Apoptosis in Normal Skin

Sir,

Apoptosis is associated with physiological or programmed cell death, in contrast with necrosis which is associated with cell injury. Some examples of systems in which apoptosis occurs are: maturation of the immune system, embryonic development, hormone deprivation of endocrine or other hormone-dependent or sensitive cells, cells responding to a mild thermal or metabolic stress, and normal tissue turnover.

In the dermatologic field, cells in the epidermis and appendages show a clear turnover. The turnover may be due to programmed cell death.

In this study, we investigated the apoptosis of skin using an in situ technique.

MATERIAL AND METHODS

Normal skin was obtained from the normal margins of excisional skin biopsy specimens from adult patients during routine surgical procedures. All specimens were placed in 10% buffered formalin, embedded in paraffin and stored until use.

We used reagents for non-isotopic DNA end-extension in situ, and other reagents for immunohistochemical staining of the extended DNA. Residues of digoxigenin-nucleotide were catalytically added to the DNA by terminal deoxynucleotidyl transferase (1), an enzyme which catalyzes a template-independent addition of deoxyribonucleotide triphosphate to the 3'-OH end of double– or single-stranded DNA. The incorporated nucleotides form a random heteropolymer of digoxigenin-11-dUTP and dATP, in a ratio that has been optimized for anti-digoxigenin antibody binding. The anti-digoxigenin antibody fragment carries a conjugated reporter enzyme (peroxidase) to the reaction site. The localized peroxidase enzyme then catalytically generates an intense signal from chromogenic substrates (3,3'-diaminobenzidine with nickel).

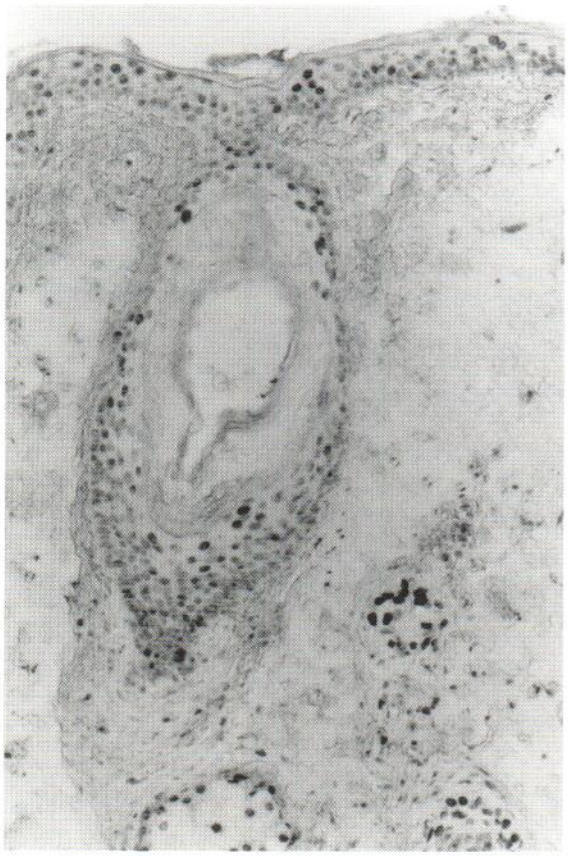

Fig. 1. Apoptotic cells are stained in black by 3,3'-diaminobenzidine with nickel.

The results are shown in Fig. 1. In the epidermis, the cells in the basal layer showed an almost negative reaction, but several cells in the granular layer showed a positive reaction. In the dermis, the cells in the hair follicle, sebaceous gland and sweat apparatus showed positive reactions, in contrast with the stromal cells which showed a negative reaction.

DISCUSSION

Skin is a dynamic organ which actively renews itself. Especially, the epidermis and appendages maintain homeostasis. Some studies have reported apoptosis of the skin during the developmental process in the embryo or fetus, similar to apoptosis in other organs during the developmental process (2), but few studies have investigated apoptosis in skin homeostasis in adults. Some studies have examined bcl-2 relative to apoptosis in the hair follicle (3–5), but few have examined apoptosis directly. Demonstrating the ladder pattern using gel-electrophoresis indicates whether apoptosis occurs but cannot show which cells are involved in apoptosis. However, the in situ method can demonstrate the cell in which apoptosis occurs. Therefore, we investigated apoptosis of adult skin in homeostasis, and the results demonstrated apoptosis in the epidermis and appendages; thus, we speculate that apoptosis is the mechanism of turnover in adult skin homeostasis. Further research into apoptosis in normal skin and that in many skin diseases is needed.

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Isotretinoin-induced Pemphigus

Sir,

A 17-year-old male patient came to us with vesicles, bullae and crusted erosions on his face, trunk, back, arms and legs. The patient also had lesions, typical of cystic acne, on his back.

Due to his cystic acne, the patient was treated initially with HCl-tetracycline and minocycline, but as he had no improvement, he started treatment with isotretinoin at a dosage of 1 mg/kg/day (a total of 90 mg/day was administered). Sixty days after the start of this treatment, blisters began to develop, first on his trunk and then on his face, arms, back and legs. The eruption was asymptomatic. After this we examined the patient. Examination revealed blisters and crusted erosions, widespread with no symptoms. Nikolsky sign was positive. The oral membrane showed no signs of the disease. The patient's general health was excellent.

A skin biopsy specimen showed intraepidermal bullae, severe acantholysis, suprabasal clefts with papillomatous projections of the dermis, typical of pemphigus (Fig. 1). Indirect immunofluorescence studies demonstrated circulating antibodies of IgG class, directed against the intercellular substance, at a titre of 1:160.

The diagnosis of drug-induced pemphigus was made and isotretinoin therapy was discontinued immediately, while treatment with prednisolone, at a dose of 75 mg/day, and azathioprine, at a dose of 200 mg/day, was started. Improvement of skin lesions was obvious within 3 weeks, with signs of healing, while complete recovery was achieved within 3 months.

The improvement of the antibody titre was also rather quick, it was negative about 6 months after the disease had started.

Prednisolone was discontinued gradually over a period of 7 months and azathioprine was administered to the patient, in total, for 9 months.

At a follow-up 52 months after the discontinuance of isotretinoin, the patient was still free of any sign of pemphigus and the antibody titre was negative.

A possible mechanism for the acantholysis in our case could be that retinoids may affect membranes, because of their lipophilic character (1). Our clues for the diagnosis of drug-induced pemphigus have been based on the following: a) the first signs of the bullous disease appeared a short period after the administration of the drug (isotretinoin); b) quick improvement (3rd week) and quick complete resolution (3rd month) of the disease, which is not very common in pemphigus; and c) despite the quick discontinuance of prednisolone and azathioprine, the patient has been free of clinical symptoms for almost 5 years and the titre of the antibodies still remains negative.

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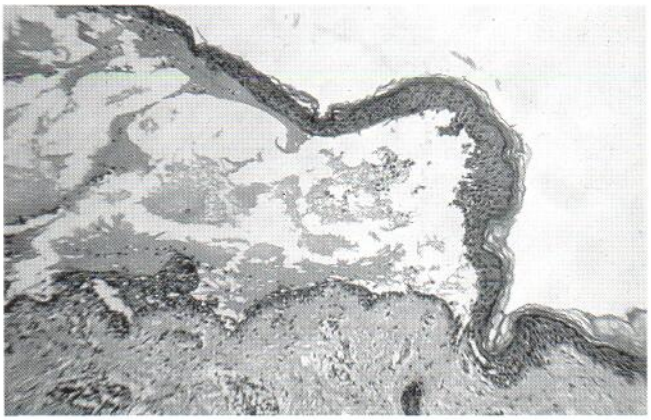

Fig. 1. Light microscopy of skin lesion (× 160).