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Epidermal keratinocyte Ia expression, Langerhans cell hyperplasia and lymphocytic infiltration in skin lesions of leprosy

T. H. REA, J.-Y. SHEN & R. L. MODLIN *Section of Dermatology, University of Southern California/Los Angeles County Medical Center, Los Angeles, California*

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SUMMARY

Epidermal changes, Ia expression on keratinocytes, Langerhans cell hyperplasia and lymphocyte infiltration were sought in skin lesions of leprosy: 15 borderline tuberculoid (BT), six borderline lepromatous (BL), 17 lepromatous (LL), 13 erythema nodosum leprosum (ENL), six Lucio reactions and nine reversal reactions. All three changes were well developed in BT and reversal reactions. ENL showed well developed keratinocyte Ia and Langerhans cell hyperplasia, but little lymphocytic infiltration. LL and Lucio tissues had some Langerhans cell hyperplasia but little or no keratinocyte Ia or lymphocytic infiltration. BL tissues were so diverse as to suggest two distinct subgroups. These findings are consistent with the hypothesis that keratinocyte Ia expression is an immunohistological sign of a cell-mediated immune (CMI) response. However, the Ia keratinocyte expression found in BL and ENL tissues appears contrary to the undifferentiated macrophages and numerous bacilli found in the lesions. Thus, if a sign of CMI, keratinocyte Ia expression is not a measure of the effectiveness of the response.

Keywords leprosy Langerhans cells keratinocyte Ia

INTRODUCTION

Demonstrations of expression of Ia (HLA-DR) by epidermal keratinocytes (Lampert, Suitters & Chrisholm 1981; Volc-Platzer *et al.*, 1984), presentation of antigen by Langerhans cells (Stingl, Tamaki & Katz 1980) and secretion of interleukin-1 (IL-1) by both cell lines (Luger *et al.*, 1981; Sauder, Dinarello & Morhenn, 1984) have established the importance of the epidermis as an active tissue in the immune response (Katz, 1985). Information concerning keratinocyte Ia expression has been of particular interest. *In vivo* the keratinocyte Ia is synthesized by keratinocytes, not shed by Langerhans cells (Breathnach & Katz, 1983; Volc-Platzer *et al.*, 1984). *In vitro* human keratinocytes can be stimulated to express Ia by incubation with interferon gamma (INFg) (Basham *et al.*, 1984; Volc-Platzer *et al.*, 1985), a potent stimulator of Ia in a variety of cell lines (Wong *et al.*, 1983). Intradermal administration of recombinant human INFg in lepromatous leprosy lesions results in Ia expression on keratinocytes and possibly bacteriolysis as well (Dr Carl Nathan personal communication). In man, keratinocyte expression of Ia has been demonstrated in three basic types of delayed-type hypersensitivity (DTH) or cell-mediated immune (CMI) reactions: chronic graft versus host reactions (Lampert, Suitters & Chrisholm, 1981; Breathnach & Katz, 1983; Volc-Platzer *et al.*, 1984), allergic contact dermatitis (Suitters & Lampert, 1982; Mackie & Turbitt, 1983) and tuberculin skin test responses (Fullmer, Modlin & Rea 1984; Scheynius & Tjernlund, 1984).

Correspondence: Dr Thomas H. Rea, USC School of Medicine-Dermatology, 2025 Zonal Avenue, Los Angeles, CA 90033, USA.

Ia expression on keratinocytes has been hypothesized to be a sign of a CMI response (Suitters & Lampert, 1982) in man, an attractive hypothesis based upon the above evidence; furthermore, it appears that keratinocyte Ia expression is induced by INFg, the terminal cytokine of the CMI cascade (Nathan *et al.*, 1983). Associated epidermal changes such as Langerhans cell hyperplasia (Mackie & Turbitt, 1983) and epidermal lymphocytic infiltration (Aiba & Tagami, 1984) are less well studied.

These recent advances, coupled with our demonstration of keratinocyte Ia in tuberculoid leprosy lesions (Modlin *et al.*, 1983a) and Ridley's observations that the epidermis is the site of earliest change in the pathogenesis of tuberculoid lesions (Ridley, 1973), have prompted us to study epidermal changes in the various granulomatous postures and reactional states of leprosy. We reasoned that such data, presented herein, are important for a better understanding of some aspects of the immunology of leprosy.

MATERIALS AND METHODS

Patients were classified according to the Ridley system (Ridley & Waters, 1969; Ridley, 1977) and reactional states were diagnosed according to criteria previously published (Rea & Levan, 1980). The classification used was that made at the time of the initial biopsy. Because no consistent differences could be found between the polar and subpolar lepromatous groups, these were grouped together as lepromatous. Tissue specimens, obtained by either 6 mm punch biopsies or scalpel excisions, were placed in OCT, snap-frozen in liquid nitrogen and stored at $-70°C$ until sectioned. As described in detail elsewhere (Modlin *et al.*, 1984), sections were sequentially stained with primary mouse monoclonal antibody, peroxidase conjugated goat anti-mouse and aminoethyl carbazol; the counterstain was haematoxylin.

Ia was sought using H4 (Dr Ron Billing, UCLA) at a dilution of 1/1000, as determined by checkerboard titration. Keratinocyte staining was scored semi-quantitatively according to reaction pattern, not staining intensity, on a scale of 0 to + + + +. Staining was judged to be + + + + if all keratinocytes stained positively, extending from the granular cell layer through the basal cell layer to the basement membrane throughout the entire section; + + + was the same as + + + + present in at least one half of the epidermis but not in all; + + staining involved only a portion of the epidermis, which proved to be the basal cell layer and the lower portion of the prickle cell layer, but was present in at least one half of the epidermis; + staining involved less than one half of the epidermis; 0 staining was scored when all of the Ia positive material was readily interpreted to be staining of Langerhans cells (Rowden, Lewis & Sullivan 1977).

Langerhans cells were sought using OKT6 (Ortho) at dilutions of 1/10 to 1/50. Langerhans cell changes were estimated semi-quantitatively by staining pattern, not intensity. 'Connecting' or 'touching' of adjacent dendritic cells was seen in approximately two out of 20 Langerhans cells in sections of normal skin. If 15% or less of Langerhans cells showed such touching, the pattern was scored as a +; 20–50% as + +; 55–80% + + +. Greater than 85% was scored as + + + +.

Ia staining on keratinocytes could be distinguished from Ia on Langerhans cells by morphological criteria as previously described (Modlin *et al.*, 1983a). The Ia-keratinocyte product uniformly filled intercellular spaces below the stratum granulosum, giving a flat, two-dimensional appearance. In contrast, the OKT6 or Ia Langerhans cell product stained cell bodies and dendrites giving an appearance of a three-dimensional structure. This was particularly evident when using a 25× or 40× ocular in conjunction with small changes in the fine focussing.

Lymphocyte infiltration into the epidermis was sought using Leu 4 (Beckton-Dickinson) at a dilution of 1/60. If only one or several cells were infiltrating the epidermis in a seemingly random manner, the intensity was judged to be a +; if infiltrating lymphocytes were regularly distributed but widely spaced, it was judged to be + +; if present as a focal, dense infiltrate or separated focal infiltrates, it was judged + + +; if the dense infiltration involved more than half the epidermis, + + + +.

The helper:suppressor (h:s) ratio in the dermis was determined using Leu-3a (anti-helper/inducer) at 1/100 and Leu-2a (anti-suppressor/cytotoxic) at 1/100 staining reactions as previously reported in detail (Modlin *et al.*, 1983a).

RESULTS

Sixty-six tissues from 60 patients were placed into one of six categories. Six patients, five LL and one Lucio, were studied again at the time the diagnosis of ENL was established. Fifteen untreated borderline tuberculoid (BT) tissues, six untreated borderline lepromatous (BL) and 17 lepromatous (LL) (15 untreated and two relapsing off treatment) were the non-reactional specimens studied. Nine tissues from reversal reaction patients (seven BL, two BT, all untreated), six untreated Lucio reaction (all LL) and 13 erythema nodosum leprosum (ENL) tissues (all LL, four untreated) were also studied.

Keratinocyte Ia. Keratinocyte expression of Ia, summarized in Fig. 1, was well developed (+++ or ++++) in all BT, eight of nine reversal reaction tissue, and was present in a majority of ENL specimens; see Fig. 2. Some keratinocyte Ia was also present in half of BL tissues. Keratinocyte Ia expression was poorly developed; 0 in 15 of 17 LL and five of six Lucio. In the LL or Lucio patients followed serially, keratinocyte Ia expression increased in two and appeared in four in association with the development of ENL; see Fig. 1.

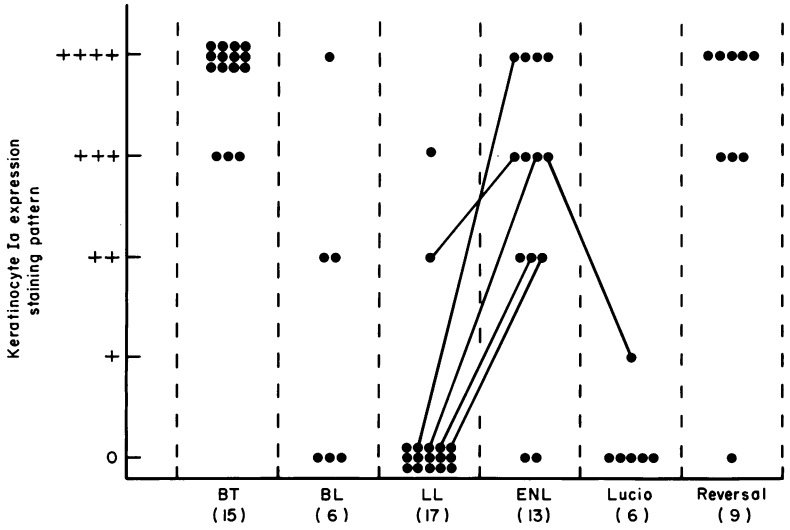

Fig. 1. A summary of Ia keratinocyte expression. Patients followed serially who developed ENL are represented by a connecting line.

Epidermal Langerhans cells. Epidermal Langerhans cell changes, summarized in Fig. 3, were well developed (+++ or ++++) in the same groups that showed strong Ia expression on keratinocyte, i.e., BT, reversal reaction and ENL (see Fig. 4) but was also well established (++ or more) in six of six BL, four of six Lucio and half of LL. Five of six LL or Lucio patients who were followed serially had a more extensive staining pattern in association with the development of ENL; see Fig. 3.

Lymphocytic infiltration. Lymphocyte infiltration of some degree was present in 10 of 13 BT specimens (two +++, two ++, six+) and in five of nine reversal reactions (three ++, two +), but was unusual in ENL (one ++, two +) and BL (one +) and was not found in LL or Lucio, with one exception.

Correlations. Among some groups the expression of all three variables was parallel, e.g., well developed in BT and reversal reactions or poorly developed in LL, but within any one group the expression of one variable did not predict the expression of others. Furthermore, in an entire group, one variable could be virtually absent and the others well developed; for example in ENL

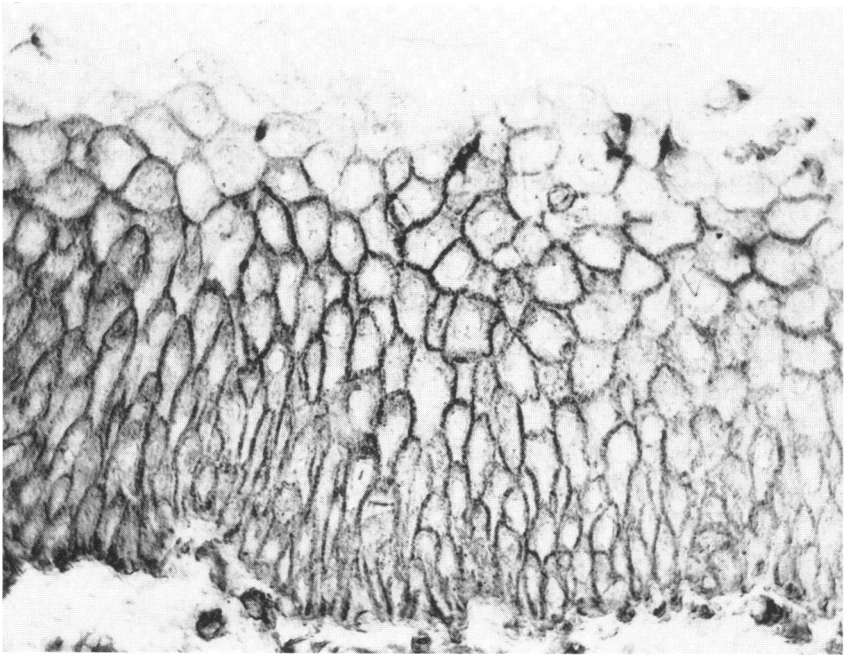

Fig. 2. Ia keratinocyte expression of + + + + intensity in an ENL lesion. The staining is continuous from the granular cell layer through the basal cell layer and extends to the basement membrane. H4 counterstained with haematoxylin, ×400.

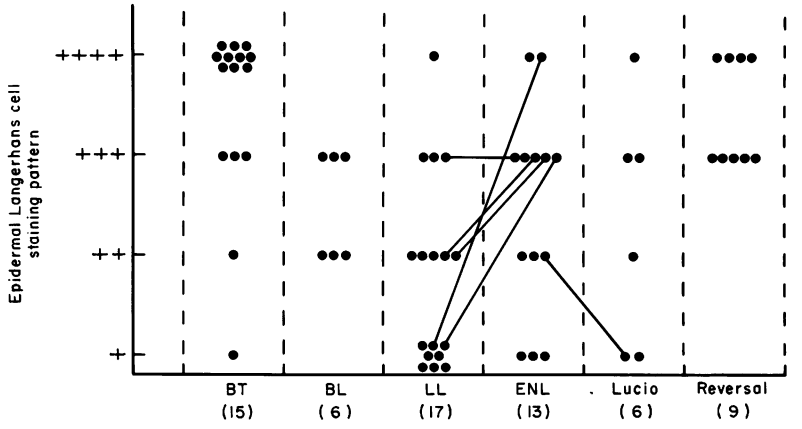

Fig. 3. A summary of epidermal Langerhans cell staining. Patients followed serially who developed ENL are represented by a connecting line.

lymphocytic infiltration was scant but keratinocyte Ia and Langerhans cell hyperplasia well expressed; also in LL and Lucio where Langerhans cell hyperplasia was not uncommon, keratinocyte expression was weak and lymphocytic infiltration rare. Among individuals there was also variation. For example, of the 33 specimens with + + + or + + + + keratinocyte Ia staining patterns, two had no Langerhans cell hyperplasia; among the 38 specimens with + + + or + + + + Langerhans cell hyperplasia, six had no keratinocyte Ia.

The tissue h:s ratio in each category was in accordance with our previously published results

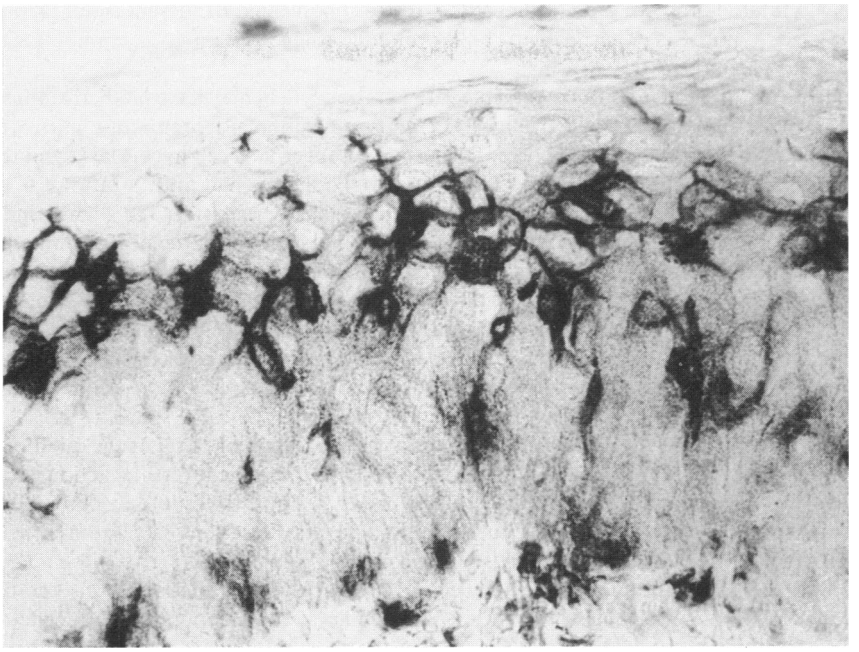

Fig. 4. Langerhans cell staining of + + + + intensity in an ENL lesion. Cells are numerous and the dendrites of adject cells appear to be touching one another. OKT6 counterstained with haematoxylin, × 400.

(Modlin *et al.*, 1983a,b, 1984) (data not shown). Ia expression was best developed in those groups having mean tissue h:s ratios of 1·7 or more, i.e. BT, ENL and reversal reactions. With the exception of BL patients, within a subgroup the h:s ratio was not predictive of variable expression; for example, in the LL patients with a tissue h:s ratio of 1·5 there was no Ia on keratinocytes and no Langerhans cell hyperplasia, but in the reversal reaction patient with a ratio of 1·2 both were + + + +. In the three BL patients with low h:s ratios (0·3 to 0·7) there was no epidermal reaction to Ia, but the three with ratios of 1·0, 3·7 and 2·0 had + +, + +, and + + + + staining patterns respectively.

DISCUSSION

Our results are consistent with the hypothesis that Ia expression on keratinocytes is a sign of a CMI response (Suitters & Lampert, 1982). The strong staining seen in BT and reversal reactions is consistent with the long-held view that these conditions represent *M. leprae*-specific CMI or DTH activity (Barnetson *et al.*, 1976; Rea & Levan, 1977), and are in accordance with the relatively high prevalence of interleukin-2-producing cells, 1/200, previously reported in BT tissues (Modlin *et al.*, 1984). Reciprocally, the near absence of staining in LL and Lucio tissue is consistent with the long-held view that there is little or no *M. leprae*-specific CMI activity in lepromatous patients and is in accordance with a low prevalence, 1/3000, of interleukin-2-producing cells in these tissues (Modlin *et al.*, 1984). The presence of keratinocyte Ia in ENL tissues is consistent with the view (Rea & Levan, 1980; Mshana *et al.*, 1982; Rea & Yoshida, 1982; Stach, Strobel & Bäch, 1982; Modlin *et al.*, 1984; Narayanan *et al.*, 1984; Laal, Bhutani & Nath, 1985; Modlin *et al.*, 1986), and is further evidence for it as well, that ENL is some sort of a CMI response; this also is in accordance with a 1/300 prevalence of interleukin-2 producers (Modlin *et al.*, 1986). Because the BL posture represents some, albeit feeble, resistance, and because epithelioid differentiation of macrophages can occur in BL tissues (Ridley & Waters, 1969; Ridley, 1973) (and was present in the one patient with + + + +

keratinocyte Ia), the presence of keratinocyte Ia expression in some BL tissues is not inconsistent with the CMI hypothesis. The diversity of BL findings suggests that it is immunologically a heterogeneous group.

Data from the three LL and Lucio patients with keratinocyte Ia may have predictive value. Two did develop ENL: see Fig. 1. The third, initially diagnosed as subpolar lepromatous, untreated, had an epidermal profile of + + + Ia expression, + + + + Langerhans cell hyperplasia and the only lymphocytic infiltration, +, observed in LL or Lucio; the dermis had an h:s ratio of 0·3. After several months of treatment, this patient developed an extensive persistent red slightly indurated and sharply marginated eruption upon the trunk and thighs which on histological examination was similar to his pretreatment biopsy except for more lymphocytes in the former; we have interpreted this to be a mild reversal reaction. Taken collectively, data from these three patients suggest that keratinocyte Ia in LL or Lucio is, when present, premonitory of a (or another) reactional state. Because patients with Lucio reactions do develop necrotic lesions with consequent denaturation of barrier function or ulceration, the observed Ia in the Lucio patient could be a result of challenge by antigens other than those of *M. leprae*.

The one reversal reaction tissue without keratinocyte expression of Ia raises the question of the sensitivity of this sign. This particular patient was considered to have a reversal reaction because of signs and symptoms of neuritis, not because of skin changes. Thus, the failure to express Ia in this particular patient is consistent with the observations that reversal reactions may be purely neural, as well as purely cutaneous or mixed (Barnetson *et al.*, 1976).

Based on our data, the most cogent objection against the hypothesis that Ia keratinocyte expression is a sign of CMI comes from the evident conflict between a putative INFg effect in the epidermis, keratinocyte Ia, in ENL and BL tissues which simultaneously show undifferentiated macrophages and abundant bacilli, a putative absence of INFg effect. This conflict could be resolved by one of several postulates: (1) a mediator other than INFg can induce Ia expression on keratinocytes; (2) keratinocytes are more sensitive than macrophages to INFg; (3) BL and ENL macrophages are pathologically unresponsive to INFg, and (4) *M. leprae* unresponsiveness is a complex phenomenon not solely explainable by failure of the CMI response and cytokine cascade. The data presented in this paper does not distinguish between these possibilities. Because ENL lesions evidently have adequate numbers of interleukin-2-producing cells to satisfy a CMI response (Modlin *et al.*, 1986), we favour the latter interpretation. In other words, the presence of IL-2 producing cells and keratinocyte Ia expression are consistent with each other and with activation of the CMI cascade, indicating that other mechanisms must at least in part account for *M. leprae*-specific CMI unresponsiveness.

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