Effects of Etretinate on Epidermal Langerhans Cell

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Summary: The effect of etretinate, applied systemically, on epidermal Langerhans cells was studied. The numbers and shapes of Langerhans cells were observed on split epidermis, and the percentage of Langerhans cells in whole epidermal cells was determined with a flow cytometrial system. On an epidermal sheet, the number of Langerhans cells changed biphasically, first the number increased, then decreased, and finally it returned to normal. The percentage of Langerhans cells was drastically decreased by etretinate and the low value was maintained during the etretinate treatment. This difference may be due to a decrease in the cell surface marker.

Key words: Langerhans cells—epidermal sheet—flow cytometry—retinoid—Ia-antigen

Introduction

Epidermal Langerhans cells (LCs), situated in the midepidermis, maintain constant shapes and numbers. Various stimuli such as UV irradiation, corticosteroids and tape stripping, can change the number and shape of the LCs (Lessard et al. 1968; Aberer et al. 1981; Berman et al. 1983). The number of stump-tail monkey epidermal LCs was reduced during the topical application of retinoic acid onto the epidermal sheet (Hachisuka and Uno, 1987). The present paper describes the effect of etretinate on the percentage of Ia antigen-positive cells in whole epidermis by flow cytometry, and compares these to the numbers in the split epidermis of guinea pig skin.

Materials and Methods

Hartley strain guinea pigs, weighing 500 g, were used in the study. Retinoid treatment was performed by oral administration of etretinate (Ro-10-9359, Roche, Basel, Switzerland) at a concentration of 10 mg/kg in 1 ml of peanut oil. The guinea pigs were separated into 6 groups and were given etretinate, daily, for 30 days. Every 5th day, the dorsal skin of 3 guinea pigs from each group was shaved with an electric clipper. The remaining hair was removed with a hair remover. Animals were sacrificed by cervical dislocation, and the dorsal skin was removed with a keratome set at 0.5 mm. The skin sample was incubated in 20 mM EDTA (pH 7.3) at 37°C for an hour. The epidermal sheet was separated from the dermis with fine forceps.

Flow cytometry:

The split epidermis was incubated in 0.25% trypsin-Hanks solution (pH 7.3) for 20 min at room temperature. After filtration through a nylon mesh, the isolated cells were washed twice with Hanks solution and suspended in phosphate-buffered saline (PBS), pH 7.2, at a concentration of 5×10^6 cells/ml. 100 μ l of the cell suspension was incubated with 5 μ l of OKIa1 (Ortho, NJ) (Stingl et al. 1978) for 30 min at 4°C. Myeloma ascites was used instead of OKIa1 for the negative control. After washing with PBS, the cells were further incubated with FITC-conjugated goat antimouse IgG (Tago, CA) for 30 min at 4°C. After again washing with PBS, the Ia antigen-positive cells were counted with a flow cytometrial system with Spectrum III (Ortho, NJ). Evaluation for threshold on the histogram was determined by comparing the fluorescence to the background fluorescence from the myeloma ascites. The threshold for the negative control was adjusted to below 2.0%. Each determination was duplicated and was based on more than 1×10^3 cells per sample.

Epidermal sheet:

The split epidermis was washed three times in PBS for 15 min and fixed with precooled acetone at -20°C for 40 min. After washing three times with PBS, the tissue was incubated with OKIa1 (1:40 dilution) at room temperature for 60 min. After another washing with PBS, the tissue was incubated with peroxidase-conjugated goat anti-mouse IgG (Tago, CA; 1:20 dilution) for 60 min at room temperature. Then the tissue was rinsed with PBS, and placed in DAB-H₂O₂ solution for 5 min and mounted on a glass slide, after washing with PBS. Ia positive-cell counts were performed using a reticle fitted into the eyepiece of the microscope. The number of cells per mm² was counted in 10 fields.

Results

Flow cytometry:

The ratio of Ia positive cells to whole epidermal cells was shown in Fig. 1. In untreated animals, 6.7% of the epidermal cells were Ia antigen-positive. The ratios of Ia antigen-positive cells in etretinate treated guinea pigs were drastically reduced to 1.9% on 5th day. This low value was maintained for 25 days. Epidermal sheet:

As shown in Fig. 1, the number of Ia positive cells was increased from the 5th to the 15th day, and then decreased at the 20th day. The dendritic processes were shortened or disappeared, and the perikaryon became oval or round. The population and morphology of the Ia positive-cells returned to normal during the etretinate treatment.

Histology:

In hematoxylin and eosin sections, the epidermis had granular and squamous layers that were increased in thickness ap-

Fig. 1. Time course of changes in the population of Ia positive cells during systemic application of etretinate, illustrated as, $\circ-\circ$, percentage of Ia antigen-positive cells in whole epidermal cells, and $\bullet-\bullet$, number of Ia antigen-positive cells in the epidermal sheet.

proximately twice, by the 15th day after beginning etretinate administration, and this thickness was maintained throughout the experiment.

Discussion

The present study demonstrates that the systemic administration of etretinate causes biphasic changes in the number of epidermal Ia antigen-positive cells in the epidermal sheet. An increase of the granular and squamous layers was observed, even by the 5th day, and continued up to the 30th day. In the epidermal sheet, the number of Ia antigen-positive cells first increased, and then decreased. Finally, the number returned to the normal level. The increase in the number of Ia antigen-positive cells may be due to migration from the dermis or to proliferation within the epidermis (Kanerva et al. 1983). On the other hand, there are several possible explanations for the reduction of Ia antigen-positive cells. First, there may be an actual decrease in the cell number. Second, the cells may have been affected functionally, as a result of alterations of the Ia antigen on the cell surface. Third, the decrease could be the result of an enhanced migration of LCS from the epidermis. In an earlier paper, the number of Ia antigen-positive cells in the epidermal sheet were counted. The increase or decrease was not expressed as a percentage of all the epidermal cells because the epidermis became acanthotic during etretinate treatment. In the present study, acanthosis was histologically observed from the 5th day. Therefore it seemed important to determine whether the change in the ratio between Ia antigen-positive cells and epidermal cells coincided with the changes in the epidermal sheet. In contrast to the results on the epidermal sheet, the percentage of Ia antigen-positive cells in whole epidermal cells was reduced at the

5th day and this low value was maintained to the 30th day. When an increase of LCS was observed in the epidermal sheet, the percentage of cells was still decreased. This difference is probably due to a decreased fluorescence of Ia antigen-positive cells below the threshold for flow cytometry. Since the Ia antigen is stable against trypsin (Stingl et al. 1978), the reduction of Ia antigen-positive cells is not due to the enzyme. Etretinate at a concentration of 10 mg/kg, which is ten times higher than the clinical dose, could destroy the Ia antigens on the LCS. Although this study does not exclude the possibility that further changes of LCS could be induced by longer administration, etretinate is assumed to have a toxic effect on the epidermal LCS.

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