Treponema Pallidum in Leukoderma Syphiliticum Demonstrated by Electron Microscopy

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Punch biopsies from syphilitic leukoderma lesions and from unaffected skin in 3 patients with secondary syphilis were studies in the transmission electron microscope. In one of the patients the pigment disorder was not preceded by any visible syphilids, and in the biopsy from the leukodermal skin in this patient Treponemata pallida were demonstrated around vessels and inside nerve fibres in which the myelin sheaths of the axons showed evidence of degeneration. In the other 2 patients the depigmented areas appeared while macular and papular syphilitic lesions were healing. In the biopsies from the leukodermal lesions of these 2 patients and from unaffected skin of all 3 patients, no treponemes were demonstrated. The study indicates that syphilitic leukoderma is not invariably a post-inflammatory phenomenon, but the pigmented skin lesions may themselves represent stigmata of an active syphilitic infection. *Key words: Nerve degeneration.*

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Leukoderma in the secondary stage of syphilis appears typically 3 to 6 months after infection. The depigmented lesions are round or oval, measuring a diameter of 1 to 2 cm. They are found predominantly around the neck ('necklace of Venus') but may involve the rest of the trunk (1), limbs, palms and soles (2). Leukoderma syphiliticum heals within a year or two. The changes are considered to develop mainly when macular or papular secondary syphilitic skin lesions are fading (3, 4). However, leukoderma syphiliticum may also occur without preceding rash (5). Hitherto, in contrast to other secondary syphilitic lesions such as roseola, papules and condylomata lata, Treponema pallidum has not been demonstrated in leukoderma.

PATIENTS AND METHODS

Patients

Case 1. A 27-year-old man attended the VD clinic because of warty lesions in the perianal area and small penile ulcers persisting for 6 to 8 weeks. During this period, he had also noted whitish, painless, non-itching spots on his trunk. The white spots had not been preceded by any skin rash, he stated. Clinical examination revealed condylomata lata in the perianal region and, in addition, eroded papules on the penis from which Treponema pallidum was demonstrated by darkfield microscopy. On the trunk and extremities, numerous non-pigmented oval-shaped spots 1 to 2 cm in diameter with a peripheral reticular hyperpigmentation were observed (Fig. 1). The Wassermann reaction (WR) in peripheral blood was 10, Treponema pallidum immobilization (TPI) test 3+, and absorbed fluorescent treponemal antibody (FTA-ABS) test 4+. A skin biopsy was taken from a leukoderma lesion on the abdomen.

Case 2. A 21-year-old man was referred because of multiple ulcers on his glans penis persisting for 6 to 8 weeks. One month earlier he had observed dispersed pink, non-itching spots on his trunk. The lesions had gradually cleared, leaving white spots. On clinical examination, eroded papules were found on the glans penis and discrete leukodermal lesions were noted on the trunk and upper

extremities. Treponema pallidum was demonstrated by darkfield microscopy of exudate from a penile papule. In blood WR was 9, TPI 3+, and FTA-ABS 4+. A biopsy was removed from a leukodermal area on his right arm.

Case 3. A 23-year-old woman was referred under the diagnosis of psoriasis. Some 7–8 months earlier she had observed non-itching red spots on her trunk and extremities. Some of the spots had gradually healed, leaving white spots in the formerly red areas. On clinical examination, annular syphilids were seen on the trunk and extremities. In addition, several leukodermal lesions were noted. Syphilis serology showed WR 8, TPI 3+, and FTA-ABS 4+. A biopsy was removed from a leukodermal lesion on her back.

In all patients, biopsies were also removed from unaffected skin on the femora.

Methods

Before biopsy the skin was cleaned with swabs containing 70% isopropyl alcohol and 0.5% chlorhexidine solution. Biopsies were taken using a 3-mm punch. Chlorethyl spray served as a surface anesthetic.

The biopsies were prepared for electron microscopy by fixation in ice-cooled 6% glutaraldehyde in 0.5 M cacodylate buffer, pH 7.3, with 7.5% sucrose. Then the specimens were osmicated and dehydrated in a series of ethanol of increasing concentrations and finally embedded in Epon 812. Ultrathin sections were placed on coated copper grids and stained with uranyl acetate and lead citrate. The grids were examined in a JEOL 100 CX electron microscope at 80 kV.

Therapy

The patients were treated with 600 000 IU aqueous procain penicillin G i.m. daily for 10 days. Therapy was followed by decreasing WR titres in all patients. One year later, the leukoderma in patient 1 had disappeared completely. In the other 2 patients, lesions were resolving during a follow-up period of 6 and 7 months.

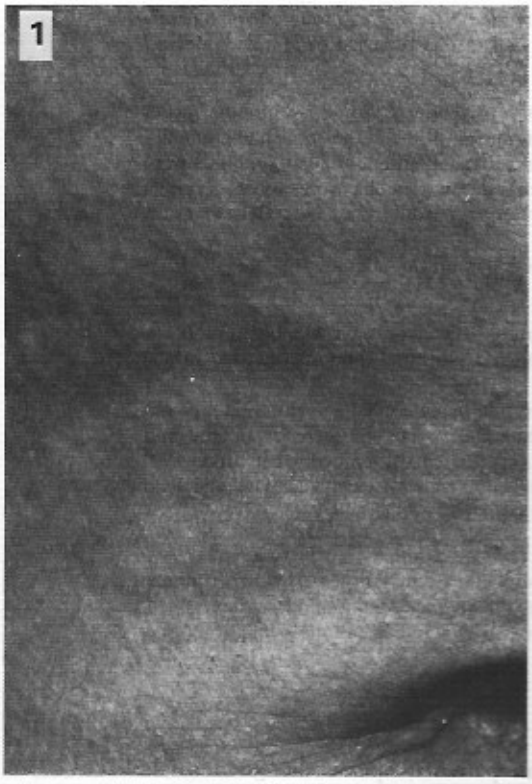
RESULTS

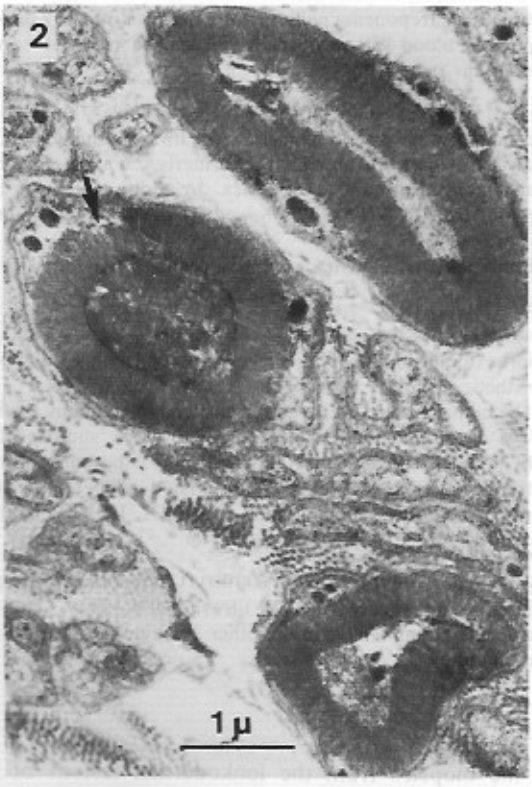
In the biopsies from the leukodermal lesions of all patients, sparse though normal-appearing melanocytes were observed in the epidermis, which, besides appeared unaffected without any treponemal structures observable. In the biopsies from lesions of patients 2 and 3 and from uninvolved skin of all patients, no evidence of inflammation and no treponemes were observed. Degenerated myelinated nerve fibres were demonstrated in the leukoderma of patient 3 (Fig. 2). In the biopsy from leukoderma of patient 1, a sparse inflammatory infiltrate consisting predominantly of plasma cells and lymphocytes was noted around the vessels in the dermal region. The endothelial cells appeared swollen. Treponeme fragments were demonstrated in each of the ultrathin sections of the dermal area. Corresponding to a visual field at 2000 magnification, 0–2 fragments were usually observed. They were demonstrated in perivascular areas (Fig. 3) and in invaginations of endothelial cells (Fig. 4) as well as inside membrane-bounded spaces in the cytoplasm of macrophages (Fig. 5). Treponemes were also demonstrated within nerve fibres, in which the myelin sheaths of the axons were fragmented and split as evidence of degeneration (Fig. 6).

DISCUSSION

The depigmented lesions in leukoderma syphiliticum are considered to be residuals of formerly active syphilitic lesions in the skin. The demonstration of Treponema pallidum in the leukodermal skin of patient 1 indicates that leukoderma may itself be a marker of an active syphilitic infection.

In syphilitic chancres (7) and in secondary syphilitic macular and papular lesions (8), Treponemata pallida have been observed inside nerve fibres close to myelinated and unmyelinated axons. The findings may explain why these syphilitic lesions are usually non-symptomatic (9). The degenerated myelinated nerve fibres and the presence of

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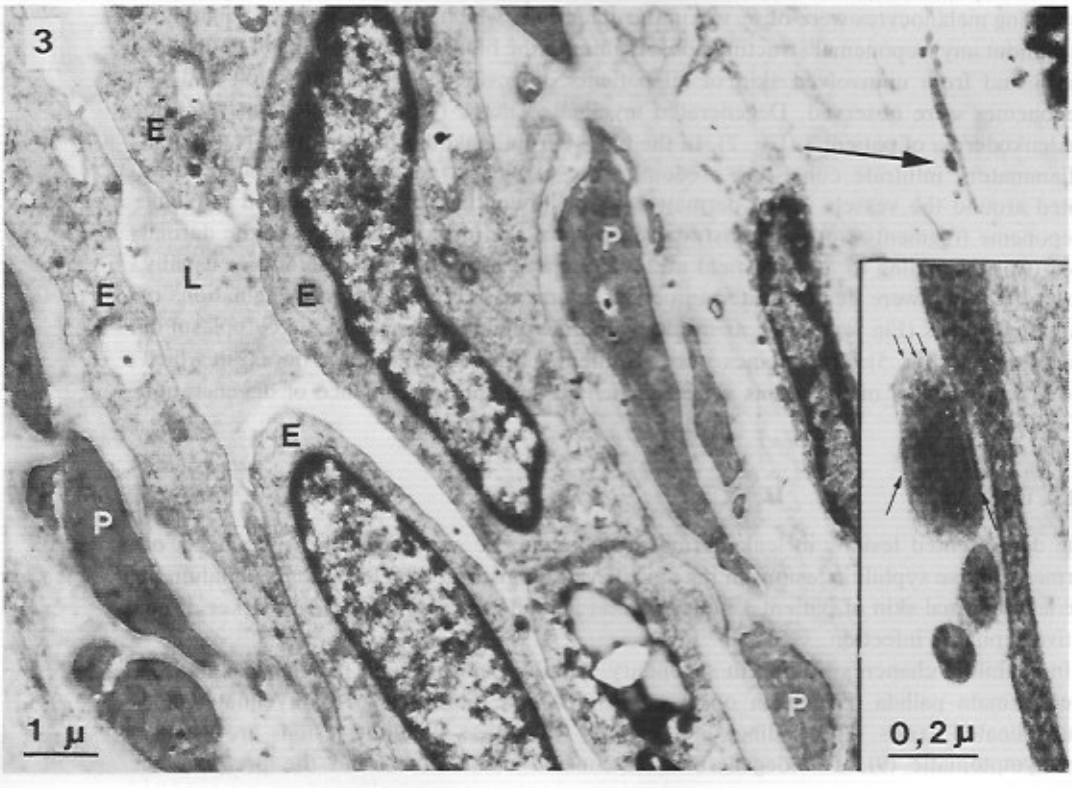
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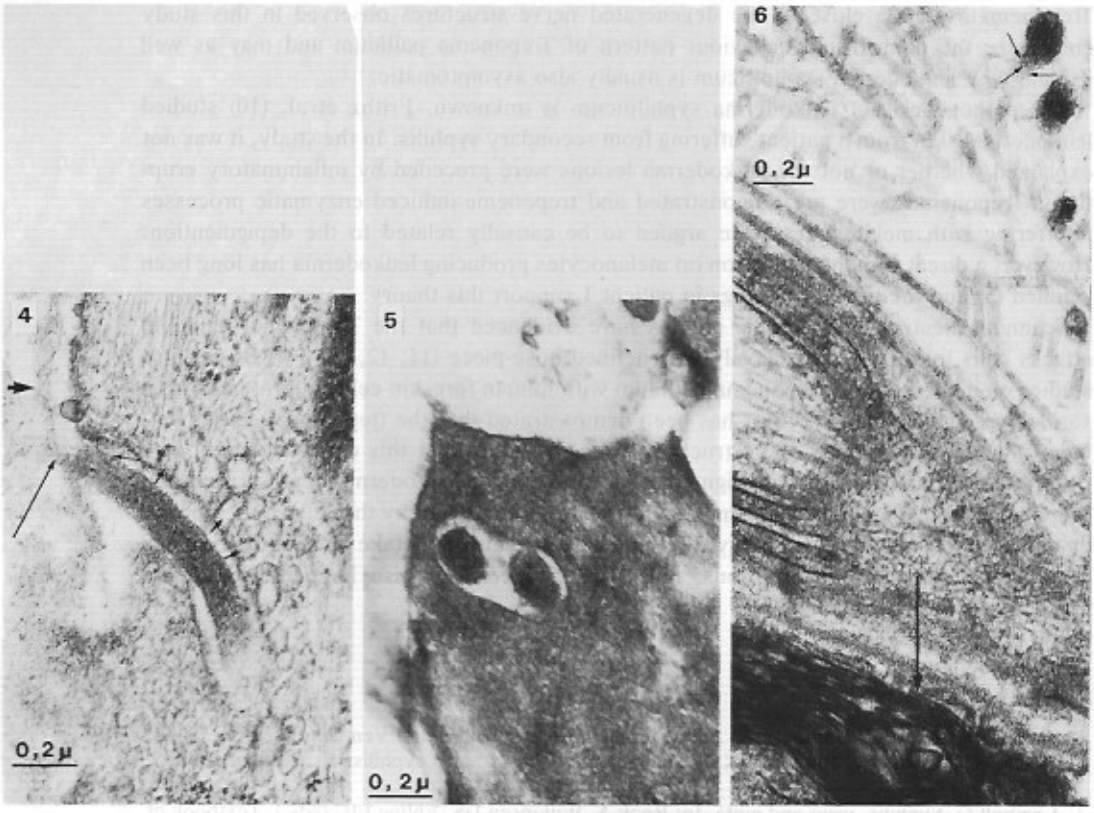

Fig. 4. Treponema pallidum in an invagination of an endothelial cell (×40 200) (patient 1). The cytoplasmic membrane is indicated by a small arrow (→). The axial filaments are fading at one end of the fragment (⟶). The basement membrane is seen (→). Pinocytotic vesicles are gathering along the invagination. The lumen of some of the vesicles (→) apparently communicates with the lumen of the invagination.

Fig. 5. Treponeme fragments inside a membrane-bounded space in the cytoplasm of a macrophage (×40 200) (patient 1). It is questionable whether the illustration represents a real intracellular position of the treponeme or a cross-sectioned treponeme containing invagination of the cell membrane of the macrophage.

Fig. 6. Cross-sectioned treponeme fragments within a nerve fibre (×40 200) (patient 1). The three partly tangentially sectioned axial filaments are indicated by small arrows (→). The long arrow indicates degenerated, lamellar and split myelin sheaths (⟶).

Fig. 1. Close-up view of syphilitic leukoderma on the abdomen of patient no. 1. Reticulated hyperpigmentations around the depigmented areas are seen. Leukoderma lesions in this patient contained treponeme fragments.

Fig. 2. Unmyelinated and myelinated nerve fibres in a leukoderma lesion of patient 3. Magnified 15 000 times. The three myelinated nerve fibres shown appear degenerated, whereas the unmyelinated nerve fibres seems utmost unaffected. The outlines of the myeline sheaths are blurred. The myelin is partly fragmented (→) and appears as a radiating striated granular substance. The radiation is probably caused by a fixation-induced shrinkage of almost totally degenerated myelinated sheaths. No treponemes were observed. The degenerations may have been induced by treponemes, which have now disappeared from the leukodermal skin.

Fig. 3. Dermal vessel (×10 000) with lumen (*L*), endothelial cells (*E*), and pericytes (*P*) (patient 1). A treponeme fragment is noted (⟶). In the inset (×60 000) the treponemal cytoplasmic membrane (⟶) and the axial filaments (→) are illustrated.

Treponemata pallida close to the degenerated nerve structures observed in this study emphasize the neurotropic behaviour pattern of Treponema pallidum and may as well explain why leukoderma syphiliticum is usually also asymptomatic.

The pathogenesis of leukoderma syphiliticum is unknown. Frithz et al. (10) studied leukodermal skin from a patient suffering from secondary syphilis. In the study, it was not explained whether or not the leukoderma lesions were preceded by inflammatory eruptions. Treponemes were not demonstrated and treponeme-induced enzymatic processes interfering with melanocytes were argued to be causally related to the depigmention. However, a direct treponemal action on melanocytes producing leukoderma has long been claimed (5) and the present findings in patient 1 support this theory.

Scanning electron microscopic studies have evidenced that the Treponema pallidum attacks cells by its morphologically well defined nose-piece (11, 12, 13, 14). By in vitro studies on incubation of Treponema pallidum with human foreskin cells and rat cells from various organ systems (15, 16) it has been demonstrated that the treponemal cell attachment induces cell damage or destruction. It seems likely that this cell attachment also occurs in vivo and that loss of pigmentation in syphilitic leukoderma could arise when Treponemata pallida attach to the melanocytes of the skin, whereby the cells are temporarily damaged or destroyed. Probably, the sequence of events may take place in roseola and in papular syphilitic skin eruptions, but it may conceivably also happen without the presence of these syphilids.

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