Langerhans' Cell and Vitiligo: Quantitative Study of T6 and HLA-DR Antigen-expressing Cells

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Epidermal Langerhans' cell (LC) densities in vitiliginous skin (VS) and normal-appearing skin (NAS) were studied in 10 patients with common vitiligo. Monoclonal mouse anti human T6 antigen IgG1 and Ia antigen IgG2 (Ortho Pharmaceuticals) were used to characterize LC. Epidermal LC densities were calculated by means of an ocular square grid and expressed per 0.1 mm^2. The results showed that LC densities of VS was similar to that of NAS (p: not significant). No differences were noted in terms of age, sex, progressing, stable or repigmenting vitiligo. We concluded that involvement of LC in vitiligo, if any, does not probably occur via a degenerating mechanism, or via variations in regional densities. *Key words: Vitiligo; Langerhans' cell; HLA-DR; OKT6.* (Received January 25, 1984.)

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Vitiligo is known to be caused by the destruction of pigment cells, but its pathogenesis has not been fully elucidated. Studies have shown that the 3 main epidermal components (melanocyte, keratinocyte and Langerhans' cell—LC) may be participating to the genesis of depigmentation in vitiligo (1–3).

In the present study, we investigated the qualitative and quantitative distribution of LC in vitiliginous skin (VS) and in normal-appearing skin (NAS).

PATIENTS AND METHODS

We selected 10 patients (6 females, 4 males) who had common vitiligo (vitiligo vulgaris). Patients with various forms of leukoderma, chemically induced vitiligo, vitiligo associated with melanomas were not included in the study. Ages ranged from 18 to 74. 2 patients had progressing vitiligo, 6 had stable vitiligo and 2 had spontaneously follicular repigmenting vitiligo. None of the patients was on any form of therapy for vitiligo.

Two punch biopsies (5 mm) were taken under local anesthesia (1% lidocaine) from each patient, one from VS and one from NAS, as far as 5 cm from the nearest white spot. No specimens were obtained from sole or genitalia.

Immunoreagents: Monoclonal (MC) mouse anti human T6 antigen IgG1, and anti human Ia antigen IgG2 (Ortho Immunobiology Ltd, Raritan, NJ) were used in the first step of immunofluorescence (IF) technique. Fluoresceine isothiocyanate (FITC)-conjugated goat anti mouse immunoglobulins (Nordic Immunology, Tilburg, The Netherlands) were used in the second step of IF technique.

Staining procedure: frozen skin sections (4 μm) were fixed in acetone (10 min at −20°C), incubated with the MC antibodies (final dilution 1 : 5) for 45 min, rinsed in phosphate-buffered saline (PBS) for 10 min twice. Sections were then incubated in FITC conjugate (dilution 1 : 20) for 45 mn, rinsed twice and mounted in buffered glycerin. The slides were viewed under a Leitz IF Orthoplan microscope and positive cells were counted by means of an ocular square grid. The dendritic cells were enumerated in 10 randomly chosen non overlapping fields. Only dendritic cells exhibiting a bright fluorescent cytoplasm were counted as positive, isolated dendrites were not scored. The mean epidermal LC densities in VS and NAS were expressed per 0.1 mm^2 and compared to control groups.

RESULTS

As shown in Table I, epidermal LC densities of VS was similar to that of NAS (*p*: not significant). No differences were noted in terms of age, sex, progressing, stable or repigmenting vitiligo.

We did not detect any differences in either the dendritic nature or fluorescent staining intensity of Ia or T6 bearing in the epidermis of VCS, NAS and control skin.

In our experience on normal skin, IF assay for LC Ia expression detected approximately 50% of the number of IF T6 antigen-bearing LC. The same discrepancies were noted in VS and NAS.

DISCUSSION

Few studies of LC have been carried out in vitiligo. LC densities have been reported to be either increased or normal in the skin lesions of vitiligo (4–6). Here, adenosine triphosphatase activity was used for the demonstration of LC in the epidermal sheets. In our study, using MC antibodies against T6 and HLA-DR (Ia-like) antigens which represent specific markers expressed only by LC and indeterminate cells in the skin (7), we have shown no increase or decrease in the concentration of LC in areas of vitiligo. No variation in T6 antigen-bearing LC densities has been noted in different anatomical regions in man, except for that of the soles or male genitalia (8). NAS from vitiligo patients could also be used as control for the study of VS, since the LC densities of NAS were similar to those of control

Table I. *Epidermal Langerhans' cell densities per 0.1 mm^2 in vitiliginous skin (VS) and in normal appearing skin (NAS)*

		Ia +	T6 +
VS	$n=10$	17.1±4.3	38.8±2.9
NAS	$n=10$	16.4±3.9	42.2±4.1
Controls	$n=10$	19.3±5.2	41.5±5.4

skin. Differences in expression of Ia and T6 antigen could be explained by a weaker Ia antigen and/or that the antisera detect two different subpopulations of LC, an indeterminate immature form of LC and a mature form of LC containing Birbeck granules.

Cellular degeneration in vitiligo is not limited to melanocyte but include keratinocyte. Recent observations have shown foci of vacuolar degeneration in basal and parabasal keratinocytes in close apposition to the pigment cell (2). The degenerative abnormalities resemble those seen during lymphocyte-keratinocyte interaction of graft-VS-host reaction (9) or during keratinocyte-LC interaction in pityriasis rosea (10). It has been hypothesized that LC in close apposition to the pigment cell could also be involved in the pathogenesis of vitiligo either by being exposed to pigment cell antigens released from dying melanocytes or from injured keratinocytes (11), or by releasing themselves lysosomal enzymes or lymphokines which may injure cutaneous pigment cells (3). Our results are in agreement with those of Haji-Abrassi et al. (12) who demonstrated that allergic contact hypersensitivity to dinitrochlorobenzene, which is highly dependent on the presence of functional LC, is normally elicited in VS.

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