JOURNAL OF CLINICAL MICROBIOLOGY, July 1995, p. 1867–1869 Vol. 33, No. 7
0095-1137/95/$04.00+0

Expression of Outer Surface Proteins A and C of *Borrelia burgdorferi* in *Ixodes ricinus*

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Received 29 December 1994/Returned for modification 14 February 1995/Accepted 27 March 1995

A total of 472 field-collected *Ixodes ricinus* ticks from southern Germany were investigated by immunofluorescence for the presence of *Borrelia burgdorferi* with a polyvalent rabbit immune serum and with monoclonal antibodies specific for outer surface proteins A and C (OspA and OspC, respectively). Borreliae were detected in 90 ticks with the polyvalent immunofluorescence assay. Infection rates in adults (females, 20.2%; males, 25.2%) were significantly higher than in nymphs (12.1%). OspA was detected in 77 ticks and OspC was detected in only 1 tick with the respective monoclonal antibodies. We therefore conclude that *B. burgdorferi* in unfed *I. ricinus* ticks usually expresses OspA and very rarely OspC.

The multisystem disorder Lyme borreliosis is the most frequent arthropodborne disease in Europe. The hard tick *Ixodes ricinus* is widely distributed in central European countries and is the primary vector of the Lyme disease agent, *Borrelia burgdorferi* sensu lato. This spirochete is maintained in nature by alternating tick-vertebrate host cycles, i.e., *B. burgdorferi* has to endure many different physiological conditions and rapid changes in its environment. This requires adaptation strategies, for example, to escape different immune mechanisms or to survive the physiological changes between the tick and host tissue. One possible mechanism is variation in expression of outer surface proteins (Osp proteins). Variation in Osp protein expression has been observed during in vitro cultivation as well as after passage of borreliae in vertebrates or ticks (10, 12, 19, 20, 24, 26).

Under culture conditions, OspC expression is detectable in about 40% of strains and OspA expression is detectable in more than 90% of strains (25). In contrast, the primary human immune response against *B. burgdorferi* is directed mostly against OspC and only rarely against OspA (2, 5, 6, 21, 26).

The aim of this study was to determine the expression of OspA and OspC of *B. burgdorferi* in unfed nymph and adult populations of *I. ricinus*. Immunofluorescence with monoclonal antibodies (MAbs) revealed that OspA, but usually not OspC, was expressed by *B. burgdorferi* in unfed ticks.

MATERIALS AND METHODS

A total of 472 unfed *I. ricinus* ticks (168 females, 147 males, and 157 nymphs), collected by flagging in spring 1994 in Munich, Germany, were investigated by immunofluorescence assay (IFA) for the presence of *B. burgdorferi* (3). Infection rates were determined by indirect IFA using rabbit antibodies raised against *B. burgdorferi* whole-cell antigens (8). Mouse MAbs against OspA and OspC were used for specific detection of OspA and OspC expression. Antibody binding was detected by using the respective fluorescein isothiocyanate-labelled anti-rabbit or anti-mouse antibodies.

MAbs. For the OspA IFA, the OspA-specific MAb L32 1F11, which recognizes an epitope conserved among *B. burgdorferi* sensu lato strains (128 tested), was used (23). For OspC detection, we combined the MAbs L22 1F8 (24) and L22 6C4 and L22 22C11 (22). With this combination of OspC-specific MAbs, we were able to detect OspC in all 36 OspC-expressing strains tested so far. Both the OspA- and the OspC-specific antibodies are nonreactive with relapsing-fever borreliae (22, 23).

Detection of borreliae in ticks. After the ticks were washed in distilled water, 96% ethanol, and phosphate-buffered saline (PBS) (pH 7.4) for 2 to 3 min, smears of dissected midgut (adults) or whole ticks (nymphs) were prepared on slides in a drop of PBS and distributed on three slides per tick. After 1 h of air drying and 15 min of methanol fixation, the three slides from individual ticks were incubated for 30 min with the following antibodies: one slide with a mixture of rabbit immune sera against *B. burgdorferi* sensu lato strains TN and PKo (25) in a final dilution of 1:100, the second slide with the OspA-specific MAb L32 1F11 (diluted 1:2), and the third slide with a mixture of the OspC-specific MAbs L22 1F8, L22 6C4, and L22 22C11, resulting in final dilutions of 1:12, 1:12, and 1:6, respectively. These dilutions correspond to the double concentration which had been shown to give optimal reactivity with the respective positive controls (see below). After three washings in PBS, the slides were incubated for 30 min with fluorescein isothiocyanate-conjugated swine immunoglobulins to rabbit immunoglobulins diluted 1:100 (Dakopatts, Copenhagen, Denmark) or rabbit immunoglobulins to mouse immunoglobulins diluted 1:50 (Dakopatts). Smears of the OspC-expressing skin isolate PKo (reactive with all three OspC-specific MAbs) and the OspA-expressing tick isolate TN (reactive with the OspA-specific MAb L32 1F11) served as positive controls. The cerebrospinal fluid isolate PKa2 (no OspC expression [24]) and the skin isolate PPop (no OspA expression [23]) were used as negative controls for the OspA- and OspC-reactive MAbs, respectively. Control slides were prepared from washed borreliae as previously described for the indirect IFA (8). For detection of borreliae, we used an immunofluorescence microscope (Leitz Laborlux 12) and 40 visual fields at a magnification of ×400. A tick smear was regarded as positive if at least three borreliae could be identified.

RESULTS AND DISCUSSION

As shown in Table 1, *B. burgdorferi* was detected in 19.1% of 472 *I. ricinus* ticks. As also previously described by other authors (for an overview, see reference 9), a significantly higher infection rate was found in adults than in nymphs. OspA expression was detected in 77 infected ticks (85.6%), corresponding to 85.9% in adults and 84.2% in nymphs. OspC expression was found in only one adult tick (1.4%), while none of the nymphs showed OspC expression. Interestingly, in the single OspC-positive tick, we found only a few OspC-expressing borreliae (three in 40 visual fields), whereas a high number of OspA-expressing spirochetes (three to five per field, corresponding to >100 in the 40 visual fields examined) was present in the same tick. The percentages of infected ticks reactive with the OspA-specific MAb were 83.8% of adult males, 88.2% of adult females, and 84.2% of the nymphs. Thus, there were no significant differences in OspA expression in infected ticks with regard to their different stages.

This study shows that OspC is exceptionally expressed only

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TABLE 1. Expression of OspA and OspC by *B. burgdorferi* in *I. ricinus*

Tick group	Total *n*	Positive results determined by:					
		Polyvalent IFA		Anti-OspA IFA		Anti-OspC IFA	
		n	%	*n*	%[a]	*n*	%[a]
Adults	315	71	22.5	61	19.4 (85.9)	1	0.3 (1.4)
Female	168	34	20.2	30	17.9 (88.2)	0	0.0 (0.0)
Male	147	37	25.2	31	21.1 (83.8)	1	0.7 (2.7)
Nymphs	157	19	12.1	16	10.2 (84.2)	0	0.0 (0.0)
Total	472	90	19.1	77	16.3 (85.6)	1	0.2 (1.1)

[a] Values given in parentheses are the percentages of Osp protein-positive ticks out of the ticks positive by polyvalent IFA.

in borreliae associated with unfed *I. ricinus* ticks, a finding also reported for unfed *Ixodes scapularis* ticks by T. Schwan (18a). In contrast, expression of OspA occurs frequently, as was previously shown for *I. scapularis* by Burkot et al. (4). While rarely expressed or not expressed at all in unfed ticks, OspC was expressed in about 40% of *B. burgdorferi* strains (15 of 34) in culture (25). The OspC protein but not OspA appears to be efficiently expressed in the human host in early disease, since OspC, and not OspA, is an immunodominant protein in this stage (1, 5, 6, 21, 26). The late occurrence of OspA-specific antibodies especially in patients with severe arthritis (11) may indicate a recurrence of OspA expression.

True antigenic variability of Osp proteins known to be involved in the pathogenesis of relapsing fever (1, 16, 17) was also considered to play a role in the pathogenesis of Lyme borreliosis. Variation in expression of Osp proteins could be detected under standard culture conditions, after reisolation from experimentally infected ticks or mammals, and at different culture temperatures (10, 12, 19, 20, 24, 26). These changes are suggested to play a major role in invasion and infection. Variation in Osp protein expression might occur in the borreliae while they penetrate different tissues: Burgdorfer et al. (3) found that ". . . spirochetes in the midgut of all 25 infected ticks reacted very strongly. In 6 of the 8 ticks with generalized infection, however, the spirochetes in tissues other than midgut reacted poorly . . .." The finding of Park et al. (14) that the OspA-specific MAb H5332 did not stain borreliae in skin biopsies from patients with erythema migrans, in contrast to the flagellum-specific MAb H9724, is an indication that OspA is not expressed in infected human tissue in early disease. These findings suggest that if *B. burgdorferi* is no longer exposed to the specific environment of the tick midgut, loss of OspA expression might occur. The question arises whether other surface proteins, for example OspC, are expressed in various tick tissues instead of OspA.

Both OspA and OspC have been effective as vaccines in animal models (7, 15, 18). Therefore, an important question is whether OspC is expressed in salivary glands, which are known to contain the infectious spirochetes, or, in other words, which proteins are expressed by the spirochetes in the salivary glands immediately prior to infection. According to Margolis et al. (13), the *ospC* gene and genes encoding purine synthesis enzymes are located on the same circular plasmid and are possibly linked in expression. This suggests that changes in OspC expression might be a consequence of adaptive responses to the tick and animal environment.

In conclusion, we showed that OspA but not OspC was efficiently expressed by tick-associated borreliae and discussed implications of this finding for pathogenesis and prophylaxis of Lyme borreliosis. However, the ticks investigated were collected in a small area near Munich, Germany. Since culture isolates may vary considerably in Osp protein expression, the possibility that Osp protein expression in tick-associated borreliae varies in different geographic regions deserves attention. The MAbs used in our study are valuable tools for such epidemiological studies, because they specifically recognize the OspA and OspC proteins of a large panel of strains from different geographic regions.

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