Frequency and specificity of the antibodies against *Borrelia burgdorferi* tested by Western blot method in patients with symptoms of arthritis

MAŁGORZATA TOKARSKA-RODAK[1], MARIA KOZIOŁ-MONTEWKA[1], HANNA FOTA-MARKOWSKA[2], DARIUSZ BIELEC[2], ROMA MODRZEWSKA[2], ANNA PAŃCZUK[3]

[1]Department of Medical Microbiology, Skubiszewski Medical University, Lublin, Poland; [2]Department of Infectious Diseases, Skubiszewski Medical University, Lublin, Poland; [3]Institute of Health, State Higher Vocational School, Biała Podlaska, Poland

Abstract

Selected, highly specific antibodies to B. burgdorferi proteins are commonly used in diagnostic serological tests for Lyme disease. From the point of view of diagnosing Borrelia burgdorferi infection, highly immunogenic antibodies to some proteins detected in vivo after B. burgdorferi transmission to the host human body seem to be very significant.

Knowledge on importance of proteins that are expressed in vivo alone has accumulated recently. The proteins are: VlsE, BBA36, BB0323, Crasp3 and pG. The main goal of this study was to evaluate the presence of IgM and IgG antibodies against expanded panel of Borrelia burgdorferi specific antigens, including antigens that are expressed in vivo alone.

The study was conducted in the group of 25 patients diagnosed with borreliosis, hospitalized in The Department of Infectious Diseases, Medical University of Lublin. The diagnosis of borreliosis was based on patient's history, clinical symptoms and ELISA serological test results. Testing for IgG and IgM antibodies to Borrelia burgdorferi was performed using Immunoblot (Genzyme Virotech) which included antigens for IgM: OspC, p39, EBV-VCA-gp 125; for IgG: VlsE, p39, p83, BBA36 (iv1), BBO323 (iv2), Crasp3 (iv3), pG (iv4).

In that group IgG antibodies against full antigens panel VlsE, p39, p83, BBA36 (iv1), BB0323 (iv2), Crasp3 (iv3), pG (iv4) were observed in 3 patients (12%). In 12 patients (48%), in addition to IgG against VlsE, p39 and p83, antibodies against BBA36 (iv1), BB0323 (iv2), Crasp3 (iv3), pG (iv4) were detected with various frequency. The remaining 10 patients (40%) tested IgG positively based on presence of VlsE and/or p39, p83. In spite of long term infection, the results revealed the presence of IgM antibodies against OspC and p39 – 16 tested patients (64%), against OspC – 5 tested patients (20%), against p39 – 1 tested patients (4%).

We conclude that:

1. *IgG antibodies to VlsE (antigen from in vivo group) are the most frequently produced during borrelia infection.*
2. *Membraneous proteins: p39 and p83 present in diagnostic serologic tests are reliable markers of B. burgdorferi infection.*
3. *In vivo proteins alone used in diagnostic tests do not guarantee reliable results that either would confirm or exclude B. burgdorferi infection.*

Key words: B. burgdorferi, VlsE, BBA36, BB0323.

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Correspondence: Małgorzata Tokarska-Rodak, Department of Medical Microbiology, Skubiszewski Medical University, Chodźki 1, 20-093 Lublin, Poland. Phone/fax number: +48 81 742 37 81, Email: rodak@vp.pl

Introduction

The immune response to chronic *B. burgdorferi* infection during late stage Lyme disese is still not well understood and generates a lot of questions and concerns. In many cases natural defense mechanisms are not able to eliminate invading pathogen which leads to a chronic, long term illness. *B. burgdorferi* developed mechanisms that allowed them to avoid complement destruction. Complement regulator-aquiring surface protein (the Crasp proteins) are responsible for the potential of complement inactivation. They can bind to regulatory proteins that activate the complement, i.e. factor H and factor H-like protein. Group Erps proteins (OspE, OspF, Elps, p21, ErpA, ErpP) demonstrate similar properties. The presence of membrane proteins that inactivate the complement cascade is one of the major factors responsible for *B. burgdorferi* transmission to the host human body [1-4]. During early disseminated phase of borreliosis as well as in its later phase other highly immunogenic membrane associated proteins are detected, e.g. BB0323, BmpA (p39), p83, BBA64, BBA66. Some of them are used to diagnose borreliosis as markers of advanced stages of the disease in laboratory diagnosis [5].

Selected, highly specific antibodies to *B. burgdorferi* proteins are commonly used in diagnostic serological tests for Lyme disease. From the point of view of diagnosing *Borrelia burgdorferi* infection, highly immunogenic antibodies to proteins detected *in vivo* after *B. burgdorferi* transmission to the host human body seem to be very significant. Knowledge on importance of proteins that are expressed *in vivo* alone has accumulated recently. They proteins: VlsE, BBA36, BB0323, Crasp3 and pG [6].

VlsE antigen is used in the majority of commercial ELISA and Western blot based assays. Other antigens are not routinely included in diagnostic panels.

The main goal of this study was to evaluate the presence of IgM and IgG antibodies against expanded panel of *Borrelia burgdorferi* specific antigens, including antigens that are expressed *in vivo* alone.

Materials and Methods

The study was conducted in the group of 25 patients diagnosed with arthritis symptoms borreliosis: 18 men (age 21-65 yrs) and 7 women (age 24-60 yrs) hospitalized in The Department of Infectious Diseases, Medical University of Lublin. The diagnosis of borreliosis was based on patient's history, clinical symptoms and ELISA serological test results. Testing for IgG and IgM antibodies to *Borrelia burgdorferi* was performed using Immunoblot (Genzyme Virotech GmbH, Borrelia LINE IgG/IgM) which included antigens:

- for IgM: OspC, p39 and EBV – VCA-gp 125 which is Epstein-Barr Virus – Virus Capsid Antigen-gp 125 used for exclusion of the first infection with EBV,
- for IgG: VlsE, p39, p83, BBA36 (iv1), BBO323 (iv2), Crasp3 (iv3), pG (iv4).

Results

Among the individuals included in the study group, 20 patients (80%) self-declared multiple incidents of tick bites without presence of typical erythema migrant rush. Five patients (20%) with one or multiple tick bites admitted that they had bull's eyes rash erythema migrans in a past. All patients reported the following symptoms: headaches, muscular pains and arthralgias (affecting shoulders, elbows, knees and wrists). Some patients presented with neurological symptoms like numbness and tingling in the legs, loss of balance, stiff neck and back pain.

In the group of patients with borreliosis and symptoms of arthritis (25 patients), IgG antibodies against *in vivo* atigens: VlsE, BBA36 (iv1), BB0323 (iv2), Crasp3 (iv3), pG (iv4) were detected with various frequency.

In that group IgG antibodies against full antigen panel VlsE, p39, p83, BBA36 (iv1), BB0323 (iv2), Crasp3 (iv3), pG (iv4) were observed in 3 patients (12%).

In 12 patients (48%), in addition to IgG against VlsE, p39 and p83, antibodies against BBA36 (iv1), BB0323 (iv2), Crasp3 (iv3), pG (iv4) were detected with various frequency.

The remaining 10 patients (40%) tested IgG positively based on presence of VlsE and/or p39, p83.

In spite of long term infection, the results revealed the presence of IgM antibodies against OspC and p39 – 16 tested patients (64%), against OspC – 5 tested patients (20%), against p39 – 1 tested patients (4%).

Table 1 shows IgM and IgG antibodies against *B. burgdorferi* detected in the patients with clinical symptoms of borreliosis (with arthritis symptoms mainly).

Discussion

Antigens recognized *in vivo* are often treated as important IgG serologic indicators in late stage borreliosis and they could be used to evaluate immune response as they relate to patient's clinical status.

Thus a serologic test with those antigens involved creates better potential to evaluate immune response with account for clinical status of the patient.

Antigen VlsE is considered a reliable serologic marker of *B. burgdorferi* infection while IgM and anti-VlsE IgG may coexist in both early and late stage of borreliosis. The detection VlsE antibodies can be performed for all *Borrelia burgdorferi* s.l. pathogenic strains with 10 times lower false positive rate than for other Borrelia antigens [7, 8].

Table 1. IgM and IgG against expanded panel Borrelia burgdorferi specific antigens in patients treated for late stage borreliosis with arthritis symptoms

N=25	Presence of IgM				Presence of IgG						
	OspC	p39 (BmpA)	VlsE	EBV	VlsE	p39 (BmpA)	p83	BBA36 iv1	BBO323 iv2	Crasp3 iv3	pG iv4
3	+	+	-	-	+	+	+	+	+	+	+
1	+	+	-	-	+	+	+	+	+	+	-
2	-	-	-	-	+	+	+	+	+	-	-
2	+	-	-	-	+	+	+	-	+	+	+
3	+	-	-	-	+	+	+	-	+	-	-
1	+	+	-	-	+	+	+	-	-	+	-
1	+	+	-	-	+	+	-	+	-	-	-
2	+	+	-	-	-	+	-	-	+	-	-
2	+	+	-	-	+	+	+	-	-	-	-
1	-	-	-	-	+	+	-	-	-	-	-
3	+	+	-	-	+	+	-	-	-	-	-
1	+	+	-	-	-	+	+	-	-	-	-
2	+	+	-	-	+	-	-	-	-	-	-
1	-	+	-	-	+	-	-	-	-	-	-

In our study IgM anti-VlsE were not detected among the patients suffering from borreliosis, which partly may be accounted for by a substantially long period between tick bites and a serological diagnostic test confirming borreliosis.

IgG anti-VlsE were detected in 22 patients (88%), and in 3 patients (12%) results were negative. Positive results were based on IgG antibodies against other *B. burgdorferi* specific antigens e.g. p39 and p83.

During *B. burgdorferi* infection in addition to VlsE antigen, other highly immunogenic proteins can be detected, e.g. Crasps (Crasp3), from Erp family (pG), and immunogenic membrane-associated proteins like BB0323 [5, 6, 9].

In the patients with history of multiple tick bites IgG anti-VlsE (22 tested patients) and BB0323 (13 tested patients) were detected.

Although *in vivo* antigens are considered to indicate late stage borreliosis, the tests conducted in persons in whom borreliosis was suspected who had erythema migrans showed the presence of IgG anti-in vivo antigen VlsE and BB0323 [10].

Our results found that in a tested panel, IgG against other *in vivo* antigens were less frequent (anti BBA36 – 28%, Crasp3 – 28%, pG – 20%) however they still may have certain diagnostic value. Despite long term infection in those patients IgM against OspC and p39 antigens were detected.

Seropositivity which resulted from *B. burgdorferi* infection may exists in both IgM and IgG classes for a long time and detection of IgM is not only indicative as reinfection [11].

Conclusions

1. Antibodies to VlsE (antigen from in vivo group) are the most frequently produced antibodies IgG during borrelia infection.
2. Membraneous proteins: p39 and p83 present in diagnostic serologic tests are reliable markers of *B. burgdorferi* infection.
3. In vivo proteins alone used in diagnostic tests do not guarantee reliable results that either would confirm or exclude *B. burgdorferi* infection.

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