IMMUNE REACTIONS OF THE GUINEA-PIG TO *M. LEPRAE*[1, 2]

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In summarizing animal work in the immunology of leprosy, Taylor [10] remarked that certain features can be studied in animals, and that dogs and guinea-pigs have been used frequently.

A review of the literature of this subject, with the help of Kefer's [7] bibliographic abstraction of the entire subject of lepromin reactions (1960), makes it clear that experimental work in guinea-pigs has been moderately conditioned by a much larger volume of work in human beings in the effort to understand the meaning of "lepromin reactions." In particular, the antigenic materials used in guinea-pig studies have been preparations of leprosy tissues treated in a variety of ways before injection into animals, usually as "lepromin."

The present work has as its thesis the thought that reactions in guinea-pigs to living preparations of *M. leprae* could provide knowledge not to be obtained from the use of any of the standard or modified lepromins, i.e., the Hayashi [6], Wade [12], Fernández and Castro [3], Dharmendra [1], and other preparations. All of these are treated chemically or thermally, and immune responses, like those studied by Taylor *et al* [11], have been to these preparations. Do fresh preparations of tissue containing *M. leprae* behave in the same way? Four experiments are recorded here.

EXPERIMENT 1

A bacillus-rich leproma was removed aseptically from a patient who had had leprosy more than 20 years. After a period of improvement on sulfone therapy, reactivation had occurred, three years before removal. No antileprosy treatment had been given in the 40 days prior to this. Half of the leproma was preserved at 4°C and the other half at 37°C for 48 hours, to permit autolysis. Cultures for contaminating bacteria at this time showed no growth. Both specimens were then minced and hand-ground in saline, the final suspensions being in the proportion of 1:30 with respect to the original tissue, but in actuality somewhat more dilute because of incomplete suspension. Both showed numerous *M. leprae* on smear.

Forty guinea-pigs in two groups of 20 were inoculated with these suspensions, the volume of 0.5 ml. being administered in two locations subcutaneously in each animal. One group received the cold-preserved material, the other the autolyzed.

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A small transient response (Fig. 1) was seen in the animals receiving the 48-hour cold-preserved leproma at about 7-9 days (A). A similar response occurred a little earlier from the 48-hour autolyzed material (B). The response was not immediate. It took a few days to appear, and may have been due in part to inflammatory changes resulting from enzymatic digestion of introduced proteins other than those within the bacilli.

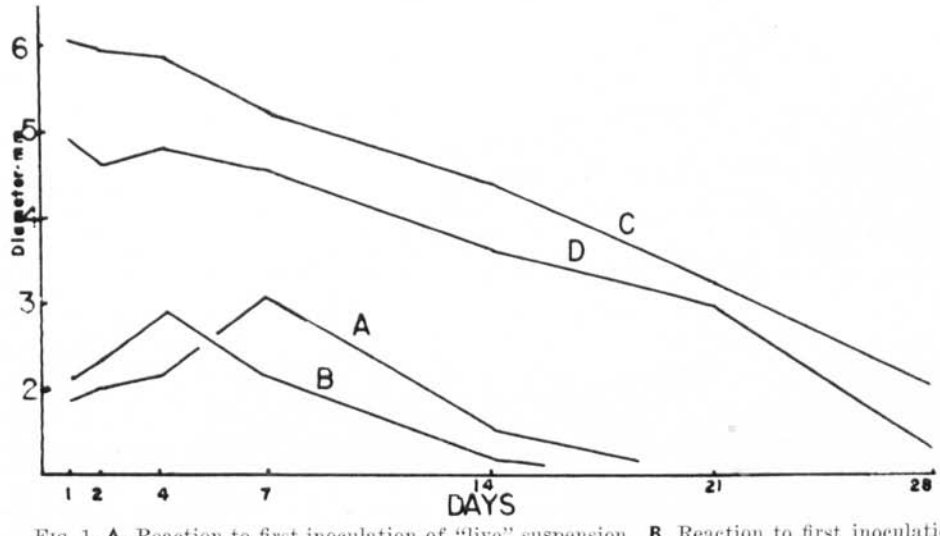

FIG. 1. **A.** Reaction to first inoculation of "live" suspension. **B.** Reaction to first inoculation of "autolyzed" suspension. **C.** Reaction at 3 weeks to second inoculation of "live" suspension. **D.** Reaction at 3 weeks to second inoculation of autolyzed suspension.

There is good agreement [11] that there is a tissue component in the lepromin reaction, although the nature of the reaction to tissue and tissue-products in the reaction is not clear. Our observations suggest that one moiety involved is a product of enzymatic protein digestion, either autolytic, or the result of host enzyme action on material inoculated. This reaction is mild, and occurs before an immune response is fully developed. It reaches its maximum in about seven days, and (in other experiments) has been observed when heat-treated tissues have been used as the inoculum. Significantly, too, it regresses more rapidly.

EXPERIMENT 2

The animals used in Experiment 1 were reinoculated with the same suspensions previously used, the suspensions having been preserved at 4° C. Again, all animals received two injections, one on each side, but the dose was smaller, 0.25 ml., and it was given intradermally. It is recognized that an intradermal inoculum of this size will, in the guinea-pig, be partly diffused into the subcutaneous area.

The results (Fig. 1) were manifestly different from the results of the first inoculation (C and D). Any "tissue response" like that occurring in the first group would be obscured, if present. The reactions de-

veloped fairly rapidly, reaching a maximum in 24 to 48 hours and regressing slowly thereafter. No Mitsuda-type response at three to four weeks was evident. If present in small degree, it is possible that it was masked by the continuing early change.

It is well shown from the many experiments of Taylor *et al.* [11] that this type of response can be elicited by a variety of prepared leprosy antigens. This particular experiment goes no further than to show that it is elicited by a single injection of lepromatous material, cold-preserved and otherwise untreated, given three weeks earlier.

A significant feature of this second inoculation was the effect upon half of the disappearing or disappeared lesions from the first inoculation. These reactivated and became larger within the first 48 hours, again gradually fading away in three to four weeks.

The prompt reactivation of these lesions resulting from the first inoculation is, perhaps, an evidence of what Taylor calls the "booster" effect of repeated inoculations. Well developed within 72 hours, even though the reactivations were not uniform and not seen in all animals, some were as marked as or even larger than they had been originally. The "booster" effect here must have resulted from an increased amount of circulating antibody stimulated by the second inoculation.

EXPERIMENT 3

In this experiment a leproma was obtained surgically from a never-treated case of leprosy. The suspension was prepared as in the first experiment, at 1:30, with some loss from sedimented coarse particles. Histologic sections of a part of the lesion showed it to contain large (almost maximal) numbers of bacilli, many in bundles. The process appeared to be very active.

An aliquot of this suspension was heated on the water-bath at 100°C for ten minutes. The unheated and the heated suspensions were inoculated (0.25 ml.) subcutaneously into separate groups of 16 guinea-pigs each. The inoculations were completed 90 minutes after the surgical removal of the leproma.

Two additional groups of animals were inoculated with a suspension of mycobacteria spp. BCG, 50 mgm./ml., the dosage being 0.25 ml. An aliquot of this suspension was heated at 100°C simultaneously with the aliquot of the suspension of leproma. These two suspensions were inoculated into two additional groups of 16 guinea-pigs each.

All animals, including those inoculated with BCG, showed a slight 7-day response, which rapidly disappeared.

Five weeks later, all animals were skin tested with (1) tuberculin (PPD, second strength), (2) the same heated suspension of leproma used for inoculation, preserved at 4°C, and (3) standard lepromin (Wade-Mitsuda).

The animals inoculated with live BCG all had positive tuberculin

reactions. The tuberculin reactions were negative in the other three groups.

The reactions of the animals inoculated with the fresh untreated leproma suspension are shown in Figure 2 (A). The response was of the delayed Mitsuda type, maximum at three weeks, although of small degree. It was slightly greater to the tests with the heated suspension than with the standard lepromin (B).

Reactions of animals inoculated with the heated suspension of leproma were too slight to chart. There was a very weak reactivity at five to seven days in those tested with the heated suspension, but none in the lepromin-tested group.

Animals first inoculated with heated BCG showed a weak response of the Mitsuda type to the later injection of the heated leproma suspension (Fig. 2, C). This was not observed in all animals, and its significance is doubtful. The live BCG provoked an early mild reaction at seven days (D).

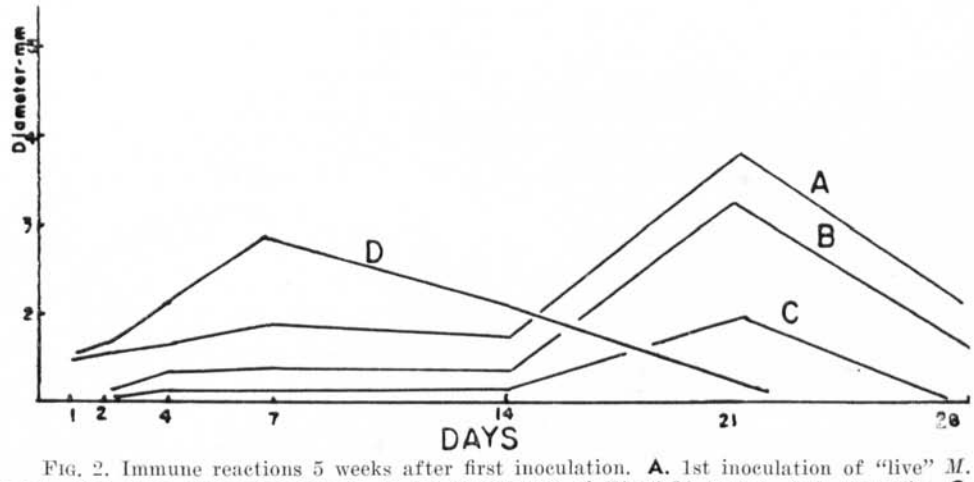

FIG. 2. Immune reactions 5 weeks after first inoculation. **A.** 1st inoculation of "live" *M. leprae*. 2nd, same suspension heated. **B.** 1st inoculation of "live" *M. leprae*. 2nd, lepromin. **C.** 1st inoculation of heat-killed BCG. 2nd, heated *M. leprae* suspension. **D.** 1st inoculation of live BCG. 2nd, heated *M. leprae* suspension.

EXPERIMENT 4

Following the death of a patient with lepromatous leprosy of long standing, who had avoided or neglected treatment for many years, the spleen, containing numerous organisms, was obtained at autopsy 5½ hours post-mortem. Portions were machine-suspended, and the heavy debris was permitted to settle for 24 hours and discarded. The supernatant was then centrifuged at 150 g. for 20 minutes, and the bottom 20 per cent also discarded. The supernatant contained large numbers of well-stained bacilli, and a fair amount of cellular debris, but few intact cells. No other bacteria were seen. The material had been handled with sterile glassware and instruments, and kept cold, but had not been aseptically removed from the body, and was presumed to be

mildly contaminated. An aliquot of the final suspension was heated at 100°C for 20 minutes.

The following day two groups of 16 guinea-pigs each were inoculated subcutaneously with 0.5 ml. of the unheated and heated suspensions, then 28 hours old.

RESULTS

There was a slight indurated reaction (2-3 mm.) at 48 and less at 96 hours at the sites of inoculation. This was gone in most animals at one week, and at two weeks the sites appeared nearly normal. No abscess formation, and no infection, occurred in either group.

At the three week reading all animals showed inflammatory lesions several mm. in diameter, and at four weeks these were elevated, bulging, and 12-20 mm. in diameter. In the animals receiving the unheated suspension, the lesions softened in their centers, and many suppurated and discharged their contents. In the other (heated suspension) group only a few suppurated, and the lesions were measurably smaller, although still very large. Microscopic preparations of intact lesions from each group of animals showed central purulent matter surrounded by granulation tissue containing poorly developed epithelioid cells. Acid-fast bacilli were numerous in the exudates, less numerous in the marginal cellular zone.

The lesions receded after five weeks and at eight weeks all had wholly regressed, with residual scarring in those which had suppurated.

All 29 surviving animals were lepromin-tested nine weeks after the original inoculation. Initial reactions were observed equally in both groups, which were largely gone at three weeks. There were no secondary Mitsuda-type responses.

DISCUSSION

The "tissue factor" is potentially more than one thing. If it is simple reaction to autolyzed cellular material, it appears early and disappears early. The violent reaction to human spleen shown at three to five weeks is probably a tissue-rejection phenomenon, which may have had its beginnings earlier than was grossly apparent. It was more intensely inflammatory when the tissue was untreated by heat or chemicals. Heating the tissue will lessen but not eliminate the reaction.

This tissue-rejection factor in the lepromin reaction will not be uniform in any series of lepromin reactions in man, because it will vary according to immune factors inherent within the population tested. It is possible that in the severe positive reactions in man the necrosis and suppuration are part of a tissue-rejection rather than of the "Mitsuda-reaction." One naturally wonders if the high incidence in man of positive reactions to lepromin made from *M. lepraemurium* represents in large part a rejection by human tissues of murine tissues. [9]

The tissue-factor is shown to be at least two-fold in nature. The

"Faria reaction," [2] and the observations of Mitsuda reactions in dogs and other animals, need seriously to be studied from this aspect. Floch, [4] and Kooij and Gerritsen [8] have also observed marked reactions to skin and liver in lepromin-positive individuals.

The reactivation of former sites by a second injection proves the existence of some circulating antibody, whether to tissue or to bacilli is not shown. Even though the first reaction had subsided, a good deal of antigenically active material remained at the site, needing only more circulating antibody to provoke further response. This suggests bacillary bodies rather than the tissue factor, as the residual antigen.

The antigenicity of whole *M. leprae* is of a low order, compared to that of *M. tuberculosis*, perhaps on a par with that of *M. phlei*. Nevertheless, it is demonstrable at this low level by a single injection of live bacilli, although the number of animals per test must be substantial, and the observations critical. It seems possible that much of the difficulty of analysis of "lepromin" activity could be controlled by using the least altered bacilli as the primary antigen. Many of the studies of leprosy antigens have not taken advantage of the fact that *M. tuberculosis hominis* in the living state has antigenic capacities exceeding those of heated preparations.

The importance of active antigen is shown by the differences between response to the second inoculations in Experiments 1 and 3.

	First	*Second*	*48-hour reaction to second*
Experiment 1	Live	Live*	Positive
Experiment 3	Live	Heated	Doubtful or absent

*Live suspension preserved at 4° C for 21 days.

Here there is a suggestion of greater antigenicity of the unheated suspended *M. leprae*. This is not an altogether original observation, because some of the experiments of Hadler and Ziti [5] suggest slight tuberculin sensitization of guinea-pigs by live *M. leprae*, although tuberculin (PPD) sensitization has not been observed here.

SUMMARY

When live preparations of *M. leprae* are used as the sensitizing antigens, weak reactions of the Mitsuda type are observed following a single immunizing inoculation. A single injection of the same suspension heated for ten minutes is ineffective. Other observations testify to a greater antigenicity of *M. leprae* minimally altered, than that of heated preparations.

The tissue-factor in lepromin reactions is seen as a dual mixture of early response to enzymatically-digested products and a later tissue-rejection immune phenomenon. When marked, it can easily mask true responses to leprosy antigens, or even prevent proper development thereof.

RESUMEN

Cuando preparaciones vivas de *M. leprae* son usadas como antígenos sensibilizantes, se observan reacciones débiles del tipo Mitsuda despues de una sola inoculación inmunizante. Una sola inyección de la misma suspensión calentada durante 10 minutos es inefectiva. Otras observaciones atestiguan la mayor antigenicidad del *M. leprae* minimalmente alterado, que aquel de las preparaciones calentadas.

En las reacciones leprominas el factor-tejido es visto como una mezcla mixta de respuesta temprana a los productos enzimaticamente digeridos y a un fenómeno de inmunidad taría de reyeccion-tejido. Cuando marcado, puede muy facilmente enmascarar las respuestas a los antígenos leprosos, o aun prevenir el propio desarrollo.

RÉSUMÉ

Lorsque des préparations de *M. leprae* vivants sont utilisées comme antigènes de sensibilisation, des réactions faibles du type Mitsuda sont observées à la suite d'une unique inoculation d'immunisation. Une seule injection de la même suspension chauffée durant 10 minutes est inefficace. D'autres observations témoignent du plus grand pouvoir antigénique de *M. leprae* lorsqu'il n'est altéré que de façon minime, par rapport aux préparations qui ont été chauffées.

L'intervention tissulaire dans les réactions à la lépromine est envisagée comme étant un mélange de deux phénomènes: une réponse précoce à des produits digérés par les enzymes, et un phénomène immun plus tardif consistant en rejet des tissus. Lorsque cette intervention est marquée, elle peut facilement masquer la réponse réelle aux antigènes de la lépromine, ou même empêcher le développement normal de cette réponse.

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