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Epidermal Changes in Reactional Leprosy: Keratinocyte Ia Expression as an Indicator of Cell-mediated Immune Responses[1]

Harry Thangaraj, Suman Laal, Irene Thangaraj, and Indira Nath[2]

Reactional states in leprosy represent clinicopathological perturbations against the background of the various disease types. Reversal or type 1 reactions are predominantly observed in the borderline forms of leprosy and manifest as local exacerbations of the existing dermal lesions. On the other hand, erythema nodosum leprosum (ENL, type 2 reaction) is seen mainly in borderline, subpolar and polar lepromatous leprosy and presents with generalized symptoms of fever, joint pains, and small, tender, erythematous, subcutaneous nodules. The causal factors precipitating reactions are ill defined. Bjune, *et al.* ([2]) have shown enhanced T-cell proliferation in type 1 reactional patients which correlated with hypersensitivity, as indicated by erythema of lesions and the presence of infiltrating granulomas. Our studies extended these observations to indicate that both peripheral blood and the lesions of borderline leprosy patients show a dichotomy of T-cell functions which were more related to the background leprosy type of the individual. While the borderline (BB) and borderline lepromatous (BL) patients showed improvement, some borderline tuberculoid (BT) patients showed deterioration of T-cell reactivity ([5]). ENL reactions, which had been thought to be due to immune-complex-mediated tissue injury ([20, 21]), have been seen in recent years to reflect the natural emergence of antigen-induced T-cell functions *in vitro*. Our studies on ENL patients showed enhanced antigen-specific T-cell-mediated proliferation, leukocyte migration inhibition, and suppressor-cell activity along with an alteration in T-cell subsets ([4]).

Lesional studies using monoclonal antibodies indicated the new entry of CD4+ helper T cells into the dermal granulomas of ENL ([6, 11]) and BB and BL patients with type 1 reactions ([11]). It would appear that T-cell perturbations in reactional states may act as natural models for understanding the immune phenomenon associated with the immune responsiveness and anergy in the leprosy spectrum. Therefore, with a view to further investigating the role of T cells, we extended our studies to include epidermal changes using monoclonal antibodies defining the phenotypic markers of T cells, Langerhans' cells, and Ia expression on keratinocytes. Our results indicate that in addition to the dermis ([6-11]) the epidermis is a seat of intense immunological reactivity during both types of leprosy reactions. Of significance was the increase in thickness of the epidermis, the Ia expression on keratinocytes, an increase in Langerhans' cell numbers, and the presence of T cells in the epidermal cell layer. Taken together, these features indicate that T-cell reactivity occurs in lesions in reactional states of leprosy. This is of particular importance at the lepromatous pole, where T-cell anergy has been consistently demonstrated in the stable form of the disease ([11]). That antigen presentation in lesions may also be influenced is indicated by an increase in the Langerhans' cell population.

MATERIALS AND METHODS

Patients. Thirty-two leprosy patients attending the Leprosy Mission Hospital, Shahadra, India, were classified according to the criteria of Ridley and Jopling ([15]).

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[2] H. Thangaraj, M.B.B.S., and I. Thangaraj, M.B.B.S., Leprosy Mission Hospital, Shahadra, Delhi, India. S. Laal, Ph.D., and I. Nath, M.D., M.N.A.M.S., M.R.C.Path., F.A.Sc., Professor, Biotechnology Division, All-India Institute of Medical Sciences, New Delhi 110029, India.

H. Thangaraj is currently at the National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

Reprint requests to Professor Nath.

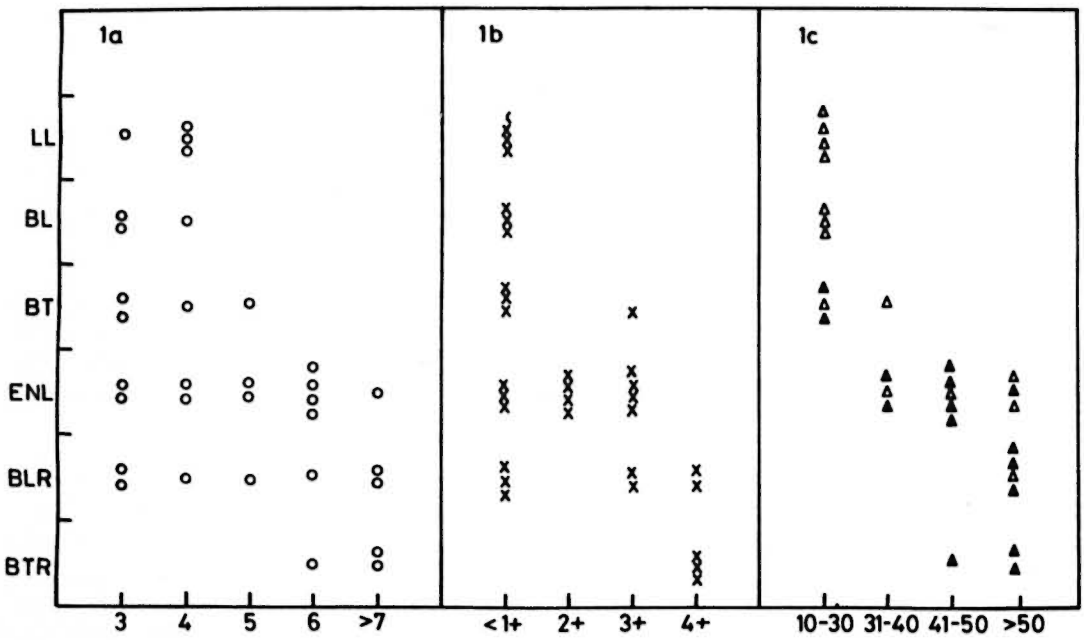

FIG. 1. Scattergram of epidermal changes observed in lesions of stable and reactional leprosy patients. **1a** shows individual data on number of cell layers observed in each biopsy. **1b** indicates the presence of Ia on keratinocytes. Scoring is as follows: <1+ = scattered Ia expression on 0 to <20%; 2+ = 20% to 50% cells in patchy areas; 3+ = 50% to 70% cells in all layers of epidermis; 4+ = Ia expression on all keratinocytes in the section. **1c** shows individual data on number of OKT6+ (CD1) Langerhans' cells among 100 epidermal cells in the presence (▲) and absence (△) of similar cells in the dermis. Results are expressed as mean of 20 high-power microscopic fields; S.D. was <10%.

These included 11 polar lepromatous leprosy (LL) patients undergoing ENL reactions (type 2) and 7 borderline lepromatous (BL) and 3 borderline tuberculoid (BT) individuals in reversal or type 1 reactional states. Control concurrent studies were conducted on 4 BT, 3 BL and 4 LL patients in the stable (nonreactional) form of the disease.

Biopsies. Punch biopsies (4 mm) of typical stable lesions and type 1 reactional lesions were taken under sterile conditions. Excision biopsies of the whole ENL lesion were undertaken. Specimens were snap-frozen using Cryowick (Arthur H. Thomas and Co., Philadelphia, Pennsylvania, U.S.A.) and stored at −70°C. Thick sections (4 to 6 mm) were cut on a cryostat (IEC CTD Harris Cryostat, U.S.A.), fixed in cold acetone for 5 min, and used immediately or after 24–48 hr storage at −20°C.

Indirect immunofluorescence. After repeated washing in Tris buffered saline (pH 7.2), the tissues were overlaid with previously determined optimal concentrations of primary monoclonal antibodies (Mabs) as described earlier (9). Langerhans' cells were identified by the characteristic morphology after staining with the definitive OKT6 as well as the general OKIa antibodies (Ortho Diagnostics, Inc., Raritan, New Jersey, U.S.A.). T cells were identified by OKT11, OKT4, and OKT8 monoclonal antibodies (Ortho). The sections were incubated with the Mabs for 1 hr at 4°C, and subsequently washed in buffered saline for 10–15 min. After blotting the excess saline on the slides, the sections were covered with FITC conjugated anti-mouse $F(ab)_2$ (New England Nuclear Corp., Boston, Massachusetts, U.S.A.) and incubated in the dark at ambient temperature for a further 45 min. The sections were then washed free of antibody as before and mounted with 90% glycerol in phosphate buffered saline (pH 7.2) containing *p*-phenylenediamine (Sigma Chemical Co., St. Louis, Missouri, U.S.A.). Fluorescing cells were viewed under a Zeiss Universal Microscope (Carl Zeiss, Oberkochen, Federal Republic of Germany) us-

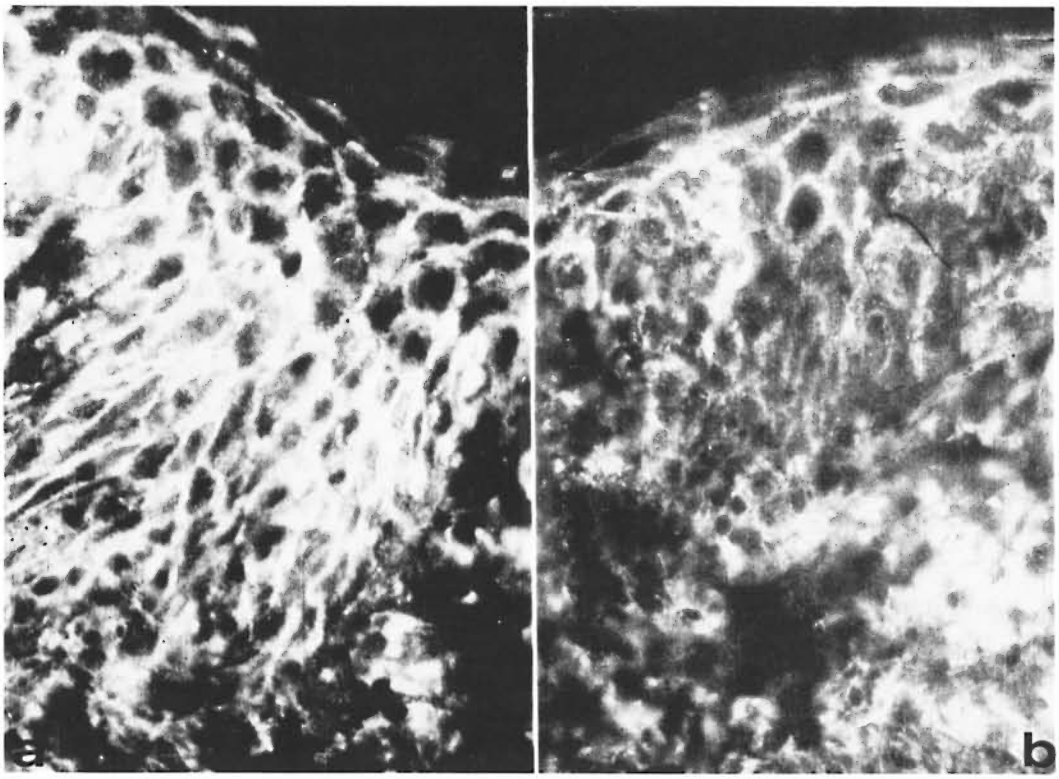

FIG. 2. Indirect immunofluorescence staining of epidermis showing the presence of Ia on keratinocyte surface of type 1 reactions of BT (**a**) and ENL (**b**) lesions (original magnification ×1360).

ing epi-illumination with an HB050 mercury lamp, excitation filter BP 450-490, and barrier filter BP 520-560. The scoring of the lesions was done as follows on 20 random high-power microscopic fields: a) Langerhans' cells were scored as a percent after counting the number of CD1+ (OKT6) cells among 100 keratinocytes; b) CD2+ (OKT11), CD4+ (OKT4), and CD8+ (OKT8) cells representing total, helper/inducer, and suppressor/cytotoxic T cells were enumerated in 20 fields; c) Ia expression on keratinocytes was arbitrarily graded on a scale of 0 to 4+ based on the extent of epidermis showing positive staining, as explained in the footnote to Figure 1b.

RESULTS

The lesions examined in this study were from patients who had given a history of reactional episodes varying from 2 to 10 days prior to biopsy. Although the numbers of patients examined were few, the borderline tuberculoid patients in type 1 reaction gave the most consistent results. The lepromatous lesions in both types of reactions showed individual variability in the degree of epidermal changes.

As compared to the corresponding stable form of leprosy, all three reactional BT patients showed marked epidermal thickening with more than six layers of epidermal cells. Both granular and basal cell layers appeared to be increased. Epidermal thickening was found in 43% of BL patients in type 1 reaction and 45% of LL patients in ENL reactions, the others being within normal range.

In accordance with earlier reports from our ([10]) and other laboratories ([14]), the numbers of Langerhans' cells were also increased in most reactional patients. This increase varied from >30% to >50% and was unrelated to the type of reaction. In both types of reactions, CD1+ cells were also seen in dermal lesions. In some patients the numbers of Langerhans' cells in the epidermis

were inversely proportional to what was observed in the dermis.

In the epidermis, scattered T cells were more consistently seen in BT and BT reactional lesions. They were few, however, and they were dispersed among the keratinocytes. BL reversal lesions and some ENL lesions also showed occasional T cells in the epidermis. As earlier reported in ENL ([11]), the dermis had T cells scattered among the macrophages.

Keratinocyte Ia was seen most in BT reactional patients and least in stable BL and LL individuals. Only 1 of 4 stable BT patients expressed 3+ Ia; the others showed lower expressions of Ia, ranging from patchy to 2+. Significantly, 4 of 7 BL patients in type 1 reaction and 8 of 11 ENL patients expressed strong Ia on keratinocytes. While all BT reactional patients showed Ia on all cell layers of the epidermis, the ENL patients had only patchy expression. The intensity of the positivity as judged by fluorescence appeared similar, but the numbers of epidermal cells showing Ia were localized. That this patchiness was not related to local Langerhans' cell hyperplasia was established by the lack of morphological criteria and the absence of dendrites in the sections staining with anti-Ia antibody (Fig. 2).

Particular efforts were made to study the relationship between Ia induction and the presence of T cells in the vicinity. However, no correlation was found between the local presence or absence of T cells and the patchy Ia expression on keratinocytes in ENL lesions of lepromatous leprosy.

It was clear that BT reactional lesions showed maximal epidermal changes, i.e., an increase in epidermal layers, Ia induction on keratinocytes, an increase in numbers of Langerhans' cells, and the consistent presence of T cells among keratinocytes. It is of importance that lepromatous lesions which do not show Ia on keratinocytes in the stable form of the disease expressed this antigen during reactional states.

DISCUSSION

Recent evidence indicates that interferon gamma (INF-γ) produced by T cells is the major molecule involved in the induction of Ia, macrophage activation, production of toxic oxygen radials, and microbicidal activity ([12, 13]). On incubation with INF-γ human keratinocytes were reported to express Ia on their surface ([18, 19]). Moreover, injection of INF-γ into leprosy lesions has also been shown to stimulate keratinocyte Ia ([12]). Similarly, induction of delayed-type hypersensitivity (DTH) after PPD injection into LL lesions also resulted in expression of Ia on epidermal cells and an increase in their numbers ([3]). Thus, the presence of Ia on cells that do not constitutively express this antigen could be indicative of production of INF-γ due to T-cell stimulation in the vicinity. In addition, Ia/MHC class II antigens are considered to be essential for the presentation of antigen to T cells ([16]).

The present study on cutaneous lesions further confirms that T-cell-mediated responses play an important role in the reactional states of leprosy ([4–6, 8, 11]). Our results indicate that both types of leprosy reactions induce changes in the epidermis. The changes taken together indicate immunological reactivity consonant with T-cell activity. As expected, tuberculoid individuals known to have a high degree of cell-mediated immunity toward *Mycobacterium leprae* show maximal and more consistent evidence of keratinocyte Ia during reversal reaction, indicating further enhancement of T-cell functions and probable local production of interferon-γ. That epidermal growth factors may also be released as in DTH reactions ([3]) is indicated by the increase in epidermal cell layers in the reactional lesions. Our earlier report ([5]), that some BT patients as a group showed a decrease in T-cell reactivity during type 1 reactions, may reflect differences in the properties of cells in the circulation and in the lesions. It is more probable that circulating functional T cells may be selectively drawn into lesions, thereby leading to a relative decrease in reactivity in blood as compared to an apparent increase in lesions.

More importantly, many anergic lepromatous patients undergoing ENL reactions also show evidence of Ia induction on keratinocytes. The expression of this antigen showed individual variation and was patchy in distribution. This is not surprising, since the lesions were biopsied at the time of examination which varied from 2 to 10 days after the appearance of the reactions. It is

probable that the lesions follow sequential changes, and the variability observed may be a reflection of the stage of pathology. Although T-cell entry into ENL lesions was established, due to individual variation no consistent geographic association could be established between the local presence of T cells and the area of the epidermis expressing keratinocyte Ia.

Langerhans' cells were increased in the epidermis of all reactional patients as reported earlier ([14]). That dynamic changes in the traffic of these cells may be taking place was indicated in some lepromatous reactional patients in whom CD1+ (OKT6) cells were also seen in the dermis. Sequential studies may clarify in which direction the Langerhans' cells migrate in reactional lesions. It is possible that a Langerhans' cell increase may promote antigen presentation to the newly migrated T cells ([1]). This, in turn, may lead to activation of T cells followed by terminal lymphokine production. INF-γ release, in particular, may be responsible for macrophage activation and the induction of Ia on keratinocytes. Histological examinations of early ENL lesions are known to show fragmented bacilli in macrophages which may be a result of enhanced microbicidal activity triggered by lymphokines. The release of bacillary antigens in the presence of pre-existing antibodies in lepromatous leprosy may promote immune complexes that have been frequently reported in ENL patients ([20, 21]). Time-related studies of the emergence of T-cell functions and immune complex deposition are required to clarify the relationship of these two phenomena.

It is of interest that although reactions are clinically separable as types 1 and 2, the lepromatous groups (BL and LL), irrespective of the reactional type, showed similar cellular and immunological perturbations in the lesions and in the circulation ([4, 5, 11]). It is possible that the difference between the reactions may lie in the degree of T-cell reactivity and the quantum of *M. leprae*-related antigens. In addition, the subsequent circulating and local immune complex deposition may be responsible for the generalized symptomatology and multiple organ involvement seen in ENL. It is intriguing that the recovery of T-cell function is of a temporary nature. The factors triggering the re-establishment of T-cell anergy following reactions would be of relevance in understanding the anergy associated with lepromatous leprosy.

SUMMARY

Significant epidermal changes were observed in lesions of leprosy patients undergoing type 1 (reversal) and type 2 (erythema nodosum leprosum, ENL) reactions. Using indirect immunofluorescence and frozen sections stained with the appropriate monoclonal antibodies, an increase in epidermal cell layers, the presence of Ia on keratinocytes, an increase in Langerhans' cell numbers, and scattered T cells within the epidermis were seen in both types of reactions. Although borderline tuberculoid patients with type 1 reactions showed the consistent presence of Ia on all keratinocytes, lepromatous patients undergoing ENL reactions showed only a patchy distribution. Taken together, these studies indicate that local T-cell activation leading to the production of terminal lymphokine, such as interferon-gamma, with subsequent induction of Ia on epidermal cells may be an important event in reactional leprosy states. It is of interest that the hitherto considered "anergic" lepromatous patients should recover temporary T-cell reactivity during the natural course of the disease.

RESUMEN

Se observaron cambios epidérmicos significantes en las lesiones de pacientes con lepra con reacciones del tipo 1 (reversa) y del tipo 2 (eritema nodoso leproso, ENL). Utilizando inmunofluorescencia indirecta y cortes congelados teñidos con los anticuerpos monoclonales apropiados se observaron, un incremento en las capas de células epidérmicas, la presencia de Ia en los queratinocitos, un aumento en el número de células de Langerhans, y células T dispersas dentro de la epidérmis en ambos tipos de reacción. Mientras que los pacientes con lepra tuberculoide limítrofe con reacciones del tipo 1 mostraron la presencia consistente de Ia sobre todos los queratinocitos, los pacientes lepromatosos con ENL mostraron sólo una distribución "en parches." Tomados en conjunto, estos estudios indican que la activación local de las células T, que culmina en la producción de linfocinas tales como interferón gamma y la subsecuente inducción de Ia sobre las células epidérmicas, puede ser un evento importante en los estados reaccionales de la lepra. Por otro lado, re-

sulta interesante que los pacientes lepromatosos tradicionalmente considerados como "anérgicos" pueden recuperar temporalmente la reactividad de las células T durante el curso natural de la enfermedad.

RÉSUMÉ

Des modifications épidermiques très notables ont été observées dans les lésions de malades de la lèpre qui étaient affectés de réactions du type 1 (reverse) et du type 2 (erythème noueux lépreux, ENL). En utilisant des épreuves d'immunofluorescence indirecte, et l'étude de coupes congelées qui avaient été colorées avec des anticorps monoclonaux appropriés, on a pu mettre en évidence, et cela dans les deux types de réaction, la présence de Ia sur les kératinocytes, une augmentation dans le nombre des cellules de Langerhans, et des cellules T dispersées dans l'épiderme. Alors que les malades atteints de lèpre tuberculoïde dimorphe souffrant de réactions du type 1 montraient la présence constante de Ia sur tous les kératinocytes, les malades lépromateux témoignant de réactions d'érythème noueux lépreux ne montraient par contre qu'une dissémination éparpillée de ces manifestations. Lorsqu'on les considère ensemble, ces études montrent clairement qu'une activation locale des cellules T, entraînant la production de lymphokine terminale telle que l'interféron-gamma, avec production ultérieure d'anticorps Ia sur les cellules épidermiques, peut constituer une étape importante des états de réaction dans la lèpre. Il est intéressant de noter que des malades lépromateux, jusqu'ici considérés comme anergiques, peuvent récupérer une réactivité temporaire des cellules T au cours de l'évolution naturelle de la maladie.

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