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Effect of Preformed Immune Complexes on the Course of *Mycobacterium leprae* Infection in Normal Mice

TO THE EDITOR:

Normal mice infected with *Mycobacterium leprae* in the foot pads show a limited multiplication. Dissemination of infection is seen in immunosuppressed animals, i.e., the thymectomized and irradiated ([9]) and nude mouse ([7]) and the nude rat ([4]). The severe immunosuppression seen in these animals does not simulate human leprosy before or during the course of the disease. Out of the many known and unknown factors operating in the causation of the depressed cell-mediated immunity in lepromatous leprosy, one is the presence of immune complexes (IC). The IC, in turn, are capable of producing specific immunodepression by blocking the recognition of *M. leprae* antigen by lymphocytes. In addition, IC might have multiple other effects on the whole array of immuno-competent cells ([2]) and, subsequently, dissemination of infection may involve other systems.

In the present study, 4-week-old, closely bred, Swiss albino mice (Lacca strain) were divided into two groups: Group I, composed of 55 normal control mice, and Group II, composed of 55 mice which were inoculated in the right foot pad with *M. leprae* (1×10^4 AFB/foot pad) obtained from biopsy specimens of untreated lepromatous patients. Both the control (NC) and infected (NI) mice were each divided into two batches, i.e., 0dIC (N = 30) and 3mIC (N = 25), depending upon the period at which IC were administered: at zero day (0d) or at 3 months (3m) post-inoculation with *M. leprae*. A large number of animals were used as a precautionary measure because of their high mortality rate. Five mice each were sacrificed periodically at 3-, 6-, and 9-month intervals from the 0dIC batches and at 6- and 9-month intervals from the 3mIC batches, since the data for the remaining period were obtained from a study conducted and published earlier ([13]) wherein the procedures for foot pad inoculation, harvesting, and counting of bacilli were also mentioned.

Freeze-dried *M. leprae* (obtained as a gift from IMMLEP) were sonicated to prepare antigen ([6]), and healthy rabbits were immunized to raise the anti-*M. leprae* serum (AMLS). The production of antibodies against *M. leprae* antigens was confirmed by immunodiffusion. The zone of equivalence (ZE) was determined as described by Roitt ([10]). The tube containing 240 μg of *M. leprae* antigen gave maximum precipitate and was taken as the ZE. Twice the ZE gave complexes in 2× antigen excess. AMLS was adsorbed with homogenized human skin tissue and also with *M. bovis* (BCG), *M. tuberculosis* (H37Rv), and *M. avium*. IC were prepared *in vitro* by incubation of the *M. leprae* antigen and the AMLS. IC in 2× antigen excess (1 mg protein) were administered in 0.3 ml of saline intravenously (i.v.) every week for 1 month starting at day 0 and at 3 months after *M. leprae* inoculation into foot pads.

The kidney, liver, sciatic nerve, skin, earlobes, snout, and tail tip of all sacrificed animals were fixed in 10% Formol saline.

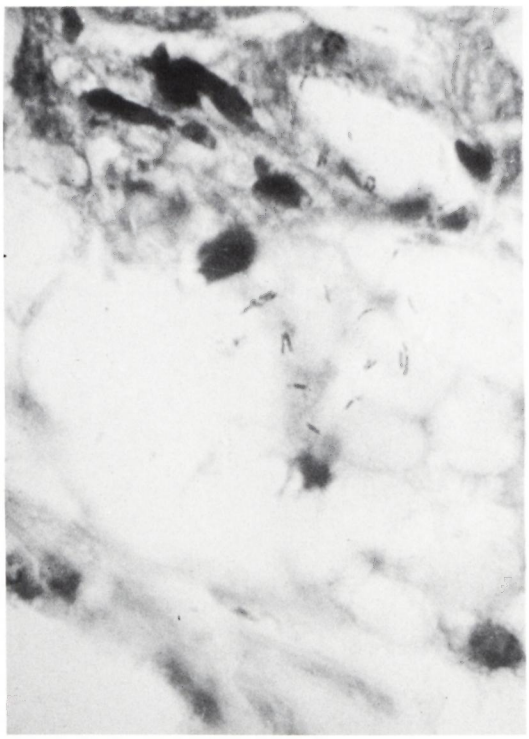

FIG. 1. Kidney section of mouse given human *M. leprae* inoculation and preformed immune complexes. Numerous AFB are seen in the renal parenchyma (Fite-Faraco ×330).

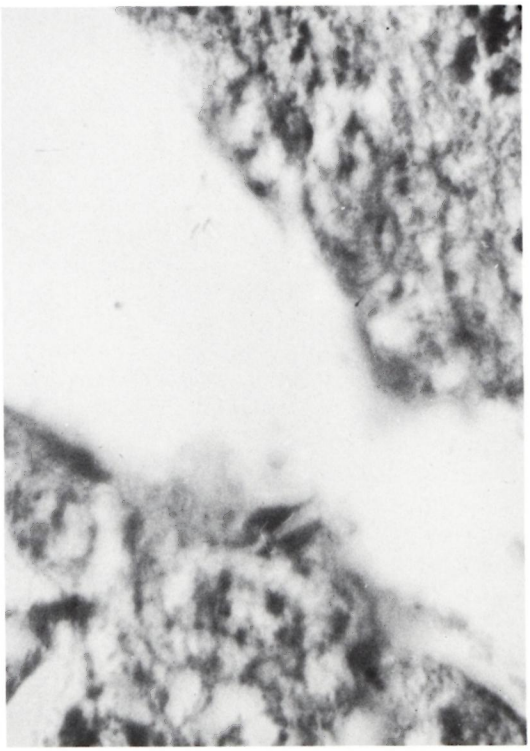

FIG. 2. Liver section of mouse infected with human *M. leprae* and given preformed immune complexes. A pair of AFB is seen in the central vein (Fite-Faraco ×330).

Skin tissue was taken from the right hind leg close to the inoculated site.

In the NI group, data for which were published earlier ([13]), acid-fast bacilli (AFB) recovered from pooled homogenate of five foot pads could not be counted since only a few bacilli were seen in the smears and none were seen in the pin-head stamps. However, there was a steep rise in the AFB counts at 6 months (3.40×10^5/foot pad) which remained stable up to the 9-month harvests (3.04×10^5 AFB/foot pad). Infection was established early in the NI-0dIC batch, i.e., at 3 months we were able to see bacilli without much difficulty (mean 8.40×10^4 AFB/foot pad). An increased number of AFB could be harvested at the 6-month sacrifice (mean 4.65×10^6 AFB/foot pad) which maintained a plateau up to 9 months (mean 1.79×10^6 AFB/foot pad). Except for the early detection of infection at 3 months, the growth pattern was similar to the NI group. Administration of IC at 3 months did not bring about any appreciable increase in bacillary counts when compared to the NI/0dIC batch at 9 months; counts at the 6- and 9-month harvests were 4.48×10^5 and 5.99×10^5 AFB/foot pad, respectively. No growth was seen in nutrient agar and Löwenstein-Jensen media inoculated with foot pad homogenates.

In the NI group a few AFB were found in 40% of the kidneys at 9 months. Sixty percent of sciatic nerves had a few AFB at 3 and 6 months. At no time were AFB detected in the skin and liver tissues. However, 40% of the animals showed solitary AFB in the earlobes and in only one animal at the nose tip at 9 months.

However, we were surprised to see numerous AFB in parenchyma (Fig. 1) and attached to walls of tubules of 20%, 40%, and 60% of the kidneys at 3-, 6-, and 9-month sacrifices, respectively. The AFB were found in both the cytoplasm of histiocytes and extracellularly. Histopathologically, interstitial nephritis, including periarteritis and periglomerulitis, was also seen

in association with AFB dissemination. A few AFB were detected in 40% and 60% of the sciatic nerves and the skin at 6 and 9 months, respectively. The skin of 20% of the animals at 3 months and the liver (Fig. 2) at each period of study were positive. Focal hepatitis with lymphomononuclear cells and histiocytes were seen in 40% and 20% of the animals at 6 and 9 months, respectively. A few AFB in the tail tip of one animal at both 3 and 6 months and two animals at 9 months were seen. In the NI/3mIC batch, a fairly large number of scattered AFB could be detected in the kidneys (20%) at both harvests. A few bacilli were also seen in 20% of the sciatic nerves and skin. A good number of AFB in the liver and a few AFB in the earlobes, nose tip, and tail tip were seen in one animal each at both 6 and 9 months. In the NC animals, no change was observed in any of the above tissues.

Very little work has been done on the role of IC in the pathogenesis of leprosy in spite of their circumstantial association with erythema nodosum leprosum (ENL) ([5, 14]). Hypergammaglobulinemia ([3]) results in the combination of specific antibodies with *M. leprae* or host antigens and consequently the production of IC. In the NI group, the infection was established at 6 months and followed a growth curve typical for *M. leprae* infection ([13]). The yields were not as high as obtained by Rees and Weddell ([9]). Variations in the maximum number of AFB obtained and time variables for the infection have been described ([11]).

AFB could be detected in the various tissues of mice given IC and *M. leprae* inoculation as early as 3 months; whereas in NI mice not administered IC, AFB were seen around 1 year as was reported earlier ([12]). IC seemed to be more effective in the production of immunosuppression when given simultaneously with *M. leprae* inoculation, as seen in the NI/0dIC batch.

From this study it may be envisaged that IC have an immunosuppressive role. Antigenically related mycobacteria may sensitize an individual to *M. leprae* antigens and/or modulate the immune response to a subsequent leprosy infection. If they are related to *M. leprae,* contact even with saprophytic mycobacteria in the environment might be important ([8]). Clarification of antigenic crossreactivity of *M. leprae* to the other mycobacteria will help in exploring the immuno-epidemiology of leprosy ([1]).

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Mycobacterium leprae, Probably a Microbe-dependent Microorganism

TO THE EDITOR:

Experimental evidence supports the recently advanced concept that *Mycobacterium leprae* is probably a unique microbe-dependent microbe ([2, 4, 6]). Results led to the formulation of a new culture medium, permitting *in vitro* multiplication of bacilli derived from armadillos infected with *M. leprae* ([3, 4]). The proposed multifactorial culture medium (MFM) was based on the recently acquired knowledge that getting *M. leprae* to grow is a multifactorial problem ([1, 3]). The slow growth of the bacilli in a deep liquid medium, however, was difficult to assess and not satisfactory for practical purposes as a tool for diagnosis, drug sensitivity, metabolic studies, and as a pharmacological model for detecting new antileprosy drugs. Experiments were aimed at achieving visible growth on solid media, accelerating growth rate, increasing yield, and providing further evidence for the validity of the claim that getting *M. leprae* to grow is dependent on a supply of growth factors (mycobactin and exochelin) from secondary mycobacteria. Results just available now bring further evidence that growth of those bacilli from armadillos infected with *M. leprae* is dependent on the above growth factors, but that a still-unidentified third growth factor might contribute to considerable yield of cell mass, and finally that a visible growth can be achieved on the surface of agar slants of the multifactorial media.

An iron-poor Sauton-Tween liquid medium contained asparagine 4 g, citric acid 2 g, K_2HPO_4 0.5 g, $ZnSO_4$ 0.04 g, $MgSO_4$ 0.4 g, glycerol 40 ml, and Tween 80 10 ml in 1 liter of distilled water. It was adjusted to pH 7.0 with NH_4OH. The solution was distributed 200 ml/flask and autoclaved for 30 min. The media were inoculated with *M. phlei* and incubated at 37°C until a thick, heavy growth developed on the surface of the medium. The flasks were sonicated in a Bronsonic sonicator (20–90 kilocycles frequency) for 10 min. The cultures were autoclaved for 30 min, and filtered while hot through Whatman 802 filter paper. The filtrate was adjusted to pH 5.8 with KH_2PO_4. Na thioglycolate 1.5 g, thioctic acid 0.05 g, and β-cyclodextrin 1.5 g were collected in a mortar, mixed, and ground for 5 min. The fine powder was dissolved in the mortar with small amounts from the filtrate and added to the collected filtrate. Ferric ammonium citrate 0.05 g and $MgSO_4$ 0.1 g were also dissolved in the filtrate. The solution was readjusted to pH 5.8 and distilled water added to the volume of 1 liter. Readjustment to pH 5.8 was required; 500 ml of the liquid MFM was autoclaved 30 min. In 500 ml of the medium 15 g agar (Difco) was dissolved and 20 ml was distributed into each 50-ml screw-cap tube. Autoclaved for 30 min, the media were cooled to make agar slants.

Armadillo-grown *M. leprae* cells were collected, prepared, and semi-purified aseptically, using the liquid MFM. The cell suspension was centrifuged at 3000 rpm for 15 min. Two 5-mm diameter loopsful of the