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ScienceDirect
International Journal of Medical Microbiology 299 (2009) 439–446
IJMM
www.elsevier.de/ijmm

Antibody responses to borrelia IR_6 peptide variants and the C6 peptide in Swedish patients with erythema migrans

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Received 5 June 2008; received in revised form 7 October 2008; accepted 13 October 2008

Abstract

The aim of this study was to evaluate the antibody responses to different VlsE protein IR_6 peptide variants and the synthetic C6 peptide in acute and convalescent (2–3 and 6 months) serum samples from Swedish patients with clinical erythema migrans (EM). Serum samples were prospectively collected from 148 patients with EM and compared to serum samples obtained from 200 healthy blood donors. The IgG responses to 3 IR_6 peptide variants originating from *Borrelia burgdorferi* (*B. burgdorferi*) sensu stricto, *B. garinii*, and *B. afzelii* were measured by enzyme-linked immunosorbent assays (ELISAs) and compared to a commercial C6 peptide ELISA. Seropositivity rate in the IR_6 or C6 peptide ELISAs ranged from 32% to 58% at presentation, 30–52% after 2–3 months, and 20–36% after 6 months. At presentation, positive antibodies in any of the 4 ELISAs were found in 66%. In 7/52 (13%), C6-negative EM cases, serological reaction was found to the *B. burgdorferi* sensu stricto-derived IR_6 peptide. In patients reporting previous LB compared to those without previous LB, significantly higher seropositivity rates were noted for all IR_6 peptides, but not for the C6 peptide. In the serology of EM in Europe, C6 ELISA does not seem to cover all cases. An ELISA using a mixture of *B. burgdorferi* sensu stricto IR_6 peptide and the C6 peptide could be of value in the serodiagnosis of LB in Europe. Further studies on combinations of variant IR_6 peptides and the C6 peptide in other manifestations of LB are needed to address this issue.

Keywords: Lyme borreliosis; Erythema migrans; Serology; ELISA; IR_6 peptide; C6

Introduction

In Europe, the causative agent of Lyme borreliosis (LB) is the tick-transmitted spirochaete *Borrelia burgdorferi* (*B. burgdorferi*) sensu lato (s.l.), consisting mainly of the 3 genospecies *B. burgdorferi* sensu stricto

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doi:10.1016/j.ijmm.2008.10.006

(s.s.), *B. garinii*, and *B. afzelii*. The most common clinical manifestation of LB is the skin rash erythema migrans (EM), followed by other manifestations such as neuroborreliosis, arthritis, acrodermatitis, lymphocytoma, and carditis (Berglund et al., 1995). The clinical manifestations of LB are classified into 3 different stages (I, II, and III) according to localisation and duration of infection. EM is an early clinical manifestation of LB and, therefore, regarded as stage I, whereas other manifestations indicate disseminated LB and belong to the later stages (II and III). The diagnosis of EM is generally based on patient history and typical skin rash. Laboratory confirmation, e.g. demonstration of anti-borrelial antibodies, is not usually recommended in the acute phase of EM due to low serosensitivity (20–50%). In stages II and III of LB, serosensitivity varies from 70% to nearly 100% (Wilske, 2005). Therefore, there is a greater potential for improvement in serosensitivity in cases of EM compared to stages II–III. Furthermore, when studying the early immune response in LB, serology in patients with EM is of particular interest. Clinically an improved serosensitivity in EM can be of use in cases of atypical rash where misdiagnosis may occur (Feder and Whitaker, 1995).

Recently, a commercial ELISA using a synthetic 26-mer peptide antigen called C6 has been evaluated and introduced in the serodiagnosis of LB (Cinco and Murgia, 2006; Smismans et al., 2006; Nyman et al., 2006; Tjernberg et al., 2007; Sillanpää et al., 2007). The C6 antigen is based on the 6th invariant region (IR_6) of the variable major protein-like sequence expressed (VlsE) by *B. garinii* (Liang et al., 1999). Although a strong antibody response has been shown against the C6 peptide regardless of the infecting strain of borrelia (Liang et al., 2000), there is evidence of variation in the IR_6 amino acid sequences between *B. afzelii, B. garinii*, and *B. burgdorferi* B31 (Göttner et al., 2004). The purpose of this study was to investigate patients with EM from an endemic area of Sweden and to compare early antibody responses to the C6 peptide and three IR_6 peptides representing the three main borrelial genospecies. In addition, we wanted to study the antibody responses over time and in relation to previously reported LB in order to determine differences in antibody response dynamics between different VlsE-based antigens.

Materials and methods

Patients with LB in Kalmar County in Sweden were prospectively included in a clinical study in 2003 (Tjernberg et al., 2007). Clinical data was recorded in a study protocol, and serum samples were drawn at the first consultation (sample I), after 2–3 months (sample II), and after 6 months (sample III) (see Tables 1 and 2).

Patients with haematological malignant disease, HIV infection, immunosuppressive treatment, or ongoing antibiotic treatment were not included in the study. Of 244 included patients, 200 completed the study by fulfilling the study protocol and 158 (79%) presented with clinically defined EM >5 cm in diameter. The remaining 42 patients presented with other manifestations of LB. Of the 158 patients, a complete set of 3

Table 1. Baseline data for patients with erythema migrans ($n = 148$). Patients were also divided into sub-groups based on self-reported previous Lyme borreliosis..

	All EM ($n = 148$)	%	Previous LB ($n = 46$[a])	%	No previous LB ($n = 97$[a])	%	p-value[b]
Age median years all (range)	58 (7–84)		61 (11–84)		54 (7–83)		0.0100
Female, number	90	61	30	65	58	60	
Age median years (range)	58 (7–84)		62 (44–84)[c]		52 (7–83)		
Male, number	58	39	16	35	39	40	
Age median years (range)	56 (11–79)		56 (11–69)[c]		56 (18–79)		
Tick bite (yes/multiple/suspected/no)[d]	64/19/48/14		20/7/14/5		40/11/34/9		
Associated symptoms, number	46	31	13	28	33	34	0.5676
Duration median days (range) of EM at presentation[e]	7 (1–45)		7 (1–30)		7 (1–45)		0.7663

n, numbers.
EM, erythema migrans.
LB, Lyme borreliosis.
[a]Previous episode of LB unknown for five patients.
[b]*p*-Values represent comparisons of EM patients with or without previous reported LB.
[c]$p = 0.0120$.
[d]Unknown for three patients.
[e]Unknown for 23 patients.