

FIBRINOID NECROSIS AND DOWNWARD MOTION OF COLLOID BODIES IN LICHEN PLANUS (APOPTOSIS)

I. Sümegi

Departments of Pathology and Dermatology, Karolinska sjukhuset, Stockholm, Sweden

Abstract. Fibrinoid necrosis which in previous investigations was demonstrated in the epithelial cells of the skin and of the liver in certain disorders, may also appear in the *colloid bodies in lichen planus*. Trichrome stainings were positive for fibrinoid, staining reactions with haematoxylin variants indicated the presence of keratin and precursors, and investigations in Wood's light permitted conclusions concerning the appearance of mixed proteins, of which keratofibrinoid seems to be the most important. Circulatory disturbances are emphasized as having an important role. The *colloid bodies* are extruded from the epidermis, according to the observations of Kerr et al., following the rules of *apoptosis*.

Key words: Fibrinoid necrosis; Colloid bodies; Lichen planus; Apoptosis

The essential change in the pathological picture of lichen planus is damage to the epidermis, in particular to the basal cell layer. Here one finds oedema, degeneration, and often strange round or oval acidophil bodies, the so-called "colloid bodies" (C.B.).

The origin and nature of the C.B. are not yet fully elucidated, although in recent years there have appeared important papers which have contributed to the better understanding of their significance (Gans (10), Thyresson & Moberger (25), Ebner & Gebhart (6, 7), Kerr, Wyllie & Currie (16), El-Labban & Kramer (8, 9), Weedon (29), and Hashimoto (11)).

The opportunity for this investigation was provided us during the course of studies on various paraproteins found in necrotic epithelial cells of the liver (23), skin (24) and around malignant tumours of various organs (22). These paraproteins consist of so-called mixed proteins; in cases of malignant tumours, of an amyloid-like substance + elastin; and in toxic liver cell necrosis, of fibrinoid which also often gave an amyloid reaction. In epithelial necrosis of the skin, we found in various der-

matoses fibrinoid necrosis and horn formation in the damaged cells. This pathological substance was named keratofibrinoid (24).

MATERIAL AND METHODS

The material investigated consisted of submitted cases of lichen planus and other dermatoses with suspected C.B. In addition to routine stainings, trichrome staining after Ladewig & Ivemark (13), Goldner, fibrin staining after Weigert, Kockel (18) and Heidenhain's iron-alum-haematoxylin (12) staining were carried out. Congo red, PAS, alcian blue, methylgreen-pyronine and PTAH were also used. Fluorescence microscopy was performed using acridine orange, thioflavine S and Rhodamine B (5) as fluorochromes. All preparations were examined by light microscopy and under Wood's light.

RESULTS

Haematoxylin-eosin

In about 33% of fairly recent cases of lichen planus, a necrotic process was found involving single cells in the epidermis. The cytoplasm stains intensely red and usually swells; within it are found fine vacuoles or granules.

The nucleus stains poorly and becomes pyknotic and adherent to the cell membrane. The nucleus is later extruded and leaves behind it a small cavity which remains visible for some time. These structures partially coalesce and often lie at the dermo-epidermal junction in small fissures or vesicles. Other cells break away and lie singly or in small groups between the normal cells. A proportion of these small bodies lie near the horny layer where they are extruded as a result of normal cell movement. The majority of the bodies move downwards (*apoptosis*) (16), are often found in the basal layer and are for the most part extruded into the underlying cellular infiltrate. This must be considered morphologically as primary keratinization or dyskeratosis of the affected cells. The structures described are designated "*colloid bodies*". Further reference is made below to *apoptosis*.

The haematoxylin-eosin preparations were examined in Wood's light. The horny layer fluoresced brilliant golden-yellow; the normal epidermis did not fluoresce. The dyskeratotic cells in the upper malpighian layer

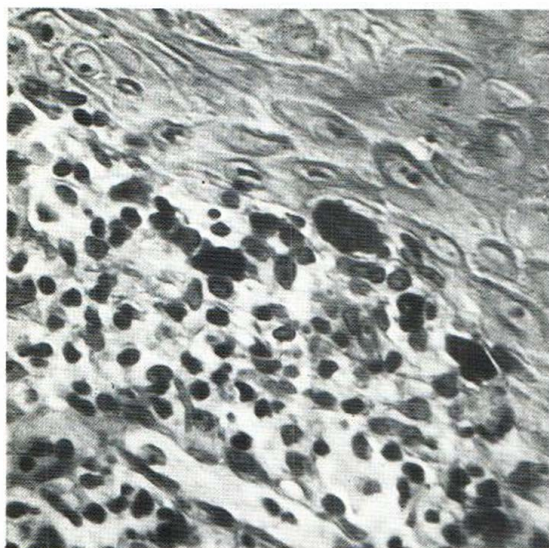


Fig. 1. Colloid bodies in the dermal-epidermal junction. Case of lichen planus. Haem.-eosin, $\times 400$.

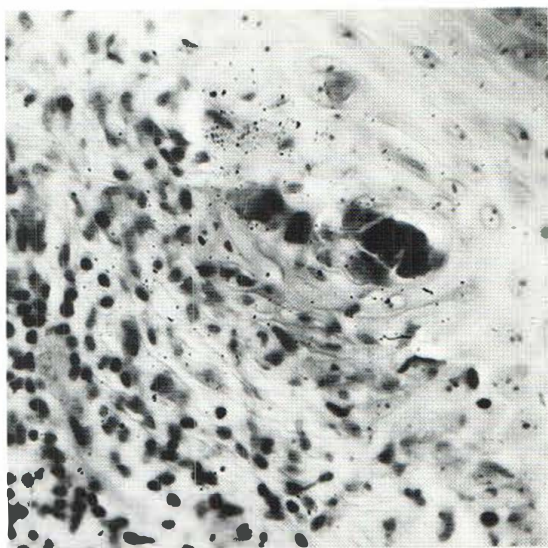


Fig. 2. Colloid bodies, partly coalescent, in the epithelial layer. Case of lichen planus. Ladewig's stain, $\times 200$.

showed the same colour as the horny layer. The C.B. lower in the epidermis and the extruded bodies showed a distinct yellow or greenish-yellow fluorescence, which became increasingly green and with progressive necrosis gradually disappeared. Thus one finds here all the transitional colours which were called "mosaic structures" or "piebald pictures" in earlier publications (23, 24). Fig. 1.

In sections studied by van Gieson's method the C.B. appeared as yellowish structures which did not fluoresce.

Trichrome stains

(a) *Ladewig's method.* With this stain, more striking changes are found. The horny layer stains a brilliant red and the C.B. assume the same colour. Some C.B. lower in the epidermis show a less intense red or bluish-red colour and in those cells which have been extruded into the exudate one sees all gradations between red and blue. The red hue slowly vanishes, becomes reddish-blue or bluish-grey and the C.B. fall victim to fully developed necrosis, appearing only as barely detectable grey structures. One often sees beautiful "mosaic structures" and also the phagocytosis of disintegrated C.B. With trichrome stains there is no fluorescence. Fig. 2.

(b) *With Goldner's trichrome method,* changes essentially similar to (a) are found. The normal horny layer and the small C.B. are stained bright red. This colour then changes into a range of shades from greenish-red, green, and greenish-grey to grey.

Methods for mucopolysaccharides and glycoproteins

The PAS reaction was almost always + to ++, with a reddish autofluorescence. Congo red was always nega-

tive, both in normal and in polarized light. In Wood's light an intensification of the autofluorescence was often observed. Staining with thionin, toluidine blue and methylgreen-pyronine (except sometimes Taft's modification) gave negative results.

Fibrin stains

With Weigert's method, the horny material is strongly positive, as is such fibrin as may sometimes be present. Rather weak staining is seen also in very early necrosis of epidermal cells (20). The fully formed C.B. do not take up the stain. With Kockel's method, the horn, all damaged cells and the C.B. are stained black or greyish-black. With Heidenhain's method only the horn stains black or very dark blue, the C.B. are at most greyish or unstained. The intensity of all staining diminished markedly lower in the epidermis and depends on the quantity of keratinoid material present. Fig. 3.

Fluorochromes

(a) *Acridine orange.* The horny layer fluoresces a glowing golden yellow, as do the subcorneal keratinizing cells. The C.B. fluoresces bright yellow with golden yellow or greenish markings and retain their colour until they are extruded downwards. In the exudate the colour becomes greenish until the fluorescence disappears. The C.B. often form beautiful mosaic structures. Fig. 4.

(b) *Thioflavine S.* The result is not very effective: Everything stains silvery-white, the C.B. are mostly sky-blue.

(c) *Rhodamine B.* The horny layer fluoresces a beauti-

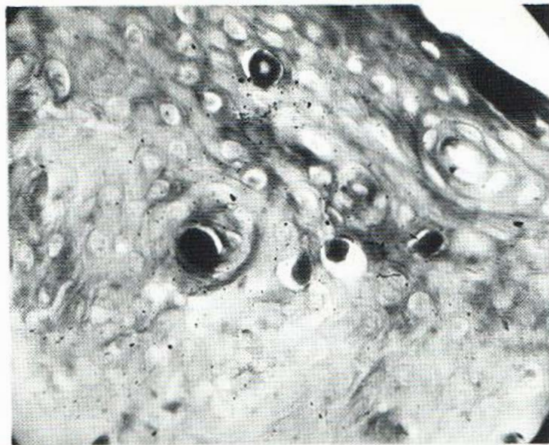


Fig. 3. Intra-epithelial colloid bodies. In the upper part, one with extruded nucleus. Lichen planus. Kockel's stain, $\times 320$.

ful reddish; the C.B. are at first golden yellow, then green before they disappear.

(d) Staining by Taft's rather fanciful method (23) shows a beautiful reddish fluorescence of the horny layer. The C.B. are lemon yellow or somewhat reddish-yellow. At lower levels they become greenish-yellow and then green until they disappear.

With staining methods (a) and (d) one sees beautiful piebald effects, which indicate the appearance of mixed proteins.

In certain skin diseases in which the occurrence of C.B. has long been known, we found C.B. particularly when the inflammatory reaction in the connective tissue was "lichenoid".

In *Lupus erythematosus* (LE) the occurrence of C.B. is "extremely rare", according to Pinkus (21). It is noteworthy that nearly all of our 12 positive cases belong to the group which Montgomery (19) distinguishes as "lichen-like LE". The infiltrate is indeed band-like but is not always sharply defined. The C.B. are found also here in the epidermis, which is however too thin to harbour many of them. They are extruded downwards through the thickened loose basement membrane. The staining and fluorescence properties are the same as in lichen planus.

Poikiloderma is the disease in which A. Civatte (13, 14) gave a detailed description of the C.B. These are mostly situated at the dermo-epidermal junction. The staining properties are the same as in LE.

In a few cases of *lichenoid toxicoderma* the findings were similar.

The following dermatoses involve cell necrosis and therefore the formation of C.B. is to be expected.

Pityriasis varioliformis acuta Mucha-Haberman. The necrotic process begins in dyskeratotic epidermal cells

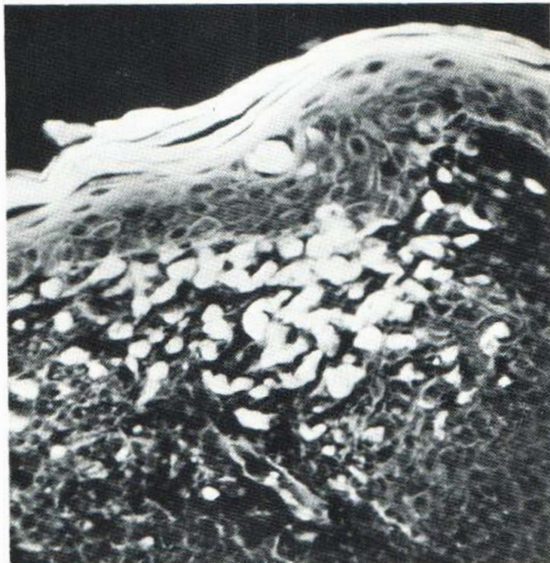


Fig. 4. Lichen planus. Extruded colloid bodies in the subepidermal infiltration. Some intra-epithelial colloid bodies have been extruded into the horny layer. Intensive fluorescence after acridine-orange staining, $\times 250$.

which, after wandering upwards or downwards, are often extruded. These cells look exactly like C.B. Their staining and fluorescence properties were also identical.

In cases of *Lyell's toxic epidermal necrolysis*, one sometimes finds dyskeratotic structures which cannot be differentiated morphologically or by their staining properties from the C.B. of lichen planus.

Bullous diseases of the herpes group show dyskeratotic necrosis of some isolated cells in the otherwise normal epidermis around the vesicle. These have pyknotic or absent nuclei, and bright red (haematoxylin-eosin) cytoplasm and may be shed into the lumen. All stains are the same as in lichen planus. These C.B. are seldom extruded downwards from the epidermis. However, they enter directly into the vesicle and thus have entirely altered kinetics and still fluoresce in the floor of the vesicle. Mention must still be made of vesicles in epidemic stomatitis, vesicles after ultraviolet irradiation of the skin (26), and actinic prokeratosis.

Also investigated were cases of actinic keratosis, Bowen's disease, keratoacanthoma and epithelial skin cancer. In all cases C.B. were to be found.

A detailed description of the histological findings would go beyond the scope of the present article but will be reported subsequently. It is interesting that the C.B., which lie immediately next to the principal pathological changes, show the same reactions as in lichen planus and that all these cases were associated with a lichenoid infiltrate.

DISCUSSION

From the findings described, it follows that the C.B. in lichen planus originate in damaged epidermal cells, of which they are indeed fragments. The process begins with premature atypical keratinization. Staining reactions soon occur which indicate a composite paraprotein, fibrinoid. One thus finds signs of keratinization and fibrinoid necrosis in cells which are undergoing complete necrosis, losing their nuclei, often disintegrating and appearing as C.B.

The significance and composition of these bodies have been disputed for many years but not conclusively resolved. A resurgence of interest began in 1957 with the works of N. Thyresson & Moberger (25) who, using ultraviolet absorption and DDD techniques were able to establish their epidermal origin. Their findings were slowly accepted as correct and displaced the misleading deliberations of authors who regarded C.B. as degenerated inflammatory cells, or degenerating plasma—or mast cells. In 1972 Kerr, Wyllie and Currie (16) established by electron microscopy the origin of C.B. from keratinocytes, which made their appearance and disappearance more comprehensible. They introduced into pathology the concept of *apoptosis*. This term means a dropping off of something from somewhere, e.g. leaves from a tree. (There is an analogy with ptosis of the upper eyelid in oculomotor palsy.) The process occurs by the elimination of redundant or damaged cells, resp. their fragments. It is found in malignant tumours (17), in the suprarenals after withdrawal of ACTH (30), in the germinative centres of lymph glands (31) and also in certain dermatoses. In the skin, the process begins with dyskeratosis of the damaged epidermal cells, which move downwards (apoptosis) and are extruded, precisely like the C.B. in lichen planus. Apoptosis plays an important role in the "mechanism to balance the cell population of diverse normal and embryonal organs, and normal, diseased and neoplastic tissues". It plays a role also in certain malformations and certain types of experimental poisoning, e.g. Thalidomide (15), or poisoning with 7-OHM-12-MBA, according to Alison (1).

The important investigations of Kerr (16) and his colleagues were extended and confirmed by Ebner & Gebhart (6, 7), El-Labban & Kramer (9) and others. Recently Hashimoto (11), likewise has studied the C.B. in the electron-microscope and in

his comprehensive work has called them "filamentous cells" on account of their strange filamentous structure. He was also able to induce C.B. formation experimentally in guinea pigs with horseradish peroxidase. Moreover he distinguished in the upper dermis two kinds of C.B.: *epidermal C.B.*, which had dropped into the dermis, and *phagocytes* containing filament aggregations. Other, older investigations also raised the question of the dual origin of C.B. and it was considered that they could be epithelial or mesenchymal structures (2, 14, 27). It is indeed probable that determined etiological factors can cause similar regressive changes in parenchymal and mesenchymal structures. However, in the investigations reported here and by using the methods described we have not obtained any definitive pictures.

As to the question of the cause of the development of C.B. and of the apoptotic process, we saw that the C.B. occurred in about one-third of the cases of lichen planus, particularly in the more acute cases. It is also remarkable that in LE the C.B. appear to be particularly numerous in the lichenoid form. In other dermatoses and in skin cancer, C.B. are also seen, particularly in association with lichenoid tissue reactions, even when the latter do not form part of the typical histological picture of the process. The morphological picture with compression of the vessels, oedema, fissure and vesicle formation, and the appearance of some enlarged cells with finely vacuolar cytoplasm, indicate a disturbance of the water balance of the epithelia and of the connective tissue. The possibility that lichenoid infiltrates have some special property cannot be excluded. The circulatory disturbances may lead to hypoxia, anoxia and cell injury with consequent dyskeratosis, more rapid keratinization and apoptosis of the injured cells, resp. the C.B. Ultimately total necrosis and resorption result.

Between the beginning of the disturbed keratinization, the total necrosis, and the elimination of the damaged cells, changes take place in the nuclei and cytoplasm which correspond to fibrinoid necrosis of the epithelial cells and are indicative of the nature of the C.B. The specified changes are (a) trichrome stainings positive for fibrinoid, (b) staining reactions with haematoxylin variants indicating the presence of keratin and precursors, and (c) last but not least, characterization by fluorochromes. These histological and histochemical findings allow us to draw conclusions concerning the appearance of

bland proteins, of which keratofibrinoid seems to be the most important.

CONCLUSION

Fibrinoid necrosis which in previous investigations was demonstrated in epithelial cells of the skin and of the liver in certain disorders can also appear in the *colloid bodies* in lichen planus. Mixed proteins are also found and appear as keratofibrinoid. Circulatory disturbances such as hypoxia-anoxia appear to have a specific significance. The colloid bodies are extruded from the epidermis according to the rules of apoptosis, as described by Kerr et al. (16). This process contributes to the maintenance of the cell balance and kinetics of the affected organs. Together with our knowledge of their ultrastructure (11) these complicated processes can now be better connected and thus the transition between life and death of the cells may be better understood.

ACKNOWLEDGEMENTS

This study has been supported by the Edvard Welander Foundation. The technical assistance of Miss Carin Lundmark and Miss Ann Sandström is gratefully acknowledged.

REFERENCES

- Allison, M. C., Kerr, J. F. R. & Currie, A. R.: The relationship of acute mesodermal cell death to the teratogenic effects of 7-OMBH-12 MBA in the foetal rat. *Br J Cancer* 26: 498-503, 1972.
- Braun-Falco, O.: See Ueki (28).
- Civatte, A.: Critères cliniques et histopathologiques des lichen planes cutanés et muqueux. *Ann Dermatol Syphiligr (Paris)* 80: 5-29, 1953.
- Civatte, A.: Atlas d'histopathologie cutanée, pp. 166-167. Masson et Cie, Paris 1957.
- Clausen, F. P. & Dabelsteen, E.: Rhodamine B method for keratinization by the use of fluorescence light. *Acta Pathol Microbiol Scand* 77: 169-171, 1969.
- Ebner, H. & Gebhart, W.: Beitrag zur Histochemie und Ultrastruktur der sogenannten hyalinen bzw. kolloiden Körperchen. *Arch Dermatol Forsch* 242: 153-164, 1972.
- Ebner, H. & Gebhart, W.: Light and electron-microscopic differentiation of amyloid and colloid or hyaline bodies. *Br J Dermatol* 92: 637-645, 1975.
- El-Labban, N. G.: Light and electromicroscopic studies of colloid bodies in lichen planus. *J Periodontol Res* 5: 315-324, 1970.
- El-Labban, N. G. & Kramer, I. R. H.: Civatte bodies and the actively dividing epithelial cells in oral lichen planus. *Br J Dermatol* 90: 13-23, 1974.
- Gans, O.: Histologie der Hautkrankheiten. Springer-Verlag, Berlin, 1925.
- Hashimoto, K.: Apoptosis in lichen planus and several other dermatoses. *Acta Dermatovenereol (Stockholm)* 56: 187-210, 1976.
- Heidenhain, M.: Technics, see Z Wiss Mikr 32: 361, 1915.
- Ivemark, B.: Barnpatologi, p. 284. Almqvist & Wiksell, Stockholm, 1971.
- Jarret, A. & Speakman, R. I.: The histochemistry of keratinization. *Br J Dermatol* 71: 277-295, 1959.
- Jurand, A.: Early changes in limb buds of chick embryos after thalidomide treatment. *J. Embryol Exp Morphol* 16: 289, 1966.
- Kerr, J. F., Wyllie, A. H. & Currie, A. R.: Apoptosis: A basic phenomenon with wide ranging implications in tissue kinetics. *Br J Cancer* 26: 239-257, 1972.
- Kerr, J. F. & Searle, J.: A suggested explanation for the paradoxically slow growth rate of basal cell carcinomas that contain numerous mitotic figures. *J Pathol* 107: 41-44, 1972.
- Kockel, N.: Technics, see Zentrabl Allg Pathol 10: 749, 1899.
- Montgomery, H.: Dermatopathology. Harper and Row, New York, 1967.
- Pearse, A. G. E.: Histochemistry. Theoretical and Applied, 2nd ed. J. and A. Churchill, London, 1961.
- Pinkus, H. & Mehregan, A. H.: A Guide to Dermatohistopathology. Meredith Corp., New York, 1969.
- Sümegi, I. & Rajka, G.: Amyloidlike substance surrounding mammary cancer and basal cell carcinoma. *Acta Pathol Microbiol Scand* 80: 185-192, 1972.
- Sümegi, I.: Fibrinoid necrosis of liver parenchymal cells. *Acta Pathol Microbiol Scand [A]* 83: 109-119, 1975.
- Sümegi, I.: Fibrinoid necrosis of the epithelial cells of the skin. *Acta Pathol Microbiol Scand [A]* 84: 468-476, 1976.
- Thyresson, N. & Moberger, G.: Cytologic studies in lichen ruber planus. *Acta Dermatovenereol (Stockholm)* 37: 191-204, 1957.
- Thyresson, N. & Wennersten, G.: Unpublished investigations (see ref. 24).
- Ueki, H.: Hyaline bodies in subepidermal papillae. *Arch Dermatol* 100: 610-617, 1967.
- Ueki, H., Wolff, H. H. & Braun-Falco, O.: Cutaneous localization of human gamma-globulins in lupus erythematoses. *Arch Dermatol Forsch* 248: 297-314, 1974.
- Weedon, D.: Civatte bodies and apoptosis. *Br J Dermatol* 91: 357, 1974.
- Wyllie, A. H., Kerr, J. F. R., Macaskill, I. A. M. & Currie, A. R.: Adrenocortical cell deletion: the role of ACTH. *J Pathol* 111: 85-94, 1973.
- Yoffey, J. M. & Courtice, F. C.: Lymphatics, Lymph and the Lymphomyeloid Complex, p. 534. Academic Press, New York, 1970.

Received March 18, 1978

I. Sümegi, M.D.
Department of Pathology
Karolinska Sjukhuset
S-104 01 Stockholm 60
Sweden