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Original Article

HISTOLOGICAL AND IMMUNOLOGICAL CORRELATES OF SUSPECTED LEPROSY LESIONS

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Thirty-two subjects with suspected leprosy lesions were investigated to assess various modalities of sensibility and sweatfunction and these were correlated with immunological and histological parameters.

It was found that pain and temperature, mediated by small unmyelinated fibres were impaired in the early lesions. Impairment of sweat function was seen only when one of the modalities of sensibility was also affected Antibodies specific to a protein (35 kDa) antigen and phenolic glycolipid 1 of Mycobacterium leprae were positive in nine and 12 cases respectively, while 15 of the 31 biopsies revealed the presence of mycobacterial antigens in these lesions. The implications of these findings are discussed.

INTRODUCTION

Early detection of leprosy is important in the control of the disease. Treatment of early lesions avoids deformities and the progress of the disease of infectious types. This is of obvious importance in a country like India where the majority of cases are paucibacillary to begin with. Diagnosis is easy in established leprosy since the cardinal signs are readily elicited (Dharmendra & Chatterji 1978). The criteria for diagnosing leprosy are loss of sensibility in a patch or enlargement of peripheral nerve trunk or a cutaneous nerve with loss of sensibility in the area of its supply or presence of acid-fast bacilli (AFB) in smears from a suspected patch or an area of skin with altered texture.

Lesions are put down as 'suspected' when touch sensibility is preserved but other modalities are impaired in a skin patch and the skin smears are negative for APB or there are areas of impairment of sensibility in the skin without a patch or nerve thickening, or, there is nerve thickening without loss of sensibility. The microscopic pathology and the immunological features of established leprosy lesions are well-recognized. However, the histological and immunological features of the lesions when leprosy is suspected are not known. In the present study we have attempted to correlate the clinical features of the first two categories of suspected leprosy lesions with some of their immunological, biochemical and histological parameters.

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MATERIAL AND METHOD

PATIENTS

The subjects consisted of 14 male and ten female adults and five male and three female children. Twenty-one of these subjects had flat, hypopigmented, ill-defined patches and ten had areas of numbness in the skin. The commonest site of such numbness was the anterolateral aspect of the thigh. All except two had single lesions.

CLINICAL EXAMINATION

The test for touch sensibility was carried out by using a painting brush no. 4. A diminution in pain (expressed as less sharp compared to the contralateral side) and loss of pain were elicited using a 26 G needle. Thermal sensibility was initially tested by using two test tubes - one with cold water and the other with tepid water. The thermal tester standardized by Srinivasan and Stumpe (1989) was used later.

SEROLOGICAL TESTS

Imrnunoglobulin M class of antibody to phenolic glycolipid 1 of *Mycobacterium leprae* was tested using ELISA as described by Cho and his colleagues (1983). This was considered to be positive if the absorbance value of the sample at a dilution of 1 in 300 was greater than 0.250. This value was derived by adding two SD values to the mean of 25 control samples. Blood from hospital volunteers served as the control. Antibody to the My2a epitope of *M. leprae* was estimated using the serum antibody competition test (SACT) as reported previously (Sinha et al 1983).

IMMUNOENZYMATIC STAINING

Immunoenzymatic staining of formalin-fixed, paraffin-embedded skin sections was performed using anti-BCG antibody (Dakopatts, Denmark) as described by Mshana et al (1982). Immunoglobulin fraction of serum from an unimmunized rabbit served as the negative control in all the samples. Antigen was seen mainly intracellularly in the macrophages of the dermal granuloma and occasionally in the Schwann cells of an infiltrated nerve. The staining was graded as doubtful (±) and 1+ to 4+ depending on the intensity and extent. Most of the positive specimens showed 1+ to 2+ grading with an occasional 3+.

The serological, histological and immunohistological examinations were performed blind without recourse to clinical details.

LEPROMIN REACTION

The Fernandez (at 24 hr) and the Mitsuda (on the 21st day) responses to Dharmendra lepromin containing $1x10^7$ *Mycobacterium leprae/* ml were measured as described earlier (Sengupta et al 1979).

SWEAT FUNCTION TEST

Prostigmin 0.02 mg was injected intradermally into the lesion and also into the contralateral site as control. Assessment of sweat secretion formed was done by the ninhydrin method (Rao et al 1987). A filter paper attached to a stamp pad was kept in contact with the test site for five minutes. The paper was then removed from the stamp pad and sprayed with 1% ninhydrin in acetone. This gives pink dots wherever there has been sweating. The colour was eluted in 75% alcohol containing copper sulphate and expressed as optical density (OD) at 540 nm. Sweat function was considered to be impaired if the OD value of the sample from the test site was less than half of the OD value from the control site.

RESULTS

Touch sensibility was intact at the affected site in all the individuals. Acid-fast bacilli were not demonstrable either in the smear or in the sections from any of the lesions. No associations could be seen when the data were analyzed in relation to age and sex of the subjects.

The patients were divided into three major categories on the basis of the sensory nervous function deficit in the lesions and the results of the various investigations in the different categories are given in Table I. The first category comprised of 20 individuals with impaired pain and thermal sensibility. The second and third categories consisted of subjects with lesions where either pain or the thermal sensibility alone was affected.

Table I. Results of investigations in 32 patients with 'suspected' leprosy lesions

Clinical category	Groups	Total no. of patients	Histology positive	Impaired sweat function	Antigen positive	PGL 1 +ve	My2a +ve	Mitsuda positive
I. Impaired pain & thermal sensibility	A	15	10	10	8	7	3	6
	B	5	5*	2	3	3	3	3
II. Impaired pain sensibility only	A	3	2	0	2	1	1	2
	B	4	1	1	2	1	--	1
III. Impaired thermal sensibility only	A	4	1	−	−	−	2	3
	B	1	−	−	−	−	−	1

Group A: Patients with skin patches; Group B: Patients without skin patches but with impaired sensibility.

* Three patients showed histological changes suggestive of regressed leprosy

Histological evidence of leprosy was seen in 12 of the 20 lesions having both pain and thermal sensory impairment. Impairment of sweat function was seen in 12 and mycobacterial antigens could be demonstrated in 11 of these 20 lesions. Antibodies to both

PGL and My2a determinant were seen in the sera of four of these individuals. Histologically, three of the lesions showed evidence of regressing leprosy and these individuals had only sensory involvement without an obvious skin patch. Interestingly, neither antigen nor antibody could be demonstrated in these subjects. Mitsuda response to Dharmendra lepromin was observed in nine of the 20 individuals.

In the second group consisting of seven subjects who had only impaired pain sensibility in the lesions (either analgesia or hypalgesia), histological evidence of leprosy was observed in three of the biopsies. Four of the lesions had evidence of the presence of mycobacterial antigens while the sweat function was affected in only one. Anti *M. leprae* antibodies were seen in the sera of four of these individuals while the Mitsuda response was positive in three of them.

The rates of positivity for antigens and antibodies were very low in the five individuals who had lesions with impaired thermal sensibility alone. Histological evidence of leprosy was seen in only one of them. However, four of them showed positive Mitsuda reaction to Dharmendra lepromin.

DISCUSSION

When the cardinal signs of the disease are satisfied, clinical diagnosis of early lesions of leprosy is possible with a high degree of concordance between clinicians (Neelan et al 1982). Thus, leprosy can be diagnosed with certainty when there is loss of touch sensibility, which is mediated by myelinated nerve fibres in a suspected lesion. It is well-known that, in leprosy, unmyelinated fibres which are responsible for pain and thermal sensibility are involved earlier than the myelinated fibres (Dastur 1955). Hence, we studied the clinical features and the immunological and histological correlates of lesions with impairment of sensory modalities mediated by unmyelinated nerve fibres alone.

It was found that a maximal positivity of 50% or more for antigen, antibody, impaired sweat function and histological positivity was observed when both the pain and the thermal sensibilities were affected. This was followed by the group with impaired pain sensibility alone. The group with only reduced thermal sensibility showed minimal positivity for antigens and antibodies. Similarly, histological evidence of leprosy or impaired sweat function could be observed only in one of them. These data together suggest that lesions with impaired pain and thermal sensibility or those with impaired pain sensibility alone are more likely to be due to leprosy than the lesions which present with only thermal fibre involvement.

Further studies are indicated to delineate the relationship between the clearance of antigen, development of antibody response and delayed hypersensitivity to *M. leprae* in such early cases.

Autonomic nerve function is thought to be impaired early in leprosy. However, the results of sweat function test performed in the present study indicate that this is not invariable.

Although the number of patients studied is small, it may be concluded that demonstration of antigen in the tissue along with demonstration of antibody in the serum in cases which show impairment of unmyelinated nerve functions is probably diagnostic of leprosy. However, studies involving larger number of cases using monoclonal antibodies and/or DNA probes specific to *M. leprae* are needed to clarify the extent and type of impaired functions mediated by unmyelinated nerve fibres in very early leprosy. This would then be of help in laying down clinical criteria to diagnose leprosy in the field without recourse to sophisticated tests involving the demonstration of antigen and/or antibody specific to *M. leprae.*

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