

Membrane-coating Granules in “Dry” Non-eczematous Skin of Patients with Atopic Dermatitis

A Quantitative Electron Microscopic Study

YLVA WERNER,^{1,2} MAGNUS LINDBERG^{1,2} and BO FORSLIND²

¹Department of Dermatology, Södersjukhuset, and ²Experimental Dermatology Research Group, Department of Medical Biophysics, Karolinska Institute, Stockholm, Sweden

Werner Y, Lindberg M, Forslind B. Membrane-coating granules in “dry” non-eczematous skin of patients with atopic dermatitis. A quantitative electron microscopic study. *Acta Derm Venereol* (Stockh) 1987; 67: 385–390.

In recent years much interest has been focused on the functions of the membrane-coating granules (MCGs). These granules seem to play an essential role in the formation of the barrier of the stratum corneum by extruding their lipid-rich content into the extracellular space of the corneocytes. The dry non-eczematous skin in atopic dermatitis has been reported to have defective barrier function. In the present study a quantitative electron microscopic analysis was made of the volume of MCGs in the transition zone between the stratum granulosum and stratum corneum in dry skin of patients with atopic dermatitis. The relative volume of MCGs was significantly greater than that in normal skin. This finding may indicate a disturbance of the “maturation” of the MCGs, leading to a defect in the barrier function in atopic dermatitis. *Key words: Lipids; Barrier function.* (Received December 29, 1986.)

Y. Werner, Department of Dermatology, Södersjukhuset, S-100 64 Stockholm 38, Sweden.

In 1957, with the development of biological application of electron microscopy, ovoid granules with a lamellar internal structure were observed in the keratinocytes of the upper part of the epidermis (1). These granules have been given a variety of names, e.g. Odland's bodies, keratinosomes, lamellar granules and—the term now predominantly used—membrane-coating granules (MCGs). Research in recent years has resulted in major progress towards the understanding of the function of these granules. The MCGs are derived from the endoplasmic reticulum (2) and they first appear at the level of the stratum spinosum. These granules have been shown to contain non-polar lipids, polysaccharides, lipases and hydrolytic enzymes (3, 4). In the upper layer of the stratum granulosum the granules fuse with the cell membrane and their lipid-rich content is extruded into the extracellular space of the stratum corneum, forming the “mortar” in a two-compartment model in which the corneocytes constitute the “bricks” (4). The extracellular non-polar lipids, rich in cholesterol and ceramides, probably provide the main barrier of the stratum corneum. Many recent investigations suggest that quantitative alterations in these lipids are responsible for both normal and abnormal stratum corneum barrier phenomena (5).

In atopic dermatitis (AD), many patients have dry-looking skin in non-predilection areas such as the back. A high frequency of bacterial infection of the skin and of non-allergic hand eczema in patients with AD are clinical evidences suggesting a defect barrier function. The aim of this study is to determine whether the production of MCGs is disturbed in dry skin of patients with AD.

MATERIAL AND METHODS

Material

Nine patients aged 18–40 years with atopic dermatitis according to the criteria of Hanifin & Rajka (6) participated in the study. They all had dermatitis on the flexures and in addition “dry-looking” skin on

the back that is—finely scaling, non-inflamed non-erythematous skin in this region (7). Nine age-matched persons with no anamnestic or clinical signs of atopy or dry skin served as controls. All persons had given their informed consent. A 3-mm punch biopsy was taken from the back of each person and was immediately prepared for electron microscopy.

Preparatory techniques

The specimens were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 4% sucrose for at least 24 h. Postfixation was carried out for 90 min with 1.5% potassium ferrocyanide and 1% osmium tetroxide in water according to Karnovsky (8). After dehydration in a graded ethanol series the specimens were embedded in Epon and ultrathin sections were cut on an LKB Ultratome. Contrast staining was performed with uranyl acetate and lead citrate. The sections were viewed in a Philips EM 301 G at 60 kV and micrographs were recorded on 35 mm film.

Quantitative analysis

Stereological analysis (9) was applied for measurement of the relative volume of MCGs in the uppermost layer of the stratum granulosum and for assessment of the relative volumes of keratohyalin granules, tonofilaments and granular material (e.g. ribosomes) in the stratum granulosum. One section from each patient was chosen at random in the electron microscope at a low magnification. For the analysis of the MCGs, a series of ten consecutive fields without overlapping were studied from the area of the upper part of the stratum granulosum and lower stratum corneum at a primary magnification of $\times 10\,000$. The micrographs were copied on photopaper at a secondary magnification of $\times 8$. A reference area comprising the upper layer of the stratum granulosum and the interspace between the stratum granulosum and stratum corneum was outlined on each micrograph (Fig. 1). The relative area and thus the relative volume of MCGs in this reference area was calculated with an area/length computer software program (Cardio 200, Kontron, France).

For determination of the relative volumes of keratohyalin granules, filaments and granular material, five micrographs at $\times 5\,100$ primary magnification were taken from the stratum granulosum in the same section as was used for the MCG analysis. A point-counting method was employed (9). A square point lattice containing 225 points ($d=1\text{ cm}$) was applied to each micrograph (secondary magnification $\times 8$).

In order to minimize the subjective influence on the quantitative assessment, all the photocopies were randomly mixed after hidden identification number had been allotted to each micrograph.

Light microscopy

Sections approximately $1\ \mu\text{m}$ thick were cut from each biopsy. They were stained with toluidine blue and evaluated in a conventional light microscope.

Statistical analysis

The Wilcoxon rank sum test (two-tailed) was used for statistical comparison of the relative volumes.

RESULTS

The result of the stereological analysis of MCGs is given in Table I. Compared with the controls, there was a statistically significant increase ($p < 0.01$) in the relative volume of MCGs in dry skin of patients of AD. No statistically significant differences were found in the relative volumes of keratohyalin granules, filaments or granular material in the two groups (Table II). Biopsies from normal skin and from dry atopic skin showed no differences in the light microscope, and in none of the biopsies were any signs of eczema observed. In both groups there was a partial loss of stratum corneum in some biopsies and these specimens could thus not be evaluated regarding the stratum corneum.

DISCUSSION

It is known that the physical properties of the stratum corneum are altered in dry non-eczematous skin of patients with AD. Increased transepidermal water loss, which indirectly reflects a defective barrier, has been noted in dry skin in AD, but also in skin with a normal appearance on predilection sites, e.g. the hands and forearms (10). A low water content of the stratum corneum in dry skin (11) and a deficient ability to bind water (12) also indicate



Fig. 1. Reference area in the uppermost layer of the stratum granulosum. $\times 80\,000$. St g=stratum granulosum, St c=stratum corneum, thick arrows=membrane-coating granules (MCG), A=reference area.

Table I. The relative volume (%) of MCGs in the reference area

	Atopic dermatitis <i>n</i> =9 (%)	Controls <i>n</i> =9 (%)
	13.2	6.5
	12.7	6.7
	8.5	8.1
	9.1	6.7
	8.3	8.7
	10.6	8.7
	11.9	5.1
	6.7	5.6
	10.0	7.6
Mean	10.1**	7.0
SD	2.2	1.3

** $p < 0.01$.

a defective barrier. At the transition from stratum granulosum to stratum corneum, major biochemical and structural changes take place. Among other things there is a shift from polar lipids (mainly phospholipids) to neutral lipids (e.g. ceramides and cholesterol) (5). During recent years it has become evident that these lipids are crucial for the barrier properties of the epidermis. From a morphological point of view the stratum corneum lipids are mainly found in the extracellular region and the volume of this extracellular space expands in the transition from stratum granulosum to stratum corneum (4). It has been shown that the MCGs play an essential role in the formation of this lipid-rich extracellular region of the stratum corneum (4, 5).

Mustakallio et al. (13) quantified the lipids of *whole* epidermis in patients with AD, and found a decrease in the content of total lipids and a relative increase in free fatty acids and sterols in eczematous skin of the forearm. Abe et al. (14) noted a decrease in squalenes and total lipids, including free cholesterol, in eczematous skin of patients with AD. However, no qualitative or quantitative data are available today concerning the extracellular lipids of the stratum corneum in dry skin of AD patients. We have approached this question from an ultrastructural aspect by performing a quantitative analysis of the volume of MCGs in the dry atopic skin.

The stereological principles used in the present quantitative analyses are based on the theory that relative areas in ultrathin sections can be translated to relative volumes (9). The

Table II. The relative volumes (%) of filaments, keratohyalin granules and granular material in the stratum granulosum

	Atopic dermatitis <i>n</i> =9		Controls <i>n</i> =9		
	Mean	SD	Mean	SD	
Filaments	25.3	2.8	25.8	4.2	NS
Keratohyalin granules	5.7	3.6	8.0	5.2	NS
Granular material	5.3	1.5	5.4	1.3	NS

NS = not significant.

MCGs are unevenly distributed in the epidermis, with an increasing number towards the stratum corneum, and the granules have a relative volume of about one per cent of the stratum granulosum in normal epidermis according to Klein-Szanto (15). As the end product of the MCGs is extruded into the extracellular space of the stratum corneum, we decided to optimize the analysis of the MCGs by defining a reference area comprising the uppermost part of the stratum granulosum cells and the intercellular space between the stratum granulosum and stratum corneum. It was found that the relative volume of the MCGs was significantly increased in this region in the dry skin of patients with AD compared with normal skin. This finding might reflect a differentiation defect or changes in the production of lipids, resulting in defective barrier function.

On the basis of histological findings it has previously been proposed that the dry skin in AD is the result of a subclinical eczema (7). Finley et al. (16) suggested that this could be the cause of a change in differentiation, leading to a defective barrier function.

We could not confirm these findings in our light microscopic analysis of semithick Epon-embedded sections. However, a comparison between histological findings in dry skin in AD has to be made with some caution as long as we are unable to define the clinical term "dry skin" objectively and thus compare the degrees of involvement of the skin sites chosen for biopsy. The area of biopsy also differs in different investigations (7, 16). In the present study we also performed a quantitative analysis of other structural markers of epidermal differentiation. No differences were detected in the contents of keratohyalin granules, filaments, or granular material (e.g. ribosomes) in the stratum granulosum between the two groups investigated. The values obtained for keratohyalin granules and filaments corresponded well with previously published data for normal skin (15). These findings would speak against the possibility of major differences in the differentiation of epidermis in our material.

In conclusion, in patients with atopic dermatitis and dry skin we have found an increased relative volume of membrane-coating granules in the uppermost part of the stratum granulosum as compared with normal skin. This finding may indicate a disturbance of "maturation" of these granules, or an alteration in the production of lipids, which could partly explain the change in barrier properties observed in dry non-eczematous skin in atopic dermatitis.

ACKNOWLEDGEMENT

This study was supported by grants from the Finsen Foundation. We are indebted to Miss Margareta Andersson for technical assistance. The computer system Cardio 200 was kindly made available by Drs Ingvar Ekberg and Christer Höglund, Department of Cardiology, Södersjukhuset, Stockholm. We are indebted to Dr Eva Brehmer-Andersson for performing the light microscopic analysis of our specimens.

REFERENCES

1. Odland GF, Holbrook K. The lamellar granules of epidermis. *Curr Probl Dermatol* 1981; 9: 29-49.
2. Wolff-Schreiner E. Ultrastructural cytochemistry of the epidermis. *Int J Dermatol* 1977; 16: 77-102.
3. Gopinathan KM, Grayson S, Elias PM. Cytochemical and biochemical localization of lipase and sphingomyelinase activity in mammalian epidermis. *J Invest Dermatol* 1986; 86: 591-597.
4. Elias PM. Epidermal lipids, membranes and keratinization. *Int J Dermatol* 1981; 20: 1-19.
5. Elias PM. Epidermal lipids, barrier function and desquamation. *J Invest Dermatol* 1983; 80: Suppl 6: 44s-49s.
6. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; Suppl 92: 44-47.
7. Uehara M, Miyauchi H. The morphologic characteristics of dry skin in atopic dermatitis. *Arch Dermatol* 1984; 120: 1186-1190.
8. Karnovsky MJ. Use of ferrocyanide-reduced osmium tetroxide in electron microscopy. *Proceedings of the 11th Annual Meeting of the American Society for Cell Biology*, 1971.

9. Weibel ER. Stereological methods. Vol. 1. Practical methods for biological morphometry. New York: Academic Press, 1979.
10. Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. *Acta Derm Venereol (Stockh)* 1985; 65: 102-105.
11. Werner Y. The water content of the stratum corneum in patients with atopic dermatitis. Measurement with the corneometer CM 420. *Acta Derm Venereol (Stockh)* 1986; 66: 281-284.
12. Werner Y, Lindberg M, Forslind B. The water-binding capacity of stratum corneum in dry non-eczematous skin of atopic eczema. *Acta Derm Venereol (Stockh)* 1982; 62: 334-337.
13. Mustakallio KK, Kiistala U, Piha HJ, Nieminen E. Epidermal lipids in Besnier's prurigo (atopic eczema). *Ann Med Exp Fenn* 1967; 45: 323-325.
14. Abe T, Ohkido M, Yamamoto K. Studies on skin surface barrier functions—skin surface lipids and transepidermal water loss in atopic skin during childhood. *J Dermatol (Tokyo)* 1978; 5: 223-229.
15. Klein-Szanto AJP. Stereological baseline data of normal human epidermis. *J Invest Dermatol* 1977; 68: 73-78.
16. Finlay AY, Nicholls S, King CS, Marks R. The "dry" non-eczematous skin associated with atopic eczema. *Br J Dermatol* 1980; 102: 249-255.