Study of certain clinical variables in patients with psoriasis and their relation to DNA content of keratinocytes

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Background: In a previous study of 26 patients with psoriasis we analyzed cytophotometrically the nuclear DNA content of the germinative compartment of involved and uninvolved skin by means of the Feulgen technique. These subjects were classified into three groups according to their DNA profile. Group 1 had a monomodal diploid profile, group 2 showed a significantly increased 2C-4C population, and group 3 demonstrated high proportions of 4C and hyperdiploid keratinocytes.
Objective: Our purpose was to analyze clinical variables implicated in the development of psoriasis in reference to the three groups.
Methods: Nuclear DNA content of each group by quantitative histochemical studies was analyzed and correlated with variables such as chronologic age, sex, age at onset, duration of flare during the study, stress, and the Koebner phenomenon.
Results: No significant differences in DNA profile were observed in the involved epidermis among the clinical variables. The only differences in the uninvolved skin pertained to the duration of the flare, where a statistically significant difference was observed between groups 1 and 3 in the basal ($p \leq 0.0459$) and suprabasal keratinocytes ($p \leq 0.06$), and in the Koebner phenomenon, which was induced in all subjects (100%) in groups 2 and 3 and in only 44% of subjects in group 1.
Conclusion: Uninvolved skin of patients with psoriasis should be included in analysis of the clinical behavior of the disease. Furthermore, the Koebner phenomenon is a good clinical indicator of the DNA profile of these subjects.
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Psoriasis is a chronic disease of complex hereditary factors and unknown cause[1-3] and is characterized by accelerated cell renewal, inflammation, and incomplete epidermal differentiation.[2, 4-6] Altered epidermal cell kinetics exists in the apparently healthy skin of these patients.[7-9] However, it is not known how these develop into skin lesions. Psoriasis can develop at any age, but in patients with a family history of this disease it tends to develop at an earlier age and to respond less well to therapy.[4, 10] Certain factors can cause a chain reaction resulting in skin lesions, such as the isomorphic phenomenon of Koebner,[11-15] stress, and social behavior.[16, 17] The isomorphic response, described in 1872 by Koebner,[18] is commonly associated with psoriasis.[19] A role for an immune mechanism has been suggested in the pathogenesis of psoriasis and in the Koebner phenomenon.[20-23]

In a study of the DNA content of keratinocytes in the germinative compartment we were able to classify patients with psoriasis into three groups.[9] Group 1 was composed of patients with a monomodal diploid profile and preferential keratinocytes with a 2C DNA content and a low proliferation activity. Group 2 consisted of patients with significantly increased 2C-4C and 4C populations and an intermediate proliferative activity. Group 3 consisted of patients with a high proportion of 4C and hyperdiploid (6C, 8C, and >8C) keratinocytes associated with

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	Involved Epidermis		Uninvolved Epidermis	
Cl. Variables	**Basal K.**	**Suprabasal K.**	**Basal K.**	**Suprabasal K.**
Age				
Sex				
Age at onset				
Duration of Disease			$X^2 = 6,1629$ (K-W) $p \leq 0,00459$	$X^2 = 5,4080$ (K-W) $p \leq 0.06$
Stress				
Koebner Phenomenon				$X^2 = 14,2867$ (K-W) $p \leq 0,0064$

Fig. 1. Relation between three established groups of patients with psoriasis according to DNA content and certain clinical variables. *Crossed box* indicates that data are not statistically significant. *K-W*, Kruskal-Wallis nonparametric test for variable quantities.

the highest proliferative activity. On the basis of these data we analyzed the relation between these groups of patients and certain clinical variables, such as age, sex, age at onset, stress, and the response to the Koebner isomorphic effect.

MATERIAL AND METHODS

As controls we used skin specimens for histochemical and quantitative studies from six healthy patients (four men, two women) between 19 and 28 years of age, with no evidence of altered epidermal cell kinetics, and 52 specimens from 26 patients (13 men, 13 women) with plaque-type psoriasis who had participated in a previous study.[9] Half the specimens from patients were from involved skin and half were from uninvolved skin. All patients were subjected to a clinical evaluation that paid special attention to the following variables concerning the evolution of the illness: the age at onset and disease duration, the existence of factors considered by the patient to be related to the appearance and successive recurrence of the illness, the influence of hormonal changes (e.g., puberty, pregnancy) and other processes (e.g., state of anxiety, infections) on its evolution, and the effect of previous treatment. Those patients who had been given any topical or systemic treatment that could influence cell proliferation were excluded.

The Koebner phenomenon was evaluated in uninvolved skin as induced by the surgical trauma and suturing of the biopsy site (braided 3-0 black silk). The koebnerization grade was measured when the sutures were removed 7 days later and every 14 days for 2 months. Thereafter, when the appearance at the biopsy site, was normal the response was considered negative, whereas a positive (to a greater or lesser degree) response was assumed if a psoriatic lesion developed in the previously uninvolved skin.

Specimens were obtained by punch biopsy from the gluteal region between 10 AM and noon (to avoid the potential influence of factors related to location and circadian rhythm) from the periphery of the skin lesion and from uninvolved skin 10 cm from the edge of the lesion. All samples were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections were cut perpendicular to the epidermal surface and processed according to the Feulgen technique.[9] Patients were classified according to DNA content profiles; the resulting relation between these groups was analyzed statistically with respect to certain clinical variables. The statistical treatment of the data consisted of univariant analysis. The chi-square test was used for variable qualities (sex, stress, Koebner phenomenon), and the Kruskal-Wallis nonparametric test was used for the variable quantities.

RESULTS

Involved epidermis

Significant differences in the clinical variables were not found between the three groups of patients.

Uninvolved epidermis

Significant differences in age, age at onset, and stress were not found in the germinative compartment in the three groups of patients.

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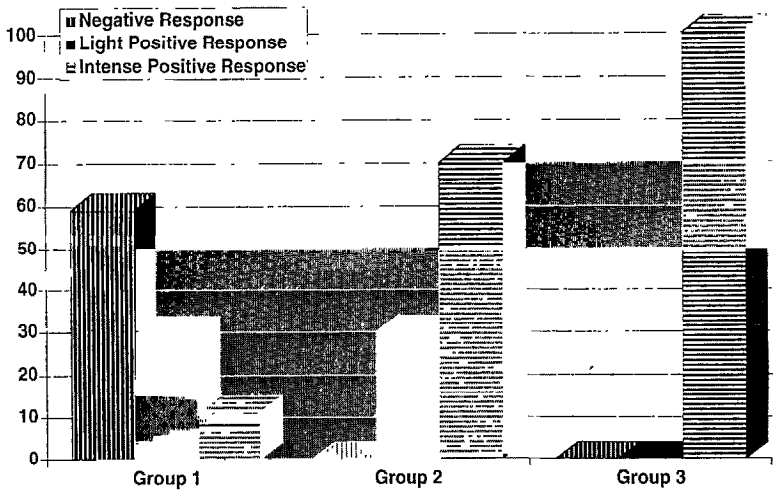

Fig. 2. Response to Koebner phenomenon in three groups of patients.

While analyzing the relation between the duration of the illness and the total DNA content in this type of skin, we found a significant difference between group 1 (monomodal diploid) and group 3 (tetraploid and aneuploid) in the basal keratinocytes ($p \leq 0.00459$) and a nearly significant difference in the suprabasal keratinocytes ($p \leq 0.06$) (Fig. 1).

We then analyzed the relation among the different groups and the response to the Koebner phenomenon. We found no significant differences in the DNA content of basal keratinocytes in the three groups but detected major differences in the suprabasal keratinocytes ($p \leq 0.0064$) (Fig. 1). When the lesion produced by the trauma of the biopsy was examined, 56% of the patients in group 1 showed a normal posttraumatic response, whereas in all patients in groups 2 and 3 a psoriasiform lesion developed to a variable degree (Fig. 2).

DISCUSSION

The study of epidermal cellular kinetics in normal skin and the processes of cutaneous proliferative activity from the effect of certain triggering factors can provide information about pathogenesis, prognosis, and possible therapeutic methods.

On assessing the relation between the three groups of patients with psoriasis and certain clinical variables that could affect the duration and development of the illness, factors such as topographic location,[24] circadian rhythm,[25, 26] and seasonal variations[24] remained constant throughout our study. We found no significant differences in the involved epidermis with respect to age and sex. This suggests that although involved skin demonstrates increased proliferative activity, it does not provide sufficient knowledge of the process and its correlation with clinical variables.

In uninvolved skin, in contrast, our study demonstrated that proliferative activity increased in proportion to the duration of the flare-up. Therefore the duration of the flare-up was shorter in subjects in group 1, whose average DNA content was similar to healthy control subjects, and longer in group 3, in which a significant increase in total DNA content was observed.

It has been suggested that psoriatic skin requires certain stimuli (e.g., stress) for alterations in its homeostasis to occur.[10, 27] In our study we found no significant differences between the three established groups and their response to stress. Nonetheless, several investigators have shown a correlation between stress and the development of psoriasis in 20% to 70% of patients.[1, 16, 17, 28] In our studies 5 of 26 patients (19.1%) associated a clearly identifiable stress with the onset of illness, and 15 patients (58%) considered stress to be a triggering factor in the flare-up of their psoriasis. Although our results suggested a trend similar to what others have observed, they did not reach statistical significance in the different groups of patients.

After cutaneous injury, cell renewal continues for a prolonged period, resulting in possible alterations in epidermal differentiation and the induction of a characteristic lesion.

Some investigators[4, 12, 14] have suggested a correlation between the age at onset of the illness and the response to the Koebner phenomenon. If psoriasis first appeared before the age of 15 years, and if previous treatment had been given, the response was positive in 75% of patients. Furthermore, if the illness first appeared after 30 years of age, and if there had been no previous treatment, the response was positive in only 5% of patients. In our study we found that 80% of patients in whom psoriasis first developed before age 15 years, and who had received previous treatment showed a positive response. In contrast, 27% of patients in whom psoriasis had developed after 30 years of age and who had had no previous treatment had a negative response, and the remaining 23% had a minimal response. The Koebner phenomenon was positive in 85% of patients who first showed signs of psoriasis between 15 and 30 years of age.

In our study we were able to demonstrate a strong correlation between the ability to induce the Koebner phenomenon and the DNA profile of the three different groups. Although the response was positive in only 44% of patients in group 1, composed of patients with a monomodal diploid profile, the Koebner phenomenon could be induced with variable intensity in all patients in groups 2 and 3. Although the percentage of positive Koebner responses reported in our study differs from that reported by others,[28] this may be a consequence of the stimulus we used to induce the phenomenon. Previous studies used other forms of trauma such as shave biopsies with a handheld keratome,[12] tape-stripping,[14, 29] freezing,[30] burning, and scratching.[4]

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