Tissue Distribution of *Treponema pallidum* in Primary and Secondary Syphilis: An Ultrastructural Study

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Background

In recent years, an increase in the prevalence of syphilis has been observed. Identification of the causative organism is nowadays more efficient with the advent of highly specific and sensitive antibodies. There are limited reports on the ultrastructure of skin lesions caused by *Treponema pallidum*. The aim of the present study has been to investigate the tissue distribution of the microorganisms in both the primary cutaneous infection and the secondary lesions.

Design

One case each of primary syphilitic chancre and secondary syphilis were included in the initial study. Subsequently, two additional cases of chancre have been added. Paraffin sections of all cases were stained with prediluted anti-*Treponema pallidum* antibody (BioCare Medical, Concord, CA). Tissue for electron microscopy was retrieved from the paraffin bloc with a tissue microarray needle (1mm). The samples were deparaffinized, fixed in osmium tetroxide and embedded in epoxy resin. Thin sections were examined under a Philips CM100 electron microscope.

Results

By light microscopy both lesions showed characteristic features: more prominent epidermal abnormalities, consisting mostly of ulceration and degeneration in the primary chancre, and a more diffuse, perivascular inflammatory infiltrate in the secondary lesion. Electron microscopy showed paradoxical results. Thus, in the chancre lesions spirochetes were located more often in the blood vessel walls and dermal tissue than in the epidermis. In secondary syphilis, they were more abundant between epidermal keratinocytes, although some could also be found in the vessel walls, amidst pericytes and endothelial cells. In all cases, they adjusted themselves to the intercellular spaces between adjacent cells. A remarkable feature was an electronlucid space around the microorganisms that allowed to identify them more easily. This could be due to a retraction artifact or represent an indirect sign of the existence of surface mucopolysaccharides. No definitive morphologic evidence of the completely intracellular location of the microorganisms was found. However, in keratinocytes and other cells many images of deep cytoplasmic invaginations that seemed to engulf the spirochetes were observed. Immunohistochemistry showed the same tissue distribution of spirochetes found by electron microscopy, revealing abundant perivascular treponema clusters in the chancre lesions, and obvious intraepidermal microorganisms in the secondary lesion.

Conclusions

Ultrastructural and immunohistochemical examination of primary and secondary syphilis lesions shows a paradoxical distribution of the causative microorganisms, as they tend to be more abundant in the blood vessels of primary chancre and in the epidermis of secondary lesions. A possible explanation for this pattern could be that in primary infection treponemes invading through the epidermis tend to concentrate around blood vessels, whereas in secondary syphilis they circulate along the bloodstream, cross the vessel walls and seem to be attracted to the epidermis. The close relationship with pericytes and epithelial cells suggests that pathogenic mechanisms similar to intestinal spirochetosis may be involved in luetic lesions.

References

1. Dettori G; Amalfitano G; Polonelli L; Rossi A; Grillo R; Plaisant P. Electron microscopy studies of human intestinal spirochetes. *Eur J Epidemiol* 1987; 3: 187-195.
2. Engelkens HJ; Vuzevski VD, ten Kate FJ, van der Heul P; van der Sluis JJ; Stolz E. Ultrastructural aspects of infection with *Treponema pallidum* subspecies pertenue (Pariaman strain). *Genitourin Med* 1991; 67: 403-407.
3. Fitzgerald TJ; Cleveland P; Johson RC; Miller JN; Sykes JA. Scanning electron microscopy of *Treponema pallidum* (Nichols strain) attached to cultured mammalian cells. *Journal of bacteriol* 1977; 130: 1333-1344.
4. Hayes NS; Muse KE; Collier AM; Baseman JB. Parasitism by virulent *Treponema pallidum* of host cell surfaces. *Infect and Immunity* 1977; 17: 174-186.
5. Konishi H; Yoshii Z; Cox DL. Electron microscopy of *Treponema pallidum* (Nichols) cultivated in tissue cultures of Sf1Ep cells. *Infect and Immunol* 1986; 53: 32-37.
6. Penn CW; Rhodes JG. Surface-associated antigens of *Treponema pallidum* concealed by an inert outer layer. *Immunol* 1982; 49: 9-16.
7. Poulsen A; Kobayasi T; Secher L; Weismann K. *Treponema pallidum* in macular and papular secondary syphilitic skin eruptions. *Acta Derm Venereol* 1986; 66: 251-258.
8. Poulsen A; Kobayasi T; Secher L; Weismann K. *Treponema pallidum* in human chancre tissue: an electron microscopic study. *Acta Derm Venereol* 1986; 66: 423-430.
9. Poulsen A; Kobayasi T; Secher L; Weismann K. Ultrastructural changes of *Treponema pallidum* isolated from secondary syphilitic skin lesions. *Acta Derm Venereol* 1987; 67: 289-294.
10. Wrzolkowa T; Kozakiewicz J. Ultrastructure of vascular and connective tissue changes in primary syphilis. *Br J Vener Dis* 1980; 56: 137-143.

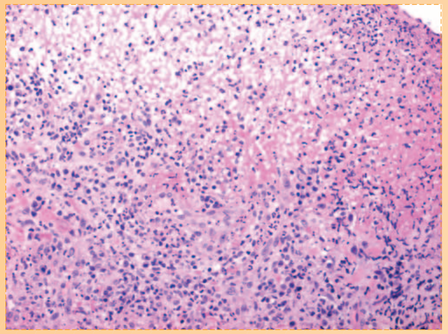

Figure 1. Chancre: epidermal ulceration and necrosis with acute inflammation. Prominent dermal mixed inflammatory infiltrate and vascular proliferation.

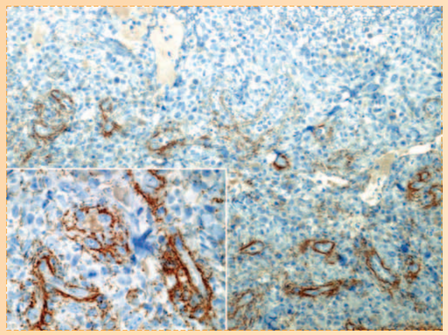

Figure 2. Chancre: anti-*Treponema pallidum* staining shows treponemes predominantly arranged around blood vessels.

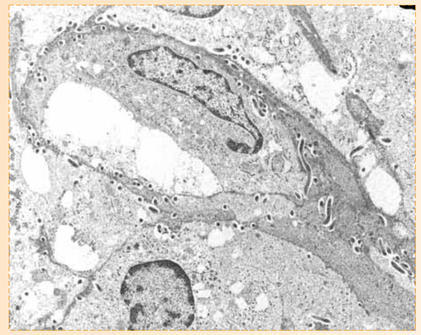

Figure 3. Chancre: capillary with multiple spirochetes attached to the membrane surfaces of endothelial cells and pericytes, apparently entering their cytoplasm.

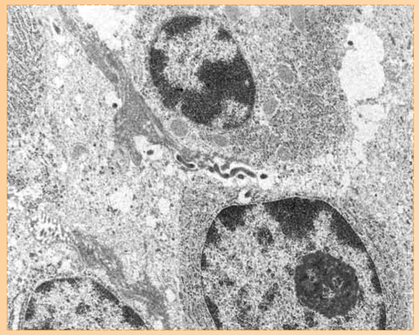

Figure 4. Chancre: view of dermal infiltrate with microorganisms located between two plasma cells.

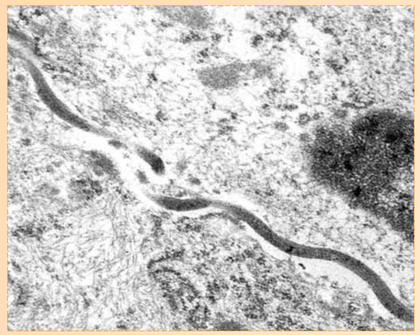

Figure 5. Chancre: detail of two spirochetes. Note the distinctive long, curved morphology and an electronlucid space surrounding the microorganisms.

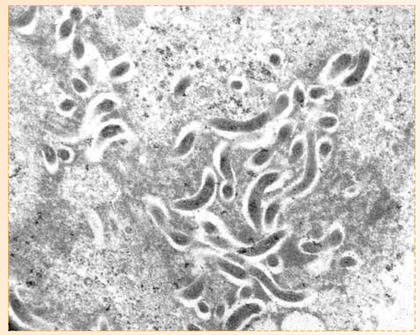

Figure 6. Chancre: many spirochetes forming a dermal cluster.

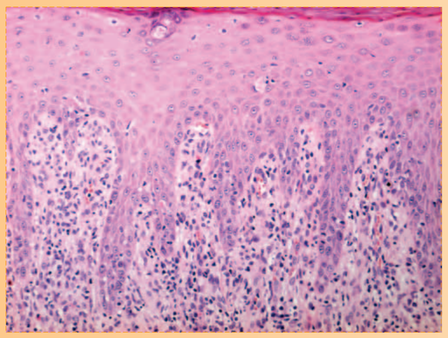

Figure 7. Secondary syphilis: epidermal acanthosis and marked dermal chronic inflammatory infiltrate, focally eroding the dermo-epidermal junction.

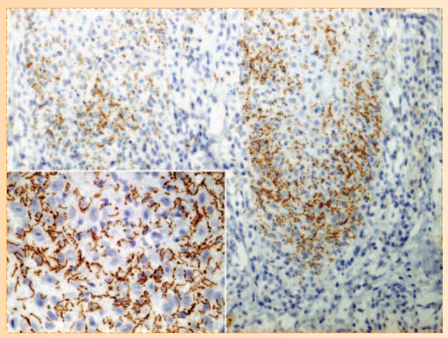

Figure 8. Secondary syphilis: immunohistochemical staining shows treponemes located predominantly but not exclusively within the epidermis.

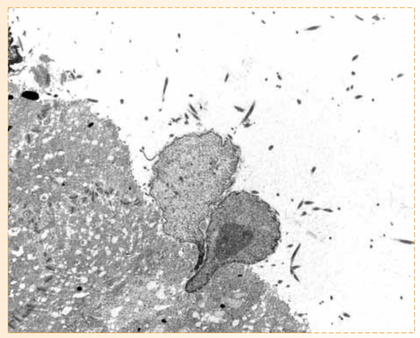

Figure 9. Secondary syphilis: intraepidermal blister containing many free spirochetes.

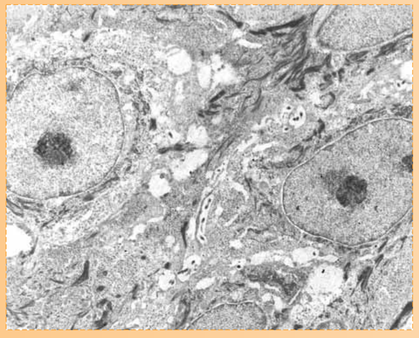

Figure 10. Secondary syphilis: the microorganisms gently adjust themselves to the intercellular spaces between keratinocytes.

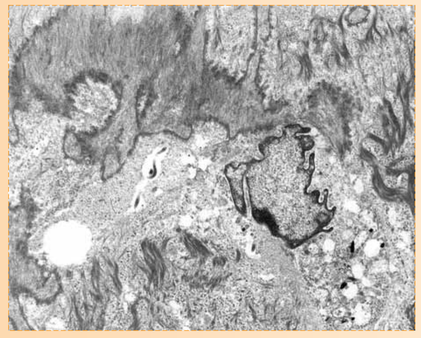

Figure 11. Secondary syphilis: upper epidermal layers showing some spirochetes that are apparently engulfed in the cytoplasm of keratinocytes.

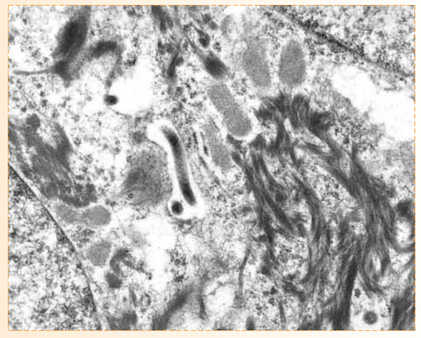

Figure 12. Secondary syphilis: close-up view of two keratinocytes and treponemes in the intercellular spaces between them

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