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Antibody response to OspC-I synthetic peptide derived from outer surface protein C of *Borrelia burgdorferi* in sera from Japanese forestry workers

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SUMMARY

The prevalence of antibodies against Lyme disease spirochaetes in serum samples from 80 forestry workers at high occupational risk of Lyme disease was surveyed by enzyme-linked immunosorbent assay (ELISA) with the OspC-I synthetic peptide. The peptide is part of the outer surface protein C (OspC) amino acid sequence located in the region conserved among *Borrelia burgdorferi sensu stricto* or *sensu lato*. Positivity for antibodies against OspC-I was observed in 25 (31·3%) of the forestry workers. Of these positive cases, 12 (15·0%) and 19 (23·8%) were positive for immunoglobulin M (IgM) and IgG antibody, respectively. Among 62 workers who were negative for IgG antibody against *B. garinii* or *B. japonica* in our previous study, 9 (14·5%) and 4 (6·5%) were positive for IgM and IgG antibody, respectively, in OspC-I ELISA. These results demonstrate for the first time that Lyme disease in forestry workers can be revealed using OspC-I ELISA. We conclude that forestry workers who show positive results for antibodies against OspC-I have very likely been exposed to Lyme disease spirochaetes, and that those who show positivity for IgM antibody against OspC-I may be in the early stage of Lyme disease.

INTRODUCTION

Lyme disease is a multisystemic illness caused by the tick-borne spirochaete, *Borrelia burgdorferi* [1]. In people at high risk of tick bites, such as forestry workers and Self-Defence Forces personnel, the prevalence of antibodies against *B. burgdorferi* is higher than in healthy individuals [2, 3]. Previously, we surveyed the prevalence of antibodies against *B. burgdorferi* B31, *B. garinii* HP3 and *B. japonica* HO14 in serum samples from 80 forestry workers in the Chichibu area of Saitama prefecture, Japan, by whole-cell enzyme-linked immunosorbent assay (wcELISA) and Western blotting (Wb) [4]. Those workers who had immunoglobulin G (IgG) antibody against *B. garinii* had frequently suffered tick bites, although no typical symptoms of Lyme disease could be confirmed. Therefore, it was suggested that these workers had previously been infected by Lyme disease spirochaetes. On the other hand, in some workers no antibodies were detectable by serodiagnosis, despite suffering tick bites. In Japan, since various species of Lyme disease spirochaetes have been isolated [5], additional investigation using other species of Lyme disease spirochaetes as serodiagnostic antigens is needed for appropriate detection of antibodies. To address this problem, we developed a serodiagnostic method using

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ELISA with a synthetic peptide, OspC-I. The OspC-I peptide is derived from outer surface protein C (OspC), which is known to be expressed in various amounts by different borrelial strains [6], and is part of the OspC amino acid sequence located in the region conserved among *B. burgdorferi sensu stricto* or *sensu lato*. Therefore, this peptide is considered to be one of the common antigenic epitopes shared by Lyme disease spirochaetes. Our previous study demonstrated that the antibody response to OspC-I was often detectable in serum samples from patients in the early stage of Lyme disease [7].

In this study, IgM and IgG antibodies against OspC-I in serum samples from forestry workers were detected for diagnosis of Lyme disease, and Lyme disease was newly found in some workers.

MATERIALS AND METHODS

Serum samples employed

Eighty serum samples were collected from forestry workers (78 men and 2 women aged 16–74 years) in the Chichibu area of Saitama prefecture, Japan (Fig. 1). These samples were analysed for the antibody response against *B. burgdorferi*, *B. garinii* and *B. japonica* by wcELISA and Wb (wcELISA/Wb) using B31, HP3 and HO14 antigens, respectively [4]. Furthermore, using a questionnaire, the forestry workers were asked whether they had suffered tick bites, and were confirmed to have no history of other spirochaetal diseases by questionnaire and serological tests. To estimate the cut-off value of the OspC-I ELISA, 30 serum samples from healthy individuals with no prior history of Lyme disease were also obtained. All the samples were stored at −80 °C until analysis.

Synthesis of peptide

The OspC-I peptide consists of 14 amino acids (ILMTLFLFISCNNS) covering positions 9–22, located in the region that is conserved among *B. burgdorferi sensu stricto* or *sensu lato* [8]. The peptide was synthesized as described previously [7], using a model 431A peptide synthesizer (Perkin-Elmer, Foster City, CA, USA) with 9-fluorenylmethyloxycarbonyl chemistry. The synthesized peptide was analysed for its composition by quantitative amino acid analysis and sequenced by automated Edman degradation with a model 477A/120A protein sequencer (Perkin–Elmer).

ELISA

The ELISA to detect antibodies against OspC-I was performed and interpreted as described previously [7, 9]. Using a peptide coating kit (Takara, Shiga, Japan), ELISA microplates were coated with 50 μl of OspC-I (10 μg/ml) in dimethylsulphoxide for 2 h at room temperature. After washing in distilled water, the plate was blocked in blocking buffer (Takara) overnight at 4 °C. Serum samples diluted in 10% Block Ace (1:100 dilution Yukizirushi, Hokkaido, Japan) were added to each well. The secondary antibodies used were horseradish peroxidase-conjugated goat anti-human IgM (1:1000 dilution, Organon Teknika, Durham, Belgium) and IgG (1:2000 dilution, Organon Teknika) in 0·01 M phosphate-buffered saline (PBS; pH 7·2) containing 2% Block Ace and 0·05% Tween 20. As a substrate, *o*-phenylenediamine (0·24 mg/ml) in 0·1 M citrate phosphate buffer (pH 5·0) containing 0·04% H_2O_2 was added. The enzymatic reaction was stopped by adding 0·1 N H_2SO_4.

The A_{492} values were measured by an ELISA reader (Sanko Junyaku, Tokyo, Japan). The cut-off was determined for each test by calculating the mean for 30 serum samples from healthy individuals plus three standard deviations. Test results were considered positive when sample sera had absorbances equal to or exceeding the cut-off.

RESULTS

Serum samples from the 80 forestry workers were analysed by ELISA with OspC-I synthetic peptides. Positive results for antibodies against OspC-I were obtained in 25 (31·3%) of the forestry workers. Of these positive cases, 12 (15·0%) and 19 (23·8%) were positive for IgM and IgG antibody, respectively, against OspC-I (Table 1). Among 18 in whom IgG antibody against *B. garinii* or *B. japonica* was detected using wcELISA/Wb in a previous study, 15 (83·3%) had IgG antibody against OspC-I. The other three showed a difference between the IgG antibody response observed by wcELISA/Wb and that observed by OspC-I ELISA. Furthermore, we confirmed that 3 (16·7%) of the above 15 individuals had IgM antibody in addition to IgG antibody against OspC-I peptide. Of the 62 workers who showed were negative

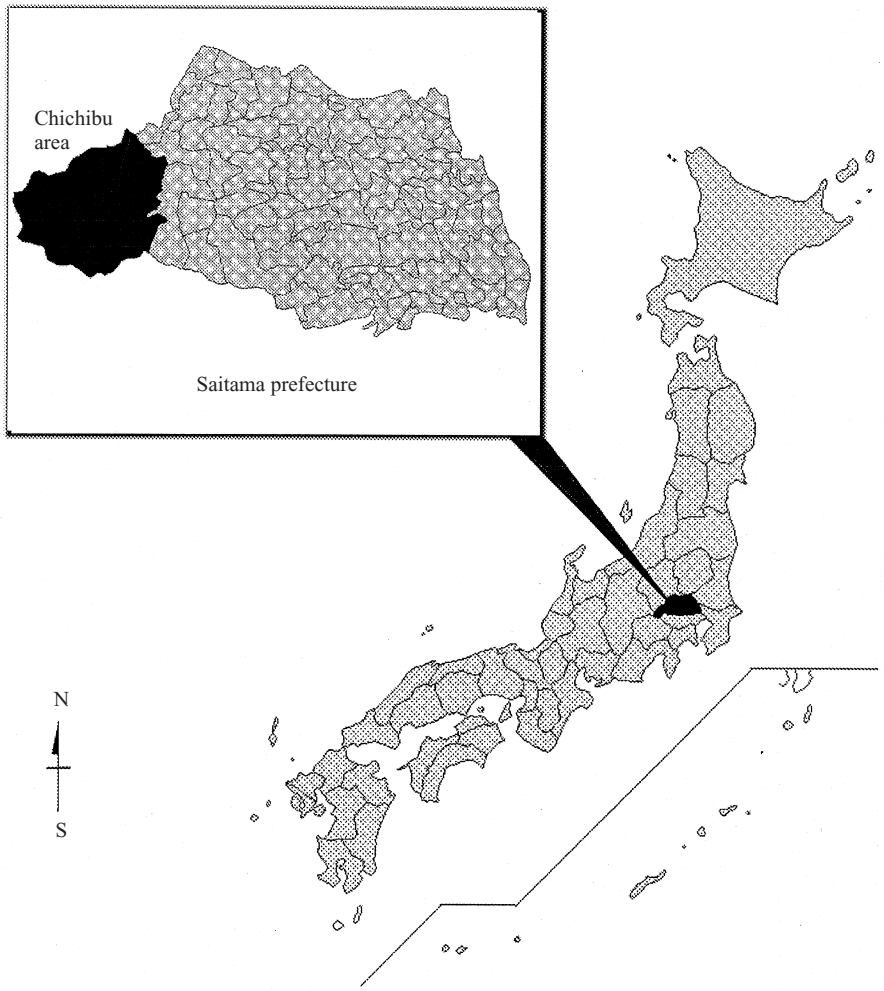

Fig. 1. Geographical location of the Chichibu area of Saitama prefecture, Japan.

Table 1. *Prevalence of antibodies against OspC-I peptide and frequency of tick bites in forestry workers*

	Total examined	Antibodies against OspC-I (%)		Antibody-positive against OspC-I/ Subjects with tick bites (%)
		IgM	IgG	
wcELISA/Wb +*	18	3 (16.7)	15 (83.3)	10/14 (71.4)
wcELISA/Wb −	62	9 (14.5)	4 (6.5)	9/10 (90.0)

* The subjects had positive (+) or negative (−) results for IgG antibody against *B. burgdorferi* B31, *B. garinii* HP3 or *B. japonica* HO14 by whole-cell ELISA (wcELISA) and Western blotting (Wb) in a previous study.

for IgG antibody against *B. garinii* or *B. japonica* in our previous study using wcELISA/Wb, 9 (14·5%) and 4 (6·5%) were positive for IgM and IgG antibody, respectively, by OspC-I ELISA, and 3 workers in these positive cases had both IgM and IgG antibody. The data of questionnaire on tick bites showed that 19 of 25 positive cases in Osp-I ELISA (76·0%) had suffered tick bites (Table 2). Of these 19 individuals, 12 (63·2%) had symptoms caused by tick bites, although they seemed to have no typical symptoms of Lyme disease. The rate of tick bites in positive cases in OspC-I ELISA was significantly higher than that in negative cases in OspC-I ELISA (76·0 *vs.* 9·1%, $P < 0{\cdot}001$). On the other hand, 14 of 18 individuals in whom IgG antibody against *B. garinii* or *B. japonica* was detected using wcELISA/Wb had suffered tick bites, and 10 of them (71·4%) gave positive results in OspC-I ELISA (Table 1). Of the 10 individuals in whom no antibody against *B. garinii* or *B. japonica* was detected using wcELISA/Wb despite suffering

Table 2 *Questionnaire survey on tick bites in positive cases in OspC-I ELISA*

	Number of answers (%)	
	Total surveyed ($n=80$)	Positive cases in OspC-I ELISA ($n=25$)
Tick bites		
Suffering	24 (30.0)	19 (76.0)
Not suffering	56 (70.0)	6 (24.0)
Symptoms caused by tick bites*		
Presence	14 (58·3)	12 (63·2)
Skin rash	7	5
Itching	3	3
Tumour	2	2
Uncertain	2	2
Absence	10 (41.7)	7 (36.8)

* The survey in cases of tick bites

tick bites, 9 (90·0 %) gave positive results in OspC-I ELISA.

DISCUSSION

The diagnosis of Lyme disease is usually based on clinical recognition of the characteristic erythema migrans accompanied by a specific antibody response to *B. burgdorferi* [1]. However, serodiagnostic confirmation of this infection is complicated by cross-reactivity with other infections [10, 11] and lack of standardization among laboratories [12]. In our previous study, the criterion for serodiagnosis was based on antibodies detectable by both ELISA and Western blotting [4]. The antigens used in this serodiagnosis system were three species of Lyme disease spirochaetes (*B. burgdorferi* B31, *B. garinii* HP3 and *B. japonica* HO14). However, since various species of Lyme disease spirochaetes have been isolated in Japan [5], additional investigation using other species of Lyme disease spirochaetes as antigens for serodiagnosis was needed for appropriate detection of antibodies. Furthermore, it was unclear whether the workers who lacked antibody against *B. garinii* or *B. japonica* using wcELISA/Wb, in spite of suffering tick bites, were really uninfected by Lyme disease spirochaetes. Therefore, we developed a serodiagnostic system using ELISA with the OspC-I peptide, which is considered to be one of the common antigenic epitopes shared by *B. burgdorferi sensu stricto* or *sensu lato*, and demonstrated that the antibody response to OspC-I was often detectable in serum samples from patients with early Lyme disease [7].

In this study, the antibody response to OspC-I of serum samples from forestry workers at high occupational risk of Lyme disease transmitted by ticks was analysed for diagnosis of the disease. Our results demonstrated that 31·3 % of the forestry workers were positive for antibodies against OspC-I. Furthermore, the rate of tick bites in the positive cases in OspC-I ELISA was significantly higher than that in the negative cases. Other earlier studies demonstrated that seroprevalence for antibodies against *B. burgdorferi* in the high risk group of Lyme disease related to exposure to tick bites [2, 13]. Our study with the OspC-I ELISA also suggested that the detection of antibodies against OspC-I related to suffering tick bites. Of the 18 in whom IgG antibody against *B. garinii* or *B. japonica* was detected using wcELISA/Wb, 15 (83·3 %) were positive for IgG antibody against OspC-I. Three cases showed a discrepancy between the IgG antibody response observed by wcELISA/Wb and that observed by OspC-I ELISA. This discrepancy may be a consequence of immunoreactivity with different epitopes from the OspC-I peptide in wcELISA/Wb. Therefore, additional serodiagnosis using other common antigenic epitopes in OspC of Lyme borrelia species should be performed for more accurate detection of IgG antibody in the above three cases. In this study, furthermore, we were able to detect antibodies against OspC-I in forestry workers who showed no antibody against *B. garinii* or *B. japonica* by wcELISA/Wb. Positivity for IgM antibody against OspC-I was seen in 90 % of workers in whom no antibody against *B. garinii* or *B. japonica* was detectable by wcELISA/Wb, despite the fact they had suffered tick bites. These results show that Lyme disease may be present in forestry workers who are apparently negative for antibody against *B. garinii* or *B. japonica* using wcELISA/Wb. Therefore, OspC-I ELISA should be able to replace wcELISA/Wb as a convenient and sensitive test for all species of Lyme disease spirochaetes.

The present results suggest that forestry workers who are positive for antibodies against OspC-I are very likely to have been exposed to Lyme disease spirochaetes. In particular, workers who are positive for IgM antibody against OspC-I may develop active disease. We recommend that forestry workers be adequately warned of Lyme disease and receive medical attention if they have tick bites and develop a subsequent rash or influenza-like illness.

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