Inability of M. leprae to grow in culture medium in vitro has been a bottleneck in leprosy research. We had reported earlier about the limited growth of M. leprae in DH medium and our inability to achieve subcultures. One reason for this is the accumulation of oxygen radicals in the growth medium and the other is existence of a possible growth factor.

In our study, normal growth of M. leprae in DH medium was obtained when inocula were from livers and spleens of infected armadillos. However, M. leprae harvested from the foot pads of nude mice failed to multiply in the same medium. Even when the inocula were from armadillo lymph nodes or from human biopsy, the growth was much slower. Furthermore, using inocula from livers and spleens of armadillos, gradual decrease in inoculum size resulted in proportionally slower multiplication.

When the DH medium was supplemented with irradiated M. leprae from livers and spleens of armadillos, nude mouse-derived M. leprae exhibited growth in DH medium similar to that obtained with armadillo-derived M. leprae. Similar results were also obtained with cell-free extracts of non-irradiated M. leprae. All these findings point to the possibility of the existence of a growth factor in armadillo-derived M. leprae.

MI50

ANERGY AND MITSUDA RESPONSES TOWARDS CHEMOAUTOTROPHIC NOCARDIOFORM ANTIGENS RUN PARALLEL TO LEPROMIN ACROSS THE LEPROSY SPECTRUM

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Chemoautotrophic nocardioform (CAN) bacteria had been repeatedly isolated from infectious human, mouse-footpad and armadillo leprosy tissues which had been found to share similar/same metabolic, physiological, enzymological, 36K DNA, lipid profile, pathogenicity and other specificities with Mycobacterium leprae. For further studies on their homology with leprosy bacillus, anergy or contrarily, Mitsuda-type responses towards 4 of these CAN - AGs and a control lepromin were tested on 93 LL, TT and borderline cases of leprosy, categorised clinically and bacteriologically. Final results were obtained for 73 cases. The antigens injected per patient varied from a maximum of 5 to a minimum of 2. The suitability standard of the control lepromin was verified first in 4 TT cases where it produced nodules (+++) ⩾10 mm diameter. Complete anergy to CAN - AGs was seen in 92/92 instances tested on 24 LL cases, while the anergy was weakly modified or unmodified in 3 other LL cases which had been vaccinated before. Concurrent studies with the same antigens tested on 33 TT cases showed clearcut, dose-dependent, Mitsuda-type late responses in 80/81 instances which included 3 cases where the control lepromin was omitted. The CAN bacteria, therefore, despite their origin from different unrelated human, mouse footpad and armadillo tissues appeared to be identical with each other and also with the leprosy bacillus, on the basis of these and other parameters. Phenolic glycolipid-I could be demonstrated among these.

OPHTHALMOLOGY

OP1

A LONGITUDINAL FOLLOW-UP STUDY OF EYE IN 649 LEPROSY PATIENTS

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Periodic examination of eyes were done for leprosy patients attending this centre. The condition of eye in 649 patients for a period ranging from 3 to 20 years (mean 8.29) are discussed. Throughout this period, 458 (70.79%) eyes were normal while the rest 191 (29.21%) were affected and majority recovered with treatment. In tuberculoid and borderline patients, lagophthalmos was the only complication. In lepromatous patients, who had monotherapy, except for fleeting scleritis/iridocyclitis no complications occurred. In lepromatous patients of short duration on MDT, complications were few and subsided with treatment. In lepromatous patients of long duration and in M.B. relapses on MDT, eye complications were more and in some scleritis/iridocyclitis lasted for 4-6 years. Even in those who were normal at the beginning of treatment, some developed scleritis/iridocyclitis after 3-5 years. Blindness was mostly due to non-leprosy causes like cataract and corneal ulcer. Lagophthalmos and corneal hyposthesia were the only causes of blindness in tuberculoid and borderline cases. In lepromatous patients on treatment, blindness occurred only in those with severe pre-existing lesions. Steroid induced cataract led to blindness in a few. Early detection of disease, management of reactions and periodic eye examination prevent eye complications.

OP2

CONSENSUAL OPHTALMOTONIC REACTION IN LEPROSY PATIENTS

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The cosensual ophtalmotonic reaction (TOR) describes the phenomenon whereby alterations of the intraocular pressure in one eye is accompanied by a corresponding pressure change in the contralateral eye. It has been postulated that the COR is mediated via a nervous reflex mechanism. In this study the COR was determined in normals and in the leprosy patients with and without ocular involvement. The theory and the potential usage of the COR in the eye clinics will be discussed.

OP3

OCULAR PROBLEMS IN CASES RELEASED FROM TREATMENT

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Successful implementation of National Leprosy Eradication Programme through proper Anti-leprosy Treatment by MDT and Monotherapy has given rise to a large number of RFI cases in India. This study deals with the remaining ocular lesions in such RFT cases. This series of 6,000 RFT cases (and equal number of PB & MB) shows the involvement of eye in 52% of cases. 70% of them had ocular problems arising directly from the disease itself and the rest developed these as a coincidental phenomenon or ageing process. This study will critically high light the WHO declaration of a disease free state after completion of therapy, though the sufferers have got specific blinding lesions or high risk eyes resulting from leprosy.

OP4

OCULAR LEPROSY IN PATIENTS WITH FACIAL PATCHES

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Facial nerve is mostly involved in leprosy and gives rise to a number of complications following peresis or paralysis of orbicularis oculi with resultant lagophthalmos. Many of the patches over the face (Primary or following Type-1 reaction) are found to be the predisposing factors to develop facial nerve damage or the involvement of the trigeminal nerve- with resultant ocular lesions or keeping the eyes at risk. This is a study of 300 cases (both PB & MB) with facial patches in search of the co-existence of ocular leprosy. The pattern of ocular leprosy is discussed in relation to the different location of the patch on the face e.g. a. specific around the globe, b. on the parotid region over the facial nerve trunk and c. other non-specific areas of the face. About 30% of the patients with specific patch around the globe were found to have ocular leprosy without severe visual disability.

OP5

A CASE ANALYSIS OF THE PATTERN OF CATARACT AND POST OPERATIVE OUTCOME OF CATARACT EXTRACTION IN LEPROSY PATIENTS AS COMPARED TO NON-LEPROSY PATIENTS.

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The ocular status of one hundred leprosy patients and one hundred non-leprosy controls coming for cataract extraction in ALERT is assessed. The visual acuity, the intraocular pressure and the state of the anterior segment will be studied. Each group will be stratified by age, sex and type of leprosy.

All patients will undergo cataract extraction and their post operative visual acuity, intraocular pressure and state of the anterior segment will be assessed and compared. Level of inflammation will be assessed by assessing the anterior segment reaction /fare and cells/. The intra operative and post operative complications will also be assessed. This will be done at discharge and one month after discharge.

Cataract extraction will be done using a cryoprobe under retro bulbar anaesthesia. Visual acuity will be taken using a Snellen's chart. Intraocular pressure will be assessed using applanation tonometry. Assessment of ocular structures will be done by a slit-lamp biomicroscope. Post operative visual acuity will be taken after correction with a +10 sphere.

In the leprosy group classification will be made into the type of leprosy /Ridley Jopling/. The activity of the leprosy will be assessed by studying the Bacteriological and Morphological indices.

OP6

RISK FACTORS FOR CATARACT IN LEPROSY PATIENTS: RESULTS FROM A CROSS-SECTIONAL SURVEY IN THE P.R. OF CHINA

Susan Lewallen, Huan-Ying Li, Lu-Fang Hu, Paul Courtright

International Centre for Eye Health, London UK

Cataract has been shown to be associated with uveitis in leprosy but no study has controlled for the effects of age or other potential risk factors to determine the principal factors associated with cataract in leprosy.

We used data from the Liangshan Leprosy Eye Survey to assess the contribution of risk factors to cataract. Among 974 patients in the survey 69 (7.1%) had monocular or binocular sight impairing cataract. Univariate analysis showed that cataract was associated with a number of other clinical eye findings (lagophthalmos, corneal surface abnormalities, corneal hypesthesia, chronic uveitis), demographic characteristics (age), and leprosy-related clinical findings (other deformities, age at diagnosis, MDT status and history of dapsone monotherapy). Using logistic regression modelling we found that chronic uveitis, age, and history of dapsone monotherapy were the factors independently associated with cataract. Independent of age, about 45% of cataracts can be attributed to chronic uveitis. Implications of these findings will be presented.

OP7

THE CONTRIBUTION OF MDT TO THE PREVENTION OF EYE DISEASE IN LEPROSY: RESULTS FROM A CROSS-SECTIONAL SURVEY IN THE P.R. OF CHINA

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The impact of MDT on the development of eye disease in leprosy remains unclear. We sought to assess the contribution of MDT on the prevention of eye disease and illustrate the hazards of pooling data from newly-diagnosed leprosy patients and patients who had a prior history of dapsone monotherapy. We used data from the Liangshan Leprosy Eye Survey to compare eye findings and other clinical characteristics in newly diagnosed MDT patients (n=292) and MDT patients on prior dapsone monotherapy (n=682).

Univariate analysis showed that newly diagnosed MDT patients were more likely to be Han Chinese, be of a younger age, be of MB disease type, have a shorter duration between onset and leprosy diagnosis, and to have a history of both reversal and ENL reactions than MDT patients with a prior history of dapsone monotherapy. Leprosy-related eye disease was recorded in 10.3% of newly diagnosed MDT patients and 23.0% of MDT patients with a prior history of dapsone monotherapy. The prevalence of eye disease remained constant in all MDT-start years (1986 through 1991), suggesting that most eye pathology found in these patients was probably present at diagnosis. Among the monotherapy/MDT patients eye pathology was most strongly associated with years on monotherapy prior to MDT. Implications of these findings will be presented.

OP8

EARLY DIAGNOSIS IRITIS IN LEPROSY PATIENTS

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Because of the redused or lack of sensations the diagnosis of iritis is very late in Leprosy patients. Quiet often this even reaches a stage where there are thick posterior synichae and complicated cataract. So the general physicians non-medical assistants should be able to diagnose at earliest to prevent complications.

Some guidelines for examination and detection of the disease will be discussed. This paper will be presented with help of slides

OP9

PUPIL CYCLE TIME IN LEPROSY

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Pupil cycle time (PCT) is an easily performed test which times the pupillary constriction and dilation stimulated by a beam of light at the pupillary edge with a slit-lamp. PCT is said to be stable in various testing conditions, repeatable to a high degree and considered to be a sensitive measure of the dysfunction of the parasympathetic efferent limb of the pupillary light reflex. Prolongation of PCT has been reported in various diseases, inferring ocular autonomic dysfunction.

Karacorlu and his colleagues reported prolongation of PCT in leprosy. They suggested that this could be an indicator of early ocular involvement, and might predict future uveitic reactions. However, the number of patients in their study was small, and many of them had pre-existing ocular complications.

We therefore recorded PCT in 361 unselected consecutive leprosy patients who had no visible pathology of the anterior and posterior segments and 173 healthy controls. The mean PCT of leprosy patients and that of healthy controls was well below those recorded by other investigators, denoting likely ethnic variation. Findings relating PCT to age, sex, type of leprosy, duration of the disease, occurrence of type I and type II reactions and the smear status of the patients will be presented and discussed.

OP10

INTRAOCULAR PRESSURE IN LEPROSY PATIENTS WITHOUT APPARENT ANTERIOR SEGMENT PATHOLOGY

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A widely prevalent notion is that intraocular pressures are generally lower in leprosy patients than in normal individuals.

Applanation intraocular pressures were recorded in 166 unselected, consecutive leprosy patients, without clinically visible anterior segment pathology, and in 111 healthy controls. The Mean(SD) intraocular pressure of the leprosy patients 13.1(2.9)mmHg was identical to that of controls 13.1(3.0)mmHg. Only 1.5% of the leprosy patient eyes had pressures of 7 mmHg or less. Correlation coefficient (r) between age, sex and intraocular pressures were not statistically significant either in leprosy patients or in the controls. No statistically significant difference in mean pressures were noted when patients were grouped according to the Ridley and Jopling classification. The duration of disease also did not alter the pressures significantly, neither did smear positivity and differing bacterial indices. Smear positive patients having the disease for more than ten years had a Mean(SD) pressure of 13.2(3.7) mmHg which was not statistically different from the pressure 13.0(2.9) mmHg of smear negative patients with the same duration of disease.

Low intraocular pressure is not that common a phenomenon in leprosy patients as is believed to be, and may not be a very useful indicator of early intraocular involvement.

OP11

VISIBLE CORNEAL NERVES IN LEPROSY

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Corneal nerve involvement is a well recognised feature in leprosy. Surveys on ocular leprosy make mention of prominent or thickened corneal nerves although these observations were likely to have been highly subjective, as no mention is made of the use of any measuring devise such as a graticule. Beading of corneal nerves, considered to be pathognomonic of ocular leprosy, has also been reported.

Corneal nerves, numbering about 70 to 80 in number, run towards the centre of the cornea from the limbus in the mid-stromal region, losing their myelin sheath within a distance of 1mm from the limbus. When viewed with a slit-lamp, they appear as thin lines which branch dichotomously. Although these nerves can be seen both in leprosy patients and in healthy individuals, the number of nerves visualized, even on careful and prolonged slit-lamp examination, varies. In order to find out whether this variation was of significance, we counted the number the visible corneal nerves, quadrant wise, in both eyes of 383 unselected, leprosy patients, who had no obvious pathology of the anterior segment, and in 213 healthy controls. Beading of nerves was also noted.

The results of the study will be presented and discussed, in relation to age, sex, type of leprosy, duration of the disease, occurrence of type I and type II reaction and smear status of the patient.

OP12

CORNEAL SENSITIVITY IN LEPROSY PATIENTS AND CONTROLS

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The corneal sensitivity (CS) of leprosy patiënts and controls has been determined. Leprosy patiënts were categorized into three groups; 1. patiënts without eye-pathology, 2. patiënts with lagophthalmos and 3. patiënts with signs of former iritis. Measurements were conducted with the corneal anaesthesiometer by Cochet & Bonnet. A correction for humidity changes during the measurements has been applied. The results showed that the CS of paucibacillary leprosy patiënts without eye-pathology is not significantly different from the control group. The CS of multibacillary patiënts, lagophthalmos patiënts and iritispatiënts

however differs significantly from the CS of both the control group and the paucibacillairy patiënts. The results support our hypothesis that a loss of corneal sensation in leprosy patiënts is mainly due to secundairy atrophy of corneal nerves or to multiple ocular pathology.

PATHOLOGY

PA1

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES OF ECCRINE SWEAT GLANDS IN LEPEOSY

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Histopathological and immunohistochemical changes of ecceine sweat glands were investigated in skin biopsies taken from four hundred leprosy cases covering the whole spectrum of the disease and including indeterminate group. The histological findings which may indicate the impairment of sweat function are 1) intraluminal retention of secretory material, 2) cystic dilation of ductal and secretory segment, 3) atrophy, vacuolation, absence and the formation of giant vacuoles in the secretory segment, 4) periglandular fibrosis of the surrounding connective tissue and 5) a decrease in the density of capillary plexus, apart from the destruction directly by inflammatory infiltration. With the immunochemical staining using antibody against neuron-specific enolase, a rich network of autonomic nerve fibers around the eccrine sweat glands could be demonstrated on paraffin embedded tissue sections. The involvement of autonomic nerve fibers was a predominant finding in all types of the disease. That the involvement was also sensitively detected in the indeterminate cases indicates that it is a hopeful approach to the diagnosis of leprosy at an early stage.

PA2

DEMONSTRATION OF PGL-I & LAM-B ANTIGENS IN PARAFFIN SECTIONS OF LEPROSY SKIN LESIONS

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An investigation on the demonstration of PGL-I and LAM-B antigens in thirty-four paraffin embedded skin biopsies taken from leprosy patients who covered the whole spectrum of the disease and in four control specimens was carried out. Neither the PGL-I antigen nor the LAM-B antigen was demonstrated in the normal skin specimens that were used as negative control; and only the LAM-B antigen appeared in the tuberculosis specimens in which the PGL-I antigen was negative. The antigens were identified as intracytoplasmic bacillary staining, in solitary, granular as well as debris patterns; and as soluble antigenic staining, in vacuolar or amorphous pattern. The PGL- I antigen was demonstrated on thirty-three samples except one TT sample and the LAM-B antigen, on all samples by the immunochemical staining technique. In addition, it is interesting to note that the immunochemical staining was able to differentiate foamy change from hydropic degeneration. We also found that the PGL-I antigen reduced after MDT treatment and increased when relapse happened while the LAM-B antigen was relatively unchanging. The results indicate that the specificity and sensitivity of the immunohistochemical staining technique used in this study are suitable for both the application of the diagnostic pathology and the research on the pathogenesis of leprosy. Particularly the immunohistochemical staining is an aid to the differentiation between reversal reaction and relapse.

PA3

IMMUNOHISTOCHEMICAL DEMONSTRATION OF PGL-I ANTIGEN IN THE SKIN AND NERVOUS SYSTEM OF LEPROSY PATIENTS

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Immunohistochemical demonstration of *M.leprae* specific phenolic glycolipid-I (PGL) antigen is important for the definite pathological diagnosis of leprosy . We could demonstrate the localization of PGL antigen as well as cross-reactive BCG antigen in formalin fixed paraffin-embedded skin, peripheral nerve and brain stem of leprosy patients.

Materials and methods: Skin biopsy of lepromatous leprosy (n=26), nervous system of clinically cured (BI- more than 10 yrs) leprosy autopsy (L: n=6, T : n=6) were immunohistochemically stained by anti-PGL monoclonal antibody and anti-BCG polyclonal antibody using ABC method.

Results and discussion: (1) PGL and BCG were clearly stained in leprosy skin biopsies. By both antibodies, solid bacilli were stained as granular pattern, and degenerated bacilli as vacuolated pattern. Even in the resorption stage and Fite's staining is negative, immunostaining remained to be positive, which indicate the efficacy of PGL immunohistochemistry for the definite diagnosis of doubtful leprosy cases using routine paraffin sections. (2) In all the autopsy cases of cured lepromatous leprosy , PGL and BCG staining was observed in sciatic nerve, dorsal root ganglia, posterior spinal roots, spinal cord (posterior horn and anterior horn neurons), medulla oblongata (mainly in ambiguus, facial, hypoglossal, cuneate and gracile nuclei), while most of the cured tuberculous leprosy were negative. These findings indicate that *M.leprae* specific antigen remains in the peripheral nerves and central motor nerves long after the clinical cure of lepromatous leprosy

PA4

TGFβ in leprosy

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Growth factors release from inflammatory cells with multiple activities such as transforming growth factor-(TGFβ) have been implicated in the progression of several inflammatory injury.This peptide is an importamt regulator of matrix formation, enhancing the syntesis of collagen, fibronectin an proteoglycans and has also been shown to be a chemoattractat for monocytes and fibroblast and has some effect as a negative immunoregulator.