Epidermal Plasminogen Activator Activity, tPA-dependent, Is a Marker of Disease Activity in Psoriasis

To the Editor:
We read with interest the article “Immunohistochemical localization of urokinase and tissue-type plasminogen activators in psoriatic skin” by Grøndahl-Hansen et al [1], in which abnormal localization of both urokinase (UK) and tissue-type plasminogen activators (tPA) in psoriatic skin is shown. This prompted us to report our autohistographic data showing that 1) psoriatic epidermis is provided with plasminogen activator activity (PAA); 2) increased epidermal PAA may be considered a marker of disease activity, and 3) autohistographically detectable epidermal PAA in psoriatic plaques is tPA-dependent.

Cutaneous PAA was estimated using Todd’s autohistographic fibrin film technique [50 mg of plasminogen-rich fibrinogen (Test Fibrinogen Behringwerke) in 5 ml of phosphate-buffered saline, pH 7.4, and 50 μl of thrombin, 250 U/ml (Thrombin Test, Behring)] [1] modified to obtain constant tissue and fibrin film thickness [3,4]. Appropriate control tests with fibrinogen-free film (Bovine Fibrinogen Fibrinogen Free, Organon Tecknika, Turn-hout, Belgium) were performed in all cases in which cutaneous fibrinolytic activity (CFA) was noted. Urokinase and tPA-dependent fibrinolytic activity (FA) was autohistographically assessed by separate preincubation, 10 min at room temperature, of each cryostat section with 2 ml of monoclonal antibodies directed respectively against the catalytic site of UK and tPA (anti-tPA and anti-UK, Monozyme ApS, D2800 Lyngby, Denmark).

In 1983 we did separate autohistographic evaluations of PAA in the epidermis and dermis (in 5 psoriatic patients, 10 healthy controls, and 5 subjects with lichen planus) to avoid the influence of any passage of activators and/or inhibitors from the epidermis to the dermis, and vice versa [5]. The separation of the epidermis from the dermis was obtained by rinsing the biopisc specimens into a 2 M sodium bromide solution for 2 h at 37°C. PAA was found in all specimens of psoriatic epidermis, but it was absent in the epidermis of the 10 healthy controls and in the epidermis of the 5 patients affected with lichen planus. In 1986 we evaluated overall CFA in 7 patients suffering from psoriasis before and after anthralin treatment [6], and in 3 healthy controls. A 6-mm punch biopsy specimen was taken from a psoriatic patch of each patient at the very beginning of the study and again 6 weeks after a single daily application of 0.1% anthralin in a petrolatum base. At the end of the 6-week period, 6 of the 7 patients demonstrated clinical clearing. In these cases, the skin biopsies were performed in previously involved areas in which minimal residual erythema was still present. In the one patient who had not responded to treatment, the biopsy was performed in the treated lesional skin. CFA was increased in all the 7 patients before anthralin treatment (Fig 1). Areas of lysis were found in the epidermis and dermal blood vessels. After the 6 weeks of treatment, no FA was evidenced in the epidermis of the 6 patients who manifested clinical clearing of the lesions, but it was demonstrated in the dermal blood vessels, with a pattern similar to that found in normal healthy skin (Fig 2).

The patient who did not respond to the treatment showed no reduction of PAA in the lesional skin.

Finally, using the same autohistographic technique (modified by preincubating anti-tPA and anti-UK monoclonal antibodies) we recently evaluated PAA in the involved and uninvolved skin of 8 psoriatic patients and in 4 sex- and age-matched controls [7]. Epidermal PAA was evidenced in all the specimens of psoriatic epidermis (Fig 3), while none was found in the uninvolved epidermis or in the control specimens.

The preincubation of the specimens with anti-tPA antibodies completely abolished epidermal PAA in the psoriatic epidermis (Fig 4), both before and after the separation of the epidermis from the dermis with NaBr, 2 M. The epidermis of uninvolved skin of psoriatic patients and epidermis from normal control subjects showed no detectable PAA either before or after incubation of the specimens with anti-tPA antibodies. Using anti-UK antibodies, psoriatic epidermal PAA was evidenced, showing the same pattern as with the

**Figure 1.** Cutaneous fibrinolytic activity in psoriasis (autohistographic method). Note areas of lysis in correspondence with the epidermis and with the dermal vessels.

**Figure 2.** Fibrinolytic activity in normal skin (autohistographic method). Areas of lysis are noted in correspondence with dermal vessels. Epidermis shows no fibrinolytic activity.
Postembedding Immunogold Labeling of Epidermis

To the Editor:
You have recently published an article by Jessen and Behnke on the nonspecific labeling of histidine-rich keratinization products with colloidal gold conjugates [1]. The main and undeniable interest of this work is that it stresses in a very spectacular way "the necessity of rigorous control experiments in immunocytochemical studies at the