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OKT6+ Epidermal Langerhans' Cell Numbers in DNCB Reactions Among Leprosy Patients[1]

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Leprosy is a chronic granulomatous disease primarily affecting the skin and nerves. The disease exhibits a spectrum with high-immune tuberculoid leprosy and low-immune lepromatous leprosy forming the two polar types. Several studies have indicated that both *in vitro* and *in vivo* cell-mediated immune responses are depressed in lepromatous leprosy ([7]). For example, tuberculoid patients exhibit a positive lepromin reaction and a good contact sensitivity reaction to dinitrochlorobenzene (DNCB) while lepromatous patients show poor responses to these antigens ([1, 3, 11]). However, there are reports of normal responses to DNCB in lepromatous patients ([8]).

Macrophages and accessory cells such as Langerhans' cells (LC) and dendritic cells are thought to play a key role in the presentation of antigen to T cells, thus leading to cell-mediated immune responses ([13]). For example, in the experimental animal, LC present in the epidermis of the skin have been shown to participate in delayed hypersensitivity reactions and in allergic contact dermatitis ([10, 12, 13]). These cells carry receptors for the Fc component of IgG, and C3 component of complement, express Ia-like antigens, and contain high concentrations of ATPase enzymes ([2, 13]). Langerhans' cells can be defined by a specific T6 marker which does not show any crossreaction with morphologically similar dendritic macrophages ([4]). This monoclonal antibody has been used previously to study the status of LC in leprosy lesions ([5]), and in *in vivo* skin reactions ([6]).

In the present communication, an assessment of the kinetics of the numbers of epidermal LC and their distribution has been made at the sites of DNCB skin reactions in untreated leprosy patients using OKT6 monoclonal antibody.

MATERIALS AND METHODS

Skin biopsies. Forty-five untreated leprosy patients were selected from the outpatient clinic of the Central JALMA Institute for Leprosy, Agra, India. The patients were classified based on the criteria of Ridley and Jopling ([9]). They were sensitized with 2 mg of 100 μl of 2,4-dinitrochlorobenzene (DNCB; Wako Pure Chemical Industries Ltd, Japan) suspended in acetone. Four weeks later, the patients were challenged with 25 μg of DNCB in 100 μl on the anterior surface of the forearm. The sensitizing and the challenge doses of DNCB were applied epicutaneously on apparently normal skin of the patients. The reaction was graded depending on the extent of erythema and degree of swelling: 1+ = mild positive, 2+ = moderately positive, and 3+ = strongly positive with edema. Sequential biopsies were collected at intervals of 30 min, 4, 24, and 48 hr in isopentane (Fluka, AG, Chemische, Fabrik CH-9470, Buchs) and frozen at −20°C for cryostat sections.

Antisera. The monoclonal antibody, OKT6 (defines cortical thymocytes and LC), was obtained from Ortho Pharmaceutical Corporation, Raritan, New Jersey, U.S.A. Sheep anti-mouse Ig $F(ab')_2$ was obtained from New England Nuclear, Boston, Massachusetts, U.S.A.

Immunofluorescence. Cryostat sections 4–5 μm thick were cut and fixed in cold acetone-chloroform mixture (1:1) for 20 min. The sections were then dried and incubated with 25 μl of a 1:5 dilution of OKT6 monoclonal antibody at room temperature for 45 min. Sections layered with phosphate buffered saline (PBS) served as controls. Subsequently, the sections were washed with 0.85% saline for 15 min, then incubated with 25 μl of a 1:80 dilution of FITC-conjugated

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sheep anti-mouse Ig $F(ab')_2$ mixed with pontochrome violet (1%) for 30 min at room temperature, and washed in 0.85% saline for 45 min. The sections were mounted in 90% glycerol-PBS and viewed by epi-illumination, using an HBO 50 mercury lamp and a Leitz inverted microscope with incident light excitation filter block no. 12 and transmitted light excitation filter block no. H(513604). The numbers of OKT6+ epidermal LC per high-power field were quantitated.

The efficacy and the optimal dilution of the monoclonal antibody OKT6 was assessed on the cryostat sections of normal skin.

A sequential histological analysis of the skin reactions was carried out from the cryostat sections stained with hematoxylin and eosin (H&E).

RESULTS

Twenty-four untreated patients with tuberculoid (BT/TT) leprosy with a negative bacterial index (BI) and 21 untreated lepromatous leprosy (BL/LL) patients with a BI of 4+ to 5+ were included for sensitization and challenge with DNCB. The patients were classified on the scale of Ridley and Jopling ([9]). Clinically, in the tuberculoid patients, the skin reaction at 30 min was negative; at 4 hr, erythema and edema were seen, and at 24 and 48 hr there was mild thickening of skin. In contrast, the lepromatous patients did not show any response (Fig. 2). Sequential histological analyses of the skin reactions showed infiltrates containing predominantly mononuclear cells seen around blood vessels and neurovascular bundles at all time intervals in both the tuberculoid and lepromatous patients. There was no difference in the quantum or composition of the infiltrate. The apparently normal skin of BL/LL patients showed very little in the way of macrophage granulomas.

Preliminary experiments were carried out on the cryostat sections of normal skin to assess the optimal dilution of the monoclonal antibody and the conditions required for staining. It was found that a 1:5 dilution of the antibody was optimal (Fig. 1). The OKT6 monoclonal antibody was gel purified and contained very low concentrations of mouse globulin.

There were no differences in the numbers or distribution of OKT6+ epidermal LC between tuberculoid and lepromatous patients. The numbers of LC in the DNCB challenge sites in both the tuberculoid and lepromatous cases were marginally low as compared to normal skin. However, this was not statistically significant. It is interesting that the lepromatous patients failed to evoke any response to DNCB at any time period, yet the numbers of epidermal LC were similar to those seen in the skin of the normal controls (Fig. 2). The intensity of fluorescence showed no variation among the controls and the two test groups.

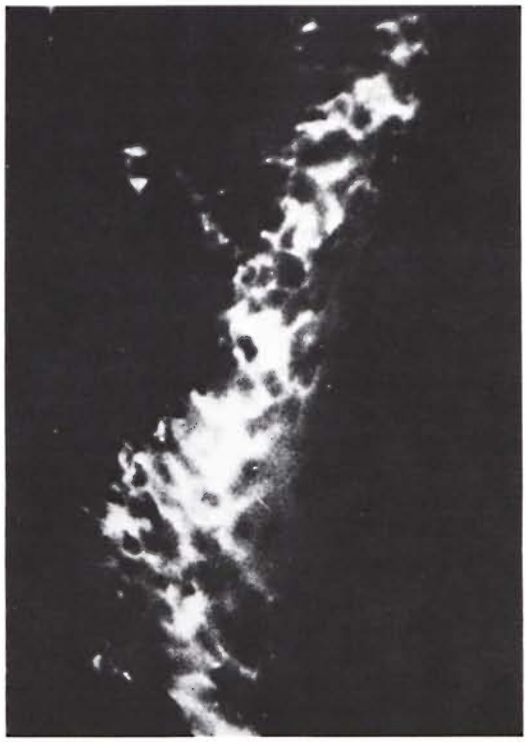

FIG. 1. Langerhans' cells in epidermis of normal skin showing intense immunofluorescent staining with OKT6 monoclonal antibody (cryostat section, counterstained with pontochrome violet ×320). A similar staining was observed in the biopsies of the DNCB-reaction sites.

DISCUSSION

Contact sensitivity skin reaction to DNCB may be a useful parameter for the assessment of the immune status of a leprosy patient. It has been reported that the DNCB skin reaction was normal ([8]) and/or depressed in patients with lepromatous leprosy ([1, 11]). This reaction appears to be me-

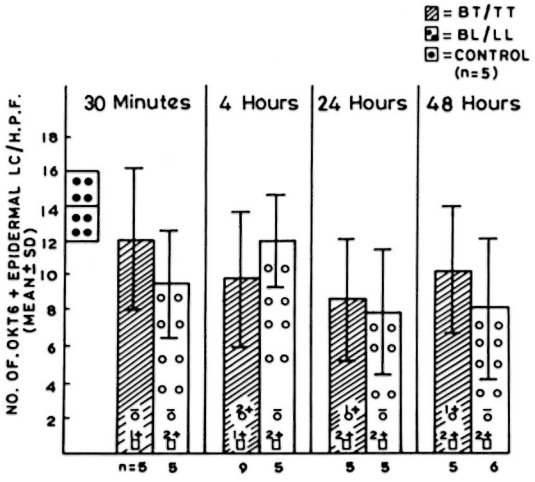

FIG. 2. Time kinetics of the numbers of T6+ epidermal LC at the sites of DNCB reaction among the leprosy patients. O = clinical grading of the reaction; □ = degree of mononuclear cell infiltration; n = number of patients.

diated by T cells ([14]). Epidermal LC have been shown to participate in contact sensitivity reactions in experimental animals ([12–14]).

The present study was undertaken with OKT6 monoclonal antibody to assess the kinetics and distribution of epidermal LC at the sites of induction of DNCB skin reactions in the untreated leprosy patients. Staining with this antibody delineates the morphology of the cells better than the conventional histochemical method (ATPase) and, thus, can be useful and specific in the quantitation of LC ([4]). Our earlier studies have shown that there are no significant differences in LC counts when they are expressed per high-power field or per 100 keratinocytes ([5]).

Clinically, the tuberculoid patients showed a positive reaction as early as 4 hr, and remained positive up to 48 hr, while the lepromatous patients failed to show any response at any time interval. These results are in agreement with previously published reports ([1, 11]). Sequential histological analyses of the skin reactions showed infiltrates containing predominantly mononuclear cells seen around blood vessels and neurovascular bundles at all time intervals in both the tuberculoid and the lepromatous patients. There was no difference in the quantum or composition of the infiltrate.

No significant difference was observed in the numbers and distribution of OKT6+ epidermal LC at the sites of either the early or the late phases of DNCB skin reactions between the tuberculoid and the lepromatous patients. Further, the appearance of the skin reactions and the quantum of infiltration did not show any correlation with the numbers of OKT6+ LC in the epidermis. In experimental animals, it has been suggested that LC are critical in determining whether exposure to potential antigens lead to contact sensitivity or tolerance. Only in the presence of normal numbers of normally functioning LC was the contact sensitivity to DNFB obtained ([14]). Despite almost normal numbers of LC (similar to that of tuberculoid patients and normal skin), the lepromatous patients in the present study failed to respond to the challenge dose of DNCB. This may be accounted for by the following possibilities: a) due to a generalized T-cell defect observed in these lepromatous patients, it may take a longer time for the immune mechanism to become activated; b) possible ultrastructural variation or functional deficit in LC might lead to slow processing of DNCB; c) reduced numbers of LC in lepromatous leprosy. The last possibility is unlikely, since our earlier observations have indicated no difference in the numbers of T6+ epidermal LC and their distribution in tuberculoid and lepromatous lesions ([5]). Further experiments need to be carried out to understand the role of Langerhans' cells in these reactions.

SUMMARY

A study was made on the Langerhans' cells at the sites of contact sensitivity skin reactions in 45 untreated leprosy patients. The skin reaction was induced by 2,4-dinitrochlorobenzene (DNCB). Langerhans' cells were quantitated using OKT6 monoclonal antibody and indirect immunofluorescence. Clinically, the skin reaction in the tuberculoid patients was positive at 4, 24, and 48 hr, while the lepromatous patients failed to respond at any of the time intervals. Sequential histological analysis of the skin reaction showed predominantly mononuclear cell infiltrates around the blood vessels and neurovascular bundles in both the tuberculoid and lepromatous patients. Time kinetic assessment showed no difference in the numbers and distribution of OKT6+ epidermal Langerhans' cells at the site of

the DNCB skin reactions among the tuberculoid and lepromatous patients. This, therefore, suggests that either there is a functional defect in Langerhans' cells or some other mechanism(s) such as a T-cell abnormality is responsible for the lack of clinical reaction in lepromatous patients.

RESUMEN

Se hizo un estudio sobre la presencia de las células de Langerhans en los sitios de reacción dérmica inducida por sensibilización por contacto con 2,4-dinitroclorobenceno (DNCB) en 45 pacientes con lepra no tratada. Las células de Langerhans se cuantificaron por inmunofluorescencia indirecta usando un anticuerpo monoclonal OKT6. Mientras que la reacción en la piel de los pacientes tuberculoides fue clínicamente positiva a las 4, 24 y 48 horas, ésta resultó negativa en los pacientes lepromatosos a los diferentes intervalos de tiempo. El análisis histológico secuencial de la reacción en piel mostró un infiltrado predominantemente mononuclear alrrededor de los vasos sanguíneos y de los manojos neuromusculares tanto en los pacientes lepromatosos como en los tuberculoides. El estudio cinético no mostró diferencias entre los pacientes tuberculoides y lepromatosos en cuanto al número y distribución de las células OKT6+ en los sitios de reacción dérmica. Esto sugiere que un defecto funcional en las células de Langerhans o algún otro mecanismo, tal como una anormalidad en las células T, son responsables de la falta de reacción clínica en los pacientes lepromatosos.

RÉSUMÉ

On a étudié les cellules de Langerhans au niveau des réactions cutanées produites par contact avec des agents sensibilisants, chez 45 malades de la lèpre non traité. La réaction cutanée était produite par le 2,4-dinitrochlorobenzène (DNCB). Le nombre de cellules de Langerhans a été estimé par une méthode d'immunofluorescence indirecte faisant appel à l'anticorps monoclonal OKT6. Cliniquement, la réaction cutanée était positive chez les malades tuberculoïdes après 4, 24, et 48 heures. Les malades lépromateux n'ont témoigné de réponse, à aucun moment. Une analyse histologique en séries des réactions cutanées a révélé une prédominance d'infiltrats à cellules mononucléaires autour des vaisseaux sanguins et des faisceaux neurovasculaires, tant chez les malades tuberculoïdes que chez les lépromateux. Une évaluation de la cinétique au cours du temps n'a pas montré de différence quant au nombre et à la distribution des cellules épidermiques de Langerhans OKT6+, à l'endroit des réactions cutanées au DNCB chez les malades tuberculoïdes, et pas davantage chez les lépromateux. On suggère dès lors que l'absence de réactions cliniques chez les malades lépromateux résulte soit d'un défaut fonctionnel des cellules de Langerhans, soit d'un autre mécanisme ou de plusieurs autres mécanismes tels que des anomalies des lymphocytes T.

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