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THE RESEARCH ACHIEVEMENTS OF THE CENTRAL JALMA INSTITUTE FOR LEPROSY, AGRA

The Central JALMA Institute for Leprosy (CJIL), Agra established in 1967 by the Japanese Leprosy Mission for Asia (JALMA) as the India Centre of JALMA was handed over to the Government of India which in turn entrusted the Institute to the Indian Council of Medical Research (ICMR) in 1976. During the last 24 years under the ICMR, the Institute has made a positive contribution to the understanding of leprosy.

The Institute fulfils its programme oriented objectives by carrying out basic and applied research on leprosy and other mycobacterial diseases in the following thrust areas : (i) Early diagnosis; (ii) improving and monitoring treatment; (iii) prevention of deformities; (iv) transmission of diseases; (v) field studies; (vi) operational research; and (vii) studies on other mycobacteria. The Institute has contributed significantly on various aspects pertaining to the host as well as pathogens. The research achievements of CJIL over the last 24 years are highlighted in this write-up.

Therapeutic Research

The Institute has been very active in improving the chemotherapy of leprosy by way of designing new drug regimens and modifying old regimens to make them more effective and need based.

Intermittent/Pulsed Rifampicin Therapy

Trials were carried out as early as in 1979 for the introduction of pulsed/intermittent rifampicin therapy which ultimately was found to be very effective, operationally feasible and cost-effective for the country[1,2]. These studies showed that pulsed administration of rifampicin could kill large proportions of viable leprosy bacilli. Pulsed therapy with rifampicin was ultimately adopted in the National Leprosy Eradication Programme (NLEP).

Primary Sulphone Resistance of *M.leprae*

During the pre-1980 period, there were several reports of increasing trends of sulphone resistance (both primary and secondary) from all over the world. The first report from India about primary sulphone resistance (supported by *M.leprae* growth in mouse foot- pad) was from this Institute[3]. Further, in the early 1980s initial intensive rifampicin therapy followed by dapsone alone was advocated and was being used extensively. However, it was noted that in many patients who had received introductory rifampicin therapy followed by dapsone, viable *M. leprae* were detected in the dartos muscle[4,5]. These studies highlighted the need for further changes towards effective therapy.

Drug Trials in Leprosy

The effectiveness of various drug regimens recommended by the National Programme and WHO was investigated at CJIL and effective regimens designed are evaluated to improve the therapy of paucibacillary (PB) and multibacillary (MB) leprosy.

PB leprosy

The WHO recommended multidrug therapy (MDT) for PB cases for 6 months has helped immensely in shortening the duration of treatment. However, persisting activity, reactions, and relapses have been observed in a section of these patients after treatment with short term regimens[6]. Studies were undertaken in these patients to reduce these drawbacks by designing new regimens as well as modifying the WHO regimen. On the basis of the results of these studies a recommendation was made that the treatment with dapsone be continued for a further 6 months. However, in order to shorten the treatment, the addition of another bactericidal drug like prothionamide/ clofazimine was tried in consultation with NLEP. The addition of either of the drugs was found to be effective in shortening the duration of treatment and removing the persisting activity, reaction and relapses seen in some patients. The clofazimine containing regimen is preferrable as it has the added advantage of administration of the same drugs in the field but for a different duration for PB and MB leprosy[7-11].

MB leprosy

For MB leprosy also, treatment with a slightly modified WHO regimen was suggested for highly bacillated lepromatous (BL/LL) patients[12]. This study showed that the loading dose of clofazimine was not necessary (as also shown earlier in human trials and animal experiments) and treatment had to be continued till smear negativity. Patients on the WHO fixed dose regimen (WHO FDT) as also those on the continuous WHO regimen were followed up to observe the relapse rate. More relapses were observed in patients on WHO FDT than in patients on continuous therapy[13]. These results indicated the need for continuing MDT and changing of drug regimen in patients with highly bacillated MB leprosy. More recently in consultation with NLEP, CJIL has developed a one year regimen in which the addition of newer drugs (ofloxacin and minocyline) in monthly supervised dosages to the current MDT MB regimen has been studied[14]. From the short term follow up data available, this regimen has been found to be effective in low bacillated cases. Further, in an attempt to reduce the duration of treatment in the highly bacillated cases, a study was conducted with the addition of immunotherapy (BCG and *Mw* separately) to the chemotherapy regimen[15]. BCG and *Mw* acted as immunomodulators and helped in reducing the duration of treatment by about 50% and also reduced the severity and duration of erythema nodosum leprosy (ENL) reactions in these patients. The addition of immunotherapy helped in both the faster killing of viable bacilli and the rapid clearance of dead bacilli from the host[16,17].

Surgical Research

Surgery is important in prevention of nerve damage as well as correcting the deformities that occur in leprosy patients. Although surgical intervention is considered necessary for the treatment of acute neural abscesses, in a study conducted at CJIL it was observed that the use of daily rifampicin and INH was useful in the treatment of chronic nerve abscesses[18]. The deformities of the hand and feet are corrected by employing routine and improved surgical procedures which help the NLEP in rehabilitating patients. Surgical decompression of the posterior tibial, ulnar and median nerves, transfer of the radial half of the flexor pollicis longus to extensor expansion for prevention of 'Z' deformity of the thumb during power pinch, surgery for foot drop by tibialis posterior transfer, reconstruction of the nose in leprosy patients and correction of claw fingers in patients using newer approaches have helped in making the feet and hands functional[19-27]. In addition, innovative techniques for restoration of abduction-opposition of the thumb[28], reconstruction of the web space depression in ulnar paralysis[29], treatment of plantar ulcers and ulcers of the heel[30,31] have been developed. These procedures have provided enhanced capabilities to treat deformities in leprosy patients.

The Institute with collaboration from other organisations developed a foot model and pressure monitoring system for better understanding and management of tarsal disintegration and plantar ulcers in neuropathic feet of leprosy patients. Irregular peaks of foot pressure while walking indicated disturbed transmission of body weight due to plantar anaesthesia, and paralysis of foot muscles have been noted and pressure points have been identified[32]. This information about disturbed foot pressures is being used to design footwear and surgical intervention for prevention and treatment of plantar ulcers.

Research on Immunology of Leprosy

Studies have been undertaken focussing on the antigenic structure of *Mycobacterium leprae, M. tuberculosis* and other mycobacteria. Studies have also been conducted to understand the host parasite interactions and techniques for diagnosis as well as classification of disease.

Standardisation of Dharmendra Lepromin

Lepromin testing using Dharmendra or Mitsuda lepromin has been an important investigation to determine the immune status of the patient so as to classify the patients. In initial studies at the Institute variability was observed in the skin delayed type of hypersensitivity (DTH) reaction in different batches of Dharmendra lepromin[33]. Lepromin test using Dharmendra antigen was therefore standardised for the first time by fixing 10 million bacterial cells per ml of lepromin[34]. This standardised leprosy antigen not only evokes an early (24-48 h) skin DTH reaction but also a good late (3 to 4 weeks) DTH reaction. This standardised lepromin has been tried at several centres and found to be an important reagent[35]. Later in studies to determine the role of soluble antigens in inducing DTH reaction, covalently coupled liposomised antigen was noted to evoke late DTH reaction[36].

Production of Monoclonal Antibodies and Their Use

Studies were carried out at CJIL to characterize *M.leprae* antigens by developing and testing monoclonal antibodies (Mabs)[37]. Out of the Mabs studied only two were found to be quasi specific[38]. One of these, MLO4 has been used for developing a test which is able to detect 90-100% patients with active BL/LL leprosy[37-39]. This test was initially standardized as a competitive inhibition assay using radiolabelled MLO4 which identifies an epitope on the 35 kDa protein of *M.leprae* and has been further improved as an inhibition ELISA[40]. This method has immense potential in detecting antibodies in healthy household contacts [41]. This test has been extensively tried and it was noted that the specific antibody levels correlate best with active infection and clinical improvement of the patient on chemotherapy in MB leprosy [42]. With regard to TT/BT leprosy the level of specific antibody has been found to correlate significantly with the number of lesions in these patients[43].

Studies have been further carried out to compare the assay with other techniques targeting antibodies to PGL-1. These anti PGL-1 antibodies have also been demonstrated in the urine of leprosy patients[44].

A significant contribution has been further made in the development of an antigen detection assay. For the first time using a novel sandwich immunoradiometric assay antigens in the serum[45], CSF[46] and urine[47] of leprosy patients have been identified by these Mabs and the procedure has been standardized. This system uses both the novel antigen (35 kDa) as well as other antigens like PGL-1. These studies have helped in understanding the immune status of the host against *M.leprae*.

Study on Immune Complexes and Reactions in Leprosy

Reactions are a serious problem encountered in the course and treatment of leprosy patients. Involvement of antigen-antibody complexes as well as DTH have been implicated in different types of reactions. In CJIL studies in this area attention had been focused on the immune complex (IC) mediated mechanism. For the first time it was reported by the CJIL scientists that there is a reduced solubilization capacity of complement in the sera of patients in reaction[48]. Taking this as a parameter it was possible to predict reaction in many lepromatous patients[49]. Further, an easy method for the demonstration of *M.leprae* antigens in ICs of leprosy patients was established[5o]. It was also confirmed that the circulating ICs are the activators of complement[50] which could suppress lymphokine generation[51] and *M.leprae* induced lymphoproliferation[52]. It has been further shown that the tissue damage in a leprosy lesion is induced by the generation of membrane attack complex due to activation of complement[53]. Recently it has been established that IgG_3 subclass antibody level against *M.leprae* and its antigens are significantly lowered in the blood of ENL patients[54,55] indicating their role in precipitation of ENL reactions.

M.leprae Components in Complement Activation

With a view to understand the role of complement activation in killing of *M.leprae*,studies have been undertaken, which clearly established that *M.leprae* and its components activated the alternative pathway of complement[56,57]. However, in spite of this activation *M.leprae* does not get killed [58], showing the inadequacy of this mechanism in causing damage to *M.leprae.*

Studies on Leprosy Granuloma

Extensive studies were carried out on the T cell subtypes of granuloma cells *in vivo* and *in vitro* to have a better understanding of leprosy lesions. Tuberculoid granulomas were found to contain more $CD4^+$ cells whereas lepromatous granulomas contained more $CD8^+$ cells. The CD4/CD8 ratio in tuberculoid lesion was 2:1 whereas it was reversed in lepromatous patients[59-61]. This signifies the presence of heightened CMI in tuberculoid disease and absence of CMI in lepromatous leprosy. Further, many of the *M.leprae* antigens (specific and cross-reactive) have been observed to be localized in leprosy lesions and could be involved in generating immune response/damage locally despite adequate anti-mycobacterial chemotherapy[62,63].

Peptides of 35 kDa Protein

In order to identify specific epitope on the 35 kDa antigen (identified by earlier Mab) studies have been carried out using synthetic peptides. These peptides of the 35 kDa protein of *M.leprae* were further selected by their binding ability with HLA-DR to find out their promiscuity. It was noted that responses to the peptide pair 206-224, differing by 4 residues between *M.leprae* and *M.avium*, involved both species specific and cross-reactive T cells. Of the cytokine responses to peptide 241-256 in the peripheral blood mononuclear cell(PBMC) cultures IFN-g production was negligible, while IL-10 responses in both patients and controls (contacts of patients) were pronounced[64]. This observation indicated that the lymphokine response of many controls may skew towards the pattern of response of lepromatous leprosy patients. Although the peptides per se do not have a significant stimulatory effect on CMI, this could be improved by strategic presentation with liposome as vesicle. Further, the study of immune responses to recombinant proteins (10, 28, 36 and 65 kDa) in patients and healthy controls revealed that IFN-gmay contribute to both pathology and protection in leprosy [65].

Local Immunity in Leprosy Lesions

In spite of establishing a specific serodiagnostic assay using Mab all leprosy patients at an early stage of the disease activity cannot be detected and so a study was conducted to understand the local immunity in skin lesions. It was noticed that there is a local *M.leprae* antibody secretion in the granuloma of both BL/LL[66] and BT patients[67]. It was also shown that both Th1 interleukins (IFN-g, IL-2) and Th2 interleukins (IL-4, IL-5, IL-10) are secreted from many of the BT lesions. This might explain the prolongation of many BT lesions even after adequate chemotherapy due to downregulation of the Th1 immune response by the Th2 interleukins.

Subclinical Infection in Leprosy using Fluorescent Leprosy Antibody Absorption Test

Identification of subclinical infection is important for understanding the dynamics of transmission and sensitization of the community to the pathogen. Further, markers of high risk of getting infection could be explored. Fluorescent leprosy antibody absorption (FLA-ABS) test which was developed at the Institute was used to screen patients and over 90% of TT/BT cases were positive[68]. More than 90% of contacts were also found to be positive indicating subclinical infection in the community at large. Further, when the results of this test were studied in combination with the lepromin skin test in a follow up study in contacts, it was noted that a large proportion of FLA-ABS positive and lepromin negative individuals developed leprosy[69,70] whereas the other groups of FLA-ABS positive and lepromin positive patients did not develop the disease.

Studies on Drug Metabolism and Related Aspects

Studies on pharmacokinetics of antileprosy drugs and drug interactions have been carried out to improve therapy. Based on studies at CJIL therapeutically significant interactions between prothionamide or clofazimine and dapsone have been ruled out[71-75]. The plasma dapsone lowering effect of continuous administration of rifampicin has been confirmed[71-74]. Pioneering studies at CJIL on the metabolism and effect of clofazimine have shown that (i) clofazimine accumulates in the icthyotic areas of skin in leprosy patients[75]; (ii) mobilization of clofazimine from the tissue depots occurs by simultaneous administration of isoniazid[71]; (iii) clofazimine therapy results in reduced skin and plasma levels of vitamin A[76], which indicate the need for its supplementation; (iv) based on the dose related faecal excretion, the monthly loading dose of clofazinine is wasteful and administration of clofazimine (50 to 100 mg) daily is adequate for therapeutic purposes and optimum absorption [73] and maintaining tissue levels; (v) significant amounts of clofazimine (@ 0.199 mg/kg/d) are ingested by infants on breast feeding by lactating mothers on clofazimine treatment. This amount constituted almost 22% of the last maternal dose of clofazimine[77]; (vi) the nature of edible oil/fat in the fatty meal does not influence the oral absorption of clofazimine and hence

its bioavailability[78]; and (vii) based on a multiple dose pharmacokinetics of clofazimine at low doses of 50 mg/ day, a steady state is likely to be reached with 30-60 daily doses of clofazimine[76,79,80].

Studies on Mycobacterial Lipids

Mycobacteria including *M. leprae* are rich in lipids. These lipids have taxonomical relevance as well as being important sites for action of various anti-mycobacterial drugs.The studies carried out at the Institute on mycobacterial lipids have resulted in significant findings on both of these aspects. Using a biphasic sequential lipid excretion system a whole range of mycobacterial cell wall lipids can be rapidly isolated from very small skin biopsy specimens from lepromatous leprosy patients or 5-10 lyophilized mycobacterial cells. Lipids purified by simple and non-invasive techniques like celite/Florisil column chromatography, have been shown to have application in mycobacterial chemotaxonomy and potential relevance in understanding the pathogenesis of mycobacterial infections and lipid biosynthetic assembly [81-84]. The studies carried out at CJIL have shown that these lipids could be detected easily from leprosy lesions. Further, taxonomic importance of mycolic acid, glycolipids and other lipids has been established for most of the mycobacteria[85,86]. Effect of several antimicrobials including rifampicin, streptomycin and isoniazid on mycolic acid biosynthesis has been demonstrated[87,88] .

Sttudies on the Metabolism of Mycobateria

With a view to understand the metabolic pathways in *M.leprae*, the Institute has carried out several investigations. These studies have shown that *M.leprae* has the enzymes of the TCA cycle and glyoxylate bypass[89]. The demonstration of the TCA cycle became the basis of the ongoing *in vitro* energy synthesis studies using different substrates and helped in establishing conditions which favoured limited *in vitro* growth of *M.leprae.* The demonstration of glyoxylate bypass has important implication in understanding the persister stage and identifying drugs which could attack at this site, preferentially[89-92].

Taxonomy

Studies have been undertaken to develop alternate taxonomic methods for investigations using enzymes, isoenzymes and protien markers. These studies revealed that LDH zymograms are useful for screening of mycobacteria at the species level[93] whereas esterase zymograms would be helpful in typing of strains of different mycobacterial species, including *M.leprae*[94-97]. A scheme for rapid identification of various pathogenic mycobacteria using these protein electrophoregrams and zymodemes has been established in this Institute. This approach has also been found to be particularly useful for analysis of isolates of *M.bovis*[98,99].

The use of immunological techniques to determine the immunological relatedness of enzymes (catalase and superoxide dismutases) in host grown microbes has been established. Using a microimmuno-precipitation technique it was established that *M.lepraemurium* has a T catalase and is closely related to *M.aviam* yet distinct from it. These techniques also established that *M.leprae* does not have significant levels of T and M catalase[99,100]. As *M.leprae* has very low levels of catalases a new reference taxonomical system based on the measurement by ELISA of immunological relatedness of superoxide dismutase of mycobacteria has been developed and its application to various pathogen including *M.leprae* is being established[101-103]. These approaches are useful for application at reference level.

Studies on Determination of Viability of *M. leprae* & Other Mycobacteria

Determination of the viability of *M.leprae* isolates has been difficult. Several years ago the Institute established a mouse foot-pad model for determining *M.leprae* growth for monitoring drug trials and for screening for drug resistance. However, as the mouse foot-pad experiments are expensive, time consuming and insensitive in low bacillary stages, there was an immediate need to develop alternative *in vitro* methods for determination of viability of *M.leprae.* CJIL has focussed on alternative methods with ATP bioluminiscence and rRNA probes. An easy, reproducible, and rapid viability determination method based on the measurement of bioluminiscence (ATP) for *M.leprae* and other mycobacteria has been developed at the Institute. Further, an optimum procedure for extraction and assay of ATP of mycobacteria including *M.leprae* has been standardised[104]. This method has been compared with some earlier described techniques and found to be highly sensitive[105 - 107]. Further, in a modified Dubos medium system in which ATP increases and limited multiplication of *M.leprae* has been achieved, an ATP decay drug screening system for screening of clinical isolates from drug susceptible strains has been standardized[104]. Using

this assay, trifluoperazines have been found to be active against leprosy bacillus[108]. Ribosomal RNA targeting probes developed at this Institute have been further standardized into microdensitometric scanning procedures and found to be useful in monitoring therapeutic trials[109]. In addition, the RT-PCR assay targeting rRNA standardized earlier has been found to be more sensitive for confirmation of diagnosis as compared to the rDNA targeting protocol.

Mouse Foot -Pad Technique : Comparison with Other Methods

This technique has been used in the Institute to monitor the viability of *M. leprae* from patients during and after treatment with various drug regimens and to know the trends of persistence/resistance. Mouse foot - pad serves as a gold standard for assessment of the viability of *M. leprae* isolates.Mouse foot-pad assay, ATP bioluminescence and PCR have also been compared in patients under treatment[109]. While all the techniques are useful in knowing the trends, ATP bioluminiscence has been found to be more sensitive than mouse foot -pad for detection of persisters in treated patients. PCR can be used for monitoring the trends only as weak signals have been found to persist in some patients after loss of viability in assay targeting DNA[107,109].

MOLECULAR BIOLOGY OF MYCOBACTERIA

Molecular biology techniques have provided capabilities in understanding the basic biology of the organism as well as the host. This understanding has helped in developing techniques for diagnosis, transmission and drug resistance. The Institute has carried out research in these areas and has contributed in several aspects:

Ribosomal RNA Based Probes

The studies carried out in the Institute have provided new insights into the rRNA genes as well as led to the development of many techniques potentially useful in research and applications. In these studies a new technique for stepwise isolation of poly (A) +RNA rRNA and DNA has been established. Further, different ribotyping (ribosomal DNA-fingerprinting) techniques based on rRNA, cDNA, PCR generated probes targeting ribosomal RNA gene region have been developed.Using the restriction fragment length polymorphism (RFLP) of ribosomal RNA genes, *M.leprae* and other pathogenic mycobacteria can be rapidly identified and characterized[110-114]. Using these techniques, rRNA gene fragments comprising homologous and specific sequence in *M.leprae, M.tuberculosis* and some other mycobacteria have been identified [115,116]. These fragments were further cloned and sequenced to identify species and strain specific sequences. Based on the information on specific sequences, two probes targeting rRNA of *M.leprae* have been developed and techniques established for clinical application[109]. Recently, two PCR -RFLP assays targeting 16S and 16-23S spacer plus flanking region have been developed which have been found to be directly applicable to clinical specimens[117-119]. These methods can be used for rapid identification and molecular typing of *M. tuberculosis*, *M. leprae* and other pathogenic mycobacteria. These studies have also provided new insights into rRNA gene structure as well as led to the development of new techniques.

Molecular Epidemiology

Mycobacterial isolates from different parts of the country deposited in the Mycobacterial Repository Centre (at CJIL) have been characterized using IS6110, ribotyping as well as random amplified polymorphic DNA (RAPD) systems. There does not appear to be much difference in RFLP types (IS6110 & ribotypes) of drug sensitive and MDR strains showing thereby that primary transmission is rather low and the major problem is random selection of drug resistance due to incorrect regimens/treatment. Analysis of IS6110-RFLP patterns of *M. tuberculosis* isolates from different parts of the country revealed 0 to 19 copies in the Indian strains. There is no major regional difference in the profile or special clustering in the MDR strains of *M.tuberculosis* from any region[120]. RAPD analysis has been observed to be promising for typing *M. tuberculosis* strains including the IS 6110 untypable strains .

Drug Resistance

With a view to understand the molecular basis of drug resistance in Indian strains of *M.tuberculosis* and pathogenic mycobacteria, studies have been carried out. In these collaborative multicentric studies (CJIL/CDFD/ NDTBC/AIIMS *etc*), both earlier known as well as novel mutations in the *rpo*B region in Indian strains of *M. tuberculosis* have been identified and probes are being developed for clinical application in tuberculosis and later in leprosy. The investigations on target sites for streptomycin (rrs and rspl) as well as quinolone (gyrA and gyrB) showed mutation in nearly 50-60% of the isolates, indicating the need to analyse other mechanisms like efflux pumps.

Clinical, Histopathological and Ultrastructual Research

Clinical studies carried out at the Institute have provided original information about various aspects of the disease, its diagnosis and possible routes of transmission.

Transmission of *M.leprae* Infection

Leprosy is known to affect the mucous membranes which is important from the transmission point of view. Several studies were undertaken to study the profile of involvement of the oral, nasal and genital mucosa. The important studies are:

Oral mucosa

Studies at CJIL have shown that the oral mucosa is commonly affected in leprosy patients. In a series of 40 patients, 53.5% of lepromatous patients had intra-oral lesions. These studies also showed that 85% of patients had acid fast bacilli (AFB) on the surface of the mouth. Positivity was higher in those who had clinical involvement inside the mouth[121-123].

Nasal breath

Studies showed that the nasal breath of 100% lepromatous (BL/LL) patients had AFB. The average count of AFB per breath was 3.3×10^4. These studies showed that nasal breath is an important mode of transmission of AFB in the environment[124].

Female genital mucosa

It has been established that female leprosy patients, particularly those with BL/LL type excrete large numbers of lepra bacilli through the genital tract. A study in BL/LL patients revealed AFB positivity in the vaginal secretions of one third of patients.

Breast milk

One of the causes of the increased incidence of leprosy among the children of leprosy patients is considered to be due to their exposure to a heavy dose of infection from their parents. In a study from this Institute, 31.5% (12 out of 38) of lactating patients were observed to secrete AFB in the breast milk. The mean count of bacilli in the milk ranged from 4.3×10^4 to 1.53×10^5/10 ml of breast milk. Positivity of AFB was shown to be 7.7, 12.5 and 58.8% in tuberculoid, borderline and lepromatous patients respectively. It was also noted that significant amounts of antileprosy drugs are also excreted in the milk after the initiation of chemotherapy thereby possibly reducing the danger of transmission of leprosy to neonates during this period [125].

Prevalence of Cardiovascular Involvement

Though leprosy predominantly involves the peripheral nerves and the skin, other organs are also involved. Earlier post-mortem studies had not shown histpathologic evidence of heart involvement in leprosy. However, the phenomenon of sudden deaths have been reported. A study was therefore undertaken on cardiac involvement and electrocardiographic changes in 430 leprosy patients. The study revealed that besides symptomatic involvement, electrocardiographic evidence of involvement of the cardiovascular system was highest in BL/LL, less in borderline (BB) and least in tuberculoid (BT/TT) patients as compared to age matched controls[126]. These differences were statistically significant.

Involvement of the Central Nervous System in LL Patients

Although it is believed that the central nervous system is not involved in leprosy, detailed neurological examination of BL/LL patients revealed a loss of brisk deep reflexes in a section of the patients (upper motor neuron type involvement) in addition to varying degrees of loss of sensations for temperature, touch and pain. In another study anti *M.leprae* antibodies could be detected in the CSF by FLA-ABS and Mab based competitive assay against defined epitopes of the 35 kDa protein and 30-40 kDa polysaccharide (lipoarabinomannan) antigens. Some correlation was observed between the upper motor signs and antibody positivity for 35 kDa and PGL-1 antigens in the CSF of these patients[127,128].

Use of Mitsuda Lepromin Reaction for Classification of Leprosy

The histology of the Mitsuda lepromin reaction has been observed to have a fair correlation with the clinical and histological classification of leprosy patients. In patients with fascial lesions or nerve involvement, the histology of the lepromin reaction may be utilized as an indicator for the classification of the disease type of leprosy patients, instead of resorting to biopsies from vital areas of the face and/or nerve.

Neural Leprosy

Pure neural type of leprosy has been reported persistantly from the Indian sub continent. Studies undertaken to compare the histopathological spectrum of nerve and skin involvement in leprosy patients showed that the affected nerves have a spectrum like that of the skin lesions. Nerve abscesses has been most commonly observed in the ulnar nerves. Different degrees of calcification were noted in some patients mostly in the ulnar nerves.

Haematogenous Spread of Leprosy

Haematogenous spread of leprosy was studied by evaluation of bacteraemia. Bacteraemia was most common in patients of BL/LL leprosy. Continuous bacteraemia was observed in a substantial number of LL patients. Occasionally, BT leprosy patients also showed continuous bacteraemia indicating a haematogenous spread of the disease [129,130].

Histological Confirmation of Indeterminate and Clinically Suspected Leprosy

Histological confirmation of non-specific and indeterminate disease was observed to be enhanced considerably by immunohistological analysis of the lesions using anti-BCG antibodies[131]. These lesions are further being analysed using *M.leprae* specific nucleic acid probes and employing *in-situ* hybridization[132].

Relation of Smear Positivity among Patients with PB Leprosy

In the management of leprosy, a rough estimation of the quantum of bacteria in a patient is made by examining slit and skin smear for the degree of bacterial positivity by determining the bacteriological index (BI). Leprosy has been classified for the benefit of the field programme as PB and MB depending on the number of skin lesions. Studies at CJIL have shown that a good number of patients diagnosed as PB clinically were AFB positive in the skin smear indicating their chances of getting inadequate treatment under the Programme and further that these patients would be prone for relapse[133].

Vascular Involvement in Leprosy

Vascular involvement in leprosy is believed to be uncommon, while nerve involvement is believed to be common. Therefore, any subcutaneous cord-like structure in a leprosy patient is taken as a nerve. However, when such cord like structures were examined under the microscope, it was noted that in some cases these were infiltrated veins. This finding indicated the occurrence of leprous phlebitis[134] in which the whole vein has been infiltrated with lepromatous infiltrate with the maximal infiltration in the intimal layer. The importance of these lesions in causing bacillaemia is obvious. Subsequent studies showed early lesions in the subcutaneous veins and it was noted that the subcutaneous veins are involved at a very early stage of lepromatous leprosy[135].

Ultrastructural Aspects

Detailed electron microscopic studies of skin and nerve granulomas have been carried out[137,138]; with special attention being given to the Schwann cells and endoneural blood vessels. In nerves from patients of tuberculoid leprosy the endothelial cells of endoneural blood vessels were hypertrophied and these cells blocked the lumen of the vessels whereas the endothelial cells of endoneural blood vessels from lepromatous leprosy patients though loaded with bacilli were not hypertrophied and the lumen was patent[138]. The Schwann cells of non-myelinated axons were frequently found affected in both types of leprosy[139]. In addition, clofazimine crystals have been demonstrated in the Schwann cells of lepromatous patients[140]. All these observations have helped in understanding the pathogenesis of the disease.

Genetic Studies

The Institute has undertaken studies to understand the genetic markers associated with susceptibility and resistance in leprosy. Association of certain dermatoglyphic patterns and leprosy has been noted[141,142]. Later on, an association between taste and leprosy type has been observed. In a field study carried out by the Institute in leprosy families, no significant association of HLA DR antigens with any of the types of leprosy has been observed[143] . Further, no correlation of HLA marker and ENL reactions in leprosy has been observed[144].

Developmental Activities

Referral Centre for Mycobacterial Diseases

The Institute has been identified as a Reference Centre for Mycobacterial Diseases for the purposes of testing of immunodiagnostics and biotechnological products. The Institute is serving as a co-ordinating centre for testing

of PCR based techniques and serological tests for tuberculosis, leprosy and other mycobacterial diseases.

Mycobacterial Repository Centre

In order to fulfill the important function of developing diagnostic and research capabilities to manage mycobacterial infections with special reference to tuberculosis in the country a DBT funded Mycobacterial Repository Centre has been established since 1995. A network from more than 28 centres / laboratories across the country has been established and about 2000 strains have been collected and characterized using conventional and reverse molecular methods. Majority of the strains have been found to be *M.tuberculosis*. Multidrug resistance (MDR) of primary type appears to be low, whereas in treatment failure cases MDR is considerably high. This Centre is serving as a source of reference Indian strains of mycobacteria to different scientists in India and also providing help in serving as a reference centre for characterizing vaccine and other strains of clinical and research interest.

CONCLUSIONS

The above account briefly summarizes the research achievements of the CJIL. Researches carried out by the Institute have provided original informations and knowledge on the therapeutic, clinical, surgical, pathological, immunological, microbiological and molecular biological aspects of the disease. In addition, the Institute through drug trials formulated new and altered drug regimens which have immensely helped the NLEP in modifying the drug regimens.

Though MDT has been responsible for significantly bringing down the prevalence of leprosy from about 20 per 10,000 to 7 per 10,000, new cases are still appearing at the same rate indicating that an active transmission of infection is occurring in the community. At this juncture of eradication of leprosy it would be of prime importance to conduct research for further reducing the disease burden in the community by identifying the reservoir of infection, understanding transmission of disease, monitoring treatment to reduce treatment failures, close surveillance of treated patients for occurrence of drug resistant relapse and by early identification of nerve damage for prevention of deformities. Basic research on understanding the host parasite relationships, search for better and effective drugs to shorten the duration of treatment, biochemical and molecular biological studies to understand drug metabolism by *M.leprae* and genetic basis of drug resistance in the bacteria need to be continued. It is expected that research carried out on these lines would bring about new findings which would further help in strengthening the leprosy eradication programme of the country.

With the changing scenario in leprosy and considering the immense potential of developing common methods for mycobacterial diseases, the Institute has recently initiated programmes on tuberculosis and other related mycobacterial infections in addition to leprosy. These efforts would further widen the scope of research of the Institute and consequently would provide opportunities for contributions from the Institute to the national health problems.

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This write-up has been contributed by Dr. U. Sengupta, Director, Central JALMA Institute for Leprosy, Agra.

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DEPARTMENT OF BIOTECHNOLOGY
MINISTRY OF SCIENCE AND TECHNOLOGY

Call for Joint Research Proposals Under
Indo-US Programme on Contraceptive and Reproductive Health Research

In pursuance of a joint statement issued in November 1997 by the Governments of India and the USA on the Expansion of Indo-US Collaboration on Contraceptive and Reproductive Health Research, the Indian scientists are invited to propose joint research projects with US scientists. The following specific areas have been identified for collaborative research emphasis:

- New reversible male contraceptive methods;
- Long-acting injectables for women;
- Barrier methods for contraception and sexually transmitted diseases prevention;
- Emergency contraceptives;
- Social and behavioural research;
- Epidemiological studies, including studies on STDs and RTIs, immunocontraception; and
- Basic, applied and clinical research in reproductive health

Expanded areas:

Microbicide and spermicide research: Development of improved microbicides/spermicides for contraception and disease prevention; effect of microbicides *eg* buffer gel, bacterial vaginosis; irritation and safety aspects of spermicides/ microbicides and behavioural response to more promising compounds into clinical trials; social, behavioural and cultural factors influencing barrier methods and to increase the potential use of microbicides; promotion of condom use and the acceptability of a range of spermicide/microbicide formulations.

Sexually transmitted diseases and reproductive tract infection research: Improved diagnostics for detection of STDs; development of instruments to improve surveillance; behavioural research; factors influencing health seeking behaviour/ accessing health care; social/cultural antecedents of sexual behaviour; factors affecting adolescent reproductive health; and role of health care providers in delivering appropriate contraceptive methods.

Twenty copies of the jointly agreed collaborative research project on specific formats would be received by the secretariat of the programme. Research proposals will be peer-reviewed in India and the United States and approved research projects would be supported appropriately on Indian and US side to meet research costs and other resources. The implementing agencies in India are the Department of Biotechnology, Ministry of Health and Family Welfare, Indian Council of Medical Research and the Council of Scientific and Industrial Research. The US implementing agencies are the National Institute of Child Health and Human Development, National Institute of Health, the U.S. Agency for International Development and the Contraceptive Research and Development Programme. The research projects would be implemented subject to necessary clearances from the Government and laws and regulations of the host and sponsoring countries. Indian investigators interested in establishing collaborative research projects with US scientists should contact for format and submission of projects to :

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