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PREVENTING NERVE DAMAGE - A STRATEGIC REVIEW Smith W.C.S.

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Introduction : Nerve damage, and the consequences of nerve damage, sets leprosy apart from other diseases. The irreversible motor and sensory impairments caused by leprosy lead to increasing secondary impairments long after the disease process has been arrested. Interventions that prevent, reverse or limit the impairments due to leprosy are therefore of the greatest priority. This review addresses the question of priorities for both research and service provision given limited resources.

Methods : The current research efforts are reviewed in terms of their likelihood of delivering significant health gain in the short and long term. Interventions to prevent nerve damage are reviewed in terms of the effectiveness, feasibility in primary care settings and cost.

Results : The majority of nerve damage occurs prior to diagnosis and efforts to improve early diagnosis and treatment have great potential for preventing nerve damage. Prophylaxis using steroids may be cost effective when targeted to groups at highest risk. Simple means of identifying those at high risk are important. Treatment of acute episodes of nerve damage and reactions is less than satisfactory since not all cases present for treatment and treatment is not always successful. Self-care has been demonstrated to be an effective means of preventing secondary tissue damage but now needs to be developed for implementation within basic health care. Although the benefit may be limited, it may, along with re-constructive surgery, be one of the few approaches open for those who have completed MDT.

Conclusions : Early case detection and MDT treatment is the most cost effective means of preventing nerve damage and the size of the potential benefit is large - this is a priority for research and interventions. Treatment of acute reactions remains a challenge while steroid prophylaxis, if effective, may offer considerable benefit when targeted to those most at risk. Self care has been shown to be effective and may, along with re-constructive surgery, be one of the few approaches available for those who have completed MDT.

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CAN RISK FACTORS IN LEPROSY BE IDENTIFIED BEFORE THEY BECOME OPERATIVE?

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Risk factors in leprosy are defined as factors responsible for reversible and irreversible nerve function impairment (NFI) leading to disability and deformity. 87 patients were inducted into a double blind clinical trial of prednisolone therapy in Type 1 reactions. It was observed that some patients developed recurrence of reactions and hence required an additional course of steroids. Inflation observed in skin lesions may indicate simultaneous sub-clinical inflammation in the nerves endangering their functional integrity. In addition, episodes of nerve inflammation can occur without skin manifestations. Identifying these phenomena through other parameters can help to selectively give higher dose or longer duration of steroids in this special group of patients. In this study, patients needing additional steroids were identified and compared with those not requiring additional steroids using a pre-determined clinical scoring method, scores being allotted to each known risk factor. Some of the known risk factors suggested for the development of Type 1 reaction and /or NFI include face patch, extent of disease, duration of disease, borderline type of disease, nerve involvement, history of reaction, duration of treatment and bacteriological positivity. Despite all the available information, there is as yet no simple test available to predict risk factor responsible for NFI. If risk factors are identified and recognised at the start of chemotherapy, adding a suitable dose of steroids along with MDT would prevent nerve damage/protect the nerve. We describe here a simple and reliable clinical scoring method to estimate the risk of development of NFI before starting chemotherapy. In this paper we recommend the use of a clinical scoring model to predict early NFI which can be used to assign patients into high risk group and plan active intervention and follow up.

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Im 104

MALARIA, LEPROSY AND MULTI-DRUG THERAPY CONTAINING DAILY 100 MG DAPSONE

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Sulphonamides and sulphones are known to have antimalarial activity. To test this, we carried out serological as well as parasitaemia assessments on 322 lepromatous leprosy patients receiving 100 mg. dapsone daily and 669 healthy subjects, living in three different districts of India, endemic for leprosy as well as malaria. Blood smears fiom 124 lepromatous patients with fever and 379 afebrile control subjects showed that the prevalence of malaria parasitaemia in both the groups was similar and varied from 25% to 30%.

However, the lepromatous patients had only P. vivax malaria; on the other hand normal controls had both P. vivax and P.falciparum infections. The ratio of the two plasmodia species in them was 9:1.

Of the 201 afebrile healthy controls, none showed parasitaemia, but 2 of the 40 afebrile lepromatous patients showed P. vivax in their blood smears. This perhaps indicated a balance between parasite survival and host immunity. Humoral antibody response was studied by IFA test in 158 afebrile lepromatous patients and 89 healthy subjects which showed humoral immune response in 19% leprosy patients and 3% control subjects. This difference might be explained by the overactive Th-2 lymphocytes in lepromatous leprosy patients with consequent anti-malaria antibody production following repeated infections.

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Im 122

SOLUBLE ICAM I AND sVCAM I IN SERA OF LEPROSY PATIENTS WITH REACTIONS

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Expression of costimulatory molecules on antigen presenting cells is one of the essential signals for activation of T lymphocytes and ultimately for an effective immune response. Soluble form of these costimulatory or adhesion molecules are released upon cytokine stimulation and can be detected in the circulation. Soluble ICAM I is reported during inflammatory conditions such as in bronchial asthma and elevated in rheumatoid arthritis(RA) compared to healthy controls. Though a few reports are there regarding sICAM I in leprosy, not much is known about soluble adhesion molecules in leprosy reactions. Reactions are clinical episodes associated with tissue damage (reversal reactions)and with systemic involvement (erythema nodosum leprosum). Since reactions further complicate the disease process and about 30 % of leprosy patients suffer from these complications we have attempted to understand the immunological phenomenon prevailing during reactions. Antibody response (total IgG and IgG subclasses) and proliferative response to different antigens of M leprae (MLSA, PGL, LAM, ML 65kDa, ML 28kDa and ML 18kDa) were analysed earlier.In this study we have estimated the soluble form of ICAM I in sera of 25 leprosy patients with ENL reactions,14 patients with reversal reactions,14 patients without reactions (4 TT/BT,10 BL/LL) and 6 healthy individuals.Soluble VCAM I molecule was estimated in sera of 35 ENL, 7 RR, 9 patients without reactions (7 BL/LL,2 TT/BT) and 6 healthy individuals. The quantitative sandwich ELISA was employed for estimation of these molecules.Soluble ICAM I is found to be significantly elevated in reversal reactional patients compared to healthy individuals. Soluble VCAM I is significantly in higher level in ENL patients in comparison to reversal reactional patients.

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Im 137

DETECTION OF DISEASE RELATED IMMUNE COMPLEXES IN THE SERUM OF LEPROSY PATIENTS

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Mycobacterium leprae antigen and antibody complexes could be detected in the serum of leprosy patients using monoclonal antibody ML-34 and anti-BCG antibodies by enzyme linked immunosorbent assay. This simplified system detects disease related complexes without the need for isolating and purifying them from the serum. Immune complexes captured using monoclonal antibody ML34 revealed positivity in 7 out of 8 neuritic, 2 out of 9 tuberculoid (TT), 5 out of 10 borderline tuberculoid (BT), 4 out of 10 borderline lepromatous(BL), and 4 out of 10 lepromatous (LL) leprosy cases. One of the controls also showed immune complex of an IgM type. Anti-BCG based IgG immune complex assay revealed positivity in 6 out of 8 neuritic, 1 out of 9 TT, 4 out of 10 BT, 2 out of 10 BL, 4 out of 10 LL leprosy cases and 2 out of 24 healthy controls. IgM type of Mycobacterial immune complexes were almost negligible. Capture of complexes using monoclonal antibody ML34 which is against lipoarabinomannan of M.leprae seems to work better than polyclonal anti-BCG antibody. The above test would be useful in Immunodiagnosis of neuritic leprosy and also in cases where antibody response is not detectable because of the formation of immune complexes.

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Im 196

LOCAL IMMUNITY IN LEPROSY - A COMPLEX PROFILE

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Several specific tests were developed for the diagnosis of leprosy. Out of all these tests PGL-I antibody and 35-kD antibody based assays were found to be most

useful in diagnosing leprosy cases. Using these assays about 90-100 percent cases of lepromatous (BL/LL) leprosy and 40-60% of tuberculoid (TT/BT) cases were detectable. It was noted that about 40% of TT/BT cases did not show any antibody level in serum even against whole M.leprae indicating that there is no significant B cell stimulation for antibody production against M.leprae. Therefore in the present study, an attempt has been made to understand the local immune response in the lesions of TT/BT cases. It was noted from oragnotypic cultures of skin lesions that locally many of the TT/BT lesions secrete antibody against M.leprae. Further, it was noted that these lesions secrete both IFN-y and IL-4 at the same level indicating both Thl and Th2 type of response in the same lesion. Further study using phenotypic markers indicated that in addition to CD4+, CD8+ T cells and macrophages many of the TT/BT lesions show the presence of B cells/ plasma cells which are responsible for a local antibody response. The present findings strongly suggest that Th2 response in these lesions is probably playing a significant role in dampening the Thl response thus allowing the lesion to linger for a long time in the skin.

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

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Type I Reaction (T1R) occurs in 30% of borderline leprosy patients and is a major cause of nerve damage which leads to disability and deformity. Although T1R reactions usually improve with steroid treatments, the biological basis of T1R is poorly understood. We have measured leprosy specific antigen-induced levels of IFN- , TNF- and IL-10 in a 24 hour whole blood assay, in TIR patients before, during and after prednisone treatment.

The levels of these cytokines were significantly increased when compared with age, sex and class matched borderline leprosy patients without T1R. Steroid treatment lowered levels of the IFN- , but levels of TNF- increased as the doses of steroids were lowered. IL-10 levels increased during steroid therapy. High TNF- levels in untreated patients (higher than 75th percentile, > 9400 pg/ml) was associated with a 5 times greater risk of reactivation of symptoms during treatment phase. High levels of TNF- , after 2-4 weeks of steroid treatment were associated with a three to five times greater risk of nerve function, impairment or failure to improve nerve function. The same patients were also at 3 times greater risk of another T1R episode within 2 months of completing treatment This study for the first time links inflammatory cytokine levels with nerve function impairment in T1R and offers a means to identify patients failing to respond adequately to steroid therapy.

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WEB SITE : LEPROSY RESEARCH SUPPORT : NIH, NIAID CONTRACT NO.1 AI-55262 AT COLORADO STATE UNIVERSITY

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We have recently developed a WEB SITE for our National Institute of Allergy and Infectious Diseases (National Institutes of Health)-supported Leprosy Contract with the following address:

http://www.cvmbs.colostate.edu/microbiology/leprosy/

The WEB SITE is divided into seven sections : Global Leprosy; Materials Available; Skin Testing; Request for Research Materials; Material Transfer Agreement; Contacts and Lab Personnel; and Links. Under Global Leprosy, the history of this NIH Contract, since its inception in 1978, is traced and its contributions to leprosy research are delineated. There are also segments on leprosy as it was, as it is today, and the continuing need for leprosy research and central resources. The Materials Available are listed and described, including whole

M. leprae, its fractions, individual proteins (native and recombinant), carbohydrates, lipids, including PGL-I and synthetic surrogates, monoclonal and polyclonal antibodies, etc. This section is supported by an experimental description of the products.

Under Skin Test Initiative, we describe the preparation of two new skin-test antigens under prescribed conditions. The composition, quality control, and efficacy in guinea pigs of these products are demonstrated with attractive graphics. The button used is Skin Testing which opens with a depiction of a Himalayan background in Nepal where the Phase II Human Study is being conducted at Anandaban Leprosy Hospital. The results of the Phase I trial on human volunteers in Fort Collins are summarized, and results from Phase II will be posted when completed.

The WEB SITE has a Leprosy Research Materials Order Form and a copy of the Biological Material Trans-

fer Agreement which must be duly completed and signed by any new investigator and a representative of her/his institute prior to the shipping of requested materials. This is a very attractive, user-friendly, and professional WEB SITE with illustrations and photographs, such that you have full information on products and the people helping you. We suggest that you Bookmark the SITE, so that you do not have to type the address each time.

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Im 382

IMMUNISATIONAGAINST MYCOBACTERIUM LEPRAE INFECTIONWITH DNAVACCINES

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The continuing detection of new leprosy cases indicates that transmission of M.leprae will continue to occur for some time. A combination of effective anti-microbial therapy of active cases and immunisation in endemic regions may be the most effective long term control measures leading to eradication of leprosy. Immunisation with M.bovis (BCG) is partially effective against M.leprae infection, however there is considerable interest in developing new subunit vaccines against tuberculosis, including DNA vaccines. Therefore we have investigated the effects of DNA plasmids as vaccines against leprosy infection. Initially we selected the immunodominant 35 kDa protein of M.leprae as the candidate antigen, as it is recognised by more than 90% of leprosy patients, with tuberculoid patients mounting a strong T cell response and lepromatous patients an antibody response. The gene for the 35 kDa protein is present in

M.leprae and M.avium, but not in members of the M.tuberculosis complex. DNA vaccine expressing the M.avium 35 kDa protein protected inbred mice against virulent M.avium infection, with greater efficacy than BCG at 4 weeks post-challenge. We then established that DNA expressing the M.leprae 35 kDa (DNA-ML35) stimulated specific IFN- T cell and antibody responses in outbred Swiss Albino mice. When challenged with viable M. leprae in the footpad, DNA-ML35-immunised mice showed significant reduction in mycobacterial replication, with equivalent protection to that induced by BCG immunisation. Mice immunised with DNA expressing the dominant M. tuberculosis Antigen 85B, which induces significant protection against murine pulmonary tuberculosis, also showed reduced growth of M.leprae. We are currently investigating ways of enhancing the protective effect of DNA vaccines against M.leprae infection. These results suggest that effective subunit vaccines can be developed against leprosy infection.

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CORRELATION BETWEEN CO-STIMULATORY MOLECULES AND T-CELL STIMULATION IN ANERZISED LYMPHOCYTES OF LEPROSY PATIENTS

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The causative agent of leprosy, M.leprae resides and multiplies within the macrophages and hence escapes the host immunity. Cell mediated immunity (CMI) plays a vital role as evidenced by BT/IT patients having high CMI and strikingly low CMI in BL/LL patients. CMI in leprosy is an enigma because it acts like a double edged sword by protecting and limiting the bacillary growth on one side and on the other hand leading to severe pathology, if turned on improperly.

Most of the invitro proliferative responses to our knowledge were done by presenting the antigen in soluble form. We believe that M.leprae reactive T cells do exist in leprosy patients but at a low frequency which can be stimulated under appropriate conditions. Hence in the present study, we attempted to restore the CMI in the anerzised T cells of lepromatous lerposy patients through immuno-manipulation thereby presenting the antigen in particulate form and combinations Ag, Ag+MDP, Ag+MDP+Trat encapuslated in liposomes or delivered in medium were studied. PBMC`s of normal, BT/TT and BL/LL were stimulated either with antigen alone or in combination of the above formulations in liposomes or in medium Ag+MDP, Ag+MDP+Trat in liposomes showed very high stimulation index compared to antigen delivered in soluble form. The results were more or less similar with all the five antigens. The culture suprenatants of the above assays collected on the 5th day were studied to measure IFN- II-2, IL-4 & IL-10 levels for assessing the CD4+, Th1 and Th2 dichotomy. IFN- (2000-9000 pg/ml) and IL-2 (40-200 pg/ml) were siginificantly high in liposomal formulations as compared to the antigen delivered in soluble form with BT/TT having highest levels fol-

lowed by BL/LL and normal individuals. Since IL-2 m RNA has short half life, we have measured IL-2 levels at different time points viz. 24 hrs, 48 hrs, 72 hrs and 120 hrs. Peak levels were observed after 24 hrs of stimulations. IL-4 (30-50 pg/ ml) and IL-10 (200-700 pg/ml) were significantly lower confirming a shift from Th2 to Th1 kind of response by this approach. There was no significant difference between the liposomal and the soluble forms as far as these two cytokines are concerned. BL/LL had slightly higher values than BT/LL folowed by normal individuals.

The study highlights the reversal of anerzid T lymphocytes to through modifying antigen presentation using some potential T cell adjuvants. Direct correlation between T cell proliferation response and lymphokine production vis a vis the role of costimulatory molecules on APC and T cells will be discussed among these patients of specturm of the disease.

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Im 71

ENHANCEMENT OF CMI IN LEPROMATOUS PATIENTS ON MDT RECEIVING HERBAL MEDICINES

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In recent years successful use of herbal medicines to treat well known diseases has been demonstrated by many workers. There is report from Chennai by Prof.Deivanayagam et al demonstrating improvement of CMI status of HIV / AIDS patients following treatment with certain herbal medicines, Therefore a drug trial in lepramatous leprosy patients using known CMI potentiating herbal medicines supplementing fixed duration multidrug therapy for a period of 12 months was conducted, The morning dose of herbal drugs will contain 1) Tinospora cardifolia 2) Terminobia chebula 3) Emblico offlcinosis. The evening dose will contain

4) Whitonia sominifera 5) Spirulina - an algae 6) Asperagus. Ten untreated patients having a bacterial index over 3+ were selected, Of them 5 were randomly allotted to a group which in addition to MDT received immunopotentiating herbal medicines for a period of 6 months, The other group receiving only MDT served as controls, An initial clinical examination, skin biopsy, skin smears and lepromin tests were done in addition to the routine blood tests before starting treatment. The tests will be repeated every 3 months until the completion of treatment at 12 months, all of them will be carefully followed for reactions, neuritis and other complications, The results of the study will be presented and discussed.

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IMMUNOLOGICAL PROFILE OF TREATED LONG SMEAR NEGATIVE LEPROMATOUS LEPROSY PATIENTS

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Lepromatous leprosy patients show suppressed cell mediated immune response (CMI) to

M.leprae whereas their humoral response is rather elevated. After clearance of bacilli following treatment upregulation of antigen specific CMI is expected. Therefore we have undertaken this study to assess the immune response of lepromatous leprosy patients who have become smear negative following treatment and have remained negative over period of time.CMI response by lymphocyte transformation test (LTT), lepromin delayed type hypersensitivity response (DTH), antibody response to 35kD antigen by serum antibody competition test (SACT) and PGL-I of M.leprae by ELISA, IgG subclasses to M.leprae and IFN , IL-2, IL-4, IL-10, IL-12 and TGF production in cell culture supernatant in response to M.leprae were studied in treated smear negative lepromatous patients and active lepromatous patients undergoing treatment. All the patients were negative to lepromin skin reaction. No difference was seen in the antibody response to 35 kD antigen among treated and active patients. Treated patients showed significantly lower IgM response to PGL-1 than active patients ($p< 0.02$). No difference in the IgG1, IgG2 and IgG4 subclass response to M.leprae was seen however IgG3 was significantly lower in treated lepromatous group than active lepromatous group. 42% of treated patients produced IFN and 31% produced IL-2, whereas none of the active patients produced IFN and 11% of them produced IL-2. On the contrary less number of treated patients produced IL-IO than active patients however there was no significant difference ($p > 0.01$). 42% of active and 50% of treated patients produced IL-12. 5% of treated patients produced IL-4 whereas 28.7% of active patients produced this cytokine. TGF production was seen in all the active patients and 77% of treated patients. This study shows that in some of the treated patients there is a tendency of upregulation of CMI.

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Im 132

IMMUNOHISTOLOGY OF THE MACULAR LESIONS OF MONOLESIONAL LEPROSY

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Patients presenting with flat, defined single lesions and, with no history of prior therapy were chosen for the study. Clinical details were recorded, skin smear for AFB done, and the lesions biopsied for routine histopathological analysis and immunostaining. Immunostaining to detect mycobacterial antigens was performed on sections wherein routine histopathology Hematoxylin and Eosin and Fite-Faraco staining of paraffin embedded sections was either non-confirmatory or equivocal.

Thirty eight subjects were chosen for the study. The cases were predominantly male adults and clustered into two main age groups of 20-28 years and 40-45 years. Fourteen (36-.84%) had contiguous nerve thickening. Only half the patients took treatment for 6 months or more; amongst them, 3 subsided, 10 had regressive lesions, and 2 developed new lesions.

On routine histopathologic examination, leprosy could be diagnosed in 23 of the 38 cases (52.63%); AFB positivity contributed to the diagnosis in 4 cases. Two cases showed features of multibacillary leprosy. On immunostaining of sections where the histological diagnosis of leprosy could not be made, mycobacterial antigenic presence was observed in 6. The overall diagnosis of leprosy in this group of patients was 68.42%.

The implications of these findings vis-a-vis single dose and short course chemotherapeutic regimens will be presented.

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Im 330

T CELL RECOGNITION OF PEPTIDES FROM M.LEPRAE 35 KDA PROTEIN IN THAI LEPROSY PATIENTS, HEALTHY CONTACTS AND NON-CONTACTS

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The objective of the study was to analyze human peripheral blood mononuclear cell (PBMC) responses to peptides from M.leprae 35 kDa protein in order to identify M.leprae-specific peptides for development of skin test reagents. The M.leprae 35 kDa protein was the antigen of interest since it has no homologue in the M.tuberculosis complex. However, there are some homologues in M.avium. The subjects enrolled in this study were either new or treated paucibacillary (PB) and multibacillary (MB) leprosy patients, healthy contacts and non-contacts. Seventy three PB and one hundred and twenty four MB leprosy patients were recruited from four leprosy clinics in Thailand. Fifty seven healthy contacts were both household and medical personnel. Fourteen non-contacts had no family history or exposure of leprosy. The peripheral blood mononuclear cells from individual subjects were tested with 12 overlapping peptides from M.leprae 35 kDa protein by lymphocyte proliferation assays.

These peptides were focalized in four areas containing residues which are distinct between

M.leprae and M.avium. The result showed that response frequencies to each peptide did not differ obviously among PB, MB patients and contact groups. However, the most stimulatory peptides from each region, recognized by 30-40% of subjects from each group, were identified. Interestingly, 3 out of 4 peptides were not recognized by any of non-contacts. Thus from this preliminary result, these 3 peptides were likely to be M.leprae-specific. However, the authors need to prove their M.leprae-specificity by developing human T cell clones and testing with their M.avium homologues. Since the frequencies of recognition to individual peptides were not high, the combination of several peptides were suggested for skin test reagents in order to increase sensitivity.

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Im 347

SEROLOGICAL DIAGNOSIS IN ACTIVE LEPROTIC AND TREATED PATIENTS

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Antibodies against M.leprae antigens have been demonstrated in sera of leprosy patients.The general view is that they occur in large amounts in sera of lepromatous leprosy patients and their frequency and activity are lower towards the tuberculoid leprosy. Many serological tests were developed to evaluate the effect of antileprotic chemotherapy, and the antigen load in the patients proved that the level of phosphoglycolipids-1 (PGL-1) in the serum might be the most reliable parameter to monitor early responses of leprosy patients to chemotherpy.

Assessment and evaluation of PGL-1 antibodies in the sera of leprosy patients were done by indirect ELISA using semi-synthetic PGL1 (ND-O-BSA) antigen.

Circulating antibodies appear to be directly dependent upon the clinical status of the patients. Circulating antibodies in LL, BL, BT, TL untreated and treated patients are estimated. The work and the results are in progress and will be statistically evaluated and presented.

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Im 364

EXPRESSION OF CC AND CXC CHEMOKINES IN LEPROSY SKIN LESIONS

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We have investigated chemokine expression in leprosy skin lesions using immunohistochemistry to detect protein production and in-situ hybridisation to detect mRNA expression in skin. A panel comprising both CC chemokines (MCP-I, RANTES and MIP-1) and CXC chemokines (IP10, MIG and IL-8) were studied. Skin biopsies from 25 leprosy patients across the leprosy spectrum, 11 patients undergoing Type 1 reversal reactions and 4 normal donors were immunostained.

Each chemokine was expressed in the majority of reactional and non- reactional skin biopsies assayed. Levels of chemokine expression did not differ between tuberculoid and lepromatous leprosy lesions. The CXC chemokines interferon gamma inducible protein-10 (IP-10) and monokine induced by interferon gamma (MIG) were expressed strongly in all lesions. The CC chemokines monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1) were expressed moderately. The CC chemokines regulated upon activation normal T cell expressed and secreted (RANTES) and macrophage inflammatory protein-1 beta (MIP-1) and the CXC chemokine interleukin 8 (IL-8) were expressed weakly in leprosy lesions. MIP-1 and IL-8 were expressed more strongly in reactional lesions than non-reactional lesions.

These results suggest that the chemokines investigated, which are known to chemoattract T lymphocytes and macrophages, are involved in assembling the cellular infiltrate found in lesions across the leprosy spectrum.

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Im 379

THE PROTEOME OF M.LAPRAE IN CONTEXT OF NEW SKIN TEST ANTIGENS

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Progress in the development of new skin test antigens is reported separately. We are also now applying dual proteomic-immunological approaches to developing reagents with greater specificity. One approach to the dissection of the cell-mediated immune response of a complex mixture of proteins is the electroelution of size fractionated proteins from preparative SDS-PAGE gels followed by protein characterization and immunological assessment. We have used this technology to produce size-fractionated cytosolic proteins from M. leprae. The individual fractions were probed with polyclonal and monoclonal antibody reagents by ELISA and 2D-PAGE Western blot to identify known proteins (or through elimination to identify novel proteins), reinforced by N-terminal sequencing and mass spectroscopic analysis. In addition, immunological responses (both antibody mediated and DTH responses) were assessed in M.leprae-, BCG-, and

M.tuberculosis- sensitized guinea pigs. Virtually all of the fractions gave sizable DTH responses in the M.leprae-sensitized guinea pigs, although the fractions containing proteins of molecular weight higher than 25 kDa produced higher responses. All fractions produced significant and lesser DTH responses in BCG-sensitized guinea pigs. To determine what specific proteins were dominant in each of the fractions, each was analyzed by ELISA and 2D isoelectric focusing gel electrophoresis. Immunogenic proteins recognized were: MMP-I; MMP-II; MCP-I/GroES; 65 kDa/GroEL-I and fragments; GroEL-2; DNAk/70 kDa; MtrA; Sod A; Ahp C; Hsp 18; L7/L12; EF-Tu; and 3 new proteins: electron transfer FixA flavoprotein subunit; RNA polymerase chain; and one with a novel protein sequence. The work to date indicates that the search for a single highly specific, highly immunogenic protein for leprosy diagnosis is probably futile; all proteins are highly immunogenic and cross-specific. Rather the current approach of using highly purified, highly immunogenic protein fractions with a differentially greater DTH response in sensitized guinea pigs, followed by testing in human populations, is the most promising approach.

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