Abnormal Sequence of Expression of Differentiation Markers in Psoriatic Epidermis: Inversion of Two Steps in the Differentiation Program?

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This immunohistologic study was undertaken to compare epidermal differentiation in normal and psoriatic skin. Although basal cells retain a normal phenotype in this disease, suprabasal layers exhibit abnormal sets of differentiation markers. The 67-kD keratin and Bd_5 antigen, which are found in normal epidermis immediately above the basal layer, appear several layers higher in involved psoriatic epidermis. On the contrary, KF_2 antigen, which is found in the upper spinous layers of normal epidermis, appears more precociously in psoriatic epidermis. Paradoxically, in this disease characterized by the absence of a granular layer, some markers specific for this layer in normal skin, such as involucrin and transglutaminase, appear in lower skin cell layers, while other granular markers, such as filaggrin, are either absent or found in the parakeratotic scales. These results point out the existence in psoriasis of a suprabasal cell population characterized by a set of markers that are never coexpressed in normal epidermis. The existence of this abnormal population of cells can be explained as the result of the inversion of two steps in the differentiation program. Thus, instead of an inability to express a given differentiation marker, psoriasis seems to be characterized by an abnormal sequence of expression of these markers. *J Invest Dermatol 90:801–805, 1988*

Psoriatic epidermis is characterized, among other pathologic changes, by a rapid keratinocyte turnover [1] and differentiation defects. When compared with normal epidermis, it shows an altered composition of keratin polypeptides [2–4], increased proteolytic activities [5,6], and alterations in cell surface glycoconjugates [7,8].

Until recently, it was assumed that psoriatic keratinocytes were following the same maturation pathway as normal keratinocytes, albeit interrupted. Psoriasis was then regarded as a skin disorder involving primarily hyperproliferation of basal cells [9], resulting in an increased turnover of the tissue and truncation of cell maturation. Recent evidence, however, has suggested that the maturation pathway of keratinocytes in active psoriatic lesions differs qualitatively from that of normal epidermis [10–14]. The purpose of this immunohistologic study was to investigate further the qualitative differences between normal and psoriatic epidermis. These new observations support the concept that instead of a block in the expression of particular differentiation markers, it is an altered sequence of expression of these markers that characterizes psoriasis.

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Abbreviations:

MoAb: monoclonal antibody
PoAb: polyclonal antibody
PBS: phosphate-buffered saline

MATERIALS AND METHODS

Skin Samples Biopsies were taken from the affected skin of 14 patients (3 female, 11 male) with psoriasis vulgaris. These patients were 25 to 80 years old and had no ongoing treatment. Recent (less than 15 days old) and stable (more than 1 but less than 3 months old) lesions were studied. Normal skin biopsies from individuals with no history of psoriasis served as controls. The biopsies were quick-frozen in Tissue-Tec OCT compound (Lab-Tek Products Division, Napperville, IL), using dry ice/acetone–isopentane and stored at −80°C until used.

Antisera KF-2 monoclonal antibody (MoAb) was a generous gift of Dr. S. Katz (NIH Bethesda, MD). Rabbit polyclonal antibodies (PoAbs) against filaggrin and involucrin were kindly provided by Dr. B. Dale (School of Dentistry, University of Washington, Seattle) and Dr. R. Rice (Harvard School of Public Health, Boston, MA). Rabbit PoAb against 67-kD keratin [15] was obtained from Dr. J. Viac (INSERM, Lyon, France) and was further purified by affinity on keratins blotted on nitrocellulose. $B.C_1$ MoAb against membrane-bound transglutaminase was a generous gift of Dr. S. Thacher (NIH). Bullous pemphigoid serum was a kind gift from Dr. J. H. Saurat (Geneva, Switzerland).

BC_2 and BC_5 MoAbs were prepared at the CIRD (Valbonne, France) by injecting SV40-transformed human keratinocytes as antigen. BC_2 MoAb is an IgG1 while BC_5 MoAb is an IgM.

Fluoresceinated conjugates were purchased from either Cappel Laboratories (Downingtown, PA) or DAKO (Sobio, France).

Indirect Immunofluorescence Technique Frozen sections at 4μm were stained with a "sandwich technique," exactly as previously described [12,14] except that in the preincubation washes, 3% bovine serum albumin was replaced by 1% dried low-fat milk

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(Régilait, Saint-Martin-Belle-Roche, France) in phosphate-buffered saline (PBS), pH 7.4.

RESULTS

A comparative immunohistologic study of normal and of involved and noninvolved psoriatic skin was performed with antibodies specifically reacting with the various layers of epidermis.

Basal Layer BC_2 is a MoAb that recognizes a glycoprotein with a molecular weight of 165 kDa [16]. In normal epidermis, it specifically decorates lateral and "apical" domains of the plasma membrane of basal cells, sparing the basal domain (Fig 1A). When reacted with psoriatic skin (Fig 1B), a similar pattern is observed, suggesting that a basal compartment is maintained in psoriatic epidermis, with polarity of the basal cells apparently preserved. It must be noted, however, that the staining intensity of involved psoriatic epidermis is reduced and that incidental suprabasal staining can be observed, probably because of tangential cryostat sectioning of the rete ridges.

Suprabasal Layers BC_5 is a MoAb that identifies a protein with a molecular weight of 62 kDa (Bernard and Darmon, unpublished result) and specifically decorates the cytoplasm of all the suprabasal cells of normal and noninvolved psoriatic epidermis (Figs 2A,C), conspicuously sparing the basal cells. When BC_5 MoAb is reacted with involved psoriatic skin, however, the staining appears several layers above the basal layer (Figs 2B,D). A double-staining experiment of the same sections with different fluorochromes confirms that in normal epidermis (Figs 3A,B), cells are either BC_2 or BC_5 positive. On the other hand, it allows identification of an intermediate cell population in psoriatic epidermis (Figs 3C,D) that is stained neither by BC_2 MoAb nor by BC_5 MoAb (dotted line). Similarly, while anti-67-kDa keratin PoAbs decorate all suprabasal layers of normal and noninvolved psoriatic skin, the staining of this keratin is progressively shifted to the upper layers of involved psoriatic skin and ultimately disappears in stable lesions (Figs 4A,B). This result confirms the biochemical finding of a reduction in 67- to 65-kDa keratins normally expressed in all suprabasal cells [2,3,4,17].

The staining obtained with both BC_5 MoAb and anti-67-kDa keratin PoAb demonstrates that the expression of early differentiation markers, that is, strictly suprabasal, can be delayed in psoriatic epidermis. The behavior of KF2 antigen(s) is just the opposite. Although KF2 MoAb stains the cell periphery, in the upper spinous layers of normal [18] and noninvolved psoriatic skin, it tends to decorate all but the basal layer in involved psoriatic skin (Figs 5A,B). The KF2 staining pattern suggested that late differentiation markers could be detected earlier in psoriatic epidermis than in normal epidermis. This conclusion was also drawn from previous studies concerning the granular layer markers involucrin [12], transglutaminase enzyme activity [14], and monoclonal anti-interleukin 2 antibody staining [19]. This is further documented in the present study, because the anti-membrane-bound transglutaminase antibody $B.C_1$ was found to stain all suprabasal layers of involved psoriatic epidermis (Fig 6B), whereas it was located in the granular layer in healthy epidermis (Fig 6A). The preceding results strengthen the idea that in involved psoriatic epidermis, the appearance of some early differentiation markers can be delayed and that some late differentiation markers can be expressed more precociously. This is not true for all granular layer markers, however. Indeed, filaggrin, which is detected in this layer in normal and noninvolved psoriatic skin (Fig 7A), is distributed higher and higher into the parakeratotic scales of psoriatic epidermis (Fig 7B), and ultimately it becomes undetectable in stable lesions.

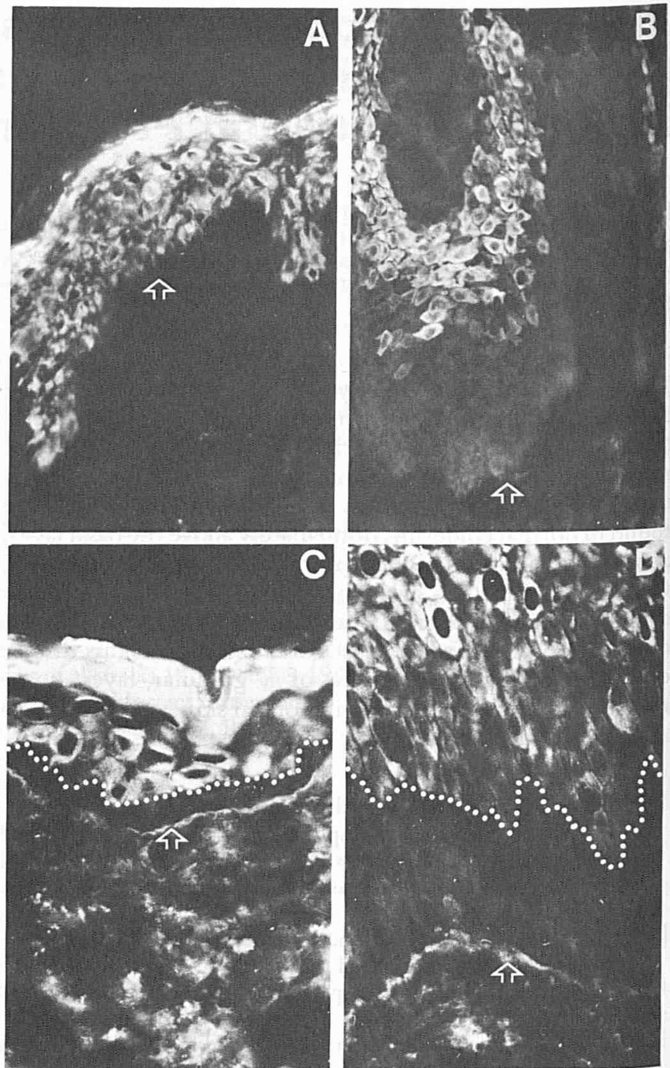

Figure 2. Immunofluorescence detection of BC_5 antigen in normal *(A,C)* and involved psoriatic skin *(B,D)*. *Arrows* indicate the basement membrane. Sections were double-stained for BC_5 and bullous pemphigoid antigen *(C,D)*. The dotted line represents the bottom limit of BC_5 staining. *(A, B)* ×200. *(C, D)* ×400.

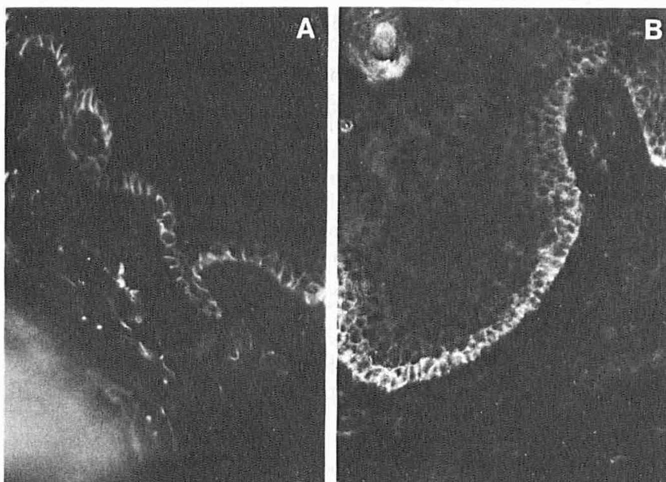

Figure 1. Immunofluorescence detection of BC_2 antigen in normal *(A)* and involved psoriatic skin *(B)*. Note the membrane distribution of the label. ×200.

DISCUSSION

The immunohistologic differences between normal and involved psoriatic epidermis observed in this study are summarized in Fig 8 together with some relevant results published previously [12,14].

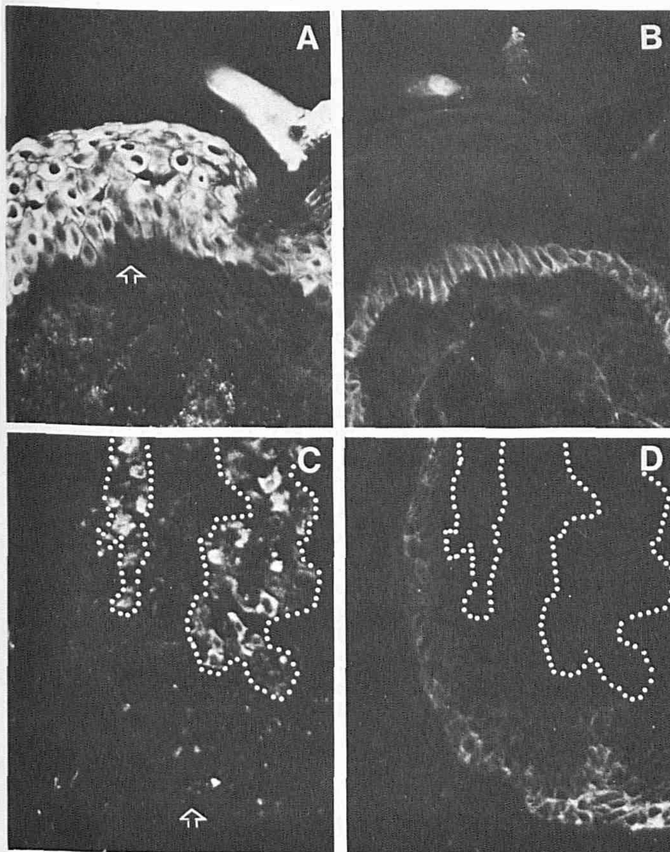

Figure 3. Double immunofluorescence detection of BC_5 *(A,C)* and BC_2 antigen *(B,D)* in normal *(A,B)* and involved psoriatic skin *(C,D)*. The dotted line represents the bottom limit of BC_5 staining *(C,D)*. Arrows indicate the basement membrane. ×200.

All the markers available were identically distributed in both noninvolved psoriatic and normal epidermis. Results obtained with BC_2 MoAb suggest that a basal–suprabasal boundary persists in psoriasis in spite of the existence of several layers of proliferative keratinocytes above the basal layer [9]. This interpretation is in agreement with results recently reported [20,21] arguing for the maintenance of an intact basal monolayer in psoriatic epidermis.

On the other hand, major abnormalities are detectable from the very first suprabasal layer (Fig 8) of psoriatic epidermis. BC_5 staining reveals the existence of an intermediate suprabasal cell population negative for this marker in involved psoriatic epidermis, which is not found in normal epidermis. Although in psoriasis the delayed appearance of reduced amounts of filaggrin confirms the absence of a typical granular layer, paradoxically several other late markers (KF2 antigen, involucrin, and transglutaminase) appear in lower skin layers in this disease. These defects thus argue against the hypothesis of a simple block in the differentiation program secondary to an increase in the germinative compartment, although the belated appearance of BC_5 antigen and 67-kDa keratin might have supported it. Neither can these alterations be directly related to the modifications of the retinoic acid concentration in psoriatic epidermis. Indeed, KF2 antigen and transglutaminase, which appear precociously, and 67-kDa keratin and filaggrin, which are belated in psoriatic skin, are all negatively controlled by retinoic acid.* One cannot exclude, however, the hypothesis of a block in the synthesis of key protein(s), that is, regulatory proteins, which may have complex consequence(s) on the expression of other genes implicated in the differentiation process.

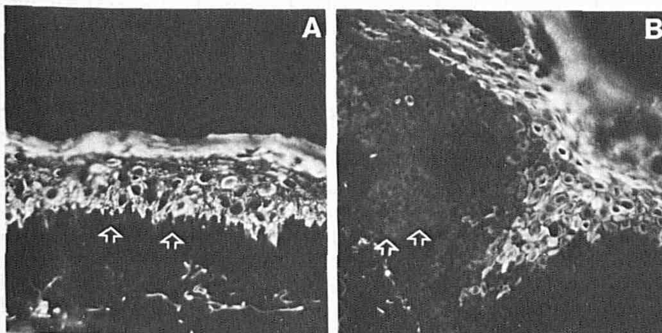

Figure 4. Immunofluorescence detection of 67-kDa keratin in normal *(A)* and involved psoriatic skin *(B)*. Arrows indicate the basement membrane. ×190.

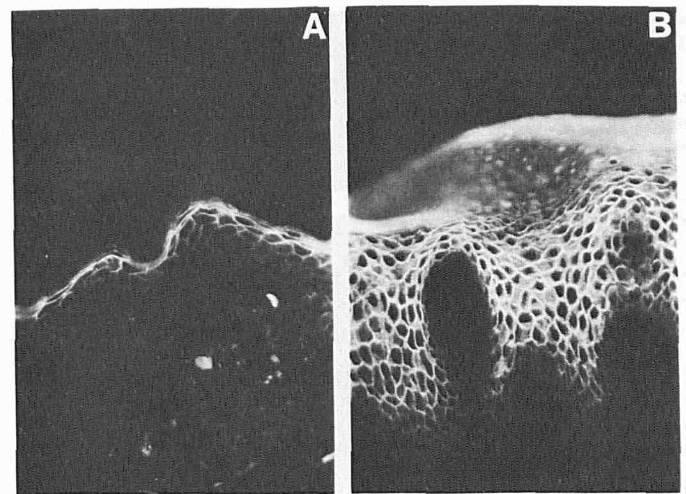

Figure 5. Immunofluorescence detection of KF2 antigen in normal *(A)* and involved psoriatic skin *(B)* ×200.

The present results show that the most characteristic defect of psoriatic epidermis is a sustained alteration in the sequence of expression of differentiation markers. Most of the abnormalities we observed are not detected in lichen planus, another parakeratotic process (B.A. Bernard, unpublished results); however, they are locally and transiently observed during the course of epidermal wound healing [22,23]. The phenotypes displayed by keratinocytes in psoriasis and during wound healing are thus somehow comparable. But in psoriasis, the control of expression is probably more complex than a simple switch between normal steady-state renewal and regeneration [23]. Actually, psoriasis is a chronic state, while wound healing is a rapidly evolving process leading back to a normal epidermis. In Fig 8, we present a model of a potential sequence of differentiation events in epidermis. Successive steps of differentiation (A,B,C,D,E) are defined by the coordinate appearance of new markers; however, the boundaries between two successive stages must be expected to be loose, as transitional cells have been observed [24]. Nevertheless, the comparison of the normal and the psoriatic sequences shows that B and C stages are inverted in psoriasis. This model fits well with several observations: (1) Psoriatic epidermis is characterized by agranulosis and parakeratosis. One easily understands that if the buildup of cellular proteins occurs in an inverted sequence, then steps that are posterior to the inversion are abnormal. In this respect, the morphology and biochemical composition of crosslinked envelopes are abnormal in psoriatic epidermis [25]. (2) Although in normal epidermis, proliferation stops after step B, as shown by experiments demonstrating that involucrin-positive cells do not replicate [24], this is not the case in psoriatic epidermis, where involucrin-positive replicating cells can be found

* D Asselineau, BA Bernard, C Bailly, and YM Darmon, in preparation.

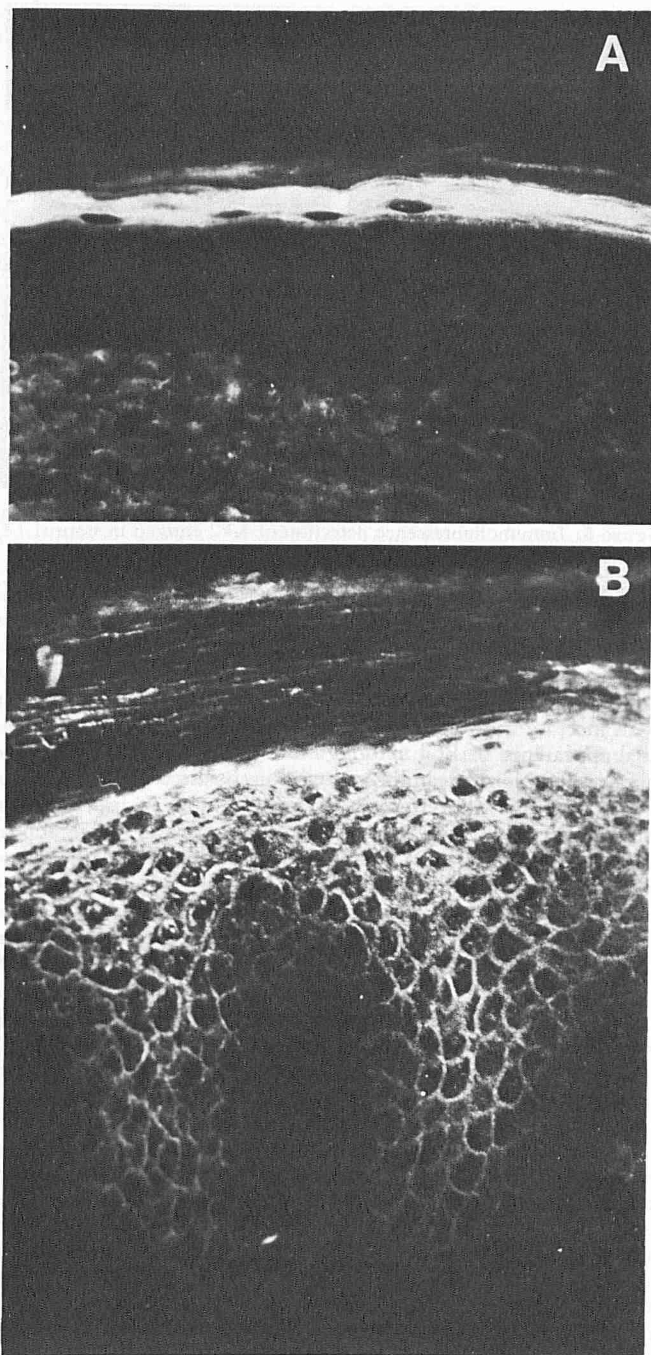

Figure 6. Immunofluorescence detection of membrane-bound transglutaminase, as revealed by BC_1 MoAb, in normal *(A)* and involved psoriatic skin *(B)*. ×570.

[26]. It is possible that a signal for mitotic arrest occurs after step B. This interpretation fits well with the hypothesis that the proliferative compartment is increased in psoriasis [9,10]. It is obvious that this interpretation of our results is still speculative. Further experiments using other markers and biochemical analysis should be performed to unravel the cellular defects resulting in the unscheduled expression of differentiation markers in psoriasis.

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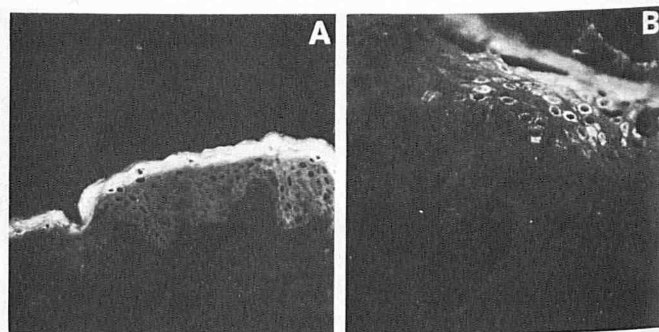

Figure 7. Immunofluorescence detection of filaggrin in normal *(A)* and involved psoriatic skin *(B)*. ×200.

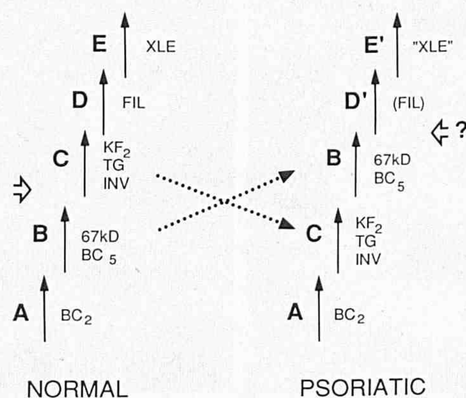

Figure 8. Sequential appearance of differentiation markers in normal and psoriatic epidermis. Note that steps B and C *(dotted arrows)* are inverted in psoriatic epidermis, the empty horizontal arrow corresponding to the arrest of proliferation. This diagram summarizes the data obtained in the present study, together with those previously published [12,14]. TG, transglutaminase; INV, involucrin; FIL, filaggrin; (FIL), reduced amount of filaggrin; XLE, crosslinked envelopes; "XLE," abnormal crosslinked envelopes.

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