OKT6 POSITIVE EPIDERMAL LANGERHANS CELLS IN TINEA CORPORIS

R B Narayanan, A Girdhar, R K Lavania and B K Girdhar

Langerhans cells (LC) were defined by indirect immunofluorescence using OKT6 monoclonal antibody in the skin lesions of 11 untreated patients with tinea corporis. The number and distribution of OKT6+ epidermal LC in the lesions was very low in comparison to that found in the normal skin. In most of the lesions, T6+LC could not be seen in the epidermis. Ia like antigens were not detectable on the keratinocytes. These observations suggest decreased CMI response to the fungal infection and that the reduction in LC may be a contributing factor in the pathogenesis of tinea corporis.

Key words : Langerhans cells, OKT6 and OKIa monoclonal antibody. Tinea corporis.

Tinea corporis is produced as a result of the combination of the destruction of the keratinocyte tissue by the invading fungus and the inflammatory response of the host.[1] It has been suggested that the cell mediated immune reaction may be involved in the pathogenesis of tinea corporis.[1] For example, a diminution of delayed dermal reaction and the lymphocyte transformation test to trichophytin (fungal antigens) has been demonstrated in the patients having tinea corporis.[1] T cell dysfunction has also been documented in tinea corporis.[1]

The role of Langerhans cells (LC) in T cell interaction has been implicated.[2] There is evidence to indicate participation of LC in the onset of contact dermatitis and other skin disorders like psoriasis and vitiligo.[2-4,6,7] In leprosy too, the participation of LC has been implicated.[5,10,17] Very little information is available on the role of LC in tinea corporis. In the present communication, the status of LC in tinea corporis has been assessed using monoclonal antibodies.

Materials and Methods

Eleven untreated patients with tinea corporis were studied. The patients had no other skin disease. A typical skin lesion was biopsied. One half of the biopsy was fixed in buffered formalin and processed for histopathological study. The sections were stained with hematoxylin and eosin, and PAS staining for the demonstration of fungus. The other half was collected in isopentane (Fluka AG, Chemische, Fabrik, CH-9470, Buchs) and frozen at —20°C for cryostat sections. Biopsy from the skin of normal individuals was used as controls, since it was not possible to obtain skin biopsies from uninvolved sites of the patients.

Monoclonal antibodies OKT6 (defining cortical thymocytes and LC) and OKIa were obtained from Orthopharmaceutical Corporation, USA; and FITC conjugated sheep anti-mouse IgF $(ab)_2$ from New England Nuclear, Boston, USA.

Immunoflurescence: 4-5μ thick cryostat sections were cut and fixed in cold acetone-chloroform mixture (1:1 ratio) for 20 minutes. The sections were then dried and incubated at room temperature for 60 minutes with 10 μl of 1:1 dilution of OKT6 monoclonal antibody and 25 μl of 1:10 dilution of OKIa antibody. Sections layered with PBS served as controls. Subsequently, the sections were washed for 10 minutes in 0.85% saline. These were then incubated with 25 μl of 1:80 dilution of FITC conjugated sheep anti-mouse IgF $(ab)_2$ mixed with ponta-

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Table I. Numbers of LCs in the skin lesions of untreated patients with tinea corporis and controls.

	Number of cases	Number of OKT6+ cells per high power field (Mean±SD)
Normal skin (controls)	5	14±2
Tinea corporis	11	Not detectable

chrome violet (1%) for 30 minutes at room temperature and washed in 0.85% saline for 20 minutes. The sections were mounted in 90% glycerol-PBS and viewed with epi-illumination using HBO 50 mercury lamp and Leitz inverted microscope with incident light excitation filter block No. 12 and transmitted light excitation filter block No. H 513604. The number of OKT6+ Langerhans cells per high power field in the epidermis was estimated.

Optimal dilutions and efficacy of OKT6 and OKIa monoclonal antibodies were assessed on cryostat sections of normal skin and skin lesions from leprosy patients. It was found that 1:1 dilution of OKT6 monoclonal antibody was optimal (Fig.1).

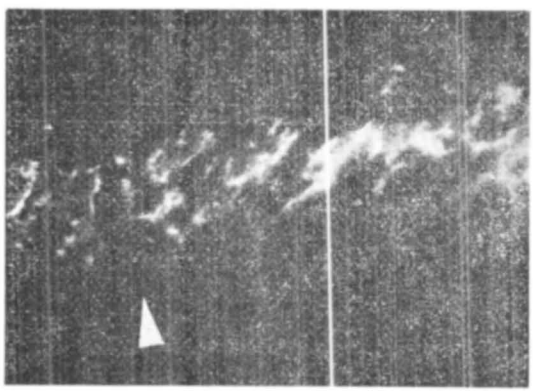

Fig. 1. LC in the epidermis (arrow) of normal skin showing intense immunofluorescent staining with OKT6 monoclonal antibody (cryostat section; counterstained with pontachrome violet X 300).

Results

Biopsies from all the patients showed histopathological features indicative of tinea corporis. Fungus was demonstrable in PAS stained paraffin sections.

The numbers of LCs in the skin lesions of untreated patients with tinea corporis and controls is shown in table I. The lesions of tinea corporis showed very few OKT6+ epidermal LC compared to the skin from normal persons. In most of the lesions of tinea corporis, OKT6+ LC could not be detected in the epidermis. The keratinocytes in the lesions of tinea corporis, also lacked the expression of Ia like antigens.

Comments

The inflammatory reaction that develops following invasion by the fungus, leads to permeation of the stratum corneum by a serum factor which kills or inhibits the fungus.[1] The sensitized lymphocytes release the lymphokines which could also directly kill the fungus. A possible dysfunction of cell mediated immune reaction normally elicited by the T cells may play a crucial role in the evolution of tinea corporis infection.[1] Patients with tinea corporis show negative delayed skin reaction to a variety of antigens indicating such a dysfunction.[1] Presence of IgE antibodies to trichophytin has also been observed in the serum of patients carrying tinea infection. LC have been shown to be involved in the presentation of the antigen to the T cells.[2] The role of LC has been emphasized in other disorders also like lichen planus.[11]

LC can be identified by a variety of techniques such as the presence of receptors for FC components of IgG, C3 component of complement, ATPase enzymes and Ia like antigens.[8] These markers may be identifying various subpopulations of LC. However, LC can be better defined by a T6 marker which is carried by all these cells.[9] This marker does not show

any cross reaction with morphologically similar dendritic macrophages.[8] More important, this marker delineates the morphology of the cells better than the conventional histochemical method (ATPase) and is far superior for the estimation of LC.[3] We have found as others have that OKIa antibody defined relatively fewer LC in comparison to OKT6 antibody and that LC count could be expressed equally accurately either as per high power field or per 100 keratinocytes.[5,10]

The number and distribution of LC in the epidermis of tinea corporis lesions was markedly reduced in comparison to that seen in the skin of normal persons. Similar findings in other conditions such as psoriasis, sarcoidosis, have been reported where reduction in LC numbers have been observed.[4,16] The uptake and the processing of the fungus may not occur in the epidermis of these lesions due to the absence of LC. LC take up the antigenic material and subsequently are involved in antigen presentation to T cells.[2,12] So, lack of LC may lead to ineffective T cell interaction resulting in poor T cell activation and consequently reduced level of cell mediated immunity in these patients. This may account for the negative skin reaction seen in these patients. It has been found in the experimental animals that contact sensitivity to DNFB could be obtained only in the presence of normal numbers of normally functioning LC.[13] That CMI level is low as evidenced by virtual absence of LC is further supported by the lack of Ia antigen expression on the keratinocytes of these lesions. It has been shown that the expression of Ia antigen on keratinocytes is a sign of cell mediated immune response.[14,15]

Perhaps the fungus itself releases cytotoxic factors which could have adverse effect on the LC in tinea corporis infection. Further work needs to be carried out to clarify these points.

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