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TISSUE REACTIONS TO LEPROMIN IN NORMAL ANIMALS*

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Reactions to lepromin are generally conceded to have importance as an aid to clinical judgment but there is controversy about their nature. Are these reactions immunologic in origin? To which main component of this complex material, bacilli or dermal tissue, does reactivity occur (1, 2, 3)? The study of such questions can best be done in animals. However, this adds the complications of the antigenic and phlogistic properties of treated human tissue components for other species and requires examination of the changes that the major individual constituents of lepromin may induce.

It is interesting to compare the histology at the site of the sensitizing injection and in the draining lymph node to that following exposure to other antigens which induce delayed hypersensitivity and antibody production (4, 5, 6, 7). This information may have practical importance also for experiments designed to determine whether sensitivity to lepromin may be transferred passively, by suggesting the optimal time for harvest and transfer of cells.

In the course of a study of reactions to lepromin, we have examined sequential events in the foot pads and draining lymph nodes of guinea pigs receiving lepromin or suspensions of dermis.

MATERIAL AND METHODS

Lepromin.—This was a purified bacillary suspension containing 4.29×10^8 bacilli per ml, as determined by a modification of Hank's method (8). It was made by enzymatic digestion of ground, epidermis-free lepromata.[1] A 1:3 dilution of this suspension was tested in lepromatous and tuberculoid patients, in healthy contacts, and in BCG immunized subjects. It produced reactions equivalent to those elicited by standard crude lepromin.

Dermis suspension.—Ground normal human dermis was prepared by the method used for making lepromin.

Vehicles.—Phosphate buffered saline, pH 7.3, with 0.5% phenol and 0.05% Tween 80 was the suspending medium for both lepromin and dermis. Incomplete Freund's adjuvant was purchased from Difco Laboratories.

Animals.—Albino, random bred guinea pigs were used, weighing from 300 to 700 grams at the beginning of the experiment. They were divided into two weight groups: 300–500 grams and 500–700 grams. Equal numbers of each were used for each experimental group.

Injections of 0.2 ml of test material were made intradermally in the right rear foot pad. The following groups were set up:

Group A: Lepromin 1:1 in incomplete Freund's adjuvant.

Group B: Dermis suspension 1:1 in incomplete Freund's adjuvant.

Group C: Diluent 1:1 in incomplete Freund's adjuvant.

Group D: Diluent alone.

There were twelve animals per group. Two from each group were examined at 3, 6, 11, 23, 42, and 112 days after injection. At the time of sacrifice, animals were weighed and the thickness of both rear foot pads was determined with calipers. Both pads were biopsied. The popliteal lymph nodes were dissected out, freed of fat, and kept in ice until weighed. Nodes and biopsies were fixed in buffered (pH 7.2) formalin, embedded in paraffin, and stained by the hematoxylin-eosin, Giemsa, Ziehl-Neelsen, and methyl green-pyronin methods (using for the latter a modification for formalin-fixed tissue (9)). Sections were made from the centers of the samples.

RESULTS

Body weights.—All animals showed moderate (about 5 per cent) loss of weight at 3 and 6 days, without significant differences among the groups. After this, all animals gained weight.

Foot pads.—Right and left pads do not differ appreciably in thickness in normal animals (Group D), nor do they show significant differences in animals of differing weights. The readings of the left (untreated) pads of all groups were similar to those of controls.

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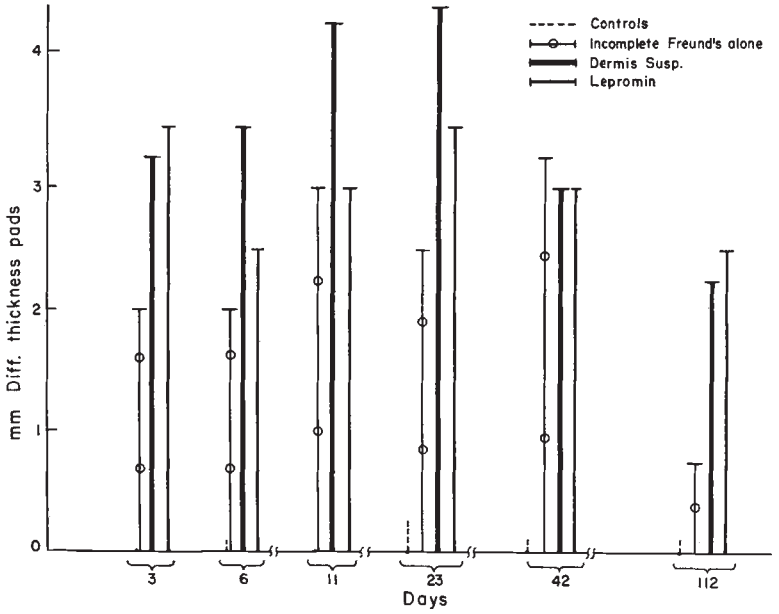
[1] Lepromatous tissue kindly supplied by Dr. Jacinto Convit, Head, División de Dermatología Sanitaria, M.S.A.S., Caracas, Venezuela.



GRAPH 1. Difference between thickness of right (stimulated) and left rear foot pads (mean values). Note the early increase in all groups, and the regression in the group that received Freund's incompete adjuvant alone.

There was no contralateral increase in thickness, as is seen, for example, in adjuvant disease in the rat.

As shown in Graph I, animals in Group C (incomplete adjuvant) developed appreciable local inflammation that reached a peak by 11 days, but tended to disappear by the end of the experiment. Local reactions in Groups A (lepromin) and B (dermis) also reached their highest levels by 11 days, but the readings remained elevated until the end of the experiment. The peak values in the dermis-injected groups were somewhat higher; but Group A animals showed firmer infiltration at the end of the experiment. In no case were there ulcerations and gross deformity as is seen when complete Freund's adjuvant is injected into the foot pads of guinea pigs.

Histology.—The left (untreated) foot pads of all animals as well as both rear foot pads of Group D (diluent alone) appeared normal (Fig. 1a). The overall histologic changes in the injected pads of groups A (lepromin), B (dermis) and C (water in oil alone) were similar (Fig. 1b, 1c), consisting of an infiltration predominantly of macrophages. With time, the macrophages increasingly appeared as pale cells with poorly defined borders and some became multi-nucleate (3 to 5 nuclei). These changes took place earlier and persisted longer in the sites injected with lepromin (group A). There was no mast cell infiltration and the scarcity of plasma cells was striking. Bacilli were identifiable in the lepromin injected sites throughout the experiment. They were phagocytosed beginning on the sixth day.

Lymph nodes.—In contrast to the thickness of the foot pad, the weight of the popliteal lymph nodes changed with the weight of the animal. This variation has been minimized by expressing results as milligrams lymph node/100 grams of body weight, since these values in normal animals tend to approach a constant. The differences in weight between left and right popliteal nodes in normal animals are insignificant. The results in the different groups are expressed as the difference between the mean values (mg lymph node/100 grams body weight) of the injected and noninjected sides. The latter did not differ appreciably from the control values (Group D).

Graph II shows that while there was a considerable increase in weight of nodes in all groups except D (diluent), Group B (dermis) generally showed higher values than did Group C (adjuvant alone), and Group A (lepromin)

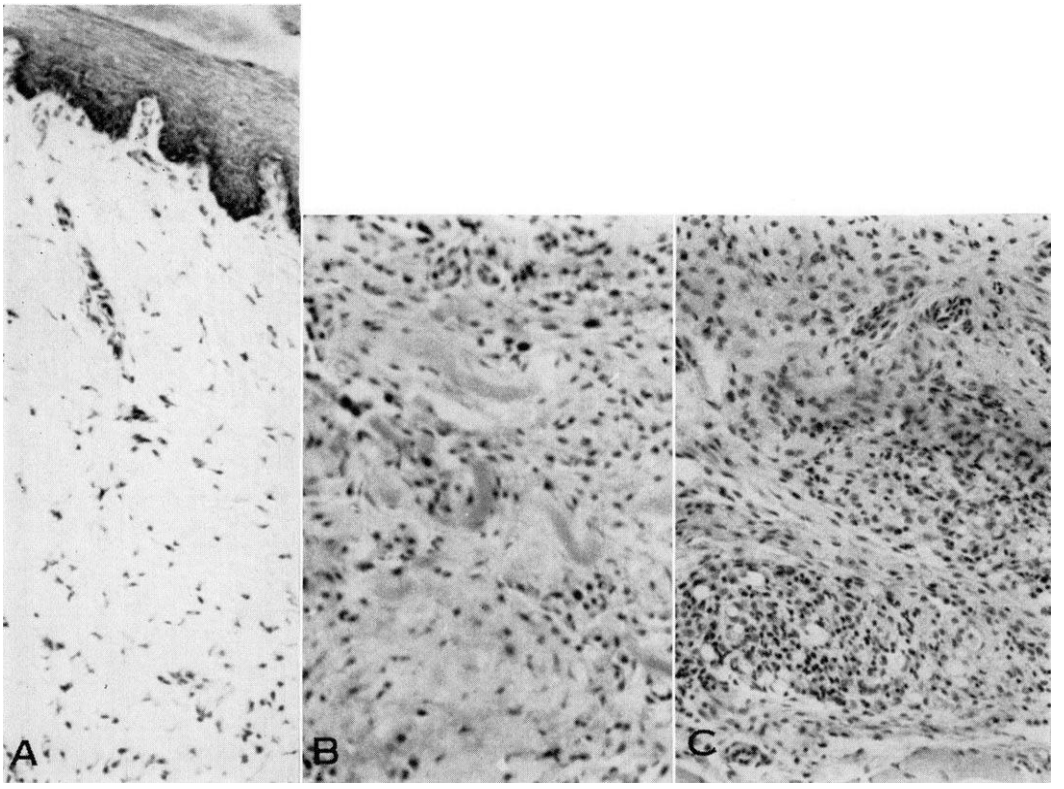
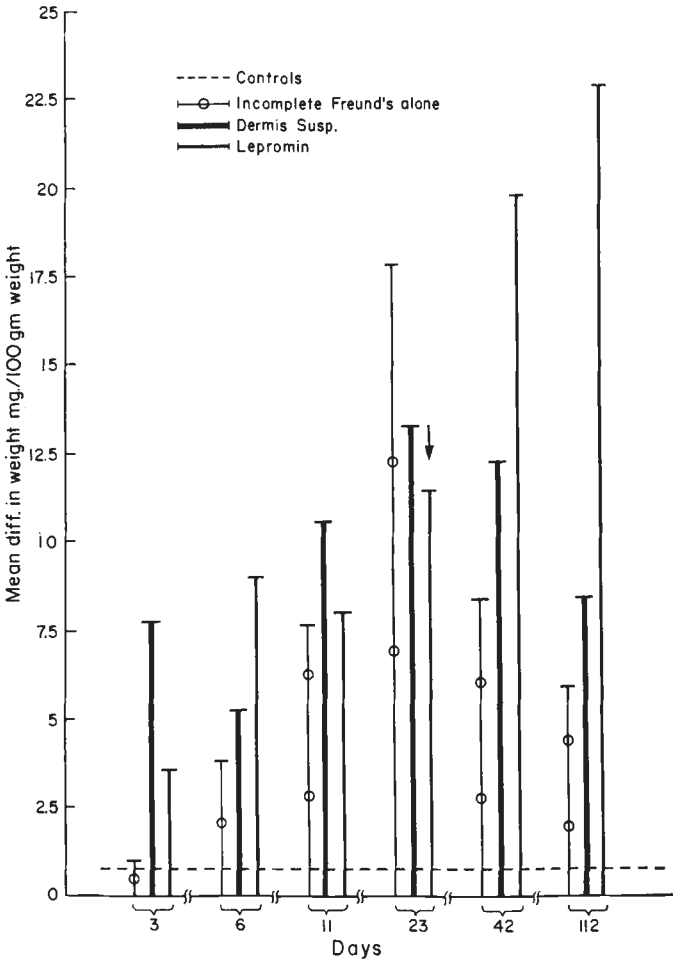


FIG. 1a. Normal foot pad: Thick epidermis; dermis rich in fibroblasts. Giemsa, × 36
FIG. 1b. Foot pad 112 days after injection of incomplete Freund's adjuvant. Predominance of collagen fibers and fibroblasts. Hematoxylin-eosin, × 36.
FIG. 1c. Foot pad 112 days after injection of lepromin: Vacuolated cell infiltrate surrounded by collagen strands. Hematoxylin-eosin, × 36.

developed the largest node response of all in the later stage of the experiment. Further, while peak values were reached at 23 days in Groups B and C, in Group A the nodes continued to enlarge throughout the experiment.

Histology of lymph nodes.—Group D (diluent): The histology of the nodes in this group is related to their weight, which varies with the size of the animal. Small nodes (<10 mg) tend to have a wide medulla and a narrow cortex with indistinct lymphoid follicles. A few polymorphonuclear cells are seen throughout the node, plasma cells appear in the medulla, and only few pyroninophilic cells occur in the cortex. Larger nodes (>10 mg) generally show a well-defined cortex with discrete follicles which can be quite rich in pyroninophilic cells and have germinal centers. These contained more plasma cells in the medulla than did the small nodes. In all of these larger nodes, there was good demarcation between cortex and medulla. There was no relation between the number of pyroninophilic cells in the node and the polymorphonuclear content, which was always small. There was no appreciable difference between injected and uninjected sides.

The left side nodes of all the remaining groups did not differ appreciably from those of normal animals of comparable weight. Thus, the significance of the histologic changes in a node on the side of injection was determined by comparison with the contralateral (left) node. In all the animals injected with emulsions (groups A, B and C) there were initial changes including the formation of oil lacunae, disruption of the general architecture of the node with blurring of the boundries between cortex and medulla, and seeming spill-over of



GRAPH 2. Differences in weight between stimulated (right) and contralateral popliteal lymph nodes. (Values expressed as the mean difference in mg/100 gm of body weight). Note the steadily increasing values in the lepromin group, contrasting with the rise and fall observed in other groups.

* One of the nodes measured here showed necrosis, which decreased its weight. Necrosis was not seen in nodes examined at any other time during the course of the experiment.

lymphoid cells from the former into the latter. Most importantly, there were increased numbers of pyroninophilic and plasma cells, the latter mainly in the medulla. Pale macrophages appeared in the medulla by the eleventh day.

The distinctions among these groups were these:

1) In the animals injected with adjuvant alone (group C) the changes were less marked, and the previously increased number of pyroninophilic cells had diminished considerably by 42 days, and had returned to normal levels by 112 days.

2) In the group injected with dermis in adjuvant (group B) the various changes were more marked than in the preceding group. The increased numbers of pryoninophilic cells, at their apex at 23 days, were still present, though markedly regressing at the end of the experiment (112 days).

3) In the animals of group A (lepromin) these changes were most intensive. The number of pyroninophilic and plasma cells reached a high point at 23 days and remained essentially unchanged thereafter (Fig. 2a, 2b). Bacilli

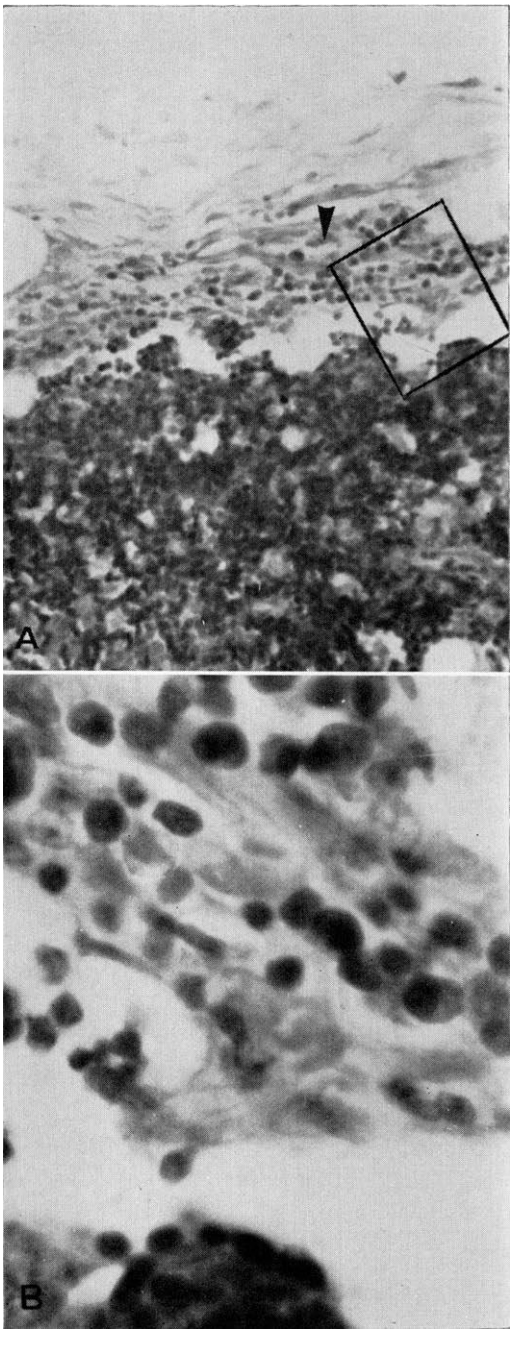


FIG. 2a. Lymph node 23 days after injection of lepromin: Cortex. Arrow indicates subcapsular accumulation of plasma cells, presumably originating from a follicle rich in pyroninophilic cells. This is an unusual occurrence. Methyl green-pyronin, × 152.

FIG. 2b. Area in rectangle in 2a, × 304.

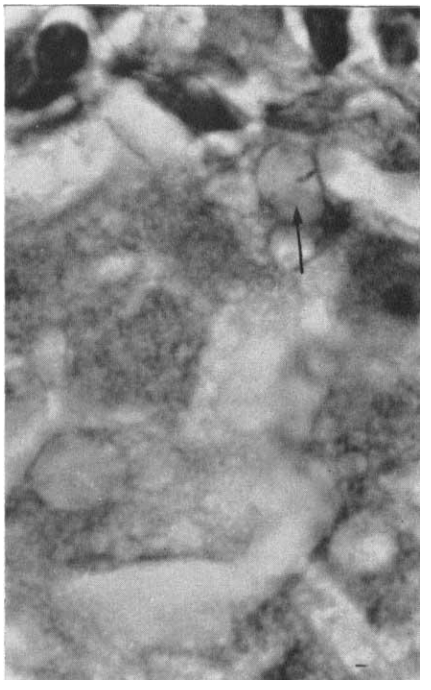


FIG. 3. Lymph node 112 days after injection of Lepromin: Phagocytosed leprosy bacillus still intact (arrow). Ziehl-Neelsen, × 400.

were identified during the entire experiment; phagocytosed by the 11th day (Fig. 3).

CONCLUSIONS

From these observations it is apparent that lepromin induced a more marked and persistent tissue response than did any of the other preparations used. This difference was particularly marked in the lymph nodes and those draining lepromin-injected sites showed a persistent increase in numbers of pyroninophilic cells in the cortex and of plasma cells in the medulla.

The lepromin-stimulated nodes never showed a massive epithelioid response. This fact, plus the persistence of the pyroninophilia, may justify the use of cells from these nodes in transfer experiments many weeks after initial sensitization, and not only during the first 2–4 weeks as is the case in tuberculin sensitivity (10).

The difference in histological response between these animals and those that receive complete Freund's adjuvant (10) may be due to the fact that the mass of bacilli in lepromin (estimated from their number) is much smaller

than that generally used for the induction of tuberculin hypersensitivity.

It can be concluded that the marked and persistent tissue response induced by lepromin is a result of the activity of its bacillary component.

SUMMARY

Lepromin and dermis suspension emulsified in incomplete Freund's adjuvant, as well as adjuvant alone and a buffered diluent, were injected into the right rear foot pads of different groups of guinea pigs. A sequential comparative study was made of the gross and histological changes produced by these substances in the foot pads and in the draining popliteal lymph nodes.

Lepromin produced the most marked and persistent response of any of the substances used. This was particularly striking in the lymph nodes, where an increase in numbers of pyroninophilic cells was conspicuous and persistent. It can be concluded that this difference was due to the bacillary component of lepromin.

The lepromin-stimulated lymph nodes did not show a massive epithelioid response or fibrosis, and had an evident increase in pyroninophilic cells as late as 112 days after inoculation. This may justify the use of cells from these nodes in transfer experiments many weeks after initial sensitization.

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