INTERNATIONAL JOURNAL OF LEPROSY Volume 52, Number 1
Printed in the U.S.A.

Free Subepidermal Grenz Zone (Band of Unna) in Lepromatous Leprosy. Histological and Ultrastructural Findings[1]

Ulrich Martens and Georg Klingmüller[2]

When lepromatous leprosy found its way into the histopathological textbooks on skin diseases, the term "band of Unna" or "free grenz zone" was mentioned for the first time in connection with the disease ([7]). The grenz zone appeared to be "free" from leprosy bacilli under the light microscope, and it was for this reason that this zone was assigned the name free grenz zone. Generally, the term is used in literature as a synonym for the subepidermal layer of collagen and connective tissue fibers in lepromatous leprosy. However, no comprehensive study of the histology and ultrastructure of this area has been undertaken until now.

The purpose of this paper is to describe the morphology of the grenz zone in lepromatous leprosy, to compare it with the subepidermal tissue in tuberculoid and borderline leprosy, and to discuss some factors which may explain the mechanism of its formation. Our own histological studies also confirmed the existence of subepidermal zones which are free of the characteristic alterations in the following diseases: lymphocytoma cutis, granuloma eosinophilicum faciei, lobomycosis, acrodermatitis chronica atrophicans, and lichen amyloidosis.

MATERIALS AND METHODS

Clinical material. Twenty skin samples were taken from 6 patients: 4 were suffering from lepromatous, 1 from tuberculoid, and 1 from borderline leprosy. The tuberculoid case was a low-resistant form and the borderline case belonged to the borderline lepromatous type. Two of the lepromatous leprosy patients were treated for many years by the Department of Dermatology, University-Skin Hospital, Bonn, Germany, so that different stages of the disease could be observed with light and electron microscopy.

Light microscopy. Hematoxylin and eosin (H&E), the Fite-Faraco method for acid-fast bacilli ([1]), and the modified method with sirius red from Junqueira, *et al.* ([3]) were used for staining. The birefringency of the collagen was enhanced with the sirius red dye. When the slide was then placed under a polarization microscope with a bright lamp, the collagen was observed to be surrounded by a dark background.

Scanning electron microscopy. The remaining histological sections had a thickness of 5 μm and were not stained, but removed of paraffin and sputtered with gold. The sections were observed under the Hitachi scanning electron microscope; photographs were taken with Polaroid instant film.

Transmission electron microscopy. Parts of each biopsy were fixed in two parts collidine buffer and one part 4% osmium tetroxide for 2 hr. Block staining was performed in 1% phosphotungstic acid and 0.5% uranyl acetate. The material was embedded in Epon 812, sectioned in a LKB ultratome with Balzer diamond knives, and double stained in uranyl acetate and lead citrate. Micrographs were taken with a Zeiss EM 9 S-2 electron microscope.

RESULTS

Light microscopy. The grenz zone appeared as a homogeneous layer of connective tissue. It contained flattened nuclei of fibroblasts and was always uniformly attached to the epidermis without any rete pegs (Fig. 1). Below the stretched collagen band lay the confluent leproma in which

[1] Received for publication on 23 August 1983; accepted for publication in revised form on 9 September 1983.

[2] U. Martens, M.D., and G. Klingmüller, M.D., Professor of Dermatology, University-Skin Hospital, D-5300 Bonn 1, West Germany.

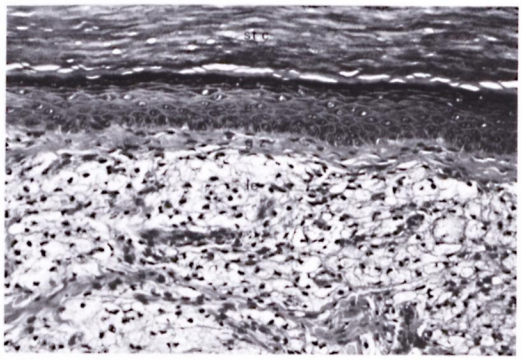

FIG. 1. A skin cross-section from the finger of a lepromatous patient showing at the top a thick stratum corneum (st c) and a stretched epidermis (e) with a grenz zone (g z) lying directly below it. In the bottom, the foamy leproma (le) is shown to consist of only a few connective tissue strands (H&E ×335).

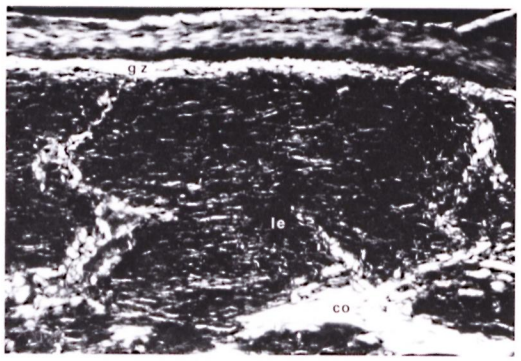

FIG. 2. Under polarized light, the grenz zone (g z) appears as a bright contrasting band. In the leproma (le) only the thin and short connective tissue fibers are visible. A thick collagen bundle (co) is situated in the middle of the corium (bottom right) (sirius red under polarized light ×310).

only short and thin connective tissue fibers were visible (Fig. 2). The capillary vessels were observed to be extremely diminished in the grenz zone. Venules and arterioles could be found between the grenz zone and the leproma.

The ratio of the widths of grenz zone to epidermis—without the stratum corneum—was measured at 84 different positions on the skin samples. The average width of the grenz zone was 35.5 ± 5.6 μm in the initial stage of lepromatous leprosy and 23.7 ± 3.1 μm in progressive lepromatous leprosy. Likewise the average width of the epidermis was greater in the initial stage of lepromatous leprosy than in the progressive stage. This implied that the width of the grenz zone decreased simultaneously with the epidermis in the progressive stage of the disease. However, the epidermis always remained thicker than the grenz zone. The absolute value of the width of the grenz zone also depended on the width of the epidermis and where the sample was taken. For instance, both the grenz zone and the epidermis from the thigh were obviously thicker than those from the ear of the same patient.

Other such transitional areas between the connective tissue and epithelium in lepromatous leprosy, such as below the epithelium of the tongue, had no grenz zone.

Scanning electron microscopy. When the connective tissue fibers of the grenz zone were magnified by the scanning electron microscope, bundles of fibrils were observed (Figs. 3 and 4). These bundles had a band-like form and had their flat sides lying on horizontal planes below the stretched epidermis (Fig. 5). This was not seen in the deeper layers of the corium or in normal skin. Only a few short, extremely thin bundles were observed to be perpendicular to

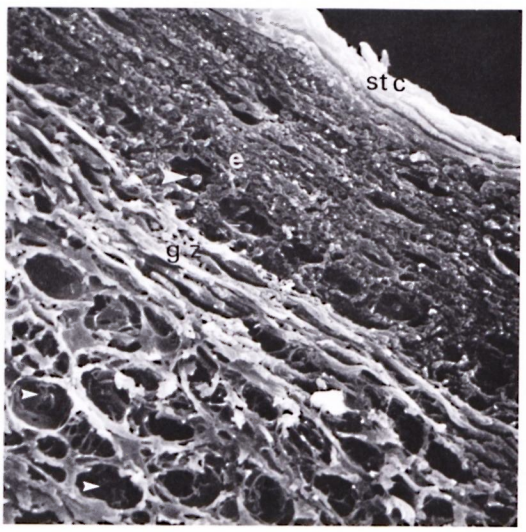

FIG. 3. The epidermis (e) lies beneath the bright statum corneum (st c). The remains of the nuclei of its basal cells are found in the cavities formed by their removal (arrow). Sectioned lepra cells form membraneous cavities containing lepra bacteria (arrows) (×1200).

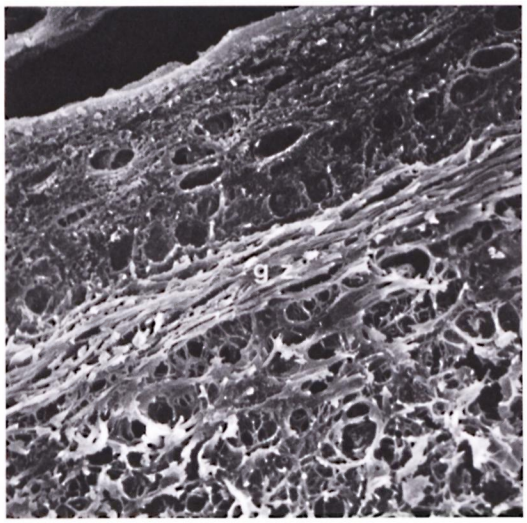

FIG. 4. The cavities in the grenz zone (g z), which were formed as a result of the removal of cells and cell extensions, are found to be thinner and stretched (arrow) compared with the cavities left by lepra cells (×840).

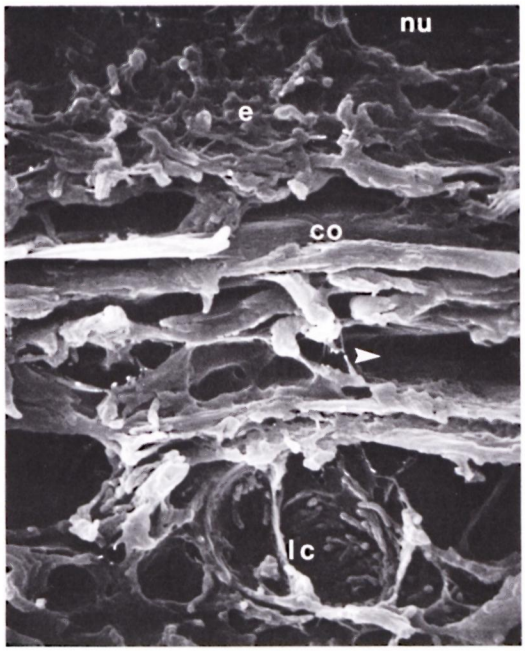

FIG. 5. The upper third of the picture shows an empty space, which contained the nucleus (nu) of a basal cell of the epidermis (e). The band-like collagen bundles (co) of the grenz zone lie horizontally with their flat sides facing towards the basal side of the epidermis. A fibroblast could have been originally situated in the small space (indicated by the arrow). The large lepra cell (lc) is found below the grenz zone (×4100).

the epidermis. Each of these bundles had a diameter of 0.2–0.3 μm, thus consisting of 5–10 elementary fibrils. The diameter of the collagen bundles increased in the grenz zone from top to bottom. But in comparison with the middle and deep corium, the band of Unna contained relatively small bundles of fibrils (0.7–1.2 μm). It was often possible to find bundles of fibrils with a width of 20 μm in the lower third of the corium outside the leproma. This was nearly as thick as the whole grenz zone of progressive lepromatous leprosy. The gap-like cavities in the connective tissue of the grenz zone were only large enough for flattened cells. The shape of the connective tissue in the grenz zone allowed maximum filling by the collagen. Examinations of the borderline leprosy case confirmed the existence of a grenz zone equivalent; this was not true in our case of tuberculoid leprosy. When the grenz zone equivalents were found to be present in our samples of borderline leprosy, they differed from the grenz zone in lepromatous leprosy in the following ways: 1) they had a larger width; 2) the transitional area between the leproma and the grenz zone was poorly defined; and 3) their widths depended on the number of epithelioid cells to macrophages in the borderline lesion, i.e., the greater the number of epithelioid cells, the wider were the grenz zone analogues.

Transmission electron microscopy. The basement lamina of the dermal-epidermal junction above the grenz zone showed no pathological changes. Most of the cells situated in the grenz zone in early and progressive lepromatous leprosy were fibroblasts. These fibroblasts were together with certain macrophages only slightly infested with leprosy bacilli. None of these cells contained giant phagolysosomes, which were characteristic of the typical lepra cells with their foamy intracytoplasmic structure. In most cases the leprosy bacilli were found alone inside the phagosomes of the grenz zone cells.

These slightly infested cells could also be seen directly below the basement lamina of the dermal-epidermal junction (Figs. 7 and 8). More cells were found in the grenz zone of the progressive stage of lepromatous leprosy than in the early stage. The ultrastruc-

FIG. 6. The basement lamina of the dermal-epidermal junction is clearly visible (arrow). One can see lengthwise stretched thin fibroblast-extensions (fi) between the collagen bundles (possibly type III and type I). The diameter of these bundles increases with increasing depth. Infested macrophages can be seen; these form the upper edge of the leproma (×7500).

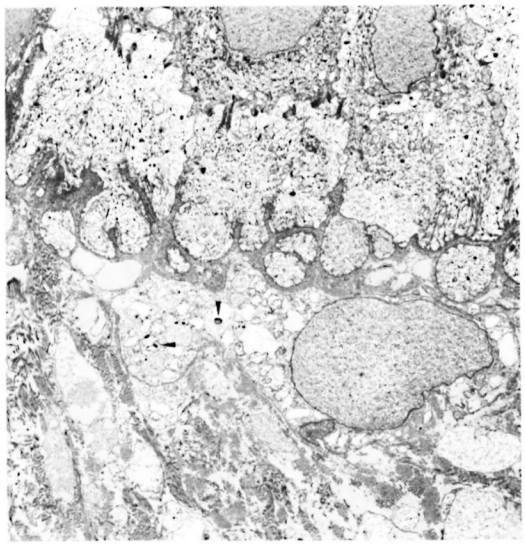

FIG. 7. Two engulfed lepra bacteria (arrows) are shown in a grenz zone cell lying directly below the epidermis (e) (×6860).

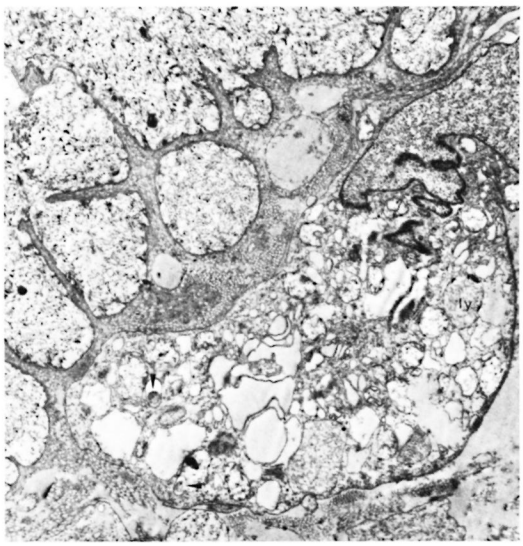

FIG. 8. An enlarged section showing the extension of a grenz zone cell with two lepra bacilli (arrows), each situated peripherally in different lysosomes (ly) (×15, 680).

tural appearance of each grenz zone cell depended on how progressive the lepromatous form of the disease was. It was, however, never identical with the ultrastructure of the cells seen in the leproma.

Leprosy bacilli were found to be rare in the lumina and in the endothelial cells of the vessels.

More than 330 measurements of collagen fibrils of the grenz zone were taken. Fibrils with a diameter of 370–420 Å appeared in the whole narrow grenz zone of patients with progressive lepromatous leprosy and in the upper third or upper half of the large grenz zone in the early stage of lepromatous leprosy. The lower part contained collagen fibrils with a diameter ranging from 600–800 Å.

DISCUSSION

The following factors could have been involved in the formation of the free grenz zone: optimal growth temperature, ultraviolet rays, lack of oxygen, mechanical compression, and immunologic factors.

Optimal growth temperature of the lepra bacilli ([6]) is primarily responsible for the distribution of the leproma in different skin areas of the body ([5]). The fact that the grenz zone has only a minimal Bacterial Index may be due to a lack of this optimal bacterial growth temperature.

Also conceivable is the inhibition of bacterial growth directly below the epidermis by ultraviolet radiation because ultraviolet rays in sunlight are found to be able to kill *Mycobacterium leprae* in bacillary suspensions ([8]).

The fact that only a few lepra macrophages were present in the grenz zone is definitely not due to the lack of oxygen since blood supply in the leproma, which is full of lepra macrophages, is hardly better than in the grenz zone. The vessels in the leproma are compressed much faster than the vessels in the connective tissue of the grenz zone. The lack of oxygen can only accelerate the narrowing of the grenz zone; if the number of vessels is diminished, the collagen production is also reduced.

The pressure exerted by the leproma on the papillary connective tissue is not responsible for the grenz zone remaining "free" but, rather, for its structure, where the parallel layers of fibrils in the grenz zone are compressed by the leproma below it.

That the fibroblasts form the main cellular fraction of the grenz zone is the explanation for the relatively low number of bacilli which were seen under the light

microscope because the fibroblasts have a low capability of phagocytosis.

Fleischmajer, *et al.* ([2]) could label fibrils which have the same diameter as the fibrils in the smaller grenz zone with ferritin-marked, type III antibodies. Fibrils with diameter-range from 600–800 Å, which are found in the lower part of the grenz zone in the early stage of lepromatous leprosy, have been identified by Fleischmajer, *et al.* with immunoelectron microscopy as type I collagen in normal and scleroderma skin. In lepromatous leprosy this could mean that under the progressive compression of the leproma, only a type III collagen layer remains which corresponds to the normal papillary dermis since Meigel, *et al.* ([4]) showed by immunofluorescence microscopy that the main part of the papillary dermis consists of type III collagen.

SUMMARY

Although the grenz zone is indeed free of typical leprosy cells with giant lysosomes, it is not free from leprosy bacilli—these are found in slightly infested macrophages and fibroblasts. For that reason, it would be much clearer if we simply called this region the subepidermal grenz zone of lepromatous leprosy. The subepidermal grenz zone is not pathognomonic of leprosy since grenz zones can be found in other diseases as well. Whereas the grenz zone is typical in lepromatous leprosy, it is not necessarily characteristic of the borderline or the tuberculoid form of the disease. The light and electron microscopical structure of the grenz zone shows that the formation of the subepidermal grenz zone cannot be explained by any particular pathogenetic principle on which therapy can be based.

RESUMEN

Aunque la zona epidérmica de grenz es realmente libre de celulas tipicas de lepra con lysosomas gigantescos, no es libre del bacilo de la lepra—estos se encuentran en los macrofugos y fibroblastos poco infestados. Por esa razon seria mucho mas claro si simplemente le llamaramos a esta region la zona subepidérmica grenz de lepra lepromatosa. La zona epidérmica de grenz no es patognomónica de la lepra puesto que esta zona también puede encontrarse en otras enfermedades. Mientras que la zona de grenz es típica en lepra lepromatosa, no es necesariamente característica de las formas dimorfa o tuberculoide de la enfermedad. La estructura microscópica de la zona de grenz no sugiere que su formación pueda explicarse por algún principio patogénico derivado de la terapia.

RÉSUMÉ

Bien que la zone limitrophe (grenz zone) soit en effet libérée de cellules typique de la lèpre avec de lysosomes géant, ce n'est pas sans de bacilles de la lèpre—celles-ci sont un peu infestées de les macrophages et fibroblasts. Pour cette raison, il serait plus clair si nous appelions cette region la zone limitrophe sub-épidermique de la lèpre lépromateuse. La zone limitrophe (grenz zone) sub-épidermique n'est pas pathognomique de la lèpre, car des zones limitrophes peuvent être observées également dans d'autres maladies. Alors que la zone limitrophe est typique dans la lèpre lépromateuse, elle n'est pas nécessairement caractéristique dans les formes dimorphe ou tuberculoide. La structure de cette zone limitrophe, telle qu'elle est observée au microscope optique ou au microscope électronique, montre que la formation d'une zone limitrophe sous-épidermique ne peut pas être expliquée sur la base d'un mécanisme pathogène particulier, qui pourrait servir à décider la thérapeutique.

Acknowledgments. The authors wish to thank Mrs. A. Schneider and Mrs. R. Speck for technical assistance.

U. Martens has contributed essential parts of his dissertation for the degree of Doctor of Medicine for this article.

REFERENCES

1. Fite, G. L., Cambre, P. J. and Turner, M. H. Procedure for demonstrating lepra bacilli in paraffin sections. Arch. Pathol. **43** (1947) 616–624.
2. Fleischmajer, F., Gay, S. T., Perlish, J. S. and Cesarini, J. P. Immunoelectron microscopy of type III collagen in normal and scleroderma skin. J. Invest. Dermatol. **75** (1980) 189–191.
3. Junqueira, L. C. U., Bignolas, G. and Brentani, R. R. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem. J. **11** (1979) 447–455.
4. Meigel, W. N., Gay, S. T. and Weber, L. Dermal architecture and collagen type distribution. Arch. Dermatol. Res. **259** (1977) 1–10.
5. Sabin, T. D. and Ebner, J. D. Patterns of sensory loss in lepromatous leprosy. Int. J. Lepr. **37** (1969) 239–248.
6. Shepard, C. C. Temperature optimum of *Mycobacterium leprae* in mice. J. Bacteriol. **90** (1965) 1271–1275.
7. Unna, P. G. Lepra. In: *Histopathologie der Hautkrankheiten. Lehrbuch der speziellen pathologischen Anatomie.* Vol. 8, chapter 2. Berlin: Verlag v. August Hirschwald, 1894, pp. 603–617.
8. Wang, H. Y. and Huang, H. S. Study on viability of *Mycobacterium leprae*: Effect of ultraviolet rays and sunlight. Chin. J. Dermatol. **13** (1980) 85–87, quoted in Int. J. Lepr. **48** (1980) 475.