MATERIAL AND METHODS
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The study has been conducted on patients suffering from various types of leprosy, admitted or attending out patient Department of Skin and V.D., M.L.B. Medical College, Hospital, Jhansi. The patients were examined in the period between July, 1985 to March, 1986. Normal healthy volunteers without immunological disorders were selected from Department of Pathology and from relatives of patients as controls.

Patients were thoroughly examined clinically for type of leprosy and findings were recorded on a predesigned proforma (Appendix - I).

COLLECTION OF BLOOD SAMPLES

Blood samples in the quantity of 10 ml with heparin as anticoagulant were collected in sterile tubes from patients with strict aseptic precautions. Routine haematological tests were done from blood collected simultaneously from each patient.

SKIN BIOPSY

Biopsy material was excised from the edge of the lesion and collected in 10% formal saline. It was dehydrated in serial concentrations of alcohol and cleaned
with xylene in place of chloroform. Tissue was embedded in paraffin (58°-60°C), blocks prepared and sections of 5 micron thickness were cut by rotatory microtome. Staining was done with haematoxylin-eosin. For demonstration of lepra bacilli modified Fite-Faraco staining was done.

**HISTOPATHOLOGICAL TYPING**

Criteria of Ridely and Jopling (1966) as given below was adopted for histopathological typing of leprosy cases.

**TUBERCULOID TYPE (TT)**

- Foci of well developed epitheloid cell granuloma with a few Langhan's giant cells, often enveloped by dense zone of lymphocytes, especially in deeper parts of dermis.
- Erosion of basal layer by granuloma.
- Nerves difficult to detect and may show caseation.
- Lepra bacilli not detectable by modified A.F.S.

**BORDERLINE TUBERCULOID (BT)**

- Narrow clear subepidermal zone above the granuloma.
- Lymphocytes plentiful and diffuse.
- Nerves swollen but recognisable.
- Lepra bacilli demonstrable 0 to ++.
BORDERLINE (BB)

- Sheets of epitheloid cells but no giant cells.
- Lymphocytes sparse and diffuse.
- Nerves showing structural disorganisation but no granuloma.
- Lepra bacilli demonstrable in grade +++ to ++++

BORDERLINE LEPROMATOUS (BL)

- Histiocytic granuloma with cells slightly epitheloid in appearance.
- Few diffuse lymphocytes.
- Nerves swollen or normal.
- Lepra bacilli demonstrable in grade ++++ to +++++

LEPROMATOUS LEPROSY (LL)

(a) Active phase
- Macrophages with some foamy changes.
- Very scanty lymphocytes.
- Nerves almost normal.
- Lepra bacilli ++++ to ++++++

(b) Regressing phase
- Foam cells with globi and much fat.
- A few lymphocytes
- Lepra bacilli +++ to +++++
REVERSAL REACTIONS

- Influx of lymphocytes
- Oedema in and around granuloma.
- Granuloma increases in volume.
- Cytological changes more towards epitheloid form.
- Number of bacilli gradually diminished.
- Foreign body giant cells present.
- In severe cases there is necrosis.
- In a patient having erythema and swelling of skin lesion often there is nerve involvement new lesions may appear. They may present tuberculoid appearance.
- Lymphocyte decrease in number.
- Granuloma spreads.
- Large giant cells of foreign body type appear.
- Cells are vacuolated (Intracellular oedema) and extracellular oedema is a constant finding.
- Bacilli appear in detectable number.

EXACERBATION NODULES

- One lesion becomes exceptionally large and loaded with many times more bacilli.
- There is healthy polymorphonuclear infiltration and cellular disintegration.
- Polymorphs are present.
- Cellular disintegration is marked.
- Later there may be significant number of lymphocytes.
- Vasculitis or vascular necrosis is prominent in some.
- In a patient having crops of small painful red nodule with lymphnode, liver, spleen enlargement and iridocyclitis,orchitis and painful enlargement of nerve seen. Temperature found to be raised.

**BLOOD EXAMINATION**

The method of Dacie and Lewis (1975) was followed for total and differential leucocyte counts. From total and differential leucocyte count, absolute lymphocyte count was calculated using the following formula.

\[
\text{Absolute lymphocyte count} = \frac{\text{Total Leucocyte Count} \times \% \text{ of lymphocytes}}{100}
\]

\[
A L C = \frac{T L C \times \% \text{ Lymphocyte}}{100}
\]

**EVALUATION OF T AND B LYMPHOCYTE**

T and B lymphocytes present in the peripheral blood were demonstrated by means of their surface receptors (Jondal et al, 1972).
The basic principle of procedure is as follows:-

I. Separation of lymphocytes.

II. Demonstration of T cells by sheep red blood cells (SRBC) rosette (E rosette).

III. Demonstration of B cells by formation of rosettes with SRBC coated with antisheep haemolysin antibody and complement (EAC rosette).

LYMPHOCYTE SEPARATION

The lymphocytes were separated by Ficoll Conray 420 density gradient centrifugation. The separation of the lymphocytes by this method was due to differences in the density of various cells in the blood.

MATERIAL REQUIRED

1. Ficoll Conray 420 solution (specific gravity 1.077) was prepared as described by Sen Gupta (1981). (11.40 gm of Ficoll dissolved in 160 ml of distilled water + 22 ml of Conray 420. Specific gravity was adjusted to 1.077 and sterilized by Seitz filter).

2. Preservative free heparin.


4. TC Medium 199 (Difco Laboratories, Detroit Michigan, USA).
5. PHA-M (Difco Laboratories, Michigan, USA).

6. Alsever's solution:
   Glucose ..... 24.6 gm
   Trisodium Citrate (Dehydrate) ..... 9.6 gm
   Sodium Chloride ..... 50.04 gm
   Distilled water ..... 1200 ml

   pH was adjusted to 6.1 with 10% citric acid.

   This was sterilized in autoclave under low pressure.

7. Phosphate Buffer Saline (PBS)

   (A) Phosphate buffer solutions
       
       0.15 M - \( \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} \) 23.4 gm/Litre in Distilled water.

   (B) 0.15 M - \( \text{Na}_2\text{HPO}_4 \) 21.3 gm/Litre

   (C) Normal saline (NaCl) 9 gm/Litre

   The pH of phosphate buffer saline was adjusted to 7.4 by mixing solution 'A' 18 ml
   Solution 'B' 82 ml

   Normal saline 100 ml was added to prepare phosphate buffer saline.

8. Glutaraldehyde 2 % solution in PBS

9. AB sera collected from persons of AB blood group.

10. Complement from guinea pig.
**METHOD**

I. 2.5 ml Ficoll solution was taken into two sterilized test tubes.

II. 5 ml of blood was poured in each test tube carefully in such a way that a layer of blood is formed. It was centrifuged immediately at 1500 rpm for 15-20 minutes.

III. After centrifugation various layers are formed which contain plasma, predominantly lymphocytes, Ficoll Conray, neutrophils and red cells from upper to lower layer respectively.

IV. After removing plasma carefully the lymphocyte layer is pippetted off and placed in 5 ml of MEM in one test tube and 5 ml of TC medium 199 in the other and washed twice, centrifuging each time at 1000 rpm for 10 minutes with MEM and TC medium, respectively.

V. The cells were suspended in MEM and viability was checked by 1% eosin in TC medium 199 and count was adjusted to 2-3 \( \times 10^6 \) cells/ml.

(B) **DEMONSTRATION OF T CELLS (SRBC ROSETTE FOR T CELLS)**

1. Sheep red blood cells (SRBC) were collected in Alsever's solution and washed thrice in phosphate buffer saline (PBS) and suspension made up to 0.5% in PBS.
2. 0.5 ml of lymphocyte suspension was mixed with 0.5 ml of SRBC and incubated for 15 min at 37°C.

3. Then it was kept at 4°C overnight.

4. 1 ml of 2% glutaraldehyde was added and test tube was kept in ice for 15 minutes.

5. Finally the wet preparation was made and stained with 0.2% methylene blue and 200 cells were counted.

Three or more SRBC adhering to lymphocytes were taken as rosette forming cells. The absolute T-cell count was calculated as below:

\[
\text{Absolute T cell count} = \frac{\text{ALC} \times \% \text{ of T cells}}{100}
\]

(C) **DEMONSTRATION OF B CELLS (EAC ROSETTE FOR B CELLS)**

1. SRBC were washed thrice with PBS and adjusted to a concentration of 5%.

2. 0.5 ml of 5% SRBC was added to 0.5 ml of antisheep haemolysin (Amboceptor) and incubated for 15 minutes at 37°C. Subhaemolytic dose of amboceptor was assessed before putting the test (Cruickshank, 1975).

3. SRBC were washed thrice with PBS and suspended with 0.5 ml of PBS.

4. 0.5 ml of 1:10 dilution of complement guinea pig added to SRBC and incubated for 45 minutes at 37°C.
5. These SRBC (ie EAC now) were washed thrice with PBS and suspended to make 0.5% concentration in PBS.

6. 0.5 ml of lymphocytes were added to 5 ml of 0.5% of EAC in PBS and incubated at 37°C for 30 minutes.

7. The solution was suspended and wet preparation was prepared and stained with 0.2% methylene blue and then 200 cells were counted.

Three or more SRBC adhered to lymphocyte were considered to be rosette.

Absolute B cell count was calculated as follows:

\[
\text{Absolute B cell count} = \frac{\text{ALC} \times \% \text{ B cell}}{100}
\]