

Host response to *Borrelia afzelii* in BALB/c mice tested by immunoblotting

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Abstract

The aim was to find if there is variability in the production of specific antibodies by BALB/c mice to certain antigens of three individual *Borrelia afzelii* (dead cell suspension) strains originally isolated from different sources. Analysis of the borrelian proteins immunogenicity was performed and determined to particular strains by immunoblotting. Some differences in production of specific IgM and IgG antibodies in each individual group of tested mice were found. The antigenic influence of OspA and OspC proteins appeared to be the most important for the induction of protective immune reaction. OspC was produced both in early and late infection of all individuals and OspA induced production of IgG antibodies in all groups of immunized mice.

Key words

Borrelia afzelii, immunization, BALB/c, WB

INTRODUCTION

Lyme borreliosis, an infectious zoonotic disease, is caused by parasitic spirochetal bacteria of the *Borrelia burgdorferi* sensu lato (Bb) complex containing at least 19 genospecies [1]. These spirochetes are transmitted by ticks, in Europe by species *Ixodes ricinus* [2]. Many scientific and medical institutions have been attempting to develop a suitable vaccine for humans based on different proteins of Bb. For that reason, the various candidate vaccines based on different proteins have been considered [3]. The influence of the genospecies proteins diversity resulted in different immunoblot patterns recommended for the diagnosis of Lyme borreliosis. Nevertheless, the situation is not so complicated. Representative panel of *B. burgdorferi*, *B. afzelii* and *B. garinii* associated strains as the most dangerous European genospecies has been analyzed for the genotype of the following proteins: OspA, OspC, p83/100, flagellin (p41) and BmpA (p39) [4]. There are other variants containing most of the recommended antigens (OspC, VlsE, p39, p83, p31, p30, p21, p19 and p17), and used in a commercial sphere [5], or home made tests based on the use of similar proteins (OspA, OspC, P39, P83/100, P66, P58, P41, P30, P17) of local strains, even coming out of Europe [6]. Especially crucial is the diversity of proteins produced by local strains of Bb and considered for diagnosis and/or vaccine development. This study focuses on the comparison of specific immune response to proteins of different strains of the same genospecies (*Borrelia afzelii*) isolated from various sources in the eastern part of the Czech Republic. The occurrence of antigen-specific antibodies and consequent importance of particular antigens is examined and discussed.

MATERIALS AND METHOD

6-week-old male BALB/c mice were inoculated with three strains of *Borrelia afzelii* obtained from three different sources: 1) *Ixodes ricinus* tick (BRZ 9) (16x passaged), 2) *Culex (Culex) pipiens molestus* mosquito (BRZ 14) (18x passaged), characterized by Žáková et al. 2008 [7], and 3) *Apodemus flavicollis* rodent (BRZ 21) (21x passaged). Each of experimental groups contained six animals. Mice were injected 3 times intraperitoneally. The injections were repeated every three weeks and each dose (300 µl) contained 7.5 mg of antigen completed with 0.1% aluxid. The dose of BRZ 14 antigens was half due to the lack of bacterium suspension. The design of immunization attempts is described in detail in a previous study about detection of the antibodies level using ELISA method [8]. After taking blood from an immunized mouse, a certain volume of the serum was stored at -20 °C. The whole cell suspensions of corresponding *Borrelia afzelii* strains were separated by gradient SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis) on 10% polyacrylamide gel (constant voltage, 200V). The proteins were transferred to a nitrocellulose membrane equilibrated with transferring buffer (192 mM glycine, 25 mM Tris, 20% (15%) MetOH, 0.1% SDS). Each step of this procedure was performed at room temperature. After blotting (constant voltage, 60V), the membrane was quickly washed three times with dH₂O for at least 5 min. Standard strips were coloured with amidoblack for 1 min. Sample strips were incubated over night with blocking buffer (PBS – buffered saline, 0.05% Tween, 3% casein). The individual antigen strips were incubated with the respective sera from immunized mice, (diluted 1:40 for IgG and 1:100 for IgM) for 1–2 hours. The strips were washed three times for 5 min each time with washing buffer (PBS, 0.05% Tween). After the washing, separately goat anti-Mouse IgG and IgM peroxidase conjugates (Sigma) diluted 1:1000 in blocking buffer were added for 1 hour. At the end of the second incubation, two washings were carried out – the first with washing buffer and the other with TBS [Tris (tris-hydroxymethylamino methan)

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– buffered saline]. The binding was visualized by addition of the chromogenic substrate DAB (diaminobenzene). Antigens with bound antibodies were identified on the basis of their Mr using Molecular Analysis software, version 1.1.1., Bio-Rad.

RESULTS AND DISCUSSION

The presented data show that immunization of BALB/c mice with *Borrelia* antigens induced the development of an immune response to all three *B. afzelii* strains. The production of IgM antibodies by mice of at least one group was induced by the following proteins: p70, Hsp66, p64, p60, p56, p48, p41, p39, p35, OspA, OspF, OspC, OspE, p17, p16, p15. For the creation of IgG antibodies, the following proteins were important: p70, p64, p49, p48, Flagellin, p39, p35, OspB, OpsA, OpsD, OspF, OpsC, OspE, p17, p16, p15 (Tab. 1).

With regard to the most important borrelian proteins, the following findings are discussed. OspA is not produced in the blood and liquor in early infection [9]. The results confirm a somewhat later IgG response to this protein. OspB (also OspA) is used by Bb predominantly for adherence in the tick's midgut [10]. Additionally, during passaging in BSK II medium, the spirochetes lose OspB and their infectivity [11]. This fact can explain the absence of antibodies to OspB in most of immunized mice because the passaged borrelian strains were used. IgM and IgG antibodies to OspC, which is the marker of early Lyme borreliosis [12], were found in all individuals. This finding confirms another study [13] describing OspC induction of both IgM and IgG antibodies response. IgG antibodies to OspD were found only in the BRZ 14 group, which correlates with the higher importance of this protein for spirochete colonization of the tick gut [14]. Erp proteins (OspE, OspF) help *Borrelia burgdorferi* to escape from the host immune response, to start the infection and to persist in the organism [15]. Table 1 shows that antibodies to OspF appeared in all tested groups, OspE only in groups of BRZ 21 and BRZ 9. The occurrence of antibodies to P91 (91.51 kDa), identified in one case, should correlate with reaction to MEP (main extra cellular protein 83–93 kDa). Intracellular persistence of Bbsl depends on the high amount of this protein [16]. P37 is an antigen that elicits an early IgM response [17]. P39 (38.4; 38.6 kDa) should correlate with the Bmp family of immunogenic lipoproteins detected in some patients [18]. Antibodies both to P37 and P39 appeared almost exclusively in the BRZ 21 group immunized with a strain isolated from *Apodemus flavicollis*, a typical reservoir of Bbsl infection [19]. In some cases, antibodies to P41 (flagellin), p60 and Hsp66 (Heat shock protein) were found. These proteins are also produced by other species of spirochetes and can cause cross-reactions [20].

This WB analysis revealed that mice immunized with the BRZ 21 antigen isolated from rodents were mostly able to induce significant production of specific antibodies. The use of different *B. afzelii* strains as antigens led to inconsistency in blot results because of variability in the expression of immunogenic proteins.

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Table 1. List of borrelian proteins in the range of molecular weight (MW) 14.4–200 kDa. Certain protein fraction corresponds to specific IgM/IgG response in different experimental groups.

Proteins (xkD-xkD)	BRZ 21		BRZ 14		BRZ 9	
	IgM	IgG	IgM	IgG	IgM	IgG
	MW (kDa)		MW (kDa)		MW (kDa)	
	204.95					
			149.31		138.02	
		120.72	120.59		126.08	
	104.18	106.24				
MEP?		91.51				
P70	70.71	68.88		70.73		
Hsp66			66.39		66.42	
P64	64.65	64.76				
				63.32		
P60				60.22	59.45	
	58.81		58.79			
		57.03				57.37
P56			55.79			
						53.02
						52.83
P49		49.95				
P48			48.54			
		44.89				
Flagellin		40.95	40.64			
P39	38.4	38.21	38.58			
P37	36.26	36.44				
OspB						35.62
		34.87				
OspA			32.87	33.4		32.72
		31.16				
OspD				28.33		
			26.86			26.8
OspF	25.98	25.92				
		24.95				
	22.12	23.32	23.51	23.61	22.22	23.16
OspC	19.79	20.87		19.67		20.92
OspE	18.33	19.31				18.75
P17			17.22			17.28
P16		16.92		16.82		
P15	15.16		15.94	15.17		15.74

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