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Psoriasis and Cyclosporin: Immunohistochemical Aspects of the Basement Membrane

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We have demonstrated both in vivo and in vitro that Cyclosporin (CsA) treatment during psoriasis induced a regression of typical keratinocyte alterations and normalization of the basement membrane (BM). It is also known that the structure of BM implies cohesion between the networks formed by laminin and type IV collagen and that these components positively influence the cytomorphosis processes of keratinocytes. According to these results, we have evaluated, by immunohistochemical study, the behaviour of laminin and type IV collagen on psoriatic skin prior to the therapy and at the end of pharmacological treatment with CsA. This study was carried out on biopsies of involved skin taken from 12 patients with severe psoriasis and with PASI between 50 and 70. Our results can be summed up as follows:

Untreated psoriasis: **absence of laminin within BM; modest staining in basal keratinocytes; intense staining in suprabasal keratinocytes; discontinuous staining of Type IV collagen in the BM.**

After treatment: **evident and continuous staining of laminin and Type IV collagen within the BM.**

The obtained results confirm the positive effect of immunomodulation determined by CsA in the regulation of the functional activity of cells implicated in BM component production. In conclusion, the authors discuss the pathogenesis of the disease.

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It is known that the basement membrane (BM) of the epidermis influences a series of fundamental biological functions of keratinocytes such as growth, differentiation, polarization, adhesion, migration and can constitute a selective filtration barrier against biologically active substances which may be of importance in the pathogenesis of hyperproliferative diseases of the skin (1–5).

Our recent ultrastructural researches (6–9) have confirmed that in psoriasis, a typical hyperproliferative disease, evident anomalies of the BM constitution are always present, and have shown that these regress, together with keratinocytic alterations, following treatment with Cyclosporin (CsA).

Following these researches and with the aim of evaluating the behaviour of the main components of BM during the thriving phase of the illness, and after the treatment with CsA, we undertook a study of immunolocalization with direct monoclonal antibodies against laminin and collagen of IV type on bioptic fragments of the involved skin from psoriatic patients before and after a therapeutic cycle with CsA.

MATERIALS AND METHODS

We carried out cutaneous biopsies in the involved skin with punch biopsies "Stiefel" (3 mm ∅) on 12 patients affected by serious and diffuse psoriasis (PASI: 50–70) before and after a therapeutic cycle with diminishing doses of cyclosporin (3 mg/kg/per day during the first month; 2 mg/kg/per day during the second month; 1 mg/kg/per day during the third month). The bioptic fragments after repeated washings

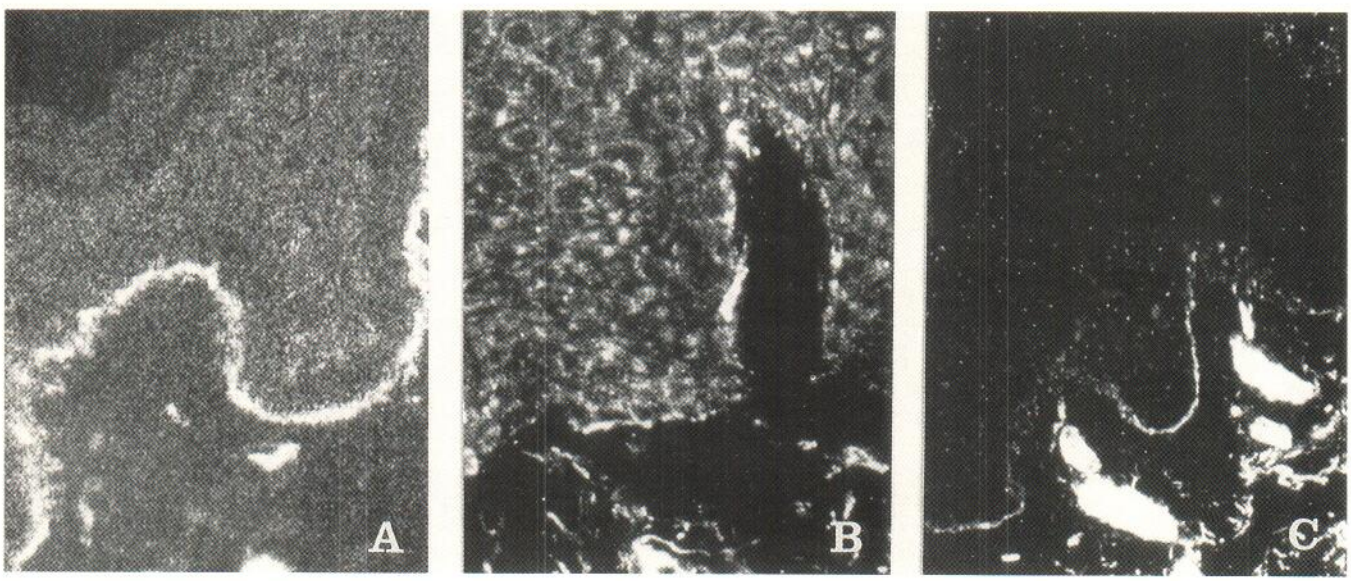

Fig. 1. Immunofluorescence staining of laminin in normal (A), involved psoriatic skin before (B) and after CsA (C). A: 400×; B: 400×; C: 200×.

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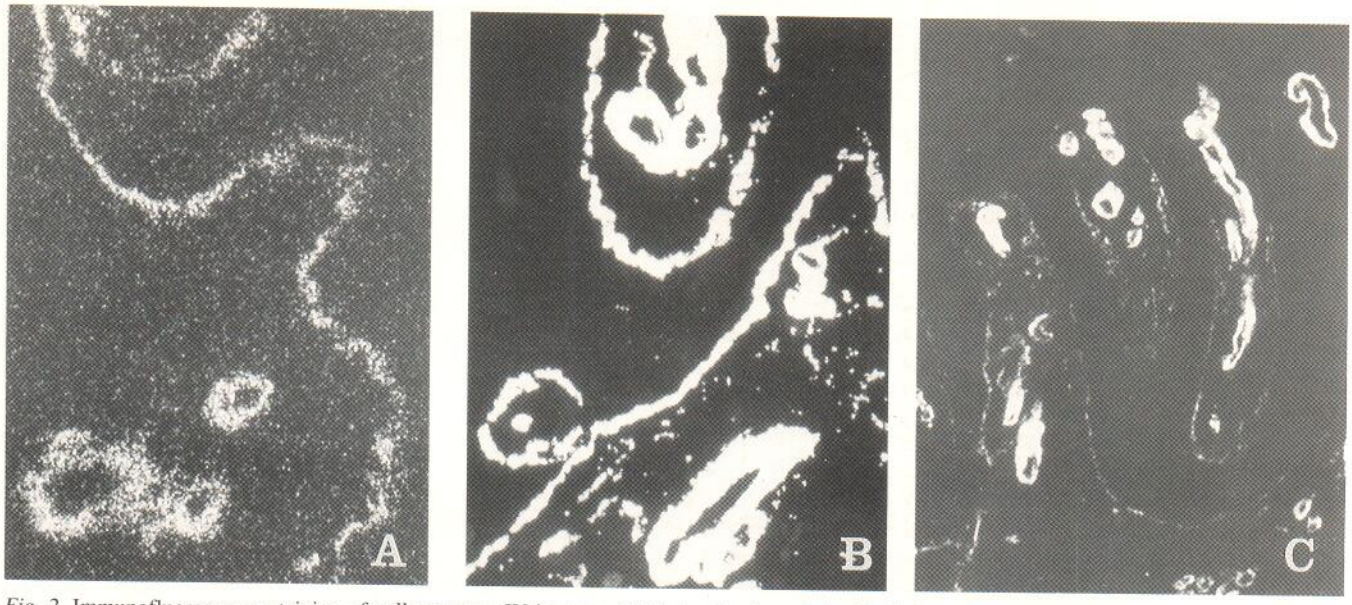

Fig. 2. Immunofluorescence staining of collagen type IV in normal (A), involved psoriatic skin before (B) and after CsA (C). A: 400×; B: 400×; C: 200×.

in phosphate 0.2 M at pH 7.4 and salt phosphate buffer, PBS were infiltrated with saccharose 2.3 M in PBS and then frozen in liquid nitrogen. Sections of about 14 μm, cut with a cryotome were gathered on gelatinized slides and tested with primary anti-laminin and anti-collagen, type IV (Sigma) at a dilution of 1:250; as a secondary antibody we used an Ig-biotinated anti-mouse in sheep at a dilution of 1:500; as fluorochrome, the Red-Streptavidin at a dilution of 1:100. The observations were carried out with a confocal microscope with laser scanning LSM 310 of Carl Zeiss.

RESULTS

Distribution of the laminin in normal and psoriatic skin. Frozen sections of normal skin stained by immunofluorescence with mouse antiserum against laminin showed a linear and continuous staining at the level of the BM and in the walls of dermic vessels (Fig. 1A). By contrast, in psoriatic skin, the staining of the BM appears completely absent in some and in others present in short segments, while it is possible to discern a positivity of the reaction in the walls of the dermic vessels and inside superbasal keratinocytes (Fig. 1B). After the treatment with CsA the laminin staining is distributed in an almost uniform way along all the BM with sporadic and short interruptions. The positivity at the keratinocytic level is no longer appreciable, while the walls of the vessels can still be discerned (Fig. 1C).

Distribution of the collagen of IV type in normal and psoriatic skin. In normal skin, staining is discerned as a continuous line at the level of the BM and in the walls of the dermic vessels (Fig. 2A). In psoriatic skin, on the other hand, staining appears less intense and clear-cut interruptions of the continuity – especially at the apex of the dermic papillae – are sometimes to be observed. The staining of the walls of the vessels is very intense and they appear tortuous and stretched (Fig. 2B). After the treatment with CsA the distribution of the staining for collagen of type IV appears almost similar to the normal skin, even if sporadic breaks are present (Fig. 2C).

DISCUSSION

From the examination of the reports it is seen that the known structural anomalies in the BM of psoriatic epidermis are in correlation with evident alterations of the molecular composition of the same; among these alterations, particular importance is attributable to the anomalous localization of the reaction of the laminin which is almost completely absent in the context of the BM, while it is positive inside the suprabasal keratinocytes.

This condition permits us to hypothesize a functional anomaly of the keratinocytes such that on the one hand they keep this glycoprotein inside the cellular body instead of releasing it, as in normal circumstances, into the extracellular matrix to consent the assemblage in the BM context; on the other hand, they are hindered in their development according to the normal scheme of epidermal differentiation.

The lack of laminin in the BM constitutes a decisively favourable condition for the manifesting of hyperproliferative phenomena which characterize psoriasis. In fact it is known that the network formed by laminin (10), in the context of the "lamina lucide", constitutes the main system of adhesion, mediated by integrins (11–12) of the basal keratinocytes to BM. The connection that is established between this transmembrane protein and the laminin fulfils the assumption why the signals that govern adhesion, migration, proliferation, polarization, cellular differentiation, biological functions these highly altered in psoriasis, have their origin.

On the other hand, the laminin, besides being connected with the basal keratinocytes, is closely connected, on the opposite side, with the components of the 'lamina densa', such as type IV collagen and heparan sulfate proteoglycan, and the normal function of the selective barrier of BM depends on the aggregation of this molecular complex (13–14). The absence of laminin, altering the normal assemblage of BM, surely facilitates the passage of the biologically active substance which, as is known, can be important in inducing keratinocytic hyperproliferation (5).

Furthermore, we also recently observed (15) a lack of laminin in the BM context in the uninvolved skin of psoriatic patients. This situation even upholds the hypothesis of an anomaly of the keratinocytes, similarly genetic, regarding the production and the liberation of laminin; therefore, the consequent lack and the alteration of the BM permeability, might explain the ease of the onset of the pathological picture in response to the variety of stimuli in subjects that bear such an anomaly.

After pharmacological treatment, the arrangement of the BM seems actually to normalize the positivity of the reaction to the laminin which is distributed in an almost homogeneous manner in the BM context; appears evident in addition to maintaining the continuity of the reaction to type IV collagen. All this confirms, at the molecular level, a morphologic normalization of the BM already observed by us by TEM (6–7) under the same experimental conditions and permits us to hypothesize a normalizing action of CsA on keratinocytic functionality. This activity of CsA, as is known (16), leads back to its capacity, of hindering lymphocyte T from producing those cytokines which, having keratinocytes has their target, alter the normal functionality and probably manifest an existing genetic anomaly.

REFERENCES

1. Timpl R, Dziadek M. Structure, development, and molecular pathology of basement membranes. Int Rev Exp Pathol 1986; 29: 1.
2. Kleinman HK, Graf J, Iwamoto Y, Kitten GT, et al. Role of basement membranes in cell differentiation. Ann NY Acad Sci 1987; 513: 134.
3. Martin GR, Timpl R. Laminin and other basement membrane components. Ann Rev Cell Biol 1987; 3: 57.
4. Briggaman RA. Biochemical composition of the epidermal–dermal junction and other basement membrane. J Invest Dermatol 1982; 78: 1.
5. Woodley DT. Importance of dermal–epidermal junction and recent advances. Dermatologica 1987; 174: 1.
6. Bruschetta D, Magaudda L, Mondello MR, Santoro G, Vaccaro M, Califano L, Cannavò SP, Guarneri B. Modificazioni strutturali ed ultrastrutturali a carico dell'epidermide psoriasica dopo trattamento con Ciclosporina. G Ital Dermatol Venereol 1992; 127: 87.
7. Guameri B, Califano I, Cannavò SP, Magaudda L, Mondello MR, Santoro G, Vaccaro M. Terapia con Ciclosporina: aspetti morfostrutturali di lesioni psoriasiche recidivanti. Dermatologia Clinica 1992; n° 2: 113.
8. Arco A, Maddalena G, Magaudda L, Mondello MR, Santoro G, Vaccaro M, Califano L, Cannavò SP, Guarneri B. Psoriasi e Ciclosporina: effetti del farmaco su culture organotipiche. Annali Italiani di Dermatologia Clinica e Sperimentale 1993; 47, 1: 21.
9. Guameri B, Vaccaro M, Califano L, Cannavò SP. Impiego ciclico della Ciclosporina nella terapia della psoriasi. Annali Italiani di Dermatologia Clinica e Sperimentale 1993; 47, 3: 233.
10. Yurchenco PD, Cheng YS, Colognato H. Laminin forms an independent network in basement membranes. J Cell Biol 1992; 117: 1119.
11. Manca V, Zambruno G, Giannetti A. Le molecole di adesione nella cute. G Ital Dermatol Venereol 1990; 123: 543.
12. Cagnoni ML, Ghersetich I, Lotti T. Le molecole di adesione nelle dermatosi infiammatorie. G Ital Dermatol Venereol 1993; 128: 1.
13. Laurie GW, Bing JT, Reiman HK, Nassel JR, et al. Localization of binding sites for laminin, heparan sulfate proteoglycan and fibronectin on basement membrane (Type IV) collagen. J Mol Biol 1986; 189: 205.
14. Le Blond CP, Inoue S. Structure, composition and assembly of basement membrane. Am J Anat 1989; 183: 367.
15. Mondello MR, Magaudda L, Vaccaro M, Cannavò SP, Guameri B, Santoro A. Comportamento dei principali costituenti della membrana basale della epidermide non coinvolta di pazienti psoriasici. Studio immunoistochimico. Atti 47° Convegno della Società Italiana di Anatomia. 1993.
16. Wong RL, Winslow CM, Cooper KD. The mechanism of action of cyclosporin A in the treatment of psoriasis. Immunol Today 1993; 14: 69.