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Interleukin-1 Decreases the Number of Ia$^+$ Epidermal Dendritic Cells but Increases Their Expression of Ia Antigen

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It is generally accepted that ETAF/IL-1 is produced in epidermis by both keratinocytes and Langerhans' cells. We have studied the density and morphology of Ia+ epidermal dendritic cells in mice after systemic or intracutaneous injection of recombinant IL-1β. We found that rIL-1β decreased the density of Ia$^+$ dendritic cells in the time period 2–7 days after rIL-1β administration. However, the remaining dendritic cells were enlarged and more arborized with increased expression of Ia antigen 1–4 days after injection of rIL-1β. The implication of the results is that ETAF/IL-1 modulates the function of Langerhans' cells through autocrine and paracrine regulation. ***Key words: Langerhans' cell; Cytokine; Time sequence; Autocrine/paracrine regulation.***

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Epidermal cell(keratinocyte)-derived thymocyte activating factor(ETAF) is the first epidermal cytokine described (1). Its activity is like that of interleukin-1 (IL-1) and recent studies indicate a close relationship at the molecular level between ETAF and IL-1 (2). The Langerhans' cells (LCs) may also synthesize a factor with IL-1 activity (3).

ETAF/IL-1 is a potent T-cell chemoattractant (4) and induces interleukin-2 receptor expression on T-lymphocytes (5). Furthermore, keratinocytes in culture are proliferating in response to ETAF/IL-1 suggesting an autocrine regulation by ETAF/IL-1 (6). In normal epidermis the LCs are the only cells which express MHC class II antigen and function as antigen presenting cells. Some in vitro experiments suggest that ETAF/IL-1 modulates the expression of T6 antigen on human LCs (7) and increase the accessory function of murine LCs (8).

The present study was undertaken to examine whether IL-1 could influence Ia expression of murine LCs in vivo.

MATERIALS AND METHODS

Animals

C3H/HeJ mice, obtained from The Jackson Laboratory, Bar Harbour, ME, USA, were outbred in our animal facilities and used when aged 8 to 10 weeks. They were kept under regular light/dark 12-hour circadian rhythm and fed pellets and water *ad libitum*.

Interleukin-1 treatment

Groups of mice (n = 3–5) were given recombinant interleukin-1β (rIL-1β) (generously supplied by Dr Robert C Newton, Du Pont Laboratories, Glenolden, PA, USA (9)) either intraperitoneally or intracutaneously in the ears in doses as stated in the text. Before injection rIL-1β (10^7 U/mg) was diluted in 0.3 M glucose. The intracutaneous injections were given into the dorsal ear skin under neurolept anesthesia, with 50 µl of rIL-1β in the right ear and the same volume of 0.3 M glucose in the left ear, which served as the control.

Visualization of Ia$^+$ dendritic cells

At the end of the experiments the mice were killed by cervical dislocation and their ears were cut off. Tissue discs, taken from the rim of the ears with a 3-mm skin biopsy punch, were incubated for 20 min at 37°C in ammonium thiocyanate solution as described (10) and the epidermal sheets peeled off from the dorsal side of the ear discs. After rinsing in Tris-buffered saline pH 7.4 (TBS) and fixation in methanol for 20 min followed by another rinse, the epidermal sheets were incubated with monoclonal mouse anti-I-A^k antibodies (Becton Dickinson, Mountain View, CA, USA) diluted 1:40 in TBS for 45 min at room temperature. After washing in TBS, fluorescein isothiocyanate (FITC)-labelled goat anti-mouse antibodies (Becton Dickinson), diluted 1:25, were applied for 30 min at ambient temperature. After rinsing, the epidermal sheets were mounted in glycerol/TBS on glass slides and studied in a Zeiss microscope fitted with incident UV-light and a camera. The morphology of the Ia$^+$ epidermal dendritic cells was studied on black and white photographs and their numbers recorded per mm^2 of epidermis.

Table I. *Groups of mice were injected intraperitoneally with 20 μg rIL-1β*

After various periods of time Ia^+ dendritic cells were determined in epidermal sheets from the ears by means of immunofluorescence

Time after rIL-1β	Ia^+ dendritic cells/mm² (mean ± SD)
Control (n=7)	1257±190
1 day (n=3)	1200±141
2 days (n=3)	570± 62***
3 days (n=3)	650± 92***
4 days (n=3)	853±104**
5 days (n=3)	940±356
7 days (n=3)	787±157**
10 days (n=3)	1037±157
14 days (n=3)	1030±132

*** = $p < 0.001$; ** = $p < 0.01$.

Statistics

Differences between group means were assessed using Student's *t*-test.

RESULTS

Effect of rIL-1β on Ia^+ dendritic cells

Time course: Following intraperitoneal injection of 20 μg of rIL-1β the presence of Ia^+ dendritic cells in the epidermis was monitored over a period of 14 days. As detailed in Table I the number of Ia^+ dendritic cells decreased significantly during a period ranging from 2 to 7 days after rIL-1β administration with a gradual restoration up to 14 days.

The morphology of the Ia^+ dendritic cells was also clearly modulated following rIL-1β injection. An increase in the intensity of Ia antigen expression was observed from 24 h onwards reaching a maximum at 2 days and a return to control levels by day 4 or 5. On day 2 after rIL-1β the Ia antigen was expressed over an enlarged cell body with elongated, branched dendrites (Fig. 1).

Dose response: In order to determine the lowest dose of rIL-1β capable of modulating the behaviour of dendritic cells in epidermis, groups of male mice were injected with four-fold dilutions of rIL-1β starting with 20 μg. The epidermal sheets were prepared for immunofluorescence studies of Ia^+ dendritic cells two days after injection. As Table II shows there is a dose response with 0.08 μg as the lowest dose inducing a significant decrease in the density of Ia^+ dendritic cells.

In this experiment we also looked at the morphology of the dendritic cells. A dose of 0.31 μg rIL-1β was required to give occasional enlarged "activated" Ia^+ dendritic cells, while 5 μg consistently produced a homogeneous picture of large "activated" dendritic cells.

Effect of intracutaneous rIL-1β

To find out whether the effect of rIL-1β was direct or was mediated via distant systemic mechanisms, a small dose of rIL-1β was injected intracutaneously into the ear skin and the dendritic cells in the overlying epidermis were studied. The dose chosen was 0.02 μg of rIL-1β, a dose small enough to avoid systemic effects as shown in the previous experiment. Accordingly, when studied two days after local injection, the ears treated with rIL-1β had a density of Ia^+ dendritic cells of 500 ± 41/mm² epidermis, while the ears from the same animals injected with the control had 875 ± 149/mm² ($p < 0.001$). The morphology of the Ia^+ dendritic cells was the same as in previous experiments with large "activated" dendritic cells in ears treated with rIL-1β but not in control ears.

DISCUSSION

The epidermal Ia^+ dendritic cells are considered to be LCs. Epidermal LCs are immunologically immature cells which change in culture by increasing the level of Ia antigen expression and by forming large

Table II. *Groups of male mice were injected intraperitoneally with various doses of rIL-1β*

Two days after injection Ia^+ dendritic cells in epidermal sheets from the ears were studied by means of immunofluorescence

rIL-1β dose(μg)	Ia^+ dendritic cells/mm² (mean ± SD)
Control (0.3M glucose) (n=7)	1257±190
20 (n=4)	624± 86***
5 (n=4)	678±133***
1.25 (n=4)	735±230**
0.31 (n=4)	791±178**
0.08 (n=4)	975±148*
0.02 (n=4)	1040±367
0.005 (n=4)	1065±158

*** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$.

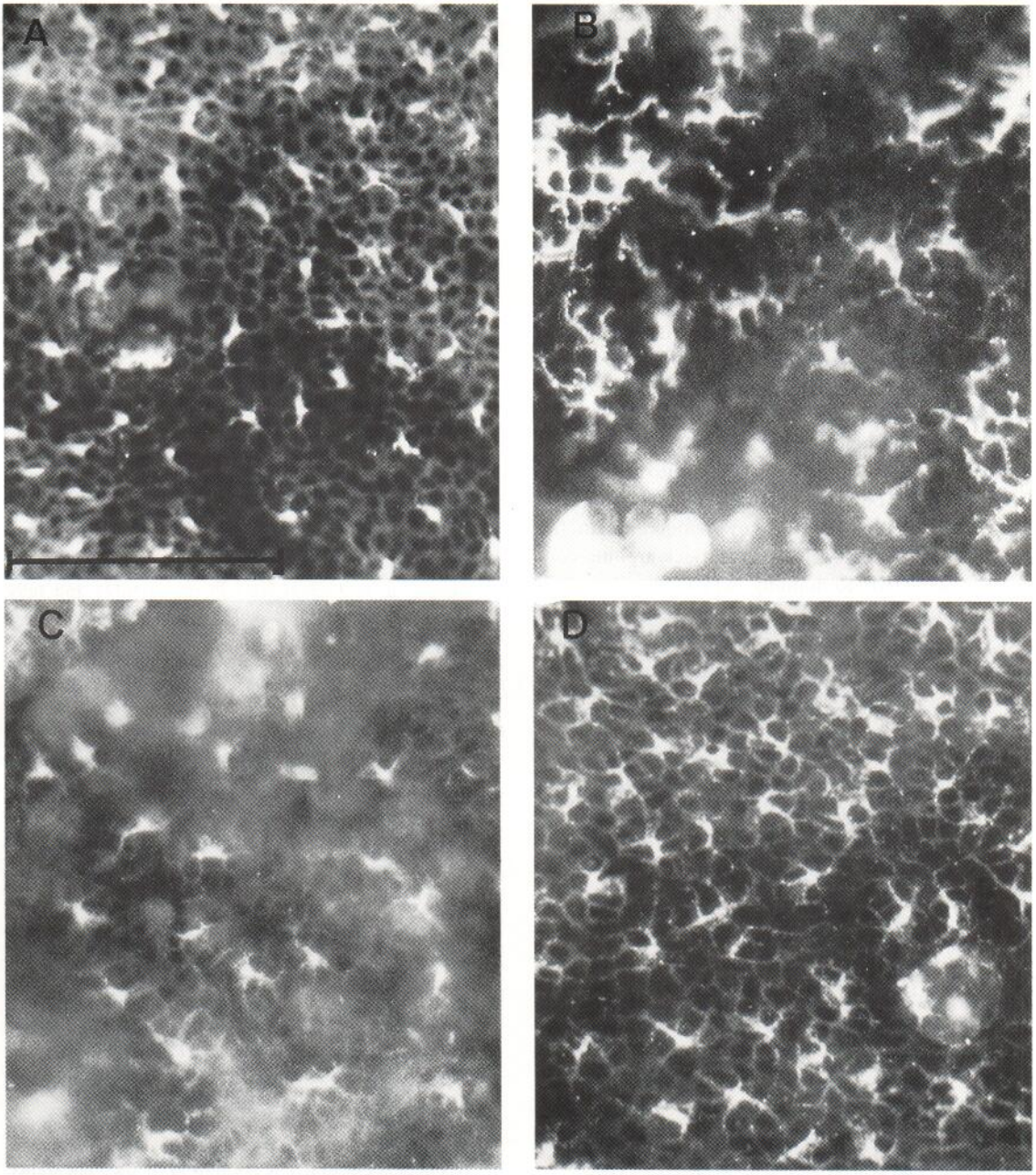

Fig. 1. Groups of mice were injected intraperitoneally with 20 μg rIL-1β and Ia⁺ dendritic cells were studied by means of immunofluorescence. *A.* Control (bar = 100 μ), *B.* 2 days, *C.* 5 days and *D.* 10 days after injection of rIL-1β.

clusters of responding T cells (11). This maturation in culture can be induced by granulocyte/macrophage colony-stimulating factor and IL-1 in concert (8). Our studies in vivo extend these findings by demonstrating that rIL-1β dose-dependently increased the Ia antigen expression of epidermal dendritic cells which were also enlarged with increased arborization. In vitro experiments show that activated LCs are far more dendritic and express Ia antigen 10 to 36 times more intensely than do normal LCs and that this activation is associated with an enhanced antigen presenting capacity (12).

It has been demonstrated that UV irradiation may enhance ETAF/IL-1 production by epidermal cells (13, 14). Previous studies also show that UV exposure in vivo may induce a biphasic response in epidermal LC function: an initial depression followed by an enhanced antigen presenting capacity (15). When this latter phase is analyzed in detail there is a decrease in the percentage of LCs and an appearance in epidermis of $T6^-Dr^+$ bone marrow-derived cells responsible for the enhanced alloantigen presentation. In our experiments we found a similar picture where normal LCs decreased in density and large dendritic intensely Ia^+ cells started to appear after 24 h, peaked at 2 days and were gone by day 5. Whether this scenario means there is a temporary activation of normal LCs or a flux of dendritic cells with heavy expression of Ia antigen into the epidermis remains to be shown. Time sequence studies of LCs after UV irradiation show a decrease in the density of ATPase positive and Birbeck granule-containing LCs (16). Remaining ATPase positive LCs were obviously enlarged, with elongated dendritic processes. This picture is consistent with our findings and suggests that UV irradiation and IL-1 modulate the morphology of the LCs through the same mechanism.

Taken together, our findings indicate that rIL-1β modulates the morphology and behavior of the murine Ia^+ dendritic epidermal cells. This implies that the immunological function of the epidermis may also be regulated through paracrine and autocrine mechanisms within the epidermis.

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