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CLINICAL INVESTIGATION

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Seroprevalence of anti-*Borrelia* antibodies among patients with confirmed sarcoidosis in a region of Japan where Lyme borreliosis is endemic

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Abstract ● Background: Sarcoidosis is a multisystemic granulomatous disease of unknown etiology, while Lyme borreliosis is a multisystemic disorder caused by *Borrelia burgdorferi*. The purpose of this study is to evaluate the relationship between sarcoidosis and Lyme borreliosis in a region of Japan where Lyme borreliosis is endemic. ● Methods: We determined the seroprevalence of anti-*Borrelia burgdorferi* antibodies as well as antibodies three Japanese *Borrelia* strains by enzyme-linked immunosorbent assay and dotblot assay using purified *Borrelia*-specific proteins in 46 patients with confirmed sarcoidosis and 150 controls (50 disease controls and 100 healthy controls) in Hokkaido, the affected region. ● Results: Fifteen patients with sarcoidosis (32.6%) tested positive for *Borrelia* spirochete in both assays, compared with two disease controls (4.0%) and two healthy controls (2.0%). The seroprevalence of anti-*Borrelia* antibodies in patients with sarcoidosis was much higher in the affected region than in the region in our previous study where Lyme borreliosis is non-endemic.
● Conclusion: In a region where Lyme borreliosis is endemic, *Borrelia* infection may be partially associated with sarcoidosis.

Introduction

Lyme disease, a tick-borne infectious disease caused by *Borrelia burgdorferi* [3, 7], is now recognized as a cause of ocular diseases [4, 5, 9, 18, 19, 23–25, 27, 28, 30, 31]. It is a multisystemic disorder including cutaneous, cardiac, arthritic and neurologic complications [2, 29]. Although ocular involvement may affect every part of the eyeball and orbit [4, 5, 9, 18, 19, 23–25, 27, 28, 30, 31], ocular manifestations, except for conjunctivitis, are uncommon even in Europe and North America [29]. In Japan, there have been fewer than 100 confirmed cases of Lyme disease since the first one was reported in 1987 [1]. There have been no reports of Japanese patients with ocular Lyme disease. Recently, a serological survey using Japanese species of *Borrelia* spirochete revealed the presence of anti-*Borrelia* antibodies in patients with uvei-

tis of unknown etiology in Hokkaido, the northern part of Japan [15, 17]. Hokkaido is known to be a region where Lyme borreliosis is endemic since the seropositivity rate in animals is high [14, 16].

Sarcoidosis is a systemic granulomatous disorder which affects the lungs, lymph nodes, skin, central and peripheral nerves, and heart. Although the major ocular manifestation is panuveitis, ocular involvement may be widespread from the conjunctiva to the optic nerve and orbit. Clinical manifestations of sarcoidosis, including ocular involvement, may share certain features with those seen in Lyme disease [2, 13]. In our preliminary study, we examined the seroprevalence of anti-*Borrelia* antibodies in patients with sarcoidosis in a region of Japan where the disease is not endemic and found it to be low [13]. In order to reevaluate the relationship between sarcoidosis and Lyme disease, the present study examined the seroprevalence of anti-*Borrelia* antibodies in patients with sarcoidosis in Hokkaido, the region where Lyme borreliosis is endemic.

Patients and methods

Serum specimens from 46 patients with histopathologically confirmed sarcoidosis living in Hokkaido were examined. Twenty-nine of the patients were women and 17 were men. Thirty-two patients had intraocular lesions: granulomatous iridocyclitis including trabecular or iris nodules and tent-like peripheral anterior synechiae (PAS), vitreous opacities taking the form of snowballs or string of pearls, retinal periphlebitis, and/or retinal exudates or hemorrhages. One hundred unrelated Japanese individuals living in Hokkaido who had no history of Lyme disease served as healthy controls. As disease controls, 20 patients from Hokkaido with conjunctivitis (10 with allergic conjunctivitis and 10 with adenovirus conjunctivitis showing adenovirus types 3, 4, 8, 19 and 37) and 30 with Behçet's disease were examined. The diagnosis of Behçet's disease was based on criteria proposed by the Behçet's Disease Research Committee of Japan [22].

Three Japanese *Borrelia* strains, *B. japonica*, *B. garinii* and *B. afzelii*, isolated from the midgut of ticks of the genus *Ixodes* in Hokkaido were used in the present study, in addition to *B. burgdorferi* (American strain, B31). At the first screening, enzyme-linked immunosorbent assay (ELISA) was carried out as described by Isogai et al. [15] to detect IgG and IgM antibodies in 100 µl of serum samples from the patients with sarcoidosis, the disease controls and healthy controls. In consideration of the positive controls used by Isogai et al. [15], the cut-off value used in the present study was 0.6 OD (optical density), the lowest possible value by which to diagnose an illness as being Lyme disease.

At the second screening, sera with positive ELISA results were also examined to confirm the presence of anti-*Borrelia* antibodies by means of dot-blot analysis using a DOTBLOT BORRELIA Kit (GenBio, San Diego, Calif., U.S.A.) as described in our previous paper [13]. A high-molecular-weight protein (HMW), flagellin protein, a 39-kDa protein (P39) and outer surface protein (Osp C) were the purified *Borrelia* specific antigens to be detected in these sera. The presence of dots against P39 and/or Osp C in addition to HMW and flagellin protein is strongly indicative of *Borrelia* infection. Analysis was based on the presence (positive dot-blot), absence (negative dot-blot) or weak presence (weakly reactive dot-blot) of dots against each *Borrelia*-specific protein with reference to a positive control sample.

The significance of the positivity of anti-*Borrelia* antibodies between the patients, the disease controls, and the healthy controls was tested by *p*-value test after chi-square analysis with Yates' correction. Relative risk (RR) was calculated from the cross-product ratio of the entries in the 2 × 2 table.

Results

The results of ELISA for anti-*Borrelia* antibodies among the patients with sarcoidosis, disease controls and healthy controls are shown in Table 1. Either an IgG or IgM antibody against *B. japonica* was detected in eight of the 46 patients with sarcoidosis (17.4%), two of the 50 disease controls (4.0%), and one of the 100 healthy controls (1.0%). Either an IgG or IgM antibody against *B. garinii* was detected in 12 of the patients with sarcoidosis (26.1%), one of the disease controls (2.0%) and one of the healthy controls (1.0%). Either an IgG or IgM antibody against *B. afzelii* was detected in 12 of the patients with sarcoidosis (26.1%) and three of the disease controls (6.0%). Either an IgG or IgM antibody to *B. burgdorferi* was detected in four of the patients with sarcoidosis

Table 1 Antibodies against *Borrelia* species in patients with sarcoidosis, disease controls and healthy controls by ELISA assay

Group	No. of patients	No. (%) of ELISA-positive[a] individuals							
		B. japonica		*B. garinii*		*B. afzelii*		*B. burgdorferi*	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Sarcoidosis	46	5 (10.9%)	3 (6.5%)	3 (6.5%)	9 (19.6%)	8 (17.4%)	9 (19.6%)	0 (0%)	4 (8.7%)
Disease controls	50	0 (0%)	2 (4.0%)	0 (0%)	1 (2.0%)	1 (2.0%)	2 (4.0%)	0 (0%)	0 (0%)
Allergic conjunctivitis	10	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adenovirus conjunctivitis	10	0 (0%)	0 (0%)	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Behçet's disease	30	0 (0%)	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Healthy controls	100	1 (1.0%)	0 (0%)	1 (1.0%)	0 (0%)	ND[b]	ND	ND	ND

[a] Cut-off value: 0.60 OD (optical density)
[b] ND: not done

Table 2 ELISA assay for *Borrelia* species in patients with sarcoidosis, disease controls and healthy controls

Assay result	Sarcoidosis (n=46)	Disease controls (n=50)	Healthy controls (n=100)
Positive IgG and positive IgM	5 (10.9%)	0 (0%)	0 (0%)
Positive IgG but negative IgM	3 (6.5%)	1 (2.0%)	2 (2.0%)
Negative IgG but positive IgM	11 (23.9%)	4 (8.0%)	0 (0%)
Positive IgG or IgM	19 (41.3%)[a, b]	5 (10.0%)	2 (2.0%)

[a] χ^2=10.9, p<0.001, RR=6.3 (sarcoidosis vs disease controls)
[b] χ^2=36.4, p<0.00001, RR=34.5 (sarcoidosis vs healthy controls)

Table 3 ELISA and dot-blot assays for *Borrelia* species in patients with sarcoidosis, disease controls and healthy controls

Assay results	Sarcoidosis (n=46)	Disease controls (n=50)	Healthy controls (n=100)
Positive ELISA but positive dot-blot	15 (32.6%)[a, b]	2 (4.0%)	2 (2.0%)
Positive ELISA but negative dot-blot	4 (8.7%)	0 (0%)	0 (0%)
Positive ELISA but weakly reactive dot-blot	0 (0%)	3 (6.0%)	0 (0%)

[a] χ^2=11.6, p<0.001, RR=11.6 (sarcoidosis vs disease controls)
[b] χ^2=25.8, p<0.00001, RR=23.7 (sarcoidosis vs healthy controls)

Table 4 Intraocular lesions observed in 15 patients with sarcoidosis who showed seropositivity for *Borrelia* species

Lesion	n	(%)
Iridocyclitis	12/15	(80.0)
Trabecular nodules and/or PAS	11/15	(73.0)
Vitreous opacity	8/15	(53.0)
Retinal vasculitis	10/15	(67.0)
Chorioretinitis	7/15	(47.0)
Macular and/or optic nerve involvement	4/15	(27.0)
No ocular lesion	3/15	(20.0)

(8.7%) and none of the controls. As shown in Table 2, either an IgG or IgM antibody against at least one of these four *Borrelia* species was detected in 19 patients with sarcoidosis (41.3%), five of the disease controls (10.0%) and two of the healthy controls (2.0%).

Significant differences were observed in the seropositivity rates between the patients with sarcoidosis and both the healthy controls ($p < 0.00001$, RR = 34.5) and the disease controls ($p < 0.001$, RR = 6.3). Furthermore, there was a significant difference between patients with sarcoidosis living in Hokkaido and those in our previous study living in the Kanto area ($p < 0.001$, RR = 12.7, data not shown) [13].

The results of both ELISA and dotblot analysis for antibodies against *Borrelia* species in the patients with sarcoidosis, the disease controls, and the healthy controls are given in Table 3. Fifteen of the patients with sarcoidosis (32.6%) were positive according to both assays, compared to two (4.0%) of the disease controls and two healthy controls (2.0%). Three patients (6.0%) in the disease control group showed weakly reactive dot-blot. There were significant differences in seropositive rates between the patients with sarcoidosis and both the healthy controls ($p < 0.00005$, RR = 23.7) and disease controls ($p < 0.001$, RR = 11.6). Again, a significant difference was observed between the patients with sarcoidosis living in Hokkaido and those living in the Kanto area ($p < 0.002$, RR = 17.9; data not shown) [13].

The following clinical features were found in 15 patients with sarcoidosis who showed seropositivity for *Borrelia* species: Ocular involvement was seen in 12 of 15 patients (80.0%), lung involvement in nine patients (60.0%), skin involvement in three patients (20.0%), and cardiac complication and facial nerve palsy in one patient each (7.0%). None of them showed joint involvement. The intraocular findings of these 15 seropositive patients with sarcoidosis are summarized in Table 4.

Discussion

A serologic survey of 46 patients with sarcoidosis living in Hokkaido was performed to study the incidence of seroprevalence for Lyme borreliosis in a region of Japan where Lyme borreliosis is endemic. It is important to show seropositivity by both ELISA and dotblot analysis in a patient, since it is known that cross-reactive anti-flagellin is found in sera from syphilis [20], infectious mononucleosis [8] (E. Isogai et al., unpublished data), and rheumatology patients as well as in sera from serologically positive *Helicobacter pylori* and parvovirus cases, (B. Kiehl, personal communication). We demonstrated in both tests that the seroprevalence of anti-*Borrelia* antibodies in sarcoidosis patients (32.6%) was much higher than that in the healthy controls (2.0%) or disease controls (4.0%), including patients with Behçet's disease and conjunctivitis. Furthermore, the seroprevalence of anti-*Borrelia* antibodies in sarcoidosis was much higher in patients living in the region where the disease is endemic (Hokkaido) than in those liv-

ing in the region where the disease is non-endemic (Kanto area) as shown in our previous paper [13]. We can therefore speculate that the reason for the difference is that the frequency of exposure to *Borrelia* spirochete is different between patients living in the region where the disease is endemic and those in the region where it is non-endemic. If so, the disease controls as well as the healthy controls in the affected region should also show the same high seroprevalence of anti-*Borrelia* antibodies observed in patients with sarcoidosis in the region where the disease is endemic. But high seroprevalence was observed only in the patients with sarcoidosis, not in the disease or healthy controls, suggesting the result cannot be explained only by regional characterization.

On the other hand, *Borrelia burgdorferi* was not a causative agent in Europe of either uveitis with an established diagnosis or unclassified uveitis [6, 26]. Therefore the possibility can not be denied that the seroprevalence of *Borrelia* species depends on the geographic environment and the method of detection [6, 15, 17, 26].

None of the 15 seropositive patients with sarcoidosis could recall a tick bite, erythema chronicum migrans or arthralgia. None of them had been diagnosed as having Lyme disease, although clinical findings of Lyme disease, including ocular manifestations such as granulomatous iridocyclitis, intermediate uveitis, vitritis and retinal vasculitis, share certain features with those seen in sarcoidosis [13]. There was no difference in the intraocular lesions observed in the seropositive patients (as shown in Table 4) and in the seronegative patients (data not shown).

The association of sarcoidosis and seropositivity for *Borrelia* species can be explained by the following hypotheses: (1) the seropositive cases are ones in which sarcoidosis is complicated by Lyme borreliosis; (2) the *Borrelia* spirochete may be one of the causative agents of sarcoidosis; (3) there might be cross-reactivity between the *Borrelia* spirochete and causative agent(s) of sarcoidosis (molecular mimicry); (4) antibodies produced to counteract the 60 kDa heat shock protein (HSP60) of *Borrelia* spirochete may be cross-reactive with human HSP [10], which would cause sarcoidosis; (5) some other strain of *Borrelia* spirochete may be a common causative agent of both Lyme disease and sarcoidosis. It is still unclear whether there are one or more causative agents of sarcoidosis, or whether the pathogenesis of the development of sarcoidosis is a one-step or multi-step immunologic phenomenon. If we hypothesize that the *Borrelia* spirochete is one of the causative agents of sarcoidosis, the fact that the rate of sarcoidosis in Hokkaido is higher than in other areas in Japan becomes interesting. Sarcoidosis is known to be a multifactorial disease and recent studies have shown that human leukocyte antigen (HLA) is associated with sarcoidosis [11, 12, 21], as one of the genetic factors. The possibility cannot be denied that *Borrelia* spirochete may be causative of sarcoidosis only in some predisposed individuals. On the other hand, it has been reported that patients with unclassified uveitis in Hokkaido have a greater possibility of getting Lyme borreliosis [15, 17].

Although we cannot distinguish at the moment whether the development of sarcoidosis is related to direct infection by the *Borrelia* spirochete or whether it is simply the result of some immune reaction, the possibility remains that the *Borrelia* spirochete might be a trigger for the development of sarcoidosis in a region where Lyme borreliosis is endemic. It is, however, also a fact that antibody positivity alone does not demonstrate causality. Further intensive studies, such as examination of histopathological specimens taken from patients with sarcoidosis, are important for clarifying the relationship between Lyme borreliosis and sarcoidosis.

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