ORIGINAL CONTRIBUTIONS

LANGERHANS CELLS IN VITILIGO

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Langerhans cells (LC) were defined by indirect immunofluorescence using OKT6 monoclonal antibody in the early lesions of 10 untreated vitiligo patients. The distribution and the numbers of OKT6+ epidermal LC in the lesions was similar to that observed in the normal skin. In the dermal infiltrates of some of the lesions, T6+ cells were visualized.

Key words : Langerhans Cells, OKT6 monoclonal antibody, Vitiligo.

Vitiligo is characterized by destruction of melanocytes and loss of pigmentation. It has been suggested that immune mechanisms may be involved in the pathogenesis of vitiligo. For example, presence of antibodies to melanocytes has been detected in the serum of vitiligo patients.[1] Autoantibodies to other organs have also been reported.[2] However, attempts to demonstrate alterations in humoral and/or cellular immune responses in vitiligo have generally been unsuccessful.

Reports in the literature indicate that Langerhans cells (LC) participate in the onset of contact dermatitis and other skin disorders like psoriasis and leprosy.[3-7] Very little information is available on the role of LC in vitiligo. In the present communication, the status of LC in vitiligo has been assessed using OKT6 monoclonal antibody.

Materials and Methods

Skin biopsies : Patients with vitiligo were selected from the outpatient clinic of the institute. The study included cases of vitiligo of recent onset (9 cases with less than 3 months duration and one case of 1 year duration). These patients were otherwise healthy. A typical skin lesion was biopsied. One half of the biopsy was fixed in buffered formalin and processed by conventional paraffin embedded blocks for histopathological analysis. The sections were stained with hematoxylin and eosin, and Van Kossa staining for melanin. The other half was collected in isopentane (Fluka, AG, chemische, Fabrik, CH-9470, Buchs) and frozen at —20°C for cryostat sections. Skin from normal individuals was used as controls. However, it was not possible to obtain skin from uninvolved sites of the same patients.

Reagents : Monoclonal antibody OKT6 (defining cortical thymocytes and LC) was obtained from orthopharmaceutical corporation, USA; FITC conjugated sheep antimouse IgF $(ab)_2$ from New England Nuclear Boston, USA.

Immunofluorescence : 4-5 μm 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 30 minutes with 1 : 5 dilution of OKT6 monoclonal 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 pontochrome

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violet (1%) for 20 minutes at room temperature and washed in 0.85% saline for 20 minutes. The sections were mounted in 90% glycerol-PBS and viewed by 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 were quantitated.

Optimal dilution and efficacy of OKT6 monoclonal antibody was assessed on cryostat sections of normal skin and skin lesion from leprosy patients.

Results

Only untreated patients of vitiligo were included in the study and in each patient only early lesion was biopsied. All the biopsies showed histopathological features compatible with vitiligo. The number of melanin positive cells in the vitiligo lesion (11 ±16) was significantly reduced in comparison to the numbers in the normal skin (80±17) (Fig. 2). This was also evident from the Van Kossa staining of paraffin sections (Fig. 1).

Preliminary experiments were carried out on the cryostat sections of normal skin to assess the optimal dilution of monoclonal antibody and the conditions required for staining. It was found that 1 : 5 dilution of the antibody was optimal. The OKT6 monoclonal antibody used, contains very low concentrations of mouse globulin.

No difference was observed in the numbers and distribution of OKT6+ epidermal LC in the vitiligo lesion and normal skin (Figs. 2 and 3). It was interesting that in some of the lesions, T6+ cells were seen in the infiltrates. These cells had dendritic appearance.

Comments

In recent years, it has been observed that the serum of vitiligo patients contains antibodies to melanocytes.[1] Uehara et al[8] have shown that vitiliginous skin does not respond to DNCB challenge in DNCB sensitized individuals where-

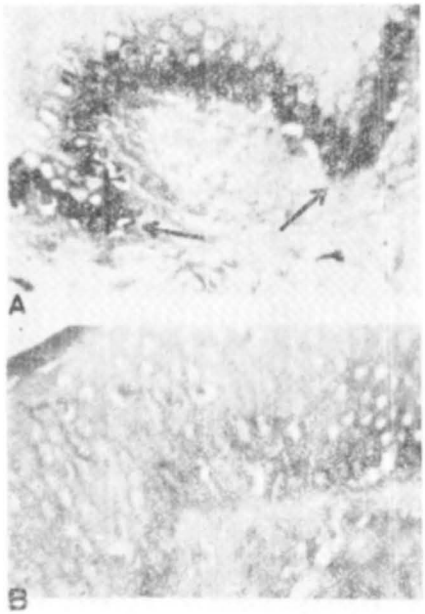

Fig. 1. Paraffin sections stained with Van Kossa stain for the demonstration of melanin positive cells (arrow). (A) Normal skin×120, (B) Vitiligo lesion×120.

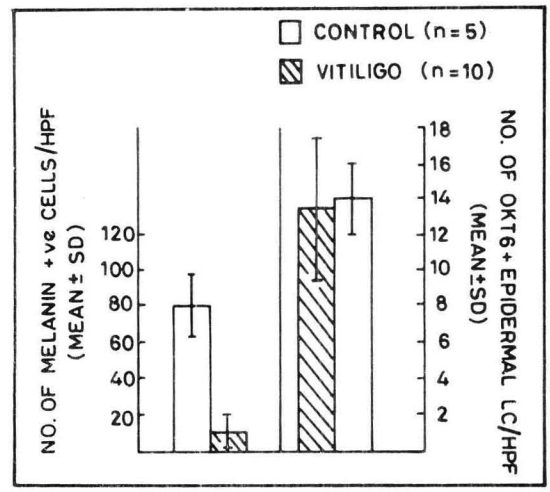

Fig. 2. Quantitation of melanin positive cells and OKT6 positive epidermal Langerhans cells in early vitiligo lesion and normal skin; n=number of patients.

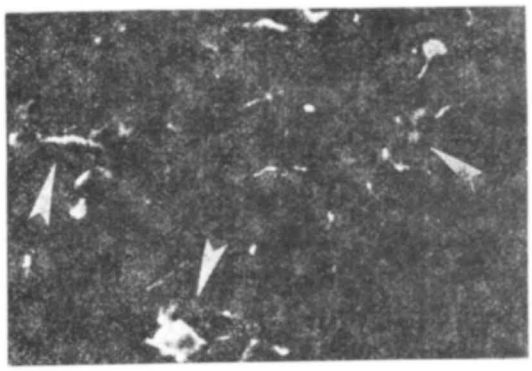

Fig. 3. LC in the epidermis (arrow) of vitiligo lesion showing intense immunofluorescent staining with OKT 6 monoclonal antibody (Cryostat section; counterstained with pontochrome violetX300).

as their response is normal when the adjoining normal pigmented skin is tested with DNCB. However, the authors have reported that their response to (injected) intradermal antigen is the same on the two sites, thus suggesting that epicutaneously applied antigen either does not reach the responsive cells or its presentation by LCs is defective. Further, the authors have shown that in addition to the absence of melanocytes, there was vacuolated degeneration of LCs and degenerative changes in keratinocytes suggesting that all the three types of epidermal cells may be involved in some way in vitiligo. Based on these observations the present study was undertaken to assess the distribution of LC in the early vitiligo lesions.

LC can be identified by the presence of receptors for FC component of IgG, C3 component of complement, ATPase enzymes and Ia like antigens. These markers may be delineating the various sub-populations of LC. However, LCs can be defined better by a T6 marker which is carried by all the cells.[10] This marker does not show any cross reaction with morphologically similar dendritic macrophages.[11] More important is that, this marker delineates the morphology of cells better than conventional histochemical method (ATPase) and is specific for the quantitation of LC.[4] Our earlier observations have indicated, (a) that OKIa antibody defines relatively fewer LC in comparison to OKT6 antibody, and (b) there is no significant difference in the LCs count whether it was expressed per high power field or per 100 keratinocytes.[6]

The numbers and distribution of OKT6+ epidermal LC in the vitiligo lesion was similar to that seen in the normal skin. These results support the previous studies where normal numbers and distribution of ATPase and Ia positive LC was observed in vitiligo.[12,13] It was interesting that in some of the lesions, T6+cells were seen in the dermal infiltrates. These cells had dendritic appearance. However, from the present study it is difficult to comment on the role of these cells.

LC have been shown to be involved in the proliferation and keratinization of the epidermis.[14] The extent of epidermal cell proliferation has been found to be inversely related to the numbers of LC.[15] So normal numbers and normal distribution of LC in vitiligo leads to normal levels of epidermal cell proliferation and function of keratinocytes thus maintaining the normal texture of the skin. It is also known that melanocytes interact with keratinocytes during pigmentation process of the skin.

The reduction or virtual absence of melanocytes in vitiligo is striking. In situations where the number of melanocytes is not drastically reduced, there may be a total absence or a poor interaction between keratinocytes and melanocytes thus causing depigmentation.

The finding of normal numbers of LCs in vitiligo is unexpected as theoretically, one would expect that vitiliginous lesions should show a decrease or damage in LC's because lack of melanin would allow ultraviolet light to permeate through the epidermis resulting in damage to these cells. LCs has been shown to be damaged

by UV irradiation.[16] Though the present investigation does not show any numerical decrease, however functional and/or electron microscopic studies need to be undertaken to see any aberation in these cells.

Acknowledgement

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