated and 19 treated) Brazilian TB patients was highly correlated in the IFN- γ ELISA. The expression of activation markers induced by ESAT-6 (%CD25/CD4 = 7.8 1.5; %CD69/CD8 = 6.4 2.0) and PeptMix (%CD25/CD4 = 8.4 1.3; %CD69/CD8 = 7.2 1.7) was also observed. The source of IFN- γ and TNF- α secreting T cells were investigated in order to identify PBMC-primed ESAT-6- and PeptMix-specific T cells. Both CD4 and CD8 T cells were responsive to this antigen in vitro. In conclusion, TB patients were able to recognize ESAT-6 and PeptMix by inducing higher IFN- γ

titers, activation molecules, and both T cell subsets cytokine secretion. Nevertheless, the proposed potential use of ESAT-6 for early TB diagnosis has to be better investigated in endemic areas

PI 9

ANTI DIABETIC AUTOANTIBODIES IN LEPROSY – AN OBSERVATION

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A number of leprosy patients attending th OPD at CLT and RI, Chengalpattu were found to be having hyperglycaemia or confirmed Diabetes. A thorough investigation of these patients showed that they were found to be having autoantibodies like GAD-65, IA-12 and ICA-512 in their serum samples. Further probe showed that these autoantibodies were present in leprosy patients without diabetes. Results are analysed and the significance of the autoantibodies in leprosy patients and their role with reference to Th-1 and Th-2 mediated immune responses is presented and discussed.

PI 10

ASSOCIATION OF AUTOANTIBODIES TO LEPROSY PERIPHERAL NEUROPATHY

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High titers of serum antibodies to neural antigens occur in several forms of neuropathy. These antibodies frequently react with glycosylated cell surface molecules, including glycolipids, glycoproteins and glycosaminoglycans. There are several correlations between antibody specificity and clinical symptoms. By extrapolation, in other neural diseases that may be of an autoimmune nature, suggestions have been made that antibodies to gangliosides may have a pathogenic significance. The ganglioside composition of the motor and sensory fibres from the human peripheral nervous system includes a wide range of glycolipids including GM1, GD1a and GD1b gangliosides. As one of the hallmarks of leprosy and leprosy reactions is neuropathy involving peripheral nerve, we decided to test serum samples from leprosy patients for the presence of antibodies to these components.

Thirty-nine serum samples from 15 leprosy patients were studied, including 5 PB and 10 MB patients. Samples were collected during and after treatment and include samples from the 5 patients that developed RR or ENL. The control group was composed of 5 contacts of leprosy patients, 5 healthy persons from the same endemic area and 10 other controls. IgG and IgM antibodies to GA1, GM1, GD1a, GD1b ganglioside and sulphatides were assayed by ELISA.

Alterations in antibody levels were not observed. Our study fails to support an enhancing role for autoimmune antibodies to gangliosides and sulphatides in leprosy neuropathy.

PI 11

ATIVIDADE DA ADENOSINA DEAMINASE NO SURTO REACIONAL

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Introdução: A adenosina deaminase (ADA), enzima - chave no metabolismo das purinas, possui importante papel no sistema imune. A atividade aumentada desta enzima ocorre tanto em afecções linfoproliferativas como em processos infecciosos, a exemplo da tuberculose. Suas isoenzimas, ADA1 e ADA2, refletem respectivamente, a atividade de linfócitos e macrófagos. Na hanseníase, a atividade linfocitária e macrófágica se relaciona com mecanismos de resistência e patogênese. Muitos pacientes com hanseníase desenvolvem periodicamente episódios inflamatórios de surto reacional, associados a lesão tecidual. O objetivo deste estudo foi investigar a atividade da ADA antes e durante a ocorrência destes episódios na hanseníase.

Material e Métodos: 44 pacientes com diagnóstico de hanseníase e apresentando surto reacional tipo 1 ou 2 foram incluídos no estudo. No grupo controle, 8 pacientes que não apresentavam surto reacional e 32 indivíduos saudáveis foram avaliados. Os valores da ADA total e de suas isoenzimas (ADA1 e ADA2) foram dosados no soro, antes e durante o surto, por método espectrofotométrico.

Resultados: Os valores da ADA total, ADA1 e ADA2 se encontram mais elevados em pacientes com hanseníase, em comparação com indivíduos saudáveis. Os pacientes em surto reacional apresentam uma tendência a mostrar uma maior atividade desta enzima, quando comparados aos pacientes sem surto. A maioria dos pacientes com surto tipo 2 apresenta um aumento da atividade da ADA, precedendo este surto em cerca de 30 dias.

PI 12

CORRELAÇÃO ENTRE BCG INTRADÉRMICO E LINFOPROLIFERAÇÃO E PRODUÇÃO DAS CITOCINAS IFN- γ , IL-12, IL-10 E IL-4 EM PACIENTES COM HANSENÍASE E EM SEUS COMUNICANTES

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O BCGid é preconizado para a imunoprofilaxia da hanseníase. Apesar de frequentemente utilizado, especialmente em comunicantes de doentes de áreas endêmicas, os seus mecanismos imunológicos de estimulação protetora são ainda pouco conhecidos para a doença. Como o desenvolvimento da hanseníase correlaciona-se diretamente com a resposta imune celular, avaliou-se a relação entre a aplicação de uma dose de BCGid em doentes e comunicantes, associando com alterações imune celulares. A avaliação foi através da linfoproliferação e da quantificação das citocinas IFN- γ , IL-12, IL-10 e IL-4. Verificou-se ainda se houve diferença entre a resposta imune celular induzida pelo BCGid em doentes e comunicantes. Foram avaliados 34 indivíduos, antes e após o BCGid, sendo 15 doentes e 19 comunicantes saudáveis. A linfoproliferação foi desenvolvida na presença de Con-A, BCG e HSP-65, durante 96 horas. Os sobrenadantes foram coletados e estocados a -70 C para dosagem de citocinas, e a proliferação foi avaliada pela incorporação de 3H-timidina. Linfócitos de doentes e comunicantes apresentaram maior proliferação na presença de Con-A e BCG. Comparando-se os resultados antes e após a aplicação de BCGid, foram notadas maiores respostas nos indivíduos submetidos ao BCGid ($p < 0,05$). Foi observado também que as células dos comunicantes pós BCGid apresentaram maior capacidade de estimulação na presença dos antígenos do BCG, quando comparadas às dos doentes ($p = 0,004$). Os resultados da quantificação das citocinas (através do Elisa) mostraram que a aplicação de BCGid leva à maior produção de IFN- γ ($p < 0,05$), sendo essa produção significativamente maior na presença do BCG em células de comunicantes que de doentes TT ($p = 0,004$). IL-12, pós BCGid, apresentou níveis equivalentes em células de doentes e comunicantes ($p = 0,1713$), frente ao estímulo BCG. Entretanto, a produção de IL-12 em comunicantes foi significativamente maior pré BCGid, na presença do BCG ($p = 0,0029$), o que não se observou entre os doentes ($p = 0,4648$). Além de IFN- γ e IL-12, BCG induziu a produção de IL-10, detectada em sobrenadantes de comunicantes em níveis significativamente maiores após o BCGid ($p = 0,0098$), frente ao BCG. Esses resultados sugerem que a resposta imune predominante induzida pelo BCGid foi do tipo protetora, associando-se à detecção de IFN- γ .

Assim, foi possível concluir que a aplicação do BCGid é útil em áreas endêmicas, pois pode induzir a resposta imunecelular específica das células do hospedeiro. A capacidade do BCGid induzir a ativação da resposta imunológica de doentes e comunicantes está associada à maior produção IFN- γ em ambos os grupos.

PI 13

CORRELAÇÃO ENTRE BCG INTRADÉRMICO E NÍVEIS DE ANTI-PGL-1 EM PACIENTES COM HANSENÍASE E EM SEUS COMUNICANTES

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O BCG intradérmico é preconizado para a imunoprofilaxia da hanseníase. Apesar de freqüentemente utilizado, especialmente em contatos de doentes de áreas endêmicas, os seus mecanismos imunológicos de estimulação protetora são ainda pouco conhecidos para a doença. Como o desenvolvimento da hanseníase correlaciona-se diretamente com a resposta imunecelular, avaliou-se a relação entre a aplicação de uma dose de BCGid em doentes e comunicantes, associando com alterações imunecelulares. Um dos métodos usados para a avaliação foi através da produção de anticorpos específicos do *M. leprae* (Anti-PGL-1). Verificou-se ainda se houve diferença entre a resposta imunecelular induzida pelo BCGid em doentes e comunicantes. Foram avaliados 34 indivíduos, antes e após o BCGid, sendo 15 doentes e 19 comunicantes sadios.

Foi utilizado o ensaio enzimático para detecção de anticorpos Anti-PGL-1 (Elisa Anti-PGL-1). Os níveis dos anticorpos Anti-PGL-1 no soro de pacientes e comunicantes foram avaliados antes e após uma dose de BCGid, sendo observados baixos níveis de anticorpos Anti-PGL-1 em tuberculóides (= 1,86), médios em borderlines (= 4,56) e elevados em virchovianos (= 15,75), correlacionando-se com as baciloscopias. Os níveis dos anticorpos Anti-PGL-1 no soro dos comunicantes foram menores do que aqueles encontrados nos doentes ($p < 0,05$). Após a aplicação do BCGid, houve diminuição significativa dos níveis de Anti-PGL-1 em doentes e comunicantes ($p < 0,0001$), o que pode sugerir que o BCG induz a ativação da resposta imunecelular (tipo Th1), potencializando a destruição dos bacilos pelos macrófagos e capacitando a defesa específica dos doentes. Os resultados tornam-se relevantes, porque até a avaliação pós BCGid, os doentes permaneceram sem tratamento específico. A redução dos níveis dos anticorpos específicos ocorreu nos pacientes, independentemente de nível baixo ou mais elevado e de forma clínica da doença.

Portanto, a capacidade do BCGid induzir a ativação da resposta imunológica de doentes e comunicantes pode estar associada à queda dos níveis dos anticorpos Anti-PGL-1, em ambos os grupos.

PI 14

CRIPTOCOCOSE EM PACIENTE DE HANSENÍASE: RELATO DE CASO

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Introdução: Doença infecciosa causada por leve-dura de distribuição universal, o *Cryptococcus neoformans*, disseminada através de dejetos de pássaros e adquirida através de inalação. A infecção cutânea em indivíduos sadios é rara. No entanto, sua freqüência aumenta em adultos com doenças sistêmicas como lupus eritematoso, linfomas, em estados de imunossupressão de origem infecciosa ou medicamentosa.

Relato do caso: Os autores apresentam caso clínico ocorrendo em paciente de 39 anos de idade, pedreiro, portador de hanseníase virchoviana tratada, mas apresentando surtos freqüentes de eritema nodoso hanseniano controlados há cerca de 6 anos com doses variadas de corticosteróides sistêmicos que há 6 meses apresentou lesão cutânea ulcerada diagnosticada como criptococose e tratada com fluconazol, tendo evoluído com cicatrização da lesão. Os autores discutem e chamam a atenção sobre a ocorrência de imunossupressão iatrogênica na tentativa de se controlar complicação importante da hanseníase que é o ENH.

Considerações finais: Trata-se de patologia cutânea incomum ocorrida por imunossupressão iatrogênica.

PI 15

CYTOKINE LEVELS CORRELATE WITH MULTIDRUG-RESISTANT PULMONARY TUBERCULOSIS IN RIO DE JANEIRO

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Introduction: Resistance of *M. tuberculosis* to antimycobacterial agents has recently received increased attention worldwide. The participation of T cell response in multidrug resistant TB is not yet

clearly understood. In addition, progression to fatal outcome in these patients might be related to enhanced inflammatory response *in vivo*.

Aim: To evaluate T cell and inflammatory response in MDR patients in comparison to TB patients not MDR.

Methods: MDR TB cases were defined as resistant to at least INH e RMP. The immune response was evaluated in 12 MDR patients who were tested negative (ELISA) for HIV. Peripheral blood was collected before the initial specific MDR treatment. For detection of cytokines, IFN γ , sTNF-RII (p75), and IL-6 were measured in PBMC cultures stimulated or not with PPD and the recombinant antigens ESAT-6 and 85B. Supernatants were harvested either after 20 or 72h (IL-6, TNF-RII) and after 5 days (IFN γ) of culture and were assayed by specific ELISA.

Results: Preliminary immunological analysis showed lower IFN levels in response to ESAT-6 in MDR patients (mean SEM = 590 223pg/ml) when compared to pulmonary TB patients (n = 50; mean = 1553 420pg/ml), and similar to the response of PPD negative healthy donors (491 74pg/ml). More interestingly, IFN γ in response to PPD in the MDR group (mean = 431 260pg/ml) was also lower when compared to both groups, TB patients (1564 110pg/ml) and controls (1332 \pm 411pg/ml). Evaluation of the inflammatory response in MDR was showed to be up-regulated, since values of soluble TNF-R in these cultures were 753 384pg/ml (72h culture) vs. 2067 923pg/ml in the TB group. In addition, for IL-6, constitutive cytokine levels were 4744 832pg/ml in the TB patients vs. 1468 878pg/ml in the MDR.

Conclusion: The data indicate that T cells from MDR patients respond to mycobacterial antigens *in vitro* at a lower extent when compared to TB patients and that inflammatory responses are also exacerbated. Follow-up studies are still necessary to determine whether worsening of clinical conditions in MDR are related to such immunological parameters.

PI 16

CYTOKINE mRNA EXPRESSION IN THE EPIDERMIS OF LEPROSY PATIENTS: DIFFERENTIAL TNF α mRNA REGULATION DURING INFLAMMATION

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Introduction: The epidermis can represent an important site of immuno-inflammatory response in the skin. In leprosy, histopathological alterations are described both in the dermis and epidermis, mainly during the reactional states (reversal reaction, RR and erythema nodosum leprosum, ENL).

Objective: To evaluate the expression of cytokines and ICAM-1 genes by RT-PCR in the epidermis of reactional leprosy patients.

Methodology: Skin biopsies were collected of the 25 reactional leprosy patients, RNA was extracted from the dermis and epidermis and RT-PCR was performed to β -actin, TNF α , IL-6, IL-8, IL-12 and ICAM-1. The amplified products were analyzed through electrophoresis in agarose gel and the radioactive hybridization was performed with specific probes to all molecules.

Results: Detection of TNF α and IL-6 mRNA in the epidermis was evidenced in all individuals during ENL and RR. IL-8 message was detected in 66.6 and 62.5% of the patients, IL-12 mRNA was present in 91.6 and 62.5% and ICAM-1 in 100 and 71.4%, respectively. In addition, when skin biopsies were obtained from the same patients before and during the reactional episode, an enhancement in cytokine mRNA, but not of ICAM-1, was noted. Seven patients were also evaluated at the onset of reaction and during anti-inflammatory treatment. In contrast to a preferential decrease in the TNF α gene detected in the dermis, during the treatment phase, a persistent/enhanced TNF α mRNA expression was detected in the epidermis in 6 out of the 7 patients assessed.

Conclusion: The present data indicate that the epidermis has an important participation in the local inflammatory response in leprosy and it seems to parallel the histological changes observed *in situ*.

PI 17

CYTOKINES QUANTIFICATION IN SUPERNATANT OF MONONUCLEAR CELL CULTURE OF PATIENTS WITH LEPROSY: PRELIMINARY RESULTS

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Objective: to evaluate the cytokine profile in the supernatant of a mononuclear cell culture of patients with leprosy, at the moment of diagnosis and six months after multidrugtherapy.

Methods: mononuclear cells from peripheral blood from 15 patients (5 LL, 7 B, 3 TT) were cultivated, in 37°C and 5% CO₂, for 24 and 48 hours in the pres-

ence and absence of PHA (8g/ml), LPS (10g/ml) and integral *M. leprae* (10 bacilli/cell). The supernatants were collected and the cytokines IL-2 and IFN γ (Th1,) IL-4 and IL-10 (Th2), IL-1 and TNF α were quantified by the ELISA technique (R&D Systems).

Results: multibacillary patients (12) produced greater levels of IL-4 before treatment (PHA= 80 ± 72 pg/ml; *M. leprae*= 4 ± 7 pg/ml; spontaneously= 10 ± 18 pg/ml) than six months after the treatment (PHA= 37 ± 55 pg/ml; *M. leprae*= 2 ± 2 pg/ml; spontaneously= 3 ± 2 pg/ml) and high levels of IL-10 before and after multidrugtherapy (PHA= 2392 ± 1673 pg/ml; *M. leprae*= 430 ± 623 pg/ml; spontaneously= 790 ± 1030 pg/ml; PHA= 2489 ± 1332 pg/ml; *M. leprae*= 564 ± 331 pg/ml; spontaneously= 811 ± 613 pg/ml, respectively).

Conclusion: the results obtained suggest that multibacillary patients produce high levels of cytokines of the Th2 pattern (IL-4 and IL-10) at the moment of disease diagnosis.

PI 18

DETECTION OF TNF α mRNA EXPRESSION BY DIRECT *IN SITU* RT-PCR IN THE PBMC OF PATIENTS WITH LEPROSY

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Introduction: TNF α is an important cytokine in leprosy pathogenesis and it has been shown to be involved in the immuno-inflammatory processes during this infectious disease.

Objective: To standardize the method of the direct *in situ* RT-PCR for evaluation of TNF α mRNA expression induced by *M. leprae* *in vitro*.

Methodology: PBMC of 7 leprosy patients were isolated by Ficoll-hypaque density centrifugation, plated on Teflon beakers and stimulated or not with LPS (10ng/ml) or *M. leprae* (1g/ml) for 1 and 3 hours. Amplification for TNF α was performed by direct *in situ* RT-PCR, using digoxigenin-dUTP.

Results: Detection of TNF α mRNA positive cells (blue-black staining) was higher in the LPS-stimulated cultures when compared with the unstimulated cells in all experiments. Similar results were found using *M. leprae* stimulated cells. TNF α mRNA expression in the PBMC from one BT patient was detected by direct *in situ* RT-PCR in the unstimulated, *M. leprae*- and LPS- stimulated cultures. It was possible to detect a qualitative difference between unstimulated and stimulated cells, which contain a higher number of positive cells (blue-black staining) as compared to the former one. Moreover, after 1 hour of stimulation, the number of positive cells was

higher than after 3 hours. The same kinetic response for TNF α mRNA expression was obtained in both, solution RT-PCR and *in situ* RT-PCR. *M. leprae*-stimulated PBMC showed higher amount of TNF mRNA 1 hour after the stimulus.

Conclusion: The *in situ* RT-PCR method will allow the more precise determination of the amount of cells that actively express cytokine message. Current experiments are being developed to determine the differential profile of TNF α synthesis by monocytes and T-lymphocytes *in vitro*.

PI 19

DIFFERENTIAL SERODIAGNOSIS OF LEPROSY AND TUBERCULOSIS BY IMMUNOBLOT BAR READING AND ELISA USING SHARED MYCOBACTERIAL ANTIGENS

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Mycobacteria are composed of complex mixture of antigens and many of them are shared by all mycobacterial species. Owing to the ubiquitous nature of mycobacteria, human sera always show serum antibody activities to shared mycobacterial (Smyc) antigens. Studies on the sero-antibody responses to Smyc antigens by immunoblot assay showed that hosts recognise different antigenic bands in the fashion of "bar" as disease specific manner. This is an important basis for discriminating leprosy patients from tuberculosis patients and patients with other types of inflammation. The group of leprosy patients were histopathologically and clinically classified. The present report is the analysis of a total of 200 patients (130 leprosy, 75 tuberculosis and 75 patients with non-mybacterial inflammation by immunoblot 'BAR' reading (ImBBR) method). The results show that 99% of LL and 77% of BL patients' sera reacted with a doublet 29/33 KD antigens whereas TT and BT sera (94% and 63% respectively) reacted to a 64/65 KD singlet Smyc antigen. Interestingly, sera of BB patients did not show any clear cut reactivity pattern rather a smeartype pattern. In contrast sera from tuberculosis patients, that were clinically and microbiologically defined, reacted with a group of Smyc antigenic bands. e.g. 58-60 KD, 38-40 KD, 18-22 KD, 14-16 KD regions. In parallel, enzyme linked immunosorbent assay (ELISA) for measuring IgG, IgM and IgA antibodies using gel purified 29/33 KD

doublet, 64/65 KD singlet and a subcellular pellet fraction (P90) Smc antigens was used. The ELISA results show that different types of leprosy can be discriminated distinctly from each other and from tuberculosis as well as from control patients. Moreover, since the immunoblots and antigen coated microtitre plates are stable at room temperature such combined assays can be used for screening population in the countries endemic for both of these pathogenic mycobacterial infection. In conclusion it appears that the combination of ImBBR and ELISA using either antigen mixtures or a panel of isolated Smc antigens will be a valuable tool for identification of potential leprosy and tuberculosis patients in endemic population.

PI 20

DIFFERENTIAL TRAFFICKING OF *Mycobacterium tuberculosis* AND *Mycobacterium leprae* IN HUMAN MONOCYTIC THP-1 CELLS

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Supra vital dye -labelled *Mycobacterium tuberculosis* and *Mycobacterium leprae* and vacuolar markers were used to study the phagosomal biogenesis in host cells (THP-1, a human monocytic cell line) by Laser Scanning Confocal microscopy (LSCM) and Electron Microscopy. Fluorescein (green), or PKH26 (red)-labeled mycobacteria and two acidotropic probes LysoTracker Red DND-99 and LysoTracker Green-26 were used to monitor the events by LSCM while Rab5 and Cathepsin D were used to identify phagosomes and lysosomes by immunoelectron microscopy. LysoTracker probes localised preferentially within lysosomal compartments whereas phagosomes were identified by transferrin receptors. Live *M. tuberculosis* co-localised with transferrin-labeled organelles upto 48hrs. Whereas *M. leprae* co-localisation with transferrin was restricted to 6 hours only. The phagolysosomal fusion event also differed with both organisms, with viability of the organisms being the pre-disposing factor during this phenomenon. Interestingly, *M. leprae* co-localised with acidic organelles up to 48 hours while *M. tuberculosis* containing phagosomes resisted fusion with lysosomes. Results indicate that although cultivable and non-cultivable mycobacteria have evolved ways to circumvent the hostile environment of the macrophage, the mechanisms employed by them are varied.

PI 21

DISTRIBUTION OF HLA CLASS II ALLELES IN LEPROSY PATIENTS OF KAZAKH POPULATION

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Genetic factors play a significant role in susceptibility to infectious diseases. A great number of investigations to study HLA-antigenic distribution in leprosy were carried out mainly on Orientals and Hindu. In Russia leprosy patients of Kazakh nationality are next to Russian patients. Meanwhile, HLA-genetic profile in leprosy patients of Kazakh population has not been studied. Our work is aimed at studying distribution of class II HLA-antigens in Kazakh population. Distribution of class II HLA-antigens of DRB1, DQA1 and DQB1 loci was defined in 52 leprosy patients and 60 healthy non-relatives of Kazakh nationality. HLA-genotyping was carried out by means of PCR. It was stated that in leprosy patients DRB1-01 and DRB1-17 antigens occurred more frequently ($P < 0.05$). Frequency of DRB1-10, -09 and DQA1-601-alleles was considerably low as compared with healthy persons. Relative risk (RR) of the disease was 2,8 and 3,6 for DRB1-01 and -17, correspondingly. The data obtained permit to consider HLA-DRB1-01 and DRB1-17 antigens as genetic markers of susceptibility to leprosy in Kazakh population. Having regard to the fact that HLA-DRB1-17 allele enters into serologically defined HLA-DR3-specificity that is defined as a marker of leprosy susceptibility in different ethnic populations, one might suggest that the highest risk of leprosy is associated with increased frequency of haplotypes with above allele. Thus, the observed peculiarities of distribution of alleles of HLA-loci strongly necessitate investigations on HLA markers of diseases in different ethnic populations. It will permit a more accurate identification of risk groups, on the one hand, and target searches for universal markers of leprosy susceptibility, on the other.

PI 22

DISTRIBUTION OF II CLASS HLA ALLELES IN RUSSIAN LEPROSY PATIENTS

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Distribution of DRB1, DQA1 and DQB1-antigens of

II class HLA was studied in 55 leprosy patients and 50 healthy non-relatives of Russian nationality. HLA-genotyping was carried out in PCR. Leprosy patients showed antigens DRB1-15, DRB1-16, DQA1-102, DQB1-602/8 and DQB1-502/4 with more high frequency ($P < 0,05$). Frequency of HLA-DQA1-301 allele was significantly low in leprosy patients as compared with healthy subjects. With correction for the number of test-antigens significant differences maintained for alleles of HLA-DQA1-102 and -DRB1-15 genes. A study of distribution of haplotypes in leprosy patients showed increased frequency of DRB1-15-DQA1-102-DQB1-602/8 and -DRB1-16-DQA1-102-DQB1-502/4 as compared with control. At the same time frequency of DRB1-11-DQA1-501-DQB1-301-haplotype was low in leprosy patients. Taking into account that HLA-DR1-15 and 16 alleles are a part of serologically detectable specificity of HLA-DR2 which was earlier defined by us as indicating leprosy susceptibility in Russian population, one might suggest that the highest risk of leprosy disease in Russian population should be associated with high frequency of haplotypes of the above alleles. The results obtained permit to consider HLA-DRB1-15 and DQA1-102 molecules as genetic markers of susceptibility to leprosy in Russian population. Thus, investigation of distribution of allelic loci of HLA-system when identifying specific haplotypes significantly increase the effectiveness of defining their associations with leprosy and, hence, permit a more accurate identification of risk groups based on genetic analysis.

PI 23

ESTUDO COMPARATIVO ENTRE REAÇÃO DE MITSUDA E FENOTIPAGEM HLA EM PACIENTES HANSENIANOS: RESULTADOS PRELIMINARES

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Sabe-se que na hanseníase as respostas imunológicas do hospedeiro determinam as formas clínicas da doença, verificadas pela reação de Mitsuda (teste incorporado como auxílio diagnóstico, principalmente, dos grupos indeterminado e dimorfo). Do ponto de vista genético, há evidências de que o complexo HLA seja o responsável pelas diferentes formas da doença, mas não existem relatos de trabalhos que descrevam tal comparação.

Desta forma, temos por objetivo verificar se existe relação entre os resultados da reação de Mitsuda, formas clínicas da doença e fenótipos HLA encontrados nos pacientes que compõe o estudo.

Realizamos, até o momento, tipagem HLA classe II

por PCR-SSP, em 75 hansenianos caucasoídes (21HT; 26 HV; 28 HD) e comparamos os dados (frequência HLA) com amostra da população caucasoíde do estado de São Paulo (n=142).

Dos 21 pacientes HT, 20 são Mitsuda positivo e apresentam frequência elevada do HLA-DR2 (52,4% × 19%); dos 26 pacientes HV, 24 são Mitsuda negativo e apresentam frequência elevada do HLA-DQ1 (73% × 50%); e dos 28 HD, 11 são Mitsuda positivo e 17 negativo, contudo, não observamos frequência elevada de qualquer fenótipo HLA.

Outro dado verificado nos pacientes hansenianos, independentemente das formas clínicas, é a diminuição do HLA-DR5 e DQ7. Acreditamos que se aumentando o tamanho da amostra, poderemos confirmar as associações observadas nesses pacientes.

PI 24

EVALUATION OF ADRENAL AND GONADAL FUNCTIONS IN LEPROSY: COMPARISON TO IMMUNE RESPONSE

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Leprosy is a chronic inflammatory disease which not only involves skin and peripheryc nerves but also endocrine organs. This disease has a clinical and pathologic spectral nature associated with distinct immunologic response. In the present study an attempt has been made to assess the functional integrity of the hypothalamic-pituitary-adrenal (HPA) and gonadal (HPG) axis and their relationships with the immune systems in leprosy. Ten multibacillary (MB, 40 3yr) and 8 paucibacillary (PB, 44 3yr) male untreated patients and 10 healthy controls (31 2yr) were evaluated. Day 1 at 9:00am: baseline plasma samples were taken for cortisol, DHEA-S, LH, FSH, testosterone, TNF α , IL1 β , IL6 and human CRH test (1g/Kg iv) was then performed. Day 2-9am, synthetic ACTH (1-24,250g) was given IV and plasma samples were collected at 60 minutes. After stimulation with hCRH the plasma ACTH and cortisol area under the curve (AUC, 15-120min) did not differ between controls and patients. Compared to controls, total and net ACTH (1-24)- stimulated cortisol levels were not different. TNF and IL6 were significantly elevated in MB and PB patients compared to controls (p0.01). IL1 beta was not different between controls and patients. Plasma DHEA-S levels were significantly lower in patients than in controls, but there was no difference between MB and PB patients. A negative correlation between DHEA-S and IL6 was observed (r-0.48; p0.01). Although plasma testosterone levels did not differ between controls and patients, LH and FSH were significantly higher in MB than in controls and PB patients. It was observed no correla-

tion between plasma ACTH or cortisol AUC and Interleukins. Regarding the HPG axis, LH and FSH levels were significantly correlated with IL6 ($r=0.46$ and $r=0.64$, respectively; $p<0.01$) and TNF ($r=0.49$ and $r=0.67$, respectively; $p<0.01$). These data suggest that DHEA-S, LH and FSH are best indicators of HPA and HPG axis function in leprosy and may be influenced by IL6 and TNF α .

PI 25

EVALUATION OF T CELL IMMUNE RESPONSE TO THE ESAT-6 HOMOLOGUE OF *Mycobacterium leprae* IN LEPROSY

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In contrast to the highly homologous mycobacterial heat shock proteins, the ESAT-6 of *M. leprae* (L-ESAT) only shows 35% identity with its homologue in *M. tuberculosis* (T-ESAT). Based on the high specificity of the T-ESAT for immunodiagnosis of tuberculosis (TB), even in BCG-vaccinated individuals, it is argued whether the ESAT homologue in *M. leprae* could provide such a diagnostic reagent for the detection of leprosy. Thus, the T cell response against the recombinant protein L-ESAT was analyzed in Brazilian leprosy patients ($n=23$), TB patients ($n=22$), and healthy controls ($n=15$). Leprosy patients were 5LL, 6BL, 6 BT, and 6 reactional (RR). PBMC derived from most *M. leprae* responding patients produced IFN following in vitro stimulation with 10g/ml of L-ESAT (mean SEM = 384 ± 125). A total of 40% lepromatous patients did respond to L-ESAT as compared to 83.3% tuberculoid, and 66.6% of the RR. Concordant responses between L-ESAT and whole *M. leprae* was found in 80% of the cases. However, TB patients (57.1%) and healthy controls either positive (62.5%) or negative (71.4%) tuberculin skin test responded equally well to L-ESAT. Among the untreated TB patients ($n=9$), 66.6% responded to this antigen as did 50% of the treated TB ($n=12$). In addition, no striking differences in IFN levels induced by L-ESAT were found between patients and healthy controls derived from a tuberculosis/leprosy-endemic area, which excludes the use of L-ESAT as a diagnostic tool for leprosy

PI 26

EVALUATION OF THE 10 MINUTES ML FLOW ASSAY USING WHOLE BLOOD

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We describe a further simplification of the ML Flow assay for the detection of antibodies to phenolic glycolipid I (PGL-I) of *Mycobacterium leprae* by using whole blood and evaluated the assay performance in the leprosy endemic area of Belem in Brazil. The agreement between results of the test performed using whole blood and sera was 85.9% (value= 0.7, SE=0.042). This simple assay is proposed for classification of leprosy patients after clinical diagnosis and identification of high-risk contacts of leprosy patients. Identifying and monitoring the contacts of leprosy patients with higher risk of developing leprosy may be a tool for the interruption of transmission of leprosy, one of the main challenges for leprosy control.

The ML 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.

PI 27

EXPRESSION OF ANTI-INFLAMMATORY AND INFLAMMATORY CYTOKINES IN THE LESIONS OF T1R PATIENTS DURING PREDNISOLONE TREATMENT

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This study investigates effect of prednisolone (30mg daily) on the expression of the cytokines in the lesions of patients with Type 1 reactions (T1R). The hypothesis that the inflammatory cytokines observed in T1R patients decrease with treatment and that the anti-inflammatory cytokines are concurrently increased has been tested.

Study: Skin biopsies were taken from 15 patients (6 BL and 9 BT) at time points (weeks 0, 1, 4 and 24) during depleting prednisolone treatment. Immunohistochemical analysis of the expression of the cytokines IFN- γ , TNF- α , TGF-1, IL-6, IL-12, IL-13, IL-10 and iNOS were determined.

Results: The inflammatory cytokines (TNF- α , IFN- γ , IL-12) levels were found to decrease with treat-

ment (significantly by 4 weeks). This study also demonstrates that the levels of the anti-inflammatory cytokines IL-10 and IL-13 decrease (significantly by 4 weeks).

Conclusion: This work suggests that prednisolone non-specifically down regulates the whole spectrum of inflammatory cytokines rather than altering the pro-/anti-inflammatory cytokine balance and that this effect is not observed in skin lesions until 4 weeks after the start of treatment.

PI 28

EXPRESSION OF PERFORIN MRNA AT THE LESION SITE IN LEPROSY REACTION

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Introduction: Perforin is a cytolytic pore-forming protein that colocalizes with granulysin in cytotoxic granules, and is responsible for the cytolytic activity of CD8⁺T cells. Perforin mRNA was detected in leprosy lesions and enhanced expression of perforin in the blood of patients with reaction (erythema nodosum leprosum, ENL and reversal reaction, RR) was also described.

Objective: To investigate the expression of perforin mRNA at the site of the leprosy lesion in patients with reaction and its induction following *M. leprae* stimulation *in vitro*.

Methods: Skin biopsies of 12 leprosy patients (10 BL and 2 LL) were collected and total RNA was extracted. For the *in vitro* experiments, PBMC was obtained from 5 patients, and kinetic cultures were established. Following RNA isolation, RT-PCR for perforin was performed, and the amplified products analyzed through electrophoresis in agarose gel.

Results: When comparing patients with reaction, ENL patients are likely to show higher relative amounts of perforin mRNA in the lesion than patients with RR. In addition, around 50% of the RR patients (n=7) expressed perforin message in the dermis, whereas, 100% of the ENL (n=3) were positive for this cytolytic mediator. Four of these patients were also evaluated during treatment for reaction and down-regulation of perforin mRNA was noted in 2 individuals. Kinetic evaluation of perforin mRNA following *M. leprae* stimulation showed to be similar for that of TNF- α as it peaks around 3h after adding the stimulus.

Conclusions: Perforin mRNA is expressed in the dermis of the reactional leprosy lesion. The present data suggest that cytotoxic mechanisms may play a role in the pathogenesis of reaction in leprosy.

PI 29

GANGLIOSIDE IMMUNO ASSAY EVALUATION FOR LEPROSY PATHOLOGICAL DAMAGE

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In some forms of motor neuron diseases and peripheral neuropathy the occurrence of high titers of antibody against Gm1 ganglioside has been described. Gm1 is an acid glycolipids composed of lipid and carbohydrate moieties. The carbohydrate portion of ganglioside contains sugars (gal and gluc) and sialic acid. The carbohydrate portion of the ganglioside could be the epitope in autoimmune reactions caused after nervous damage. Leprous neuritis caused by *Mycobacterium leprosy* is the most common peripheral neuropathy in developing countries. Since lipids such as gangliosides, cerebroside and sulfatides are known to be immunogenic and are present in peripheral nerve and perhaps the nerves are the first system to be attacked by *M. leprae* presenting along of the time irreversible damage, we investigate the existence of an auto antibody response to ganglioside in leprosy patients using Gm1 antigen (monosialoganglioside). Elisa Anti-Gm1 antibodies (IgG, IgM and IgA) were measured in sera from leprosy patients, household contacts and healthy individuals. Comparison of anti-Gm1 IgG, IgM and IgA rates between leprosy patients and healthy individuals did not show significant statistical difference (p>0.05). Antibodies levels were very low, 84% (85/101) of leprosy sera showed $\Delta E < 0.1$ and only 3% (03/101) presented $\Delta E > 0.1$. With Household contacts and Normal results were similar: 43% (21/49) showed $\Delta E < 0.1$ and 8% presented $\Delta E > 0.1$. Most of the sera presented high background; this could be, perhaps, by the use of detergent tween 20 in the block and washing solution. In fact Anti-Gm1 antibodies do not have value for evaluate pathological damage in leprosy.

Support: CNPq, FAPERJ

PI 30

HEPATITIS B AND C INFECTION AMONG LEPROSY PATIENTS ATTENDING THE SANATORIUM OF FONTILLES (SPAIN)

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A possible association between infection by hepatitis viruses B (HBV) and C (HCV) and leprosy has been

proposed. Hepatitis B (HBV) and hepatitis C (HCV) viruses are transmitted by blood (transfusions, par-enteral injections) possibly sexual contacts and probably other unknown routes. They can cause chronic liver disease. Populations with increased risk of these viral infections, specially patients with hemophilia and on hemodialysis have been identified. Patients with leprosy possibly also form a high risk group because of skin lesions, blood transfusions and confinement in institutions during prolonged periods of time. Some consider that the 2 polar forms of leprosy (tuberculoid and lepromatous) provide a model of interaction between cellular immunity and the hepatitis viruses.

In this study, the distribution of HBV and HCV virus markers were evaluated in 214 leprosy patients mostly long term institutionalised in the Sanatorium of Fontilles and compared with matched controls, using the same protocols required for screening of blood donors. Initially, two third generation microparticle enzyme immunoassays and positive results were confirmed by PCR methods.

The HBsAg and HCV positivity rates were 6% and 35% respectively, significantly higher than in the corresponding control groups (2% and 3.5%). The influence of possible risk factors (blood transfusion, confinement in leprosaria during prolonged periods of time, open skin lesions etc.) on this group of patients is discussed.

PI 31

HUMAN T CELL RESPONSES TO PEPTIDES OF THE *Mycobacterium leprae* 45-KDA SERINE-RICH ANTIGEN

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In order to identify T cell epitopes within the *Mycobacterium leprae* 45-kDa serine-rich antigen, T cell responses to overlapping 17-mer peptides encompassing the whole antigen were analysed in non-exposed UK controls, Pakistani leprosy patients and tuberculosis patients in both the UK and Pakistan. This antigen has been described as *M. leprae*-specific, although it has a hypothetical homologue in *Mycobacterium tuberculosis*. Peripheral blood mononuclear cells were stimulated with peptide for 5 days and

IFN- γ measured in supernatants by ELISA. Some peptides were more frequently recognised by T cells from tuberculoid leprosy patients than those from UK controls, suggesting that such T cell epitopes might have diagnostic potential. Short-term cell lines and flow cytometry confirmed specific T cell recognition of these peptides. However, T cells from many tuberculosis patients also recognised these potentially specific peptides suggesting that there could be a true 45-kDa homologue present in *M. tuberculosis*, or that tuberculosis patients living in a leprosy-endemic area have also been exposed to *M. leprae*.

PI 32

IDENTIFICATION AND CHARACTERIZATION OF THE ESAT-6 HOMOLOGUE OF *M. leprae* AND T CELL CROSSREACTIVITY WITH *M. tuberculosis*

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The present study describes the identification and characterization of *M. leprae* ESAT-6 (L-ESAT), the homologue of *M. tuberculosis* ESAT-6 (T-ESAT). T-ESAT-6 is expressed by all pathogenic strains belonging to the *M. tuberculosis* complex, but absent from virtually all other mycobacterial species, and is a promising antigen for immunodiagnosis of TB. Therefore, we have analyzed whether L-ESAT-6 represents a similarly powerful tool in leprosy, by examining T cell responses against L-ESAT-6 in leprosy patients, TB patients and exposed or non-exposed healthy controls from leprosy/TB endemic and nonendemic areas. L-ESAT-6 was recognized by T cells from leprosy patients, TB patients, TB patients' contacts and healthy individuals from a TB/leprosy endemic area, but not by non-*M. tuberculosis*, non-*M. leprae*-exposed individuals. Moreover, *M. leprae*-unresponsive leprosy patients failed to respond to L-ESAT-6. A very similar pattern was seen in case of T-ESAT-6. These results show that L-ESAT is a potent *M. leprae* antigen that stimulates T cell-dependent IFN- γ production in a large proportion of *M. leprae*-exposed individuals. Moreover, our results suggest the existence of significant cross reactivity between T- and L-ESAT-6, which has implications for the use of ESAT-6 as diagnostic tool for diagnosis of leprosy and TB in areas endemic for both diseases.

PI 33

IMMUNE SERIC PARAMETERS IN LEPROSY REVERSAL REACTION

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Introduction: It is well known that reactions are commonplace occurrences during the course of leprosy disease representing a clinical challenge in view of the immune response involved. In terms of immunology, the acute clinical episodes taking place in some chronic diseases are attributed to an exacerbation of the immune-inflammatory response expressed through seric and cellular markers, which are clinically evaluated, for example, by way of the erythrocyte sedimentation rate (ESR), reactive protein C, and nuclear activity factor. Other markers, like Neopterin, β 2-microglobulin, and tumor necrosis factor (TNF) have recently been shown to also be significant in this context. In leprosy, however, while the Lepromin test and the linfoproliferative assay are capable of confirming exacerbation of the immune response, no clinical or laboratory evidence has yet been reported to uphold this claim.

Objective: To evaluate the serum markers used to assess immunological activity during and after reversal reaction (RR).

Material and Methods: The first reactional episodes of 21 multibacillary (MB) leprosy patients who developed RR during specific MDT treatment were studied. The patients were classified according to the Ridley and Jopling criteria, having been submitted to routine clinical, histopathological, bacteriological, and immunological tests at diagnosis and then at the onset of a reactional episode. Neopterin and β 2-microglobulin levels, tumor necrosis factor, and soluble TNF- α receptors 1 and 2 were likewise assessed at the beginning of the RR episode and after treatment.

Results: Slightly over 90% (90.5%) of the first reactional episodes occurred during the first year of treatment; and the great majority of the patients (71,4%) experienced only one. During reaction, increased levels of the studied markers, which declined after treatment, were observed. Increased values of neopterin (66,6%) were seen more frequently during RR than were other markers, such as β 2-microglobulin (62%). The levels of these two markers showed a statistically significant ($p= 0,007$ and $p=0,01$, respectively) regression pattern subsequent to RR treatment.

PI 34

IMMUNOEPIDEMIOLOGICAL MONITORING OF LEPROSY

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Identification of risk groups for leprosy disease among contacts and population of regions with sporadic cases of leprosy is rather topical. Survey was carried out of 316 household contacts with leprosy cases and 516 inhabitants of Astrakhan region where sporadic cases of leprosy are registered. Epidemiological monitoring involves specific serological diagnosis (ELISA) and skin tests. In DIS-BSA-based ELISA anti-PGL-1 antibodies and antibodies against *M. leprae* sonicates were determined. DTH response to lepronin (Leprosy Research Institute, Astrakhan, Russia) and leprosin A (WHO Bank) was determined. As controls 150 volunteers out of inhabitants of non-endemic for leprosy regions of Russia were used. Among 316 contacts 39 subjects showed positive serological results (12,3%), and among 516 inhabitants anti-*M. leprae* antibodies were observed in 9 cases (1,7%). Levels of anti-PGL-1 antibodies ($0,23\pm 0,05$) and anti-*M. leprae* antibodies ($0,27 \pm 0,12$) in contacts significantly differed from indices in control subjects ($0,08 \pm 0,04$ and $0,13 \pm 0,01$, respectively). Serologically positive contacts were investigated each 6 months during 2 – 2,5 years. Contacts with permanently high titers of antibodies against *M. leprae* were given additional 6-months' course of preventive therapy resulting in serological conversion in some of them. Among 138 contacts 28% gave a positive reaction to lepronin and 35% - to leprosin A (coincidence for the two antigens – 81%). Whereas among 419 inhabitants positive reactions to lepronin were observed in 21% and to leprosin A – in 18% (coincidence for the two antigens – 88%). Thus, the data of serological monitoring and skin testing of household contacts and general population of leprosy endemic regions favor for identification of risk groups and might serve as additional characteristic of epidemiological situation in a region.

PI 35

IMMUNOLOGICAL PROPERTIES OF THE *Mycobacterium leprae* HLP PROTEIN

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Mycobacterium leprae Hlp (histone-like protein) is a cationic protein that is able to bind the laminin α 2 and other extracellular matrix proteins present on the surface of Schwann cells (Marques *et al.*, Microbes and Infection. 2:1407, 2000). Besides its potential role as a *M. leprae* adhesin, it has been reported that

a Hlp found in a number of *Streptococcus* species may play a role in the pathogenesis of bacterial-induced tissue inflammation (Choi *et al.*, Clin. Immunopathol. 76:68, 1995). In this context, it has been speculated that Hlp can accumulate within the basal lamina of infected nerves inducing tissue damage in leprosy. To elucidate its potential role in the immunopathogenesis of leprosy, the present study investigated the immunological properties of Hlp and its expression in infected tissues. Hlp was able to elicit high levels of IFN- γ secretion in mononuclear cells isolated from borderline tuberculoid leprosy patients. Experiments are under way to determine the capacity of Hlp to induce TNF- α secretion in the same cultures. Additionally, the presence of antibodies specific to Hlp was detected in leprosy patient's sera, indicating that this protein is able to induce both cellular and humoral immune responses in *M. leprae* infected individuals. Immunohistochemical analysis using a specific antibody anti-Hlp showed that the protein is expressed in the cutaneous infiltrates of leprosy lesions as Hlp-positive phagocytosed materials inside macrophages. These data indicate that Hlp is immunogenic and could contribute to tissue inflammation in leprosy. Currently, we are analysing the presence of Hlp in nerve biopsies to define its potential role on the persistent inflammation and delayed sequelae observed following *M. leprae* endoneural infection.

Supported by CNPq and NIH.

PI 36

IMPORTÂNCIA DAS PROVAS INFLAMATÓRIAS INESPECÍFICAS NA EVOLUÇÃO DO TRATAMENTO DA HANSENÍASE MULTIBACILAR

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O sistema imune responde geralmente a agentes lesivos animados, microorganismos, com a produção de anticorpos e células sensíveis. Anticorpos são proteínas originadas na progênie dos linfócitos B, os plasmócitos, caracterizados como globulinas, e portanto pela alteração dos seus níveis plasmáticos podem caracterizar a presença de infecção, embora se compreenda de forma inespecífica.

Uma infecção pode ser rastreada quanto à sua evolução através da repetição destes exames que podem evidenciar a melhora ou não do processo infeccioso, no caso a hanseníase multibacilar.

Nosso serviço pela simplicidade do armamentário diagnóstico laboratorial disponível tem lançado mão

daquilo que chamamos de "provas funcionais de proteínas inflamatórias", acrescidas do leucograma para evidenciar leucocitose.

Os resultados obtidos mostram que de uma forma mais simples, em serviços mais periféricos de controle da Hanseníase, este rastreamento pode ser uma forma a mais de consolidar a alta clínica.

O estudo foi realizado na Unidade Básica de Saúde de Itapevi, estado de São Paulo, onde foram acompanhados 23 pacientes multibacilares. Foram solicitadas provas inflamatórias durante o curso de tratamento. De acordo com o estudo pudemos evidenciar que os únicos exames que parecem estar relacionados com critérios para alta de multibacilares são proteína C reativa (positiva em 40% dos casos) e VHS (positiva em 60%)

PI 37

INDUCTION OF CELL DEATH (APOPTOSIS) BY TNF α AND TGF β IN A HUMAN SCHWANN CELL LINE *IN VITRO*

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A major complication in leprosy is the development of deformities along the chronic course of disease. In the present study, we used a human malignant Schwann cell line to characterize mechanisms of cell death *in vitro*. The ST88-14 tumor cell line was established from malignant schwannoma of neurofibromatosis (NF1) patient. The cells (SC) were grown in complete RPMI medium. The purity of Schwann cells was assessed by morphologic examination through Wright Giemsa, toluidine blue staining and S-100 protein. In the present study, we investigated physiological and morphological characteristics of ST8814. Cytokine gene expression and secretion was assessed by RT-PCR and ELISA respectively. Constitutive mRNA (for TNF α , TNF-R1, TNF-R2, IL-8, ICAM-1 and *c-fos*) was present in these cultured cells. Albeit TNF α gene expression was detected, no TNF α protein was observed in culture supernatants. However, soluble TNF-R1 and TNF-R2 were released in the culture medium. FACS analysis demonstrate, for the first time, expression of both TNF-R on the human SC surface. When SC were cultured in the presence of TNF α (10ng/ml) and TGF \downarrow (40ng/ml), around 30% of cell death was detected *in vitro*, a 3 fold enhancement when compared to the unstimulated cultures. No significant effect was noted when either TNF or TGF \downarrow were used as the stimulus. In order to determine whether the synergistic effect of TNF and TGF \downarrow leads to apoptosis *in vitro*, adherent and free cells in the culture medium were stained with propidium iodide and analyzed by flow cytometry.

etry. Preliminary results demonstrate an increase in the subdiploid peak in cells cultured in the presence of TNF/TGF as compared to the controls. The present data indicate that expression of the TNF and TNF-R genes in the SC may have implications in the pathogenesis of nerve damage in leprosy and induction of cytokine-mediated SC death *in vivo*.

PI 38

INHIBITION OF *M. leprae*-INDUCED APOPTOSIS AND CYTOKINES PRODUCTION BY THALIDOMIDE AND ANALOGUES

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One of the clinical manifestations of leprosy is the erythema nodosum leprosum (ENL) characterized by enhanced TNF levels. IFN and IL-12 were shown to be up-regulated during the reactions and are likely to be involved in the high TNF production in response to *M. leprae* *in vivo*. The use of thalidomide (THAL) in the treatment of several pathologies has been described by several authors. The search for new stable analogues with increased effectiveness and lower side effects has been requested. In order to conceive the mechanisms of THAL action, the *M. leprae*-induced production of IL-12 and TNF (secretion and mRNA synthesis), and its impact on cell death was investigated in cells culture. PBMC was stimulated with irradiated *M. leprae* (10g/ml) in the presence of THAL and analogues (25g/ml). RT-PCR performed of cultured cells 3h after stimulation displayed a decreased TNF and IL-12 mRNA by effects of THAL and N-hydroxyphthalimide (NH). After 20h of culture, TNF α production (2306 190pg/ml, n=5) was decreased by THAL (64 \pm 12%), NH (81 \pm 8.7%) and N-butylphthalimide (NB, 48 \pm 4.4%). N-methyl-ethylketonephthalimide (NK) showed no effect so far. Analysis of intracellular cytokine staining demonstrated an enhancement in TNF positive cells for both CD4⁺ and CD8⁺ T subsets induced by *M. leprae*, which was higher in the former group (3.7 \pm 0.8% Vs. 1.3 \pm 0.3%). In the presence of THAL, CD8⁺ T cells seem to be more affected than CD4⁺ T cells. When incubated with THAL, analysis of the rate of apoptosis induced by *M. leprae* in monocytes showed a reduction of 58,3%. Our preliminary data indicate that THAL, NB and NH lead to inhibition of pro-inflammatory cytokines on both monocytes and T cells, and the potential role to modulate *M. leprae*-induced features is evidenced.

PI 39

INVOLVEMENT OF B CELLS IN LOCAL IMMUNITY OF LEPROSY PATHOLOGY

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Mycobacterium leprae responsive T cell subsets are regarded to control the clinical and immunological spectrum in leprosy. On the otherhand, the longlasting *m.leprae* specific antibodies are diagnostically important, despite approximately 60% of paucibacillary (PB) patients can not be diagnosed by sero antibody assays. Low level of systemic antibody in PB patients can be due to the absence of optimal antigenic load in circulation that is needed for stimulating circulating B cells. Moreover, it is not understood why the PB lesions show continued clinical activity long after stopping the treatment. Since it is believed that appropriate quantum of immunologically defined both Th1 and Th2 cells exist in these PB patients, it is not known whether subsets of B cells are locally present. Further it remains unknown whether these also present B cells can be activated by the *in situ* persistence of *m.leprae* antigens and thus causing reactivation of the disease. Interestingly role of specific T cells in skin inflammation is studied exclusively but the participation of B cells in skin has not been studied.

In this study, we report the presence of B cell subsets as identified immunohistochemically by means of monoclonal antibodies e.g. CD20, CD79 and Syndican-1(CD138), that are involved in local interaction with residual *m.leprae* antigens and other immune cells e.g. T cells and subsets of antigen presenting cells (APC). We analysed the immune infiltrates and antigen expressions in both skin and nerve biopsies of leprosy patients originating from Brazil, India and The Netherlands. Our results show that all the subsets of B cells e.g. CD20⁺/CD79⁺/CD138⁻, CD20⁺/CD79⁺/CD138⁻, CD20⁺/CD79⁻/CD138⁻ and CD20⁺/CD79⁻/CD138⁺ cells are present in varying proportion and distribution in both skin and nerve lesions. The often presence of these B cells and persistent presence of *m.leprae* antigens are seen microscopically interacting with T cells and APC in both nerve and skin. We hypothesise that locally produced antibodies by these B cells may play an important effector role together with T cells and APC in the dynamicity of leprosy pathology.

PI 40***M. leprae* INDUCED APOPTOSIS PARALLELS A DOWN REGULATION OF CD14**

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Until very recently, the function of CD14 was thought to be limited to innate immunity, as the major endotoxin receptor. Nowadays, a role for CD14 in the regulation of monocyte apoptosis is being reported. It has been shown that down regulation of CD14 or its removal triggers apoptosis, whereas up-regulation promotes survival. Since our previous results indicate that *M. leprae* induces monocyte-derived macrophages (MDM) apoptosis in a dose dependent manner, the expression of CD14 in MDM stimulated with increasing concentrations of *M. leprae* or LPS was monitored by flow cytometry. When dead *M. leprae* (1g/ml) or LPS were added to cultures for 2 days an up-regulation of CD14 and an increase in cell viability was observed. However, when higher amounts of the bacteria (10 or 20g/ml), reported to induce apoptosis, were used, a down regulation of CD14 expression was noted after the same period of culture. When live *M. leprae* was used a similar profile in CD14 expression was detected. Our results indicate a selective and progressive CD14 down regulation, which parallels apoptosis induced by dead *M. leprae* in MDM, suggesting that CD14 down regulation is an early signal of cell death, as previously reported for MTB.

PI 41**MATURE DENDRITIC CELLS INFLUENCE TH1/TH2 CYTOKINE PROFILES IN STABLE LEPROMATOUS LEPROSY PATIENTS**

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The role of dendritic cells (DC) in immune response to *M. leprae* antigen was investigated in lepromatous leprosy patients. Dendritic cells (DC) are unique antigen presenting cells specialised in antigen capture and triggering adaptive immune responses. Immature DCs act mainly to capture antigens whereas mature DCs acquire high levels of MHC class I/II

and co-stimulatory molecules, present antigens and initiate T cell and B cell responses. The role of both populations of DCs in Th cell differentiation in leprosy was evaluated. Co-expression of Th cytokines IFN γ and IL4 and regulatory cytokines IL10 and IL12p40 was compared in antigen stimulated peripheral blood mononuclear cells (PBMCs), T-cells reconstituted with autologous monocytes (Mo), T-cells reconstituted with immature DC (high intracellular MHC II, low CD83 and p55) and T-cells reconstituted with mature DC (CD11C+, high surface MHC II, high CD83 and p55) by conventional and Real Time fluorogenic based RT-PCR (Reverse Transcription Polymerase Chain Reaction) and by ELISA. Reconstitution of purified T-cells with autologous Mo and immature DC resulted in down regulation of IL4 and IL10. On the other hand reconstitution of purified T cells with mature DC resulted in upregulation of IFN γ and dysregulation of IL4. The fact that stimulation of different populations of DCs could alter the cytokine profile in reconstituted cultures, suggests that they may have a varied influence on the immunological stability of this disease.

PI 42**MODULATION OF CYTOKINE RESPONSE TO POLYCLONAL AND MYCOBACTERIAL STIMULI BY THE PHENOLIC GLYCOLIPID-I(PGLI)**

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Previously, PGLI has been shown to inhibit T cell activation parameters and to enhance TNF- α production by *M. leprae*-engulfing mononuclear phagocytes. In order to further assess the role of PGLI in cytokine production, peripheral blood leukocytes from healthy volunteers, tuberculoid and lepromatous leprosy patients were stimulated *in vitro* (ConA, *M. Leprae*, PPD), in the presence of PGLI, and the levels of 6 cytokines (IFN- α , TNF- α , IL-2, IL-4, IL-5 and IL-10) were evaluated in the culture supernatants, using the cytokine bead array flow cytometric method and ELISA. In the presence of PGLI, IFN- γ levels were reduced/absent in ConA and *M. Leprae*-stimulated wells, but unaffected or increased with PPD. PGLI reduced TNF- α in response to ConA, but markedly enhanced this cytokine concentration when added to *M. Leprae*-stimulated wells. PGLI inhibitory actions were not associated to increase in IL-10 levels. The observed effects of PGLI were seen in the patients and healthy volunteers. Taken together these observations show that PGLI effects change with different stimuli, perhaps reflect-

ing a different sensitivity to PGLI by the leukocyte subsets and/or pathways involved in cytokine production induced by ConA and these mycobacterial stimuli.

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PI 43

NÍVEIS DE ÓXIDO NÍTRICO EM PLASMAS DE PACIENTES HANSENIANOS

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As infecções causadas por parasitos intracelulares obrigatórios tais como o *Mycobacterium leprae* são contidas pela imunidade celular. Os pacientes portadores desta infecção apresentam um variado espectro de manifestações clínicas, o que reflete o estado imunológico em que se encontram. Pacientes multibacilares podem apresentar alta bacteremia em virtude de uma resposta imune celular deficiente. Por sua vez, pacientes paucibacilares apresentam baixa bacteremia associado a uma resposta imune celular eficiente. A produção de reativos intermediários do oxigênio e do nitrogênio por macrófagos ativados é crucial para o desenvolvimento da imunidade contra microrganismos intracelulares. Este estudo tem por objetivo quantificar os níveis de óxido nítrico (NO) em plasmas de pacientes portadores das diferentes formas clínicas da Hanseníase. A quantificação de NO será realizada indiretamente através da medida de nitritos e nitratos. Metodologia: 50 L das amostras de plasmas diluídos em água foram incubados overnight com uma mistura contendo FAD, NADPH e nitrato redutase. As amostras foram desproteinadas por ZnSO₄. A seguir, 100 L das amostras em duplicatas foram misturadas com 100 L do reagente de Griess e a absorbância determinada em leitor de ELISA. Os resultados expressos em M/mL foram obtidos pela extrapolação de uma curva padrão com NaNO₂. Resultados: os níveis de NO encontrados nos plasmas dos pacientes foram: paucibacilares: 218,8 M/mL (n=3), multibacilares: 183,4 M/mL (n=10) e controles saudáveis: 217,7 M/mL

(n=10) respectivamente. Conclusão: os resultados sugerem não haver diferenças significativas nos níveis séricos de NO entre pacientes hansenianos e controles saudáveis.

Apoio Financeiro: FAPEMIG

PI 44

PARACOCIDIOIDOMICOSE E HANSENÍASE: RELATO DE CASO

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Paciente masculino, negro, 44 anos, natural de Teófilo Otoni- MG, procedente de São Paulo.

Procurou nosso serviço em Dezembro de 1999 apresentando dermatose localizada em ângulo esquerdo da boca e língua caracterizada por exulceração com cerca de 2 cm de diâmetro, de limites irregulares, mal delimitadas em cuja superfície se observava exudato seropurulento e crostas hemáticas.

O diagnóstico de Paracoccidiodomicose foi confirmado pelo exame micológico direto e cultura para fungos.

Foi introduzido o tratamento com cetoconazol, porém o paciente abandonou o tratamento.

Em Julho de 2001 procurou o serviço de Otorrinolaringologia com queixa de rouquidão progressiva e perda ponderal de 5 kg. Na mucosa nasal foi observado lesão ulcerosa infiltrativa e exsudativa.

Na mesma época retornou ao nosso serviço.

Ao exame dermatológico observou-se: dermatose disseminada caracterizada por nódulos e placas que variavam de 2 a 7 cm de diâmetro. Face infiltrada, nódulos em pavilhões auriculares e madarose.

Na região de sulco nasogeniano e axila direita nódulos ulcerados com cerca de 2 cm de diâmetro.

Baciloscopia com bacilos álcool ácido resistentes isolados e em globias.

Exame micológico direto e cultura para fungos positivos para Paracoccidiodomicose.

Exame anti-HIV negativo.

Iniciou-se tratamento com poliquimioterapia esquema multibacilar para Hanseníase Virchowiana e Itraconazol 100 mg ao dia para Paracoccidiodomicose.

Trata-se de uma associação rara de duas doenças infecto contagiosas, com mecanismos imunológicos distintos, onde o paciente apresenta a forma crônica multifocal da Paracoccidiodomicose com resposta Th1 e Hanseníase Virchowiana com resposta Th2.

PI 45

PREVALÊNCIA DE ANTICORPO ANTICARDIOLIPINA NAS DIFERENTES FORMAS CLÍNICAS DE HANSENÍASE

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Os Anticorpos Anticardiolipina (AA) são autoanticorpos associados com trombose vascular e aborto de repetição, porém vários relatos têm associado com doenças infecciosas como a Hanseníase. O objetivo deste estudo foi avaliar a prevalência dos AA nas diferentes formas clínicas de Hanseníase. Foram estudados 42 pacientes, sendo 26 do sexo masculino e 16 do sexo feminino, com idades entre 17 e 77 anos com média de 48 anos. Todos pacientes eram portadores de Hanseníase confirmados pela clínica, baciloscopia e biópsia de pele. As manifestações clínicas da Síndrome do Anticorpo Antifosfolípido (SAF) não foram encontradas. Os pacientes foram submetidos a classificação de Ridley-Jopling: 9,5% indeterminado(I), 16,6% tuberculóide(T), 16,6% dimorfo tuberculóide(DT), 7,1% dimorfo dimorfo(DD), 4,7% dimorfo virchowiano(DV) e 45,2% virchowiano (V). A avaliação dos níveis de AA no soro foi realizada pelo método ELISA (Enzyme-linked Immunosorbent Assay). Foi utilizado na análise estatística o teste exato de Fisher com $p < 0,05$ e IC 95%. Os dados obtidos foram comparados com grupo controle formado por 100 doadores do banco de sangue de estudo prévio da instituição. A prevalência global dos AA foi de 47%, sendo significante $p < 0,0001$ em relação ao grupo controle, sendo encontrado positividade na forma I (25%), T (28,5%), DT (28,5%), DD (33,3%), DV (50%) e na forma V (68,4%). Os pacientes com baciloscopia positiva apresentaram significância estatística quando comparados com os negativos $p < 0,01$. Conclui-se que os pacientes com Hanseníase apresentam alta prevalência de AA, sendo as formas com baciloscopia positiva mais prevalente que as formas com baciloscopia negativa.

PI 46

REAÇÃO DO GRANULOMA *IN VITRO* COM ANTÍGENOS DO *Mycobacterium leprae* E CÉLULAS MONONUCLEARES DO SANGUE PERIFÉRICO DE DOENTES COM HANSENÍASE

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As razões para diversidade da resposta imune nas formas polares da doença frente ao mesmo agente agressor, *Mycobacterium leprae*, não estão completamente elucidadas. Buscamos desenvolver um modelo de granuloma *in vitro* com antígenos do *M. leprae* e células mononucleares do sangue periférico de doentes com as formas polares da hanseníase e avaliar as diferenças na produção de citocinas e de óxido nítrico em culturas de células. Foram selecionados, 10 doentes com hanseníase sem tratamento, atendidos no HC-FMRPUSP: Grupo V (virchowianos polares e borderline-lepromatosos) e Grupo T (tuberculóides polares e borderline-tuberculóides). Procedeu-se o isolamento de células mononucleares do sangue periférico utilizando-se centrifugação sob gradiente de Ficoll-Hypaque. Foram realizadas culturas duplicatas (2×10^6 linfócitos/ml): controles, com meio de cultura ou *beads* isolados; com antígenos de 28kD ou de 36kD do *M. leprae* conjugados a *beads* de poliacrilamida e com a presença ou ausência de aminoguanidina. As placas foram incubadas a 37°C em atmosfera úmida contendo 5% de CO₂, durante 21 dias e, colhidas amostras do sobrenadante no 7º e 21º dias. A pesquisa das citocinas IL1, IL6, IL10, IL8, TNF, IFN no sobrenadante foi feita pelo método ELISA e a atividade da enzima NO sintase foi avaliada pelo ensaio modificado da citrulina. Ao 7º dia, observou-se aumento significativo da produção de IL-6 e IL10 nas culturas controles e com antígenos no grupo V, comparada à do grupo T. Não se detectou produção de TNF em nenhum grupo neste período. Ao 21º, os grupos V e T produziram IL6 de modo similar, na presença ou ausência de antígenos nas culturas. Entretanto, no grupo V, observou-se redução da produção de IL10 nas culturas com antígenos. A produção de TNF tende a ser mais acentuada no grupo T e reduzida com a presença de qualquer um dos antígenos testados, em ambos os grupos. Com a presença de antígenos, a produção de IL1 foi reduzida no 21º dia. Não se detectou a atividade da enzima NO sintase nas culturas. A IL6 atua preferencialmente em células B, proporcionando a produção de

anticorpos, e a IL10 exerceria efeitos imunossupressores. No modelo desenvolvido, o aumento destas citocinas no grupo V e a inibição da produção de TNF e IL1, na presença de antígenos, são indicadores da imunomodulação. Vale ressaltar, que o modelo de granuloma *in vitro* mostrou-se útil no estudo da imunomodulação na hanseníase com amplas perspectivas de aplicação experimental

PI 47

REGULATION OF MACROPHAGE BCL-2 GENE FAMILY EXPRESSION DURING *M. leprae*-INDUCED APOPTOSIS: INVOLVEMENT OF BAX AND BAK

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Bcl-2 protein family regulates cell death. This family has antiapoptotic (Bcl-2 and Bcl-xL) and proapoptotic (Bad, Bax and Bak) proteins. These proteins form homo and heterocomplexes that can, among other functions, regulate mitochondria release of the cytochrome C. Mycobacterias were described to induce apoptosis in mononuclear phagocytes *in vivo* and *in vitro*. Our previous results demonstrate that *M. leprae* can also induce apoptosis in leprosy patients monocytes in a dose dependent manner *in vitro*. In order to investigate the molecular mechanisms underlying the apoptotic pathway in this model, RT-PCR was used to evaluate the expression of some members of the Bcl-2 family, namely Bax- α and Bak. Initial experiments demonstrated that *M. leprae* could modulate the mRNA expression of some proapoptotic genes. Cells stimulated with low concentration of *M. leprae* (1g/ml), not able to induce macrophages apoptosis, also did not induce expression of Bak or Bax. However, cells stimulated with 10 or 20g/ml of *M. leprae* showed increased expression of both genes and exhibited a significant enhancement in the rate of apoptosis. LPS, an inhibitor of macrophage cell death, did not induce large amounts of these genes. Bad expression was not detected in either culture. These results suggest that Bcl-2 family proteins may be involved in *M. leprae*-induced cell death *in vitro*.

PI 48

SELECTIVE INCREASE IN THE CD4+ T CELL ACTIVATION THRESHOLD AS A POTENTIAL MECHANISM FOR THE BIOLOGICAL ROLE OF THE PHENOLIC GLYCOLIPID-I (PGL-I) IN LEPROSY

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Pathogens frequently synthesize immune response modifiers to enhance their survival in the host microenvironment. In order to evaluate the role of PGL-I, a *M. leprae* (ML) glycolipid, as a T cell function modifier, PBLs from healthy volunteers and leprosy patients were stimulated *in vitro* in the presence of PGL-I. Flow cytometry of the cultured PBLs demonstrated PGL-I inhibition of CD28 expression in CD4+, but not CD8+T cells. The induction of CD69 and CD25 in ConA or anti-CD3 stimulated T cells was markedly reduced by PGL-I. The proliferative responses, IL-2/TCGF and TNF bioactivities, as well as IFN- γ levels (ELISA) were also reduced by PGL-I. All the effects of PGL-I were seen in healthy volunteers and in patients across the spectrum of leprosy. PGL-I actions occurred only at suboptimal levels of stimulation, being reversed by increasing the stimulus. This observation suggests that this glycolipid increases the threshold for T cell activation. The inhibition of CD28 expression/function in CD4+ T cells and, as a consequence, less effective sorting of activation-associated molecules in the immunological synapses is a potential mechanism for PGL-I action on T cell activation. These effects of PGL-I can lead to pathogen-specific anergy and defective effector function against ML, if associated to conditions allowing the initial survival of ML in an infected individual.

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PI 49

SERODIAGNOSIS OF *Mycobacterium leprae* INFECTED INDIVIDUALS IN HIGHLY ENDEMIC AREAS OF MYANMAR

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This study was carried out on serodiagnosis of *M. leprae*-infection in highly endemic areas of Myan-

mar. It was studied on the residents of Kyanbokone (A-village) and Konethandin (B-village) in Bago Division and NTP-BSA ELISA was done on their sera. The IgM and IgG seropositives in A-villages were 59/263 (22.43%) and 34/263 (12.93%) in the first year, 41/223 (19.21%) and 46/223 (20.63%) in the second year. The same in B-village is 41/115 (35.65%) and 9/115 (7.82%) in the 1st year. The age group of 10-19 year-old was the commonest one in age specific distribution of anti IgM and IgG antibodies to NTP-BSA antigen. Some villagers who presented with high titre of antibodies should be followed up considering the indication of chemoprophylaxis. Through the follow up study of *M. leprae*-infection in particular populations, some valuable information about the process from infection to development of disease can be expected.

PI 50

SERUM CYTOKINES AND MONOKINES IN LEPROSY AS A MARKER FOR MONITORING REVERSAL REACTIONS

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During the course of leprosy episodes, two types of "reactions", classified either type-I (reversal reaction: RR) or type II (erythema nodosum leprosum: ENL) occur among 30-40% of the patients. The immunopathology of leprosy is primarily due to immune interaction between subsets of T cells, macrophages/relevant antigen presenting cells and *M.leprae* antigens. Such interactions produce Th1/Th2 cytokines, activated macrophage products and monokines, which act as molecular signals for communication between immune cells and the organ specific cells, which play a pivotal role in the dynamics of host immune response and tissue damage. The latter, in case of leprosy, is the destruction or dysfunction of peripheral nerves and deformity. *In vitro* and *in vivo* studies have been used to delineate the immunologic aspects of leprosy and the reactional states. In this respects serum cytokine and monokine levels had been considered as useful markers for monitoring the leprosy patients during the course of the disease. In this study we measured, by either ELISA or radioimmunoassay, serum levels of T cell cytokines: IFN- γ , TNF- α , TNF- α R (p75, p55), IL-4, IL-5 and neopterin in seven leprosy patients from Phillipines before treatment and thereafter for 1-6 months when patients developed clinical signs of RR with the expectation that serum cytokine

and monokine profile at the onset of reaction may provide a clue for early prediction of reactions to be experienced by the patients. The results show that at the onset of RR and/or one month after, 6/7 patients showed significantly increased levels of neopterin and in some cases remained high even after being treated with immunosuppressive drugs. On the other hand serum IFN-, IL-4, IL-5 showed inconsistent increase in not more than 2/7 patients. Interestingly, levels of TNF- γ , TNF-R were increased in 6/7 patients either at the onset or after 1 month of the onset and remained high above the normal value. It appears from this pilot study that measurement of serum neopterin and TNF- α , TNF- α R could be valuable in monitoring the RR patients during the treatment.

PI 51

SPECIFICITY OF PGL-I SEMI- SYNTHETIC ANTIGENS IN LEPROSY SEROLOGY

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Several serological tests to detect *M. leprae* infection have been developed using PGL-I, a cell wall component specific for *M.leprae*. Semi-synthetic derivatives were produced by either directly linking a disaccharide group to BSA, leading to disaccharide BSA (DBSA), or by linking the synthesised sugar groups (natural di- or tri-saccharides [ND or NT]) to BSA with an octyl (O) or a phenolic linker (P).

We evaluated whether the antibody response to PGL-I was specific for *M.leprae* and determined whether serum samples gave similar results with different PGL-I-based semi-synthetic antigens.

143 serum samples from different groups were tested using the native PGL-I and three semi-synthetic antigens (ND-O-BSA, DBSA and NT-P-BSA). ND-O-BSA was more often positive than the others. All antigens were compared with ND-O-BSA. DBSA gave the highest agreement and PGL-I the lowest. There was no disagreement in the patients group with OD values above 1.5 and the groups formed by contacts and non contacts of leprosy patients with negative ND-O-BSA. The group composed of non-contacts with positive ND-O-BSA results gave the lowest agreement between antigens, namely 62, 83 and 94% when compared with DBSA, NT-P-BSA and PGL-I, respectively.

Comparison between different batches of ND-O-BSA gave a strong suggestion that non-specific binding in a particular batch could be occurring. This re-

sults indicate that a rigorous quality control of the antigen should be performed.

PI 52

SPINDLE CELLS AND MACROPHAGES IN HISTOID LEPROSY

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Purpose: Histoid form of leprosy is a rare manifestation of lepromatous form. The histological aspect is the presence of a pseudocapsule, with fusiform cells surrounding this structure. The aim of this study was to demonstrate that this spindle cells express the same antigen as the foam cell and that this form are related to high positivity of acid fast bacilli.

Methods: We have performed an histological (HE and Fite-Faraco stain) and immunohistochemical study (anti-CD4, anti-CD8, anti-CD68 and anti-BCG) in 8 patients, presenting clinical criteria for histoid leprosy.

Results: In HE stain, the infiltrate was mainly constituted by Virchow's cells. The spindle cells were present in four patients. In only two cases, we could distinguish pseudocapsule in peripheral localization of the macrophagic granuloma. We observed a relationship between globi and presence of spindle cells, by Fite-Faraco stain. Virchow's cells were related to isolated and granular bacilli. The expression for BCG antigen was also strong in every patients. CD4+ and CD8+ cells were diffusely distributed in the infiltrate, without any typical pattern. CD68 antigen expression was strong in Virchow's and spindle cells. The expression for BCG and CD68 antigen was positive in the cytoplasm of fusiform cells, constituting the pseudocapsule.

Conclusion: Spindle cells are typical of this resistant form of leprosy. Expressing the same antigen (CD68+) in Virchow's and spindle cells, we could consider that these spindle cells have macrophagic function.

PI 53

STUDIES ON REGIONAL IMMUNITY USING EX-VIVO EXPLANT CULTURES OF SKIN LESIONS IN RELATION TO LEPROSY PATHOLOGY

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Prevention of acute reactions in leprosy is the principal goal to be tackled for the containment of leprosy in foreseeing future. Understanding the local immunity on skin lesions is therefore, a pre-requisite so that a more direct approach for early diagnosis of leprosy lesions can be developed. At present, several serodiagnostic tests using antibody titres are used for leprosy diagnosis. Although these tests can diagnose 90-100% of multibacillary (MB) leprosy, they failed to detect 40-60% cases of paucibacillary (PB) patients, but 40% of these PB patients show serum antibody to *m.leprae* antigens. In addition, several investigators used serum cytokine levels as the marker for disease activity but with limited value, particularly in the context of discriminating leprosy from other inflammatory skin diseases.

We established organotypic cultures of full thickness skin to study the local production of anti-*m.leprae* antibodies and cytokines in the lesions of paucibacillary (PB), multibacillary (MB) patients with and without leprosy reaction. These presently studied PB and MB patients were histopathologically classified as BT/TT and BL/LL respectively. Kinetics of antibody production and cytokines in the culture supernatants were analysed.

Results: show that production of antibody peaked at 48 hours in all leprosy lesions but negative in all control specimens. Such antibody production could not be seen when the biopsies were autoclaved before culturing. On the otherhand kinetics of IFN-, TNF- in the same supernatants that peaked at 24 hours were significantly more pronounced in PB lesions and lesions with reversal reactions than those in MB and control specimens. However, there was no difference in the local production of IL-4 and IL-10 among the specimens. Interestingly, IL-6 production peaked at 48 hours that was variable but pronounced in the lesions of PB patients although statistically not different from those in MB lesions.

Conclusion: The present data taken together with the phenotypes of *in situ* immune-infiltrates in PB lesions suggest that a combined role of locally produced antibody and T cell response is important in leprosy pathology.

PI 54

T- AND B-CELL RESPONSES TO *Mycobacterium leprae* HOMOLOGUE OF ESAT-6 IN LEPROSY PATIENTS AND CONTROL INDIVIDUALS

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ESAT-6 (early secreted antigen target 6 kDa protein) has been described as an immunodominant antigen in the context of *Mycobacterium tuberculosis* infection in animal models and humans. ESAT-6 generates CD4+ Th1 cells and antibody production, thus showing promise as a specific diagnostic tool for active tuberculosis. It was recently demonstrated that *M. leprae* expresses *in vivo* an ESAT-6 homologue sharing only 36% identity with its *M. tuberculosis* counterpart. The present study investigated the specific T- (IFN- γ secretion) and B- (IgG antibody) cell responses to *M. leprae* ESAT-6 in the context of leprosy. T-cell response to *M. leprae* ESAT-6 was measured in peripheral blood mononuclear cells (PBMC) from leprosy patients, *M. leprae*-exposed individuals, tuberculosis patients, and healthy individuals from an endemic area. Cells were stimulated with the antigen and secreted IFN- γ levels were quantified by standard ELISA in cultured supernatants. Initial results show that *M. leprae* ESAT-6 was recognized by cells from leprosy patients (6/9), exposed individuals (8/12), tuberculosis patients (1/2), and healthy individuals (2/2) from an endemic area. IgG antibody response was observed in lepromatous leprosy patients (11/13), tuberculoid leprosy patients (6/8), TB patients (1/10), and exposed individuals (14/18). Currently, *M. leprae* ESAT-6 peptides are being analyzed for IFN- γ induction in PBMC. These preliminary results indicate that ESAT-6 induces T- and B-cell responses in most leprosy patients and healthy exposed individuals. The potential cross-reactivity with *M. tuberculosis* ESAT-6 together with the positive responses observed in individuals from an endemic area suggest limitations on the use of *M. leprae* ESAT-6 in a specific diagnostic test for leprosy.

(Research supported by the NIAID, NIH).

PI 55

THALIDOMIDE CAN CO-STIMULATE OR SUPPRESS CD4+ CELLS' ABILITY TO INCORPORATE [H³]-THYMIDINE – A DEPENDENCE ON THE PRIMARY STIMULANT

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Thalidomide is the treatment of choice for erythema nodosum leprosum and has immunomodulatory properties. To assess if the stimulant and/or thalido-

midide could modify the synthesis of IL-2, IFN- γ and incorporation of [H³]-thymidine, peripheral blood mononuclear cells (PBMC) were incubated for three days in the presence or absence of thalidomide and Staphylococcal enterotoxin A (SEA), anti-CD3, Con-A or PHA.

Regardless of the mitogen used to stimulate the PBMC, the thalidomide-treated-PBMC produced more IL-2 than controls. Thalidomide enhanced IFN- γ synthesis in the Con-A and anti-CD3-stimulated PBMC. It suppressed the ability of SEA and PHA stimulated PBMC to incorporate [H³]-thymidine; whereas it enhanced incorporation of [H³]-thymidine in PBMC's stimulated with anti-CD3.

When the PBMC were enriched for CD4+ or CD8+ cells, the SEA-stimulated CD4+ cells responded far better than the CD8+ cells in the synthesis of IL-2 and incorporation of [H³]-thymidine. In CD4+ cells thalidomide acted as a co-stimulant with SEA to enhance the synthesis of IL-2, but it suppressed incorporation of [H³]-thymidine. In the anti-CD3-stimulated-thalidomide treated cultures of PBMC enriched for CD4+ or CD8+ cells, thalidomide acted as a co-stimulant to enhance the synthesis of IL-2 and incorporation of [H³]-thymidine.

Thalidomide cooperated with all of the mitogens to enhance T-cell synthesis of

IL-2; however, depending on the stimulant, thalidomide could suppress or enhance cellular incorporation of [H³]-thymidine. The SEA-stimulated cell targeted by thalidomide to suppress incorporation of [H³]-thymidine was CD4+. CD4+ and CD8+ cells stimulated with anti-CD3 were enhanced by thalidomide in their ability to synthesize IL-2 and to incorporate [H³]-thymidine.

PI 56

THALIDOMIDE DID NOT MODIFY LEPROSY PATIENTS' CELLS' ABILITY TO PROLIFERATE IN RESPONSE TO *M. leprae* ANTIGENS

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The immune response in reversal reaction, (RR) and in erythema nodosum leprosum (ENL) is characterized *in vitro* by an enhancement in lymphocyte blast transformation to *M. leprae*. As thalidomide is effec-

tive treatment for ENL, this study assessed the effect of thalidomide on these phenomena. Mononuclear cells from patients attending the clinic at ALERT and healthy staff were exposed for 5 days to integral *M. leprae*, or a modified Dharmendra antigen, or a preparation of PPD from *M. tuberculosis*. The cultures were treated with thalidomide. In one set, thalidomide was added once at the initiation of the culture, and in another set it was added for a second time (2x), 24 hr prior to harvesting the cells.

The mononuclear cells, in the absence of thalidomide, from the healthy staff (N=11), borderline tuberculoid patients (BT, N= 14) and the BT patients in RR (BT/RR, N=11) responded best to PPD > Dharmendra > *M. leprae*. The cells from patients who were being treated with prednisone to suppress ENL (N=7) did not respond well to the *M. leprae* antigens. Thalidomide (2x) enhanced proliferation to PPD in the ENL group (paired t-test, p=0.02). No significant changes occurred for the other groups. Comparing PPD-stimulated cells treated with thalidomide once to those treated with thalidomide twice, thalidomide (2x) suppressed incorporation of [³H]-thymidine by the PPD-stimulated cells in the healthy staff group (p= 0.04). In the Dharmendra-stimulated cells from the healthy staff thalidomide significantly suppressed TNF- α (p=0.01). A mixed effect was seen within and between the other groups, but there was a trend for thalidomide to suppress of TNF- induce by the *M. leprae* and PPD antigens.

PI 57

THE EFFECT OF THE ACTIVITY OF MICROSOMAL ENZYMES AND ACETILATION ON METHEMOGLOBIN RATE IN LEPROSY PATIENTS

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As it is known, dapsone at certain doses may induce hemolysis, especially in persons with glucose-6-phosphate dehydrogenase (G6PDH) deficiency, occurring in about 10% of leprosy patients. However, DDS-induced hemolysis might be due to other factors among which peculiarities and intensity of drug metabolism, including rate of sulphone acetylating and hydroxylation, play an important role. Patients with lepromatous leprosy were given various schemes of MDT with dapsone 100 mg daily as a main component. Activity of microsomal enzymes by the time of antipyrine half-secretion (T1/2) and acetylation rate of sulfadimazine was studied. All the patients studied had no G6PDH-deficiency. It was observed that in patients showing rather high activity of microsomal enzymes (T1/2 =12,5 h in average) blood methemoglobin rate was significantly higher

(P<0,05) than in those with low activity of these enzymes (T1/2=23,5 h in average). Though methemoglobin rate in the most patients did not exceed 1,5%, it approached 2,5-3,9% in persons with a combination of low acetylating rate and high activity of microsomal enzymes. It might be a consequence of increase in derivatives of N-hydroxylation of dapsone with methemoglobin-forming properties in persons with predominance of oxydative phenotype of xenobiotic biological transfor.

PI 58

THE REPORT FOR THE SKIN SMEARS QUALITY CONTROL ON LEPRESY IN SICHUAN PROVINCE IN THE PAST 15 YEARS

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The skin smears quality control on leprosy was implemented in the leprosy epidemic counties in Sichuan Province, in order to improve the quality of skin smears and implementation of MDT. 10% of skin smears, came from the leprosy epidemic counties, were selected randomly with double-blind method and evaluated in smears, stain and diagnosis in Sichuan Leprosy Laboratory on the basic of the criterion of the skin smears quality on leprosy in the Handbook of MDT on Leprosy. Meanwhile, the skin smears came from Sichuan Leprosy Laboratory were also checked and contrasted by the paramedical workers. In the past 15 years, the skin smears quality control was implemented and the quality of skin smears was improved between 17 and 97 leprosy epidemic counties in Sichuan. 4529 pieced of skin smears were checked. The average qualified rate of smears, stain and diagnosis was 96.88%, which was 86.97% in 1986. The implementation of skin smears quality control could improve professional level of paramedical workers and the quality of leprosy control

PI 59

TOLL-LIKE RECEPTOR 2 ON HUMAN SCHWANN CELLS.

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Nerve damage is a characteristic clinical feature of leprosy. We investigated the ability of human

Schwann cells to participate in microbial recognition according to their expression of Toll-like receptor 2 (TLR2). In this paper, FACS analysis of a human Schwann cell line ST88-14 and immunohistochemistry of leprosy skin lesions demonstrate expression of TLR2 on the surface of human SC (double-fluorescence labeling showed colocalization of a Schwann cell marker, neural cell adhesion molecule (NCAM) and TLR2). Given that TLR2 mediates recognition of microbial lipopeptides, we engineered a synthetic lipopeptide comprising the first six amino acids of the putative *M. leprae* 19 kD antigen. Acti-

vation of the human Schwann cell line with the *M. leprae* lipopeptide triggered an increase in the number of cells with condensed nuclei and evidence of DNA fragmentation, characteristics consistent with cell death. Hoescht stain and 7-AAD showed a 2 or 3 fold enhancement in the cell death when compared to the unstimulated cultures. The ability of *M. leprae* components to induce apoptosis of Schwann cells through Toll receptors might provide a mechanism for nerve damage in leprosy in the absence of inflammation.

MICROBIOLOGY & MOLECULAR BIOLOGY

PM & BM 1

A HISTOLOGICAL AND BACTERIOLOGICAL ASSESSMENT OF LEPROSY PATIENTS WITH < 5 LESION

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Aim: To study the histological and bacteriological features of leprosy patients with 5 or less than 5 lesions and relate it to clinical features.

Methods: 76 consecutive leprosy patients (M 57 F 19) who had 5 lesions were included in the study. Clinical features were recorded, slit skin smears and skin biopsies were done on all patients. A nerve biopsy was performed (radial cutaneous or Sural nerve) in 18 patients who had a clinically thickened cutaneous nerve.

Results: Out of the 76 patients, 28 patients had single skin lesions, 17 had 2 lesions, 13 had 3 lesions, 5 had 4 lesions and 2 had 5 lesions. The clinical diagnosis was TT leprosy in 4, BT in 68 and indeterminate in 4. Slit skin smears were positive in only 1 BT leprosy patient.

Histological examination revealed features of TT leprosy in 2 patients (2.6%), BT leprosy in 42 patients (55.3%), BL in 4 patients (5.3%), indeterminate leprosy in 16 (21%) and non-specific inflammation in 12 (15.8%). Acid fast bacilli ranging from a bacterial index of granuloma (BIG) of 1+ to 4+ were present in 10 of the skin biopsies (13.2%). The cutaneous nerve biopsies in 16 of the 18 patients (88.8%) revealed features of BT leprosy consisting chiefly of lympho-epithelioid granuloma. 12 of these nerve (66.7%) revealed AFB in them with a BI ranging from 1+ to 4+.

Conclusion: The findings from the study indicate that the number of lesions does not determine the type or extent of the disease.

PM & BM 2

A HISTOPATHOLOGICAL STUDY OF TYPE II (ENL) REACTION IN LEPROSY

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Type II lepra reaction produces a defined clinical picture of painful and tender erythematous nodules which are described as 'Erythema Nodosum Leprosum'. The histology of these lesions have been variously described. The aim of this study was to document the different components that constitute a histological diagnosis of ENL and their consistency of occurrence in each lesion.

A detailed study was made of 22 skin biopsies from ENL lesions. A histological diagnosis of 'LL in ENL' was made in 11 biopsies (50%). The most consistent feature noticed in these 11 biopsies was the presence of foamy macrophage granulomas in a pale oedematous dermis. The oedema was more prominent in the upper dermis and was associated with dilated vascular channels. Neutrophilic infiltrate was a consistent finding in 9 biopsies and vasculitis in 8. Plasma cells were present in 5 and panniculitis was noticed only in 1 biopsy.

Acid-fast stain revealed predominantly beaded and granular bacilli in the macrophages, nerves, smooth muscle and in the sub epidermal zone. Bacilli were also seen in the endothelial cells in 2 biopsies and in the wall or lumen of the blood vessels in 2 biopsies.

In the remaining 11 biopsies although the patient was clinically diagnosed as LL in ENL the histology did