CONCISE COMMUNICATION

No evidence of skin infection with *Chlamydia pneumoniae* in patients with cutaneous T cell lymphoma

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Recently, *Chlamydia pneumoniae*-specific DNA and antigens were reported in the skin of patients with Mycosis fungoides (MF), the most common form of cutaneous T-cell lymphomas. In order to revalidate these data we analyzed skin sections of patients with MF for the expression of three different chlamydial antigens and *C. pneumoniae* DNA by immunohistochemistry and PCR according to previously described protocolls. Neither C. pneumoniae-specific DNA sequences nor antigens were detected in any of the skin biopses from 24 MF patients tested, suggesting that further studies are needed to establish any pathogenetic relevance of *C. pneumoniae* in MF.

Keywords Mycosis fungoides, cutaneous lymphoma, *chlamydia pneumoniae*, infection

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Chlamydia pneumoniae is a common cause of community-acquired pneumonia, pharyngitis, and bronchitis [1,3]. In addition, *C. pneumoniae* has attracted attention because of its possible association between a previous or persistent *C. pneumoniae* infection and a number of different diseases including multiple sclerosis, Alzheimer's disease, chronic fatigue syndrome and arteriosclerosis [3,4,5]. Recently, *C. pneumoniae*-specific DNA and antigens were reported in the skin of patients with mycosis fungoides (MF), the most common form of cutaneous T-cell lymphoma [6,7]. In order to revalidate these data we analyzed skin sections of patients with MF for the expression of three different chlamydial antigens and *C. pneumoniae* DNA by immunohistochemistry and PCR according to previously described protocols [4,8,9].

Formalin-fixed and paraffin-embedded tissues were collected from eight male (mean age, 58 years; range, 42–74 years) and 16 female (mean age, 67 years; range, 40–73 years) MF patients. The clinical diagnosis of MF (13 patients in patch stage, six patients in plaque stage and five patients in tumor stage) was confirmed in all cases by routine histology, immunohistology, and by identification of the dominant T-cell clone using a standard PCR technique [10]. Immunohistochemistry for the detection of *C. pneumoniae* antigens was performed on paraffin-embedded 7-μm-thick skin sections. We used an indirect avidin-biotin complex immunoperoxidase method including the following primary monoclonal antibodies that have been proven effective for immunohistochemistry [1]: a *C. pneumoniae*-specific antimajor outer membrane protein A (ompA) mAb RR402 (dilution, 1:5; DAKO, Hamburg, Germany), a genus-specific antiheat shock protein 60 mAb A57-B9 (dilution, 1:250; Dianova, Hamburg, Germany) and a species-specific antilipopolysaccharide mAb ACI (dilution, 1:1; PROGEN, Heidelberg, Germany). After deparaffinization and before staining with primary antibodies, tissue sections underwent an 800-W microwave treatment in citrate buffer. Peroxidase was visualized with AEC Substrate System (DAKO); the sections were counterstained with Mayer's hematoxylin. Tissue sections of an atherosclerotic plaque from a patient with coronary heart disease were used as positive control. Subsequently, stained sections were examined by two independent investigators using a light microscope. DNA was extracted from tissue material

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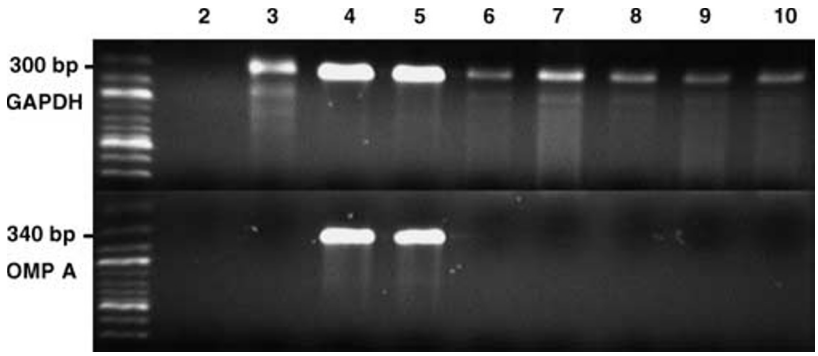

Figure 1 PCR analysis of skin lesions from representative patients with mycosis fungoides. Lane 1, molecular weight marker; lane 2, negative control (water without DNA); lane 3, normal human skin; lane 4, *C. pneumoniae*-infected Hep-2 cells; lane 5, coronary atherosclerotic plaque; lanes 6–10, skin lesions from patients with mycosis fungoides (upper part: 300-bp amplification product of the GAPDH gene; lower part: 340-bp amplification product of the ompA gene).

using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according the manufacturer's protocol. *C. pneumoniae* DNA was detected by PCR using an ompA sense (5′–CGC TTT GAT GTT TTC TGT ACT T−3′) and ompA antisense (5′–AAG CAA CGC CTT TAT AGC CC−3′) primer pair which yield a 340–bp product [11]. Each PCR step consisted of 60 cycles of 20 s at 94°C, 20 s at 56°C, and 40 s at 72°C [12]. Extracted DNA from a coronary artery plaque and genomic DNA from *C. pneumoniae*-infected Hep-2 cells were used as positive controls. In addition, amplification of the GAPDH gene was used for DNA extraction control. Amplified products were detected by using polyacrylamide gel electrophoresis, ethidium bromide staining, and UV translumination.

Neither *C. pneumoniae*-specific DNA sequences nor antigens were detected in any of the skin biopses from 24 MF patients tested. On the other hand, DNA extraction was sufficient in all cases, as indicated by successful GAPDH gene amplification, and *C. pneumoniae*-specific DNA sequences were amplified from a coronary artery plaque as well as from *C. pneumoniae*-infected Hep-2 cells as a positive control in all experiments performed [Figure 1]. The suggestion of *C. pneumoniae* infection as a risk factor for MF is as yet based on a single report and has to be reproduced by other investigators. We found no evidence that *C. pneumoniae* exists within the skin of CTCL patients and suggest that further studies are needed to establish any pathogenetic relevance of *C. pneumoniae* in MF.

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