Sequential Histological Study of Lepromin Reaction[1]

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The lepromin test, after its initial description by Mitsuda in 1919 ([4]), has been extensively used throughout the world for assessment of the immune status of leprosy patients. Although several antigens are being used to elicit this reaction, in India the Dharmendra antigen ([2]) (obtained after chloroform and ether extractions of bacilli from skin nodules) is the one most widely used in leprosy patients and in the general population. Scant information is available about the nature of the reaction and the morphological changes taking place in the skin after the intradermal inoculation. The present study has been planned to observe the sequence of changes seen during the lepromin reaction in various types of leprosy and to attempt to understand the significance of these changes in the context of the immune status of the leprosy patient.

MATERIALS AND METHODS

Fifty-five leprosy patients were the subjects of the study. They included 12 cases of lepromatous leprosy (LL), 5 of borderline-lepromatous (BL), 16 of borderline-tuberculoid (BT), and 22 of tuberculoid (TT) leprosy classified clinically and histologically. There were only 2 females, the remaining 53 being males: 18 were in the age group 20–29; 26 were aged 30–39; 6 were aged 40–49; and 5 were 50 or older.

Dharmendra antigen, prepared by the method standardized at this Institute ([8]), was used for the skin test. The antigen was injected intradermally on the volar aspect of the forearm or on the back, well away from any lesions present. The reaction was measured at the following intervals: 24, 48, 72 hr and 5, 7, 8, 12, 14, 15, 20, 21, and 28 days after the injection. The erythema was measured with a caliper, taking the average of the greatest and least diameters. The induration was measured by the thickness of skin pinched by the caliper and subtracting from it the thickness of nearby normal skin. Biopsy specimens of representative samples of the skin reactions at different intervals of time ranging from 24 hr to 28 days were obtained. In LL cases a biopsy specimen was not obtained after eight days since there was no visible reaction. Some patients had more than one skin test, and it was possible in them to repeat the biopsy. However, the majority of the patients had only one skin test with Dharmendra antigen. The sequence of events was therefore built up by observations on the biopsy materials obtained from different patients at succeeding intervals of time. A total of 77 biopsy specimens were collected—12 from LL cases, 5 from BL cases, 35 from BT cases, and 25 from TT cases. The biopsy specimens were fixed in Zenker-formol solution for 18 hr, processed, and embedded in paraffin. Five micron sections were stained with hematoxylin and eosin (H&E) and Fite's modification of the Ziehl-Neelsen stain. The sections were examined without referring to the case notes so that a personal bias of the pathologist was avoided.

RESULTS

Erythema (Fig. 1)

In TT cases, significant erythema appeared by 24 hr and then disappeared slowly over the next week. This pattern was also seen in the BT cases, with the erythema being slightly more at all times and also persisting for a longer time period. In LL and BL cases, the erythema was insignificant.

Induration (Fig. 2)

In TT cases, maximum induration was seen at 24 hr, tapered off over the next week,

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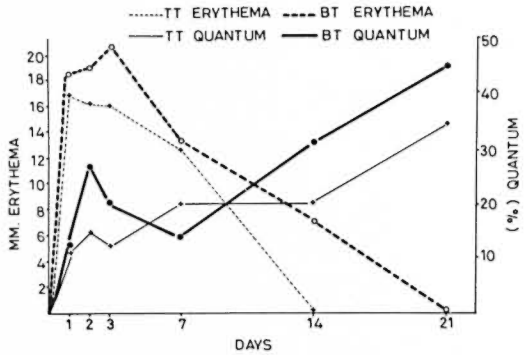

FIG. 1. Correlation of erythema and quantum of infiltration in TT and BT cases.

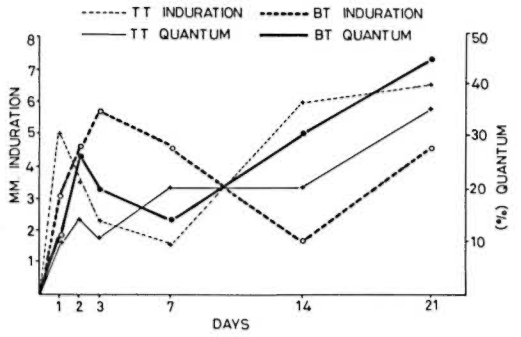

FIG. 2. Correlation of induration with quantum of infiltration in TT and BT cases.

and rose again to a peak by the 21st day. In BT cases, although a similar trend was followed, there was a time lag with the first peak at 48 hr. BL and LL cases did not have any significant induration.

Quantum of exudate

The amount of infiltrate was semi-quantitatively assessed in terms of the proportion of the dermis occupied by the exudate cells. The maximum amount of infiltration in the early reaction was seen in the BT group cases (Figs. 1 and 2) at the end of 48 hr. The quantum of exudate was less in the BT group at 24 hr and was less in the LL group when compared to the BL, BT, and TT cases. Subsequently, in LL and BL cases the amount of cellular exudates tapered off to insignificant levels by the end of the first week. In the BT and TT cases, after a slight decrease, the quantum of exudate rose to a peak at the end of the third week, corresponding with the late reaction (Figs. 1 and 2).

Edema

Edema at the microscopic level, as assessed by separation of collagen bundles, was a significant finding in the early reaction in all types of cases, particularly at 24 hr. Subsequently, it gradually diminished. In TT and BT cases, there was again a slight rise commencing from the 12th day onward during the course of the late reaction.

Location of exudate

The infiltrating cells were mainly localized in the superficial dermis. However, there was always a clear subepidermal zone separating the epidermis from the exudate cells, even in tuberculoid cases. In some cases the exudate was also found to extend deeper into the dermis, but in no case was the subcutis involved.

Distribution of exudate cells

The arrangement of cells could be described as three types: a) scattered, when the cells were diffusely strewn in the dermis; b) loose clusters, when they were generally found to aggregate loosely, and c) focal, when they were found in compact aggregates with a tendency to get circumscribed. In LL and BL cases, arrangement types seemed to be mostly either scattered or loose clusters in the first 72 hr (Fig. 3) with a tendency to aggregate subsequently into loose clusters. In the case of BT leprosy, there was better aggregation, with the cells forming either loose clusters or foci. On the other hand, in the tuberculoid cases there was an attempt at focalization even within the first 24 hr. By 72 hr the cells were essentially focal in distribution (Fig. 4). With the onset of the late reaction the influx of new cells resulted in loose clusters which also focalized by the end of the third week.

Relation to skin structures

The exudate was generally found around neurovascular bundles. The appendages were infiltrated in most of the cases with varying numbers of cells. An interesting finding was the relationship of the exudate to nerve twigs (Fig. 5). In 39 out of the 77 biopsies, prominent nerve sections were seen

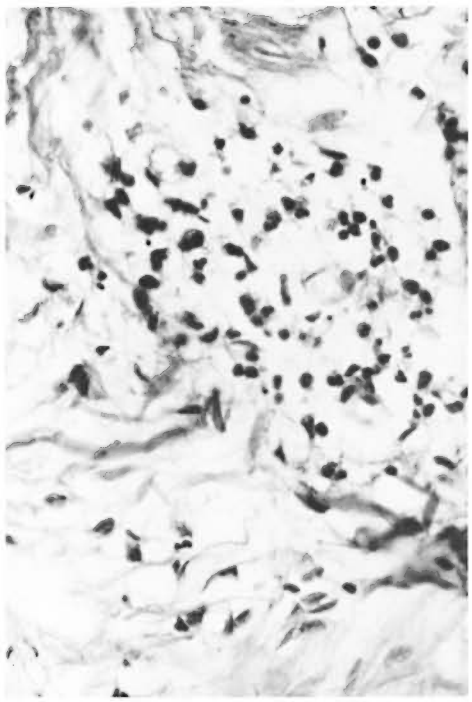

FIG. 3. Photomicrograph of a loose cluster of polymorphs and lymphocytes around a dermal blood vessel seen at 24 hr in a case of lepromatous leprosy (H&E ×630).

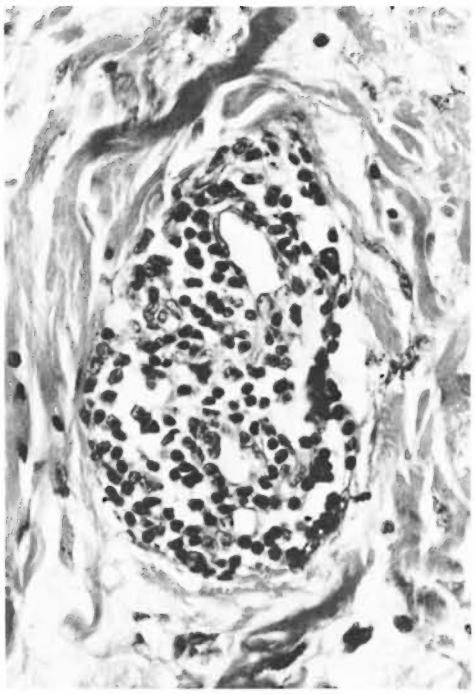

FIG. 4. Photomicrograph of reaction at 48 hr from a TT case showing evident focalization of lymphocytic infiltration (H&E ×630).

surrounded by cells which were mostly mononuclear. In the remaining 38 biopsies, nerves were not seen in the sections examined. In other words, in all cases where nerves were seen in the sections, exudate cells were found to surround them. The cells were present in close proximity to the nerves, without actually infiltrating or destroying them.

Composition of exudate cells (Tables 1–6)

24 hours. At the end of 24 hr the predominant cells that appeared at the site of lepromin reaction in all leprosy types were neutrophils (polymorphs) and lymphocytes (Fig. 6). This was seen not only in BT and TT cases, but also in LL cases (Fig. 3). In the LL cases the proportion of polymorphs and lymphocytes was about equal. There were slightly more polymorphs in the BT type, and in the TT type the lymphocytes were in higher proportions. Lymphoblasts were seen, particularly in the tuberculoid cases, and plasma cells in small numbers were seen in all the cases. An interesting finding was the occurrence of eosinophils, seen particularly in BL and LL cases.

48 hours. At the end of 48 hr polymorphs were still persisting in the LL and BL cases in almost equal proportions with lymphocytes. In the BT cases, the predominant cells were lymphocytes, and this was more obvious in TT cases (Fig. 4). Very small numbers of plasma cells were seen in all the types. Eosinophils persisted in the LL, BL, and BT types, with a large proportion in the LL types.

72 hours. At the end of 72 hr the polymorphs were not seen any more in any of the specimens examined, and lymphocytes were the predominant cells in all the cases. However, in the LL, BL, and BT types there were, in addition, some plasma cells. Small numbers of eosinophils were found in LL and BL types.

5/7/8 days. Lymphocytes were still the predominant cells in all types. A significant feature was the occurrence of epithelioid cells in BT and TT types (Fig. 7). Occasional eosinophils were still seen in the BT types,

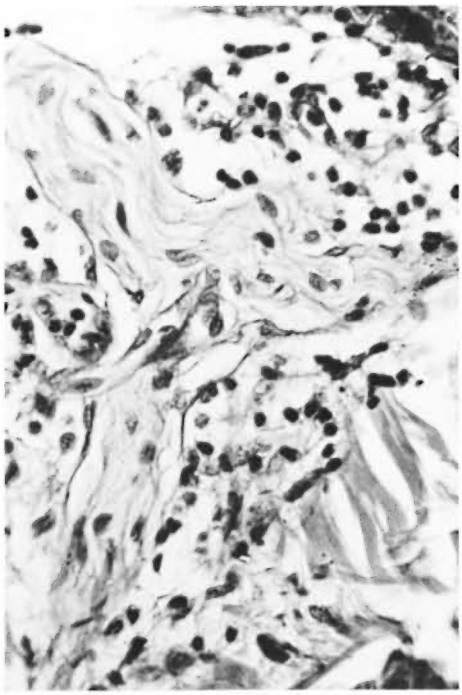

FIG. 5. Photomicrograph of reaction at 24 hr in a TT case showing infiltration of perineural areas by lymphocytes (H&E ×630).

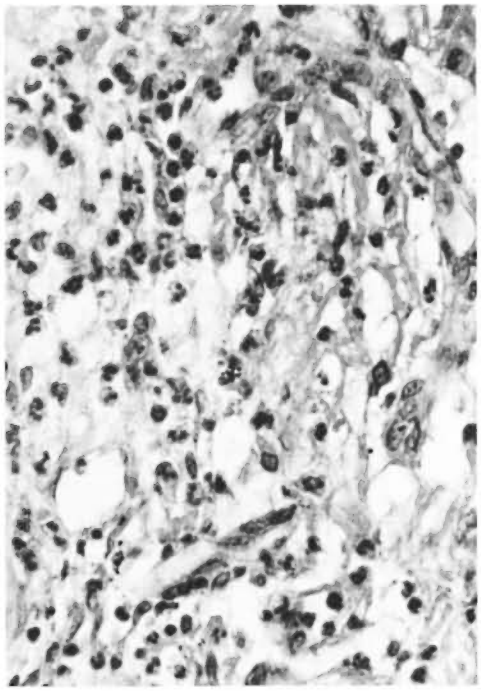

FIG. 6. Photomicrograph showing polymorphs in the infiltrate at 24 hr in a TT case (H&E ×630).

and occasional mast cells were seen in some of the sections examined.

12/14/15 days. The picture now was similar to that at the end of one week in the BT and TT types. Biopsy specimens were not available from the LL and BL types since biopsies were not performed because of the absence of any visible reaction.

20/21/28 days. The picture at the end of the third and fourth weeks was that of a chronic granuloma with epithelioid cells, lymphocytes, and giant cells. These were seen as focal collections and the picture was quite similar to that of tuberculoid granuloma.

Thus, the temporal distribution of the various cell types in the reaction was as follows:

Polymorphs were present at 24 hr and persisted up to 48 hr especially in the LL,

TABLE 1. *Proportionate distributions of inflammatory cells. 1. Neutrophils.*

Type of leprosy	Time after Dharmendra lepromin applied (days)					
	1	2	3	7	14	21
LL	1.8[a]	2.0	0	0	N.D.[b]	N.D.
BL	N.D.	1.1	0	0	N.D.	N.D.
BT	2.6	1.3	0	0	0.3	0
TT	1.1	0	0	0	0	0

[a] Mean values of cell frequencies on the following scale: 0 = no cells; 1 = few cells; 2 = moderate number of cells; 3 = predominant cell type.
[b] N.D. = Not done.

TABLE 2. *Proportionate distribution of inflammatory cells. 2. Lymphocytes.*

Type of leprosy	Time after Dharmendra lepromin applied (days)					
	1	2	3	7	14	21
LL	1.5[a]	1.5	2.2	2.0	N.D.[b]	N.D.
BL	N.D.	1.5	3.0	3.0	N.D.	N.D.
BT	1.6	2.3	3.0	2.7	2.0	2.0
TT	2.1	3.0	2.5	3.0	2.5	2.2

[a] Mean values of cell frequencies on the following scale: 0 = no cells; 1 = few cells; 2 = moderate number of cells; 3 = predominant cell type.
[b] N.D. = Not done.

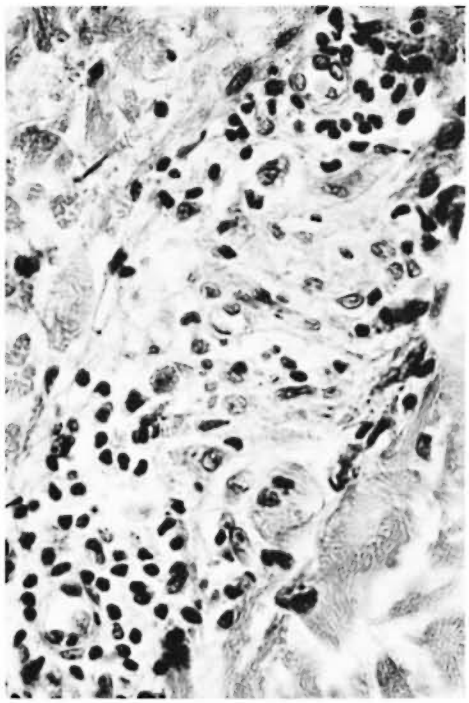

FIG. 7. Photomicrograph showing epithelioid cells in the infiltrate at 7 days in a TT case (H&E ×630).

BL, and BT cases. Plasma cells were seen in all types in varying numbers at 48 hr and 72 hr. Eosinophils were seen throughout the early reaction, particularly in the LL and BL cases.

Lymphocytes were present in good numbers at 24 hr, and at 72 hr they were the predominant cell type seen. Even in LL and BL cases, although the total quantum of exudate was less than that seen in BT and TT cases, the presence of lymphocytes was seen throughout the period of early reaction. In TT and BT cases the lymphocytes were always the predominant cells after 24 hr.

Epithelioid cells made their appearance by the end of the first week in the BT and TT cases.

TABLE 3. *Proportionate distribution of inflammatory cells. 3. Lymphoblasts.*

Type of leprosy	Time after Dharmendra lepromin applied (days)					
	1	2	3	7	14	21
LL	0.4[a]	0	0	0	N.D.[b]	N.D.
BL	N.D.	0	0	0	N.D.	N.D.
BT	0.8	0.7	0	1.4	0	0
TT	1.2	1.4	2.0	0	0.5	0

[a] Mean values of cell frequencies on the following scale: 0 = no cells; 1 = few cells; 2 = moderate number of cells; 3 = predominant cell type.
[b] N.D. = Not done.

TABLE 4. *Proportionate distribution of inflammatory cells. 4. Plasma cells.*

Type of leprosy	Time after Dharmendra lepromin applied (days)					
	1	2	3	7	14	21
LL	0.3[a]	0	0.8	1.0	N.D.[b]	N.D.
BL	N.D.	0.8	0.5	1.0	N.D.	N.D.
BT	0.4	0.5	0.5	0	0	0
TT	0.4	0	1.0	0	0	N.D.

[a] Mean values of cell frequencies on the following scale: 0 = no cells; 1 = few cells; 2 = moderate number of cells; 3 = predominant cell type.
[b] N.D. = Not done.

TABLE 5. *Proportionate distribution of inflammatory cells. 5. Epithelioid cells.*

Type of leprosy	Time after Dharmendra lepromin applied (days)					
	1	2	3	7	14	21
LL	0[a]	0	0	0	N.D.[b]	N.D.
BL	N.D.	0	0	0	N.D.	N.D.
BT	0	0.3	0.5	1.7	2.5	3.0
TT	0	0	0	1.5	2.5	3.0

[a] Mean values of cell frequencies on the following scale: 0 = no cells; 1 = few cells; 2 = moderate number of cells; 3 = predominant cell type.
[b] N.D. = Not done.

TABLE 6. *Proportionate distribution of inflammatory cells. 6. Eosinophils.*

Type of leprosy	Time after Dharmendra lepromin applied (days)					
	1	2	3	7	14	21
LL	0.5[a]	2.0	0.2	0	N.D.[b]	N.D.
BL	N.D.	1.0	0.5	0	N.D.	N.D.
BT	0.1	0.7	0	0.1	0	0
TT	0	0	0	0	0	0

[a] Mean values of cell frequencies on the following scale: 0 = no cells; 1 = few cells; 2 = moderate number of cells; 3 = predominant cell type.
[b] N.D. = Not done.

DISCUSSION

The lepromin test is characterized by two types of reaction: the early reaction seen between 24–72 hr, and the late reaction between 21–28 days ([7]). The term "early reaction" was originally introduced because this was the first of the two types of skin reaction. However, it creates a confusion in view of the present terminology for allergic skin reactions because this reaction is virtually the delayed type of hypersensitivity reaction (Type IV of Coombs and Gell[1]).

The early reaction is characterized by a well-defined area of erythema, edema, and induration. It has been the practice of several groups of workers in India, including the authors, to measure both erythema and induration as parameters to assess the intensity of the reaction. One of the objectives of the present study was to find out which of these parameters better reflects the intensity of the reaction. Presuming that the quantum of exudate (expressed as the proportion of dermis occupied) reflects the intensity of the reaction, a correlation has been sought between the quantum of exudate on the one hand and erythema and induration on the other. In TT and BT cases, both erythema and induration correlate well with the quantum of exudate in the first 72 hr (Figs. 1 and 2). Subsequently, both parameters decrease and during the development of the late reaction, it is the induration which rises concurrently with the quantum of exudate to a peak at the end of the third week. Thus, in the early reaction, both erythema and induration are good parameters of the reaction and as such support our practice to record both. However, in the late reaction it is only the induration that is to be measured.

The present study has revealed certain characteristic histological features. There is a collection of exudate cells with varying amounts of fluid, mainly in the superficial part of the dermis. The subepidermal zone which is characteristically infiltrated in tuberculoid types of leprosy is not involved in the course of the lepromin reaction in any type of leprosy. It is also interesting to note that even in the BL and LL cases where the lepromin is graded as negative, a good number of exudate cells were seen to collect in the dermis in the first 72 hr. However, while there is an increase in exudate cells in BT and TT cases subsequently, there is a sharp diminution in the BL and LL cases. Also, there is a striking difference in the distribution and type of cellular response. The exudate cells in the first 24 hr are found loosely scattered in all types of leprosy. In the BT and TT types they tend to aggregate into focal clusters; while they remain as loose collections or as scattered cells in the BL and LL cases. Since in the lesions of TT and BT cases the cells of the granuloma form focal collections, it is presumed that focalization of the cells reflects a good cell-mediated immune response.

The exudate is generally found around neurovascular bundles and skin appendages. A striking finding was the focal collection of cells around nerves without any significant infiltration of the nerves themselves. This finding of constant nerve involvement would raise the possibility that nerve tissue is in some way associated with the immunological process of the lepromin reaction. Further work needs to be done to find out the possible role of nerves in delayed hypersensitivity reactions in leprosy, particularly in view of the special predilection of *Mycobacterium leprae* for nerves ([3]).

Lymphocytes are the predominant cells seen in the course of the early reaction in the TT and BT cases. This reflects the Coombs' Type IV delayed hypersensitivity reaction in these types of leprosy. However, the presence of a good number of polymorphs could be an indication of a concomitant Arthus-like reaction along with the Type IV reaction. The activation of the complement system through the alternate pathway by *M. leprae* could also be responsible for the polymorph influx in the early reaction ([6]).

A consistent observation in the present study is a stronger response in the early reaction in BT cases compared to the TT cases, taking into consideration the erythema, induration, edema, and quantum of exudate. No published data are available on the comparison of intensity of lepromin reaction between TT and BT cases. Myrvang, *et al.* ([5]) in a study of lymphoblast transformation found that in both TT and BT types there were strong responders as well as nonresponders. Thomas, *et al.* ([9]) have reported stronger lepromin reaction in TT cases. In

another concurrent study of reactional states in leprosy, we have consistently found a strong early reaction in BT cases, generally stronger than in TT cases (unpublished data). This suggests that there is a stronger element of hypersensitivity in BT cases which could lead to acute nerve damage. This fact should be borne in mind by clinicians when dealing with BT cases with strong early lepromin reactions.

SUMMARY

Skin testing with Dharmendra antigen was performed on 55 patients with TT, BT, BL, and LL types of leprosy and the reaction measured at different intervals from 24 hr to 28 days. At various time intervals, a biopsy specimen was taken from the reaction site. In TT and BT cases, the erythema was maximum at 48 hr; while the induration was maximum at 21 days. The sequence of the histological changes was built up on the observations made from different cases at varying intervals. The quantum of cellular exudate was high in TT and BT cases as compared to BL and LL cases. The cellular distribution showed loose scattering of cells in the LL and BL types and attempts to form tight clusters in the TT and BT cases. Neutrophils were predominant during the first 48 hr, particularly in the LL, BL, and BT types. By 72 hr the cells were mainly lymphocytic. A tendency for the lymphocytes to cluster around nerve twigs was seen in the TT and BT cases. In the early reaction the quantum of exudate correlated both with erythema and induration; while in the late reaction, it correlated with induration only. The intensity of the early lepromin reaction was more in BT than in TT leprosy, while the induration in the late reaction was more in TT than in BT types. The significance of these findings is discussed.

RESUMEN

Se hicieron pruebas intradérmicas con el antígeno de Dharmendra en 55 pacientes con lepra de los tipos TT, BT, BL o LL, y los resultados se midieron a diferentes intervalos desde las 24 horas hasta los 28 días. A diferentes periodos de tiempo se tomaron biopsias del sitio de reacción. En los casos TT y BT, el eritema fue maximo a las 48 hs mientras que la induración fue maxima a los 21 días. La secuencia de cambios histológicos se construyó a partir de las observaciones hechas en los diferentes casos a intervalos variables. La cantidad de exudado celular fue grande en los casos TT y BT y menor en los casos BL y LL. La distribución celular mostró una dispersión laxa de las células en los tipos LL y BL, y una tendencia a formar grupos apretados de células en los casos TT y BT. Los neutrófilos fueron las células predominantes durante las primeras 48 hs particularmente en los tipos LL, BL y BT. Hacia las 72 hs, las células fueron principalmente linfocíticas. En los casos TT y BT se observó una tendencia de los linfocitos a agruparse alrededor de los haces nerviosos. En la reacción temprana la cantidad de exudado correlacionó tanto con el eritema como con la induración mientras que en la reacción tardía sólo correlacionó con la induración. La intensidad de la reacción temprana a la lepromina fue mayor en la lepra BT que en la lepra TT mientras que la induración en la reacción tardía fue mayor en TT que en BT. Se discute el significado de estos hallazgos.

RÉSUMÉ

Chez 55 malades atteints de lèpre et appartenant au type TT, BT, BL et LL, on a procédé à une épreuve cutanée avec l'antigène de Dharmendra; la réaction a été mesurée à différents intervalles entre 24 heures et 28 jours. A différents moments, une biopsie a été pratiquée à l'endroit de la réaction. Chez les cas TT et BT, l'érythème atteignait son maximum après 48 heures, alors que l'induration quant à elle était maximum après 21 jours. La séquence des modifications histologiques a été reconstituée à partir d'observations faites chez différents malades, à différents intervalles. La quantité d'exsudats cellulaires était élevée dans les cas TT et BT par comparaison aux malades appartenant aux types BL et LL. La distribution cellulaire a montré un éparpillement lâche des cellules dans les cas LL et BL, mais par contre, une tendance à former des amas serrés dans les cas TT et BT. Les neurtrophiles étaient prédominants au cours des premières 48 heures, particulièrement chez les malades LL, BL et BT. Après 72 heures, les cellules lymphocytaires prédominaient. On a observé chez les malades TT et BT une tendance des lymphocytes à se grouper autour des filets nerveux. Dans la réaction précoce, la quantité d'exsudats était en relation à la fois avec l'érythème et avec l'induration, alors que dans la réaction tardive, on ne notait une corrélation qu'avec l'induration. L'intensité de la réaction précoce à la lépromine était plus prononcée dans les cas de lèpre BT que dans les cas TT alors que l'induration notée pour la réaction tardive était plus marquée dans les cas TT que dans les cas BT. La signification de ces observations est discutée.

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