

THE DEPOSITION OF IMMUNOGLOBULINS AND COMPLEMENT
IN STRATUM CORNEUM IN MICROSCOPIC LESIONS
IN PATIENTS WITH ACTIVE PSORIASIS:
THE RELATIONSHIP TO HYPERPROLIFERATION

Anders Johannesson,¹ Hans Hammar¹ and K.-G. Sundqvist²

¹Department of Dermatology, Karolinska sjukhuset, Karolinska Institutet, Stockholm, and ²National Bacteriological Laboratory, Stockholm, Sweden

Abstract. The morphology of the horny layer was utilized to determine the age distribution of corneocytes in early psoriatic lesions and in their vicinity. By using the age distribution the number of corneocyte layers found in any time interval could be obtained and expressed as the rate of corneocyte layers formed. Deposits of immunoglobulins G, M and A and C3 were also age-distributed in a similar way. In the period during which the horny layer was formed, variation in the rate of corneocyte formation could be related to the appearance of immunodeposits. Hyperproliferation was found to precede deposits of immunoglobulins G, M and A and C3 by one to several days. Deposits were examined both visually and by means of microfluorometry, with the same result. The specificity of the deposits was established in two ways. A Fab₂-anti-immunoglobulin conjugate was used to detect a possible Fc receptor binding. This was not demonstrated. Albumin was used as a plasma filtrate marker. Albumin was not found in the horny layer but was abundant in the intercellular spaces in the rest of the epidermis. It was concluded that the immunodeposits found in the early lesions of psoriasis are a secondary phenomenon during the initiation of a psoriatic lesion.

Key words: Psoriasis; Hyperproliferation in epidermis; Immunodeposition in the horny layer; Initiation mechanism in psoriasis

The initiation of a psoriatic lesion is suggested to be mediated by an autoimmune mechanism, since immunodeposits in the horny layer are found in early lesions (2). Previously, Krogh and Tönder (4, 5) have found circulating immunoglobulins directed towards antigen determinants in stratum corneum, while activation of C3 was observed by Tagami & Ofuji (7). The hypothesis has been proposed that an immune complex reaction takes place in the horny layer, thus initiating the psoriatic lesion. Hyperproliferation of the epidermis is thought to be a secondary repair process.

In the present work the proposed sequence of

events in the epidermis of minute psoriatic lesions was tested: Is hyperproliferation secondary to immunoglobulin deposition, or not?

We have recently shown that the structure of the preserved horny layer can be used to outline the speed of its formation in nearby areas (3). The corneocytes in the horny layer are of varying age, with the youngest in its basal part and the oldest in its superficial part. Therefore, the age of a particular corneocyte can be determined from the site it occupies within the horny layer. The number of corneocyte layers found during any interval of time of this age distribution gives the rate of corneocytes formed during this period. Comparison of a minute psoriatic lesion with its vicinity is used to obtain the relative rate of corneocyte formation (3). This is a quotient of how many times more the rate is in the lesion than it is in the non-involved epidermis. The deposition of immunoglobulins and complement in the horny layer can be examined to obtain its age distribution by the same procedure as indicated above. This can be compared with the relative corneocyte formation rate in order to answer our question.

MATERIAL AND METHODS

Patients. Sixteen patients with active psoriasis supplied 28 biopsies. The patients were 24 to 46 years old. Nine were females. All had guttate or nummular lesions, which were disseminated, untreated and at an acute stage. The thigh skin was carefully examined under a magnifying glass, $\times 3$ – 10 , for detection of minute lesions located at a distance from visible lesions (>5 cm) and clearly within interfollicular fields. Without anesthesia a punch biopsy 1–2 mm thick and 2–3 mm in diameter was excised, frozen immediately on solid carbon dioxide with the dermal side down, transported in a cold environment and kept in a deep-freezer at -90°C until sectioned.

Table 1. Number of psoriatic lesions with deposits in the horny layer

An early lesion had normal-looking corneocyte layers on top of the parakeratotic area. At least one of these layers could be traced out to the adjacent normal epidermis and was found not to be altered in morphology throughout. A late lesion had areas of parakeratosis out to the rim of the horny layer

Conjugate	Type of lesion	
	Early	Late
IgG	13	13
IgM	7	5
IgA	9	6
C3	8	5
No deposits	2	0
No. of patients	15	13

The histological techniques and the analysis of the sections for measurement of the relative corneocyte formation rate (RCR) was done as described recently (3). In the material of serial sections the measurement of the corneocyte formation rate was done on 3-4 consecutive sections selected just before and after the 6 used for immunofluorescence to detect Ig or C3 deposits. The sections were 6 µm thick. The size of the lesions varied between 0.2 and 0.8 mm in diameter.

Immunofluorescence (IF) of the horny layer. The non-fixed cryostat sections were placed on microscope slides. In six biopsies some sections were kept at 30°C for 2 days to fix the section firmly to the glass; others were used directly after thawing. The final result was the same with these two procedures. The first mentioned procedure was carried out in only 4 patients.

Deposits of IgG, IgM, IgA and C3 were detected by means of direct immunofluorescence using fluorescein isothiocyanate (FITC)-conjugated antisera against the appropriate human serum proteins. The conjugates used had the following characteristics. FITC-sheep anti-human IgG. (Wellcome): F/P molar ratio 3.25; protein concentration 8.0 mg/ml; antibody protein 1.4 mg/ml; plateau end-point in a standard performance test 1:400; working dilution 1:10. FITC-sheep anti-human IgG (WHO): F/P molar ratio 3.90; protein concentration 6.5 mg/ml; plateau end-point 1:400; working dilution 1:10; specific antibody concentration 1.69 mg/ml. FITC-sheep anti-human IgM (Wellcome): F/P molar ratio 3.2; protein concentration 7.2 mg/ml; plateau end-point 1:200, working dilution 1:10. FITC-sheep anti-human IgM (WHO): F/P molar ratio 3.8; protein concentration 6.1 mg/ml; plateau end-point 1:200; working dilution 1:10, specific antibody concentration 0.67 mg/ml. FITC-sheep anti-human IgA: (Wellcome) F/P molar ratio 4.2; protein concentration 8.7 mg/ml; working dilution 1:10. FITC rabbit anti-human C3 (Behringwerke). F/P molar ratio 2.9; protein concentration 4.9 mg/ml and working dilution 1:5. Fab₂ sheep anti-human Ig: F/P molar ratio 8.6×10^{-3} , protein concentration 6.4 mg/ml, working dilution 1:10.

In order to detect albumin a rabbit anti-human albumin serum of our own production was used. The reactivity of this serum was detected by a FITC-conjugated sheep anti-rabbit immunoglobulin with F/P 3.2 and protein concentration 5.4 mg/ml and working dilution 1:10. In the direct staining procedure the cryostat tissue sections were reacted with the conjugates for 30 min at room temperature. The sections were then washed for 5 min three times in phosphate-buffered saline (PBS (Na₂HPO₄ 8mM, KH₂PO₄ 1.5 mM, NaCl 0.14 M, KCl 3 mM) pH 7.4). In the indirect staining used to detect albumin the tissue sections were reacted for 30 min with the anti-albumin serum diluted 1:20 or 1:40, washed three times in PBS followed by incubation with the conjugate for 30 min followed by three washes in PBS.

Fluorescence microscopy and microfluorometry

The stained tissue sections were evaluated in a Leitz Dialux 20 microscope using incident light illumination, a 50 W high pressure mercury lamp and equipped with Leitz filter block K. Photographs were taken with a Leitz automatic camera using Kodak Ektachrome film.

Localization of Ig deposits. The IF stained section was easily recognized to be morphologically very similar to the RCR sections. This means that the horny layer on top of the microscopic lesion and at the nearby normal epidermis corresponded completely in the two sections of IF and RCR compared. The localization of the IF deposits was therefore measured by a simplified procedure with the aid of an ocular micrometer. The distances between the surface of the horny layer (point A) down the most superficial part of the deposits (point B) and to the border of Stratum Malpighii (point C) were measured, as well as the whole thickness of the horny layer at the reference area of nearby normal epidermis. The proportion of the lengths between points A and B and between points B and C was used in the RCR slides to indicate the corneocyte layer where IF staining first appeared.

The intensity of fluorescence. In different areas of the tissue section fluorescence was measured using a Leitz photometer coupled to a Leitz orthoplan microscope. These measurements were performed in the same way as previously described in detail for intensity measurements in single cells (6).

In each section the horny layer was measured in the centre of fluorescent immunodeposits, in an area superficial to and adjoining deposits but devoid of visible fluorescent material and also in the adjacent normal horny layer (Fig. 1). The circular aperture of the incident light had a diameter of 3 µm in the plane of the section. This indicated about 10 corneocyte layers in the normal stratum corneum and about half that in lesional skin.

Specificity of IgG binding was controlled by using the FITC-Fab₂-anti-Ig conjugate. Four biopsies showing IgG deposition in parakeratotic areas was tested with the Fab₂ conjugate. Identical stains were obtained on consecutive sections with the two conjugates.

RESULT

The result of the Ig and C3 deposits is summarized in Table 1. Deposits were present only in

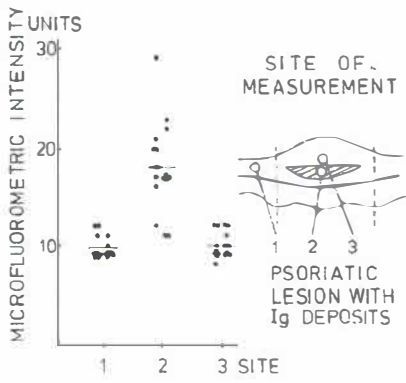


Fig. 1. Analysis of immunodeposits in the horny layer by microfluorometry. In the sketch the hatched area indicates visible immunodeposits. The circles show positions for measurements. The shutter has an aperture $3 \mu\text{m}$. Site 3 is taken 2–3 layers of corneocytes on top of visible deposits in order to reveal unnoticed fluorescence. Low intensity at site 2 is due to 2 cases of a narrow band of fluorescence not completely filling the aperture of the instrument. The two rows of values per site indicate duplicate measurements.

parakeratotic areas of the horny layer. In areas where thickened non-nucleated corneocytes (3) were present and in normal-looking parts of the horny layer, no deposits were seen.

According to the morphology of the minute lesions these were divided into an early and a late group. The early lesions were defined as containing a normal-looking part of the horny layer on top of the parakeratotic part.

In the 13 early lesions with deposits, the part of the horny layer on top of the deposit was 2.5 (range 1.65–5.0) times thicker than that of whole, normal-looking epidermis used as the nearby reference. The early lesions were analysed in detail to estimate the relative corneocyte formation rate (Fig. 2). The appearance of Ig or C3 deposits was designated as day 0. Prior to this day hyperproliferation was present up to 4 days. The two lesions which did not contain any deposits had on average a maximum relative rate of corneocyte formation 13.5 times that of the adjacent normal-looking epidermis (range $\times 10.5$ – 15.5). Deposition in an early and a late lesion is shown in Figs. 3 and 4, respectively. Five lesions with positive reactions for Ig deposits were used to reveal deposition of albumin. In stratum corneum the indirect IF staining using the anti-albumin serum was negative in all cases. In stratum Malpighii it gave a speckled intercellular fluorescence. The microfluorometric measurement was done on IgG-positive slides, one from each of 8 patients with early lesions. The horny layer in the non-involved

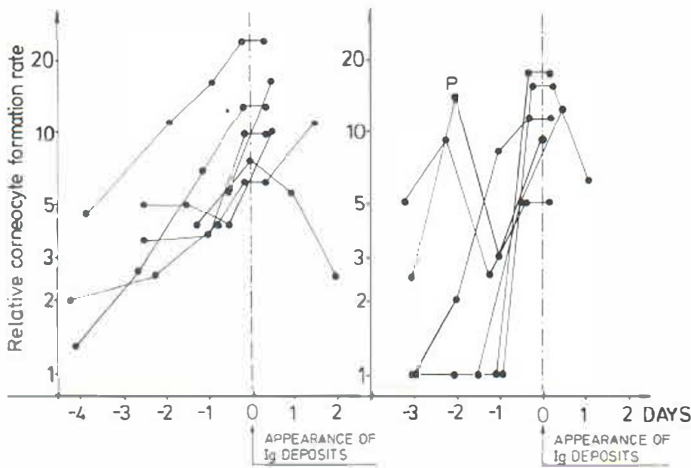


Fig. 2. Relative corneocyte formation rate in early psoriatic lesions and its relation to the appearance of deposits of IgG in the horny layer. The relative rate of corneocyte formation is defined in the introduction and in (3), expressed as the number of corneocyte layers formed in the lesion vis-à-vis each layer formed in non-involved skin. The age distribution of corneocytes in the horny layer was used to date the appearance of immunodeposits. The time basis on the abscissa is taken from earlier studies indicat-

ing a rate of corneocyte formation of two layers per day in non-involved psoriatic skin (3). The lesions fall into two groups, one with a slower onset of the increase in formation rate (left panel) and another with a sudden onset (right panel). In the latter situation the rate peaked (P) in advance of deposits in two cases. An increased rate of corneocyte formation precedes immunodeposits by one or several days in both groups.

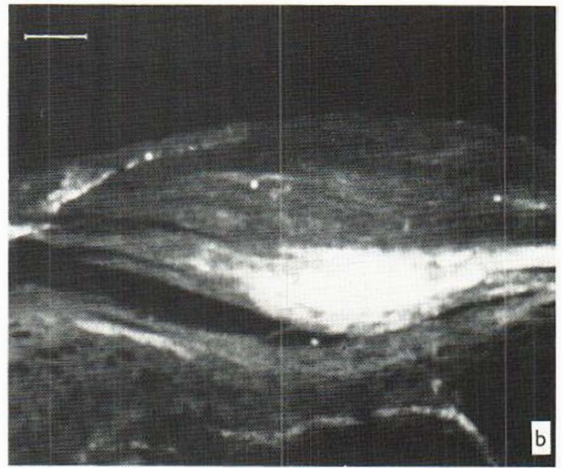
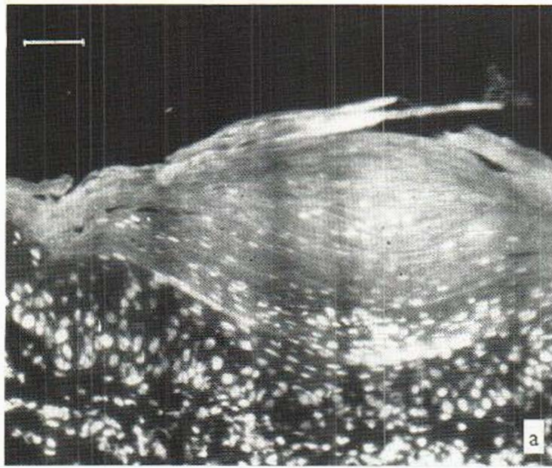


Fig. 3. Histology of an early lesion (a) Fluoresceinisothiocyanate and 4-4-diamidinodiphenylamine stained section to reveal corneocyte membranes and nuclei. A sparse parakeratosis is present from the mid-portion of the horny layer and increases towards the Malpighian

layer. (b) The same lesion with a faint IgG deposit located in the bottom part, coinciding with severe parakeratosis. The relative corneocyte formation rate ranged from $\times 4.5$ up to $\times 16$ in the area of the horny layer formed prior to that where the deposit is located. Bar equals $50 \mu\text{m}$.

skin was compared to that of the lesion (Fig. 1). In arbitrary units the mean fluorescence of the non-involved horny layer was 9.7 units. In the area of Ig deposits it was 18.0 units and just superficial to observed deposits it was 10.0 units. An analysis of variance gave a highly significant difference between the area of deposits vis-à-vis those without visible deposits ($F=268$, degrees of freedom 1/24; $P<0.001$). The area outside the lesion and that superficial to visible deposits did not differ ($F=0.29$, degrees of freedom 1/24; $P>0.5$). The error in the

double determinations of the fluorescence expressed as coefficient of determination was 12.9%.

DISCUSSION

The finding of the deposition of Ig or C3 in very early lesions in psoriasis, shown in the Table, was also noted by Jablonska et al. (1). The deposits are interpreted as specific, since they do not occur outside areas with parakeratosis. The controls made with the Fab_2 anti-Ig conjugate support this conclu-

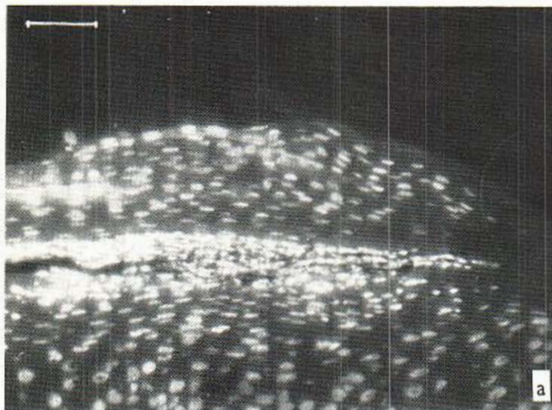


Fig. 4. Histology of a late lesion. (a) and (b) refer to the same stainings as in Fig. 3. Parakeratosis and IgG deposits coincide. The maximum relative corneocyte formation

rates in the two areas with severe parakeratosis were $\times 7.0$ and $\times 12.5$ for the superficially and the more basally located areas, respectively. Bar equals $50 \mu\text{m}$.

sion, as this conjugate yielded the same staining as the class-specific conjugate. In the Fab₂ anti-Ig conjugate the Fc part of the immunoglobulin is detached and therefore cannot react with an Fc receptor on the surface of the cells but only with the Fab portion of bound Ig molecules. The results of the indirect IF staining of albumin indicated that albumin is excluded from the horny layer in the examined sections but was abundant in the rest of the epidermis. Albumin was used as a marker of the intercellular plasma filtrate and differed from the results of the immunodeposits, again indicating specificity of the latter in the lesion. The strict localization of the deposits to parakeratosis has not been too well remarked on in recent literature (1, 2).

The division of the material into early and late lesions is arbitrary. Lesions in which parakeratosis is shown to appear up to the surface clearly is not newly formed. Therefore, we decided to indicate lesions as early only when they had a normal looking horny layer near the surface. We cannot exclude that in these lesions there might be already desquamated parts which have had an immunoreactive region. This is hardly likely, however, since normal corneocytes near the surface could be traced as a continuous layer from the non-involved area into the lesion. Near the surface several of the early lesions had an increased rate of corneocyte formation (Fig. 2.). The reason for this is that the top layer of corneocytes—which could be traced without difficulty in the RCR slides—was located just beneath the surface. Since one layer gives only a time coordinate, two must be retrieved before the rate measure is available. Our conclusion is that the lesions used are young, but we cannot state their age accurately in every case.

The results indicate that IF deposits are often delayed several days after initiation of hyperproliferation. This was expected, as IF deposits coincide with parakeratosis, which is known to be preceded by hyperproliferation for one or several days (3). One of our cases (Fig. 2) had a normal rate which suddenly increased from $\times 1$ up to $\times 17.5$. The deposits did not appear until half of the parakeratotic layers had been formed, however.

These interpretations are founded on the assumption that the observer can decide whether immune

deposits exist or not, by visual inspection. The results of the microfluorometry measurement support the interpretation and deny that the observed deposits are preceded by small, imperceptible immune deposits. From our results it can be concluded that IF deposits are a secondary phenomenon to other events precipitating hyperproliferation in the initial psoriatic lesion.

ACKNOWLEDGEMENT

This study was supported by the Swedish Psoriasis Association, the Swedish Medical Research Council (12X-5665) and contributions from the Foundations of O. E. and Edla Johansson, Welander, Finsen, Karolinska Institutet and the Swedish Medical Association.

REFERENCES

- Jablonska, S., Chorzelski, T. P., Beutner, E. H., Jarzabec-Chorzelska, M., Chowanec, O., Maciejowska, E. & Rzeska, G.: Autoimmunity in psoriasis. II. Immunohistologic studies on various forms of psoriasis and the Koebner phenomenon. *In* Psoriasis. Proceedings of the 2nd International Symposium (ed. E. M. Farber & A. Cox), pp. 73–80. Yorke, New York, 1976.
- Jablonska, S., Chorzelski, T. P., Beutner, E. H., Maciejowska, E., Jarzabec-Chorzelska, M. & Rzeska, G.: Autoimmunity in psoriasis. Relation of disease activity and forms of psoriasis to immunofluorescence findings. *Arch Dermatol Res* 261: 135–146, 1978.
- Johannesson, A. & Hammar, H.: The rate of corneocyte formation in microscopic lesions in patients with active psoriasis: Description of a new method and the finding of a highly increased but pulsative rate. *Br J Dermatol* 1981. *In press*.
- Krogh, H. K.: Antibodies in human sera to stratum corneum. *Int Arch Allergy* 36: 415–426, 1969.
- Krogh, H. K. & Tönder, O.: Antibodies in psoriatic scales. *Scand J Immunol* 2: 45–51, 1973.
- Sundqvist, K. G.: Quantitation of cell membrane antigens at the single cell level. *Scand J Immunol* 2: 479–494, 1973.
- Tagami, H. & Ofuji, S.: Leukotactic properties of soluble substances in psoriatic scales. *Br J Dermatol* 95: 1–8, 1976.

Received May 26, 1981

Anders Johannesson, M.D.
Department of Dermatology
Karolinska sjukhuset
S-104 01 Stockholm
Sweden