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SHORT REPORTS

The Majority of Epidermal Merkel Cells Are Non-proliferative: A Quantitative Immunofluorescence Analysis

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Although epidermal Merkel cells (MC) are able to form synapses and synthetize neuromediators, they can be considered as being of epithelial nature because of the presence of cytokeratins in their cytoskeleton and desmosomes on their membranes. Since epidermis is an epithelium undergoing permanent renewal, it was important to determine whether MC were able to renew, as neighbouring keratinocytes do. This was investigated by studying whether S phase nuclei could be found in cells bearing a specific MC marker. The technique consisted of injecting rabbits with bromodeoxyuridine (BrdUrd) and performing double immunofluorescence on skin sections with the antikeratin number 8 monoclonal antibody (MAb) TROMA-I and anti-BrdUrd MAb. The results show that, in contrast to the neighbouring epidermal cells, the great majority of MC were found to be devoid of BrdUrd labelling, indicating that most of these cells are unable to divide, or divide very rarely. (Received April 14, 1987.)

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The developmental origin and the histogenesis of Merkel cells (MC) have recently been questioned (1, 2). MC, which are present in the basal layer of mammalian epidermis, possess neural characteristics, such as the ability to synthetize neuromediators and form synapses. For this reason, MC have long been thought to be of neural crest origin (3). Recent studies, although not solving the question of their developmental origin, have shown that MC contain keratin intermediate filaments (4) and that they are able to form desmosomes (5), as do authentic epithelial cells. Most epithelial cells divide actively (6), while cells involved in synapse formation (neurones, sensory cells, muscle cells, etc.) do not, certainly because their function requires stability of neuronal connections. Because of this apparent contradiction between the epithelial nature and the function of MC, we investigated whether these cells are able to divide and renew as do the neighbouring keratinocytes (7), or are permanent, as the neurones with which they form synapses.

METHODS

Rabbits

Animals were New Zealand white male rabbits weighing 2.5 to 3 kg.

BrdUrd injection

Animals were injected locally with 100 mg of BrdUrd (Sigma, St. Louis, USA) in 1 ml saline, 4 h prior to sacrifice. Samples were then excised and frozen.

Rabbit lip staining

MC in the rabbit lip can be identified by labelling with the anti-keratin number 8 MAb TROMA-I, as previously described (5). The specificity of this staining has been controlled by immunoelectron microscopy. The staining obtained with TROMA-I is unaffected by previous incubations (in 4 N HCl, 0.1 M sodium tetraborate, pH 8.5, and 0.5% Tween 20/PBS) of the anti-BrdUrd labelling (8). Rabbit lips and rabbit epidermis from two different animals were studied.

Procedure

Cryostat sections (4 μm) were incubated in 50 μl of PBS, pH 7.2, for 10 min, then treated with 50 μl of 4 N HCl at room temperature for 5 min. To neutralize the acid, the sections were washed for 5 min in 50 μl of 0.1 M $Na_2B_4O_7$ and treated with 50 μl of 0.5 Tween 20/PBS. After three washes in PBS, pH 7.2, for 5 min each, the sections were incubated with anti-BrdUrd FITC MAb (Becton Dickinson; dilution 1 : 10) for 30 min at room temperature. They were then washed in the same buffer as before and incubated in TRITC conjugated goat antirat immunoglobulins (Ig) for 30 min. They were finally washed in PBS, pH 7.2. and treated with TROMA-I MAb (dilution 1:30) for 30 min at room temperature.

All preparations were mounted in PBS buffered glycerol and examined by epi-illumination with a Zeiss Universal microscope. Photographs were taken with a Zeiss MC camera system and Kodak Ektachrome 400 film.

Split rabbit epidermis and rabbit lip epithelium

Merkel cells can also be identified by TROMA-I on split rabbit epidermis (RE) and rabbit lip epithelium (RLE) after chemical association by NaBr or EDTA. In these experiments, RE and RLE were split mechanically from their underlying epidermis after an incubation in EDTA during 1 h at 37°C. After washing in PBS, pH 7.2, for 5 min each, the epithelial sheets were applied on a glass slide and then treated as described. In this case, however, the incubation time for the two MAbs (TROMA–I and anti-BrdUrd) was increased to 2 h.

Control

After using the anti-BrdUrd FITC, the sections were treated for 30 min at room temperature with a normal mouse serum diluted at 1 : 10 in PBS, pH 7.2, to eliminate a cross reactivity between conjugated mouse and rat Igs.

RESULTS

As shown on Fig. 1, TROMA-I MAb stained the cytoskeleton of some epidermal cells in a filamentous pattern in red fluorescence (TRITC). As shown in previous studies (5), this marker makes possible the identification of MC. The green (FITC) staining pattern obtained with BrdUrd MAb was nuclear and had a granular appearance. Quantitative observations were performed in the rabbit lip epithelium and epidermis from horny skin in two different animals. A total of 1 100 MC was scored. Only one cell among 1 100 TROMA-I-positive cells showed a reactivity for the anti-BrdUrd MAb. Fig. 1 shows this single cell presenting a double labelling. In addition, 1 000 BrdUrd-positive cells were carefully examined, either on cross sections or on split preparations. None of them showed any reactivity for TROMA-I as expected for adult keratinocytes.

DISCUSSION

The use of anti-BrdUrd MAb makes possible the detection, in vivo, of cells which have been able to incorporate BrdUrd after injection in the animal, or, in vitro, after addition to the culture medium. The labelled cells are thus actively synthetizing DNA and are presumably in the S phase (8).

The usefulness of this technique should be stressed. It is faster than autohistoradiography, easy to perform, and combined with the use of a cell-specific marker, it allows to evaluate by immunofluorescence the cell renewal of a given cell type. It has already been

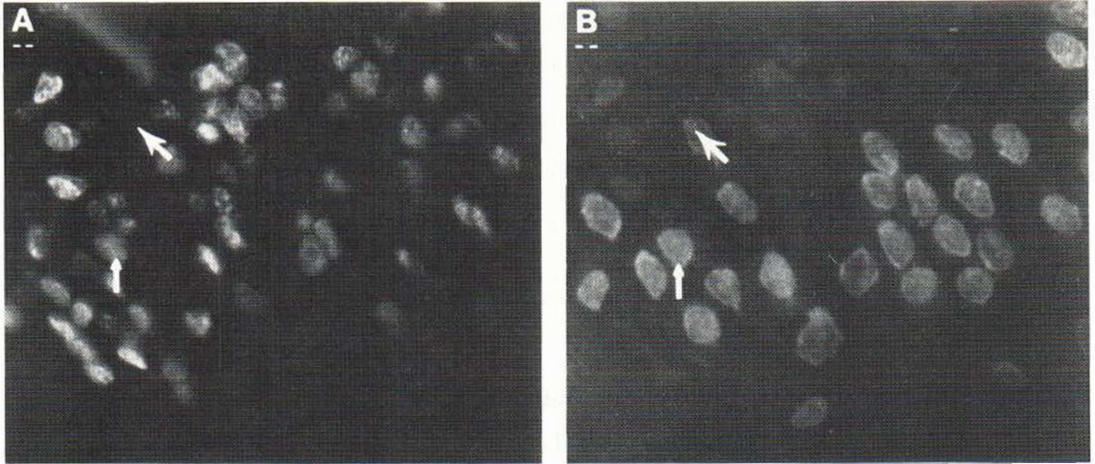

Fig. 1. Double labelling on a 4 μm cryostat section of a rabbit lip with A: anti-BrdUrd MAb and B: TROMA-1 MAb. The large arrow shows an example of Merkel cells negative for anti-BrdUrd. The small arrow shows the only double-labelled cell found among 1 100 scored. Some other cases of apparent double staining correspond to superpositioning of distinct cells.

applied to different cell types in the skin (9, 10, 11). However, one of its limitations is the drastic chemical procedure needed to denature DNA, which can alter the antigens used to identify a cell type in a double staining immunofluorescence procedure. Fortunately, this was not the case for the cytokeratin number 8 used in these studies as a marker for MC.

From our observations, the great majority of MC do not incorporate BrdUrd in vivo in adult rabbit skin and lip (12). Results presented here show that the rate of DNA synthesis by MC is virtually nil (at the most 0.1%). This contrasts with the high fraction of BrdUrd-incorporating cells (2–5% in vivo) (6, 7) of neighbouring keratinocytes and dermal fibroblasts.

Although MC have an epithelial phenotype and location, they are more closely related to neurones and cells of sensory receptors from a tissue kinetic point of view. This property is probably related to the fact that synapse formation is incompatible with frequent cell renewal.

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T-Helper Cell Activation in Bullous Pemphigoid

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In 10 untreated patients suffering from acute bullous pemphigoid the number of peripheral blood T cells (CD 3), T suppressor (CD 8) and T helper cells (CD 4) as well as activation antigens (DR and Tac) bearing lymphocytes was evaluated by monoclonal antibodies. While the pan-T cell population (CD 3) and T suppressor subpopulation (CD 8) were normal, the T helper subpopulation (CD 4) and the number of DR and Tac positive lymphocytes were significantly increased in the acute stage of bullous pemphigoid when compared to the age- and sex-matched controls. Phenotypically those DR positive cells belonged to the pan-T cell population (CD 3) at 66% and to the T helper subpopulation (CD 4) at 53% respectively. Under treatment with immunosuppressants the cell counts returned to normal suggesting a T helper cell activation to be involved in the acute stage of bullous pemphigoid. *Key words: Lymphocyte subpopulations; Activation antigens.* (Received April 8, 1987.)

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Bullous pemphigoid (bP) is a blister forming skin disease of autoimmune nature characterized by the deposition of immunoglobulins and/or complement at the basement membrane zone (BMZ) and by freely circulating BMZ antibodies, mostly of the IgG class. No further deviations of the humoral immune system up to now is detected. Concerning the cellular immunity it is known, that the peripheral blood lymphocytes (PBL) of bP patients exhibit a normal distribution of T and B cells (1, 2, 3) and that neither a lack of suppressor cells nor an increase in the helper cell activity is found in functional assays (4, 5). Activated lymphocytes and their products may play an important role in the blister formation, because lymphokines with lymphocyte chemotractant (6) and lymphotoxin-like activities (7) have been detected in the blister fluid of bP. The proportion of lymphocyte subpopulations and activation antigens bearing lymphocytes are still quite unclear in bP patients. The aim of the present study was to quantify some of the defined subpopulations of PBL and to identify the phenotype of activation antigens bearing lymphocytes by monoclonal antibodies in bP patients before and under treatment with immunosuppressants.

PATIENTS

The patients were studied in the acute stage of bP (before treatment) as well as under therapy with immunosuppressants. All patients exhibited subepidermal blisters in histology and IgG and/or com-