

SHORT REPORT

Anti-*Borrelia burgdorferi* immunoglobulin seroprevalence in pulmonary sarcoidosis: a negative report

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Anti-Borrelia burgdorferi immunoglobulin seroprevalence in pulmonary sarcoidosis: a negative report. H. Martens, B. Zöllner, G. Zissel, D. Burdon, M. Schlaak, J. Müller-Quernheim. ©ERS Journals Ltd 1997.

ABSTRACT: The aetiology of sarcoidosis is still unknown. An infectious micro-organism as causal agent for this disease could not be identified, but high titres of antibodies against *Borrelia burgdorferi* were detected in Chinese studies implying a causality with this disease. These findings, however, could not be reproduced by other researchers. The aim of this study was, therefore, to evaluate the possible role of these spirochetes in the pathogenesis of sarcoidosis by serological examinations.

Sixty sera of patients suffering from sarcoidosis were examined for anti-*B. burgdorferi* immunoglobulin by enzyme-linked immunosorbent assay (ELISA). ELISAs for these antibodies show a high sensitivity, but a low specificity; therefore, a specific immunoblot was used to confirm positive results.

Initially, 8% of the patients were reactive in the ELISA, and 20% of these could be confirmed by immunoblot. Therefore, the prevalence for *B. burgdorferi* antibodies in sarcoidosis patients was 1.6%. This result did not differ significantly from the prevalence of *B. burgdorferi* antibodies in 1,000 regular blood donors of the city of Hamburg (7% reactive in the ELISA, 38% confirmed *via* immunoblot, prevalence 2.7%).

The hypothesis of causality between a *B. burgdorferi* infection and sarcoidosis cannot be confirmed by this data.

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Sarcoidosis is a systemic granulomatous disease of unknown aetiology, affecting mainly the respiratory tract and the hilar lymph nodes [1]. At present, there is discussion of the requirement for an initiating antigen for the formation of granuloma, and there seems to be a T-cell-mediated immunological response against an antigen or a group of antigens [2]. Several microbic and organic antigens have been implicated as a causal agent in sarcoidosis, but no definite pathogenic role could be demonstrated [3-7]. Mycobacterial deoxyribonucleic acid (DNA), for example, was detected using the polymerase chain reaction (PCR) in biopsies with sarcoid lesions. Other authors, however, could not confirm these results, as recently reviewed [8]. Furthermore, elevated titres of antibodies against *Mycoplasma pneumoniae* have been described [3].

The hypothesis that *Borrelia burgdorferi* could be seen as a causal infectious agent for sarcoidosis was first mentioned in 1989 in epidemiological studies [9]. High levels of antibodies were discovered in Chinese patients *via* immune fluorescence testing (IFT) (81.8% positive) [10], and enzyme-linked immunosorbent assay (ELISA) (78.3% positive) [11]. On the basis of these data, a causality between a *B. burgdorferi* infection and sarcoidosis was assumed. Other research workers could not confirm

these results [12, 13]. Up to this day, inconsistent results exist concerning the role of *B. burgdorferi* as a causal agent for sarcoidosis. The aim of this study was, therefore, to evaluate the possible role of these spirochetes in the pathogenesis of sarcoidosis, by serological tests using a specific immunoblot.

Methods

Sera of 60 patients with the diagnosis of sarcoidosis according to defined criteria were examined [1]. The enzyme immunoassay "Enzygnost Borreliosis" (Behring, Marburg, Germany) was used for the indirect detection of immunoglobulin G and M (IgG and IgM) antibodies. This ELISA has a sensitivity of 90% and a specificity of 72% [14]. This low specificity results from a high prevalence of positive antibody titres within the whole population. The discovery of antibodies does not correlate with the clinical manifestation of Lyme borreliosis. Saprophytic spirochetes and other bacteria having common antigenicity with *B. burgdorferi* may be responsible for these high antibody titres [15]. Additionally, cross-reactions with the bacteria responsible for the endemic recurrent fever have been described [16]. To

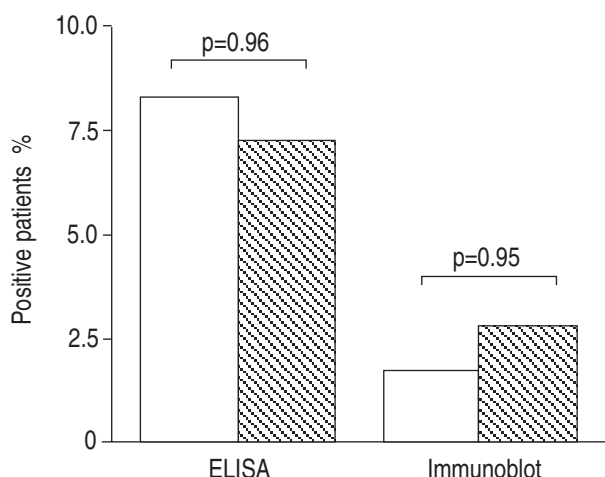


Fig. 1. — Seroprevalence of anti-*Borrelia burgdorferi* immunoglobulin in the study populations tested by enzyme-linked immunosorbent assay (ELISA) and immunoblot. □ : sarcoidosis patients (n=60); ▨ : blood donors (n=1,000).

confirm positive results obtained via ELISA, a specific immunoblot was used. With this test, the specificity for IgG could be increased to 95%, and for IgM antibodies to 100% [17]. *Borrelia* antigens were obtained from *B. garinii* bread in Kelly medium, and applied to a 10% sodium dodecyl sulphate (SDS) gel charged with 150 V. Subsequently, a Western blot on nitrocellulose was performed, and then incubated with sera from the patients [17]. One thousand regular blood donors of the University Hospital Eppendorf, Hamburg, tested using the same methods, served as the control population [18].

Statistical analysis

Chi-squared test was used to search for significant differences between the study groups. The sample size chosen was big enough to yield a positive result if the seroprevalence in the sarcoidosis population was at least doubled.

Results

In the ELISA, initially 8% of the patients were reactive (four patients IgG-positive, one patient IgM-positive), and 20% of these could be confirmed by immunoblot (IgG-positive). Consequently, the prevalence of *B. burgdorferi* antibodies in patients with sarcoidosis amounted to 1.6%. Three patients showed a nonspecific polyclonal IgM reactivity in the immunoblot. These results do not differ in a statistically significant manner ($p=0.95$) to the prevalence of *B. burgdorferi* antibodies within the control population (7% reactive in the ELISA, 38% confirmed by immunoblot, prevalence 2.7%) (fig. 1).

Discussion

When testing for anti-*B. burgdorferi* immunoglobulin, both the IFT and the ELISA produce false-positive results not only in healthy persons, due to saprophytic

spirochetes and other bacteria with common antigenicity to *B. burgdorferi* inducing cross-reacting antibodies, but also in patients with autoimmune diseases, infectious mononucleosis, endemic recurrent fever, and other diseases caused by spirochetes [16, 19–22]. Confirmation of positive test results by other methods (immunoblot, isolation and culturing of *B. burgdorferi*, or DNA detection by PCR) should, therefore, be obtained and is generally recommended [17, 23]. Data gained without a confirmatory test are only of limited value in interpretation. Conclusions concerning the pathogenic significance of *B. burgdorferi* should only be based on data utilizing a confirmatory test, as performed in the present study.

Assuming that at least a doubling of the anti-*B. burgdorferi* seroprevalence in a sarcoidosis population is of immunopathogenetic relevance, the sample size of our study and control populations were large enough to ascertain such a difference, with a probability of error <0.05 . The fact that a significant elevation could not be found argues against the hypothesis of a causal connection between a *B. burgdorferi* infection and sarcoidosis. Methodological factors, such as washing mistakes, wrong conjugate dosage, or nonspecific reactions due to microbe contamination, could explain the high prevalence of *B. burgdorferi* antibodies in sarcoidosis patients in the other studies [10, 11]. Additionally, 15 patients out of 33 were recruited from rural regions, with a possible endemic *Borrelia* infection. Information concerning the origin of the control population was not mentioned [10]. A study from Italy examining patients from a *B. burgdorferi* endemic area failed to identify anti-*B. burgdorferi* immunoglobulin in 21 patients with sarcoidosis [13].

The fact that we and others could not find an association between *B. burgdorferi* seroprevalence and sarcoidosis does not necessarily mean that the associations published previously [10, 11] are false, ultimately due to methodical pitfalls. A protective immunity against *B. burgdorferi* is mediated by type 2 T-helper (Th-2) lymphocytes, which preferentially express interleukin-4 and interleukin-5 [24]. Thus, in areas with an elevated *B. burgdorferi* prevalence, a high Th-2 and a low type 1 T-helper (Th-1) lymphocyte activity has to be assumed in the general population. This might establish a predisposing factor for the acquisition of sarcoidosis, leading to the association published in China. In Germany, however, with a *B. burgdorferi* seroprevalence of about 2.0% [18], such an association cannot be expected and was not found.

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