

Inability of outer-surface protein C (OspC)-primed mice to elicit a protective anamnestic immune response to a tick-transmitted challenge of *Borrelia burgdorferi*

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A one-inoculation regimen of recombinant outer-surface protein C (OspC), which has been demonstrated to elicit protective immunity against a tick-borne challenge of *Borrelia burgdorferi*, was administered to outbred mice. Following seroconversion, the serum antibody titre against OspC was allowed to wane with time until there was little or no detection of anti-OspC antibodies by immunoblot. The mice were then challenged with an infectious dose of *B. burgdorferi* by tick transmission. Eleven of 12 OspC-primed mice subsequently became infected by *B. burgdorferi*, demonstrating that a protective anamnestic response was not generated in these mice following the introduction of infectious OspC-expressing spirochaetes.

Received 6 September 2002

Accepted 30 January 2003

INTRODUCTION

Protective immunity against Lyme borreliosis, an infection caused by the tick-borne bacterial pathogen *Borrelia burgdorferi*, has been demonstrated to various degrees by immunization with several surface antigens (Probert *et al.*, 1997; Hagman *et al.*, 1998; Hanson *et al.*, 1998; Exner *et al.*, 2000; Fikrig *et al.*, 2000). The borrelial outer-surface proteins (Osps) that have been studied most extensively for their protective capabilities are OspA and OspC (Fikrig *et al.*, 1990; Schaible *et al.*, 1990; Simon *et al.*, 1991; Preac-Mursic *et al.*, 1992; Gilmore *et al.*, 1996; Zhong *et al.*, 1997). OspA is the immunogen currently used in the Lyme disease vaccine for humans (Sigal *et al.*, 1998; Steere *et al.*, 1998).

Within the non-feeding tick, OspA is localized on the surface of *B. burgdorferi*, which resides primarily in the midgut. During the 3–4-day feeding stage of the *Ixodes* tick, the antigenic surface structure of the borreliae undergoes a change whereby a subpopulation of the organisms synthesizes OspC with concomitant reduction of OspA (Schwan *et al.*, 1995). Thus, as bacteria migrate from the midgut to the salivary glands in preparation for transmission to a mam-

malian host, the borrelial population displays heterogeneity in its surface-protein phenotypes; infecting spirochaetes become OspA-negative, while a small percentage are OspC-positive (Ohnishi *et al.*, 2001). As the invading borreliae lack OspA, Lyme disease patients do not normally elicit antibodies against OspA early in infection. Therefore, vaccination with recombinant OspA antigen is prophylactic and relies on the production of OspA-specific borrelicidal antibodies in the host. These antibodies are taken in with the bloodmeal during tick feeding and eradicate the spirochaetes in the tick before they shed their OspA antigenic coat, thereby preventing bacterial transmission to the host (de Silva *et al.*, 1996).

OspC, like OspA, has been demonstrated to elicit both active and passive protective immunity in experimental animals, although its mechanism of protection appears to be slightly different. During the normal tick-to-host transmission stage, a subpopulation of *B. burgdorferi* that has migrated to the salivary glands expresses OspC to the exclusion of OspA (Schwan *et al.*, 1995; Ohnishi *et al.*, 2001). Thus, following successful mammalian infection, host anti-OspC antibodies are among the first to be detected, indicating expression of OspC by invading borreliae (Dressler *et al.*, 1993; Padula *et al.*, 1994). A study from our laboratory has shown that *B. burgdorferi* migration to the tick salivary glands is diminished while ticks feed on OspC-immunized mice, thus

Abbreviation: Osp, outer-surface protein.

Confocal micrographs of *B. burgdorferi* in the salivary glands of a tick are available as supplementary material in JMM Online.

effectively preventing transfer of infectious organisms to the host, although viable borreliae remained in the midguts of the fed ticks (Gilmore & Piesman, 2000). The conclusion was that anti-OspC antibodies, which were present in the blood-meal, selected against OspC-expressing borreliae within the tick and prevented their transmission. That study also indicated a putative function for OspC in the process of *B. burgdorferi* mobilization from midgut to salivary glands and subsequent infectious transmission.

Host antibodies against OspC are made early in infection and are therefore important in the serodiagnosis of Lyme disease. OspC has indeed been detected on infectious borreliae in the tick salivary glands prior to transmission, and on organisms in the skin of mice infected by tick-bite (Leuba-Garcia *et al.*, 1998; Ohnishi *et al.*, 2001). Previous studies have singularly addressed OspC protection against infection when the experimental animals have been immunized and boosted and contain high antibody levels at the time of challenge. The current study tested whether surface-localized OspC on the invading organisms would trigger a host-mediated protective secondary immune response in primed animals in which the circulating antibody level had waned. The results, which define these mechanisms of immunity, provide information relevant to the design of OspC-based vaccines and the biology of tick-to-host transmission.

METHODS

Mouse immunization. Fifteen 6-week-old, female, specific pathogen-free, outbred mice (Institute for Cancer Research, Philadelphia, PA, USA, maintained at the Division of Vector-Borne Infectious Diseases) were immunized with a single injection of soluble *Escherichia coli* lysate that contained recombinant OspC, cloned from *B. burgdorferi* strain B31, which had been shown to be protective as described previously (Gilmore *et al.*, 1996). A control group of five mice was immunized with *E. coli* lysate that contained plasmid vector only. A second control group of four mice was immunized with the protective recombinant OspC lysate and boosted at 2 weeks, with subsequent tick challenge approximately 1 month later. The latter control group reproduced the protective capability of the antigen (three of four mice were protected) under the conditions used when protection from challenge had been demonstrated previously (Gilmore *et al.*, 1996). Each mouse was immunized subcutaneously with 100 µg protein (of which recombinant OspC was approximately 1–10% of the total), mixed 1:1 with Inject Alum aluminium hydroxide adjuvant (Pierce Biotechnology).

Western blotting. Immunoblots on fractionated proteins from *B. burgdorferi* strain B31 were performed with MarDx MarBlot strips (Trinity Biotech) using mouse serum samples at 1:100 dilution, followed by incubation with 1:1000 dilution of goat anti-mouse alkaline phosphatase-conjugated IgG (Kirkegaard and Perry Laboratories), according to the manufacturers' directions.

Mouse challenge with tick-transmitted *B. burgdorferi* and tick dissection.

Mice were challenged with an infectious dose of *B. burgdorferi* strain B31 by tick-borne transmission (10 ticks per mouse) as described previously (Gilmore *et al.*, 1996; Dolan *et al.*, 1997). At 72 h after tick infestation, two ticks from each mouse were removed; the salivary glands were immediately dissected, placed on microscope slides and allowed to air-dry briefly. Whole glands were fixed in 4% paraformaldehyde in PBS for 1 h. Fixed slides were washed extensively in PBS and stored at –20 °C. Immediately after removal of the salivary glands, the tick midgut was placed on a separate slide and allowed to air-dry. The midgut smear was fixed in acetone for 10 min and stored at –20 °C. These slides allowed for the eventual observation of borrelial OspC synthesis within feeding ticks by immunofluorescent staining, as necessary. The remaining ticks were left to finish feeding on the mice and were collected at repletion. These ticks were stored at –80 °C until potentially needed for further midgut stainings. Salivary glands in replete ticks deteriorate rapidly and were therefore not removed. At 4 weeks post-challenge, ear biopsies were taken from each mouse and cultured for the presence of *B. burgdorferi* as described by Sinsky & Piesman (1989).

Immunofluorescent staining. *B. burgdorferi* in the tick salivary glands and midguts was stained as follows. The fixed tissue slides were incubated first with a mouse anti-OspC mAb (Mbow *et al.*, 1999) for 1 h, followed by rinsing in three changes of PBS. Next, co-incubation with goat anti-mouse Alexa Fluor 594-conjugated IgG (Molecular Probes) and polyclonal rabbit anti-*B. burgdorferi* fluorescein-conjugated antiserum (Jackson ImmunoResearch Laboratories) was performed for 1 h, followed by PBS washes as before. The dried slides were overlaid with a drop of ProLong Antifade solution (Molecular Probes) and a coverslip. Samples were observed by confocal microscopy on an Olympus FVX-IHRT Fluoview confocal laser scanning microscope.

Ethics. The use of animals in this research complied with federal guidelines and the protocol was reviewed and approved by the Animal Use and Care Committee of the DVVID.

RESULTS AND DISCUSSION

Mouse seroconversions to recombinant OspC

Twelve of the 15 mice seroconverted against OspC after 2 weeks, as determined by immunoblot, and a robust IgG response was observed approximately 8 weeks post-immunization (Fig. 1a). Three mice did not demonstrate seroconversion by this assay, but remained in the study nonetheless. None of the 15 mice in the test group was given a booster immunization. Periodic bleedings were taken in the weeks following the primary immunization, and were tested qualitatively by immunoblotting to determine the presence or absence of anti-OspC antibody in individual mice. An observable drop in anti-OspC antibody levels was not seen until approximately 1 year post-immunization (Fig. 1b). As previous studies in our laboratory showed that host anti-OspC antibodies prevent transmission of *B. burgdorferi* on tick-borne challenge (Gilmore & Piesman, 2000), it was essential to allow the circulating antibody in the blood to drop to low or undetectable levels to assess the effect of borrelial infection on the OspC-primed host's immune response.

Mice were bled 3 weeks prior to tick-borne challenge. At the

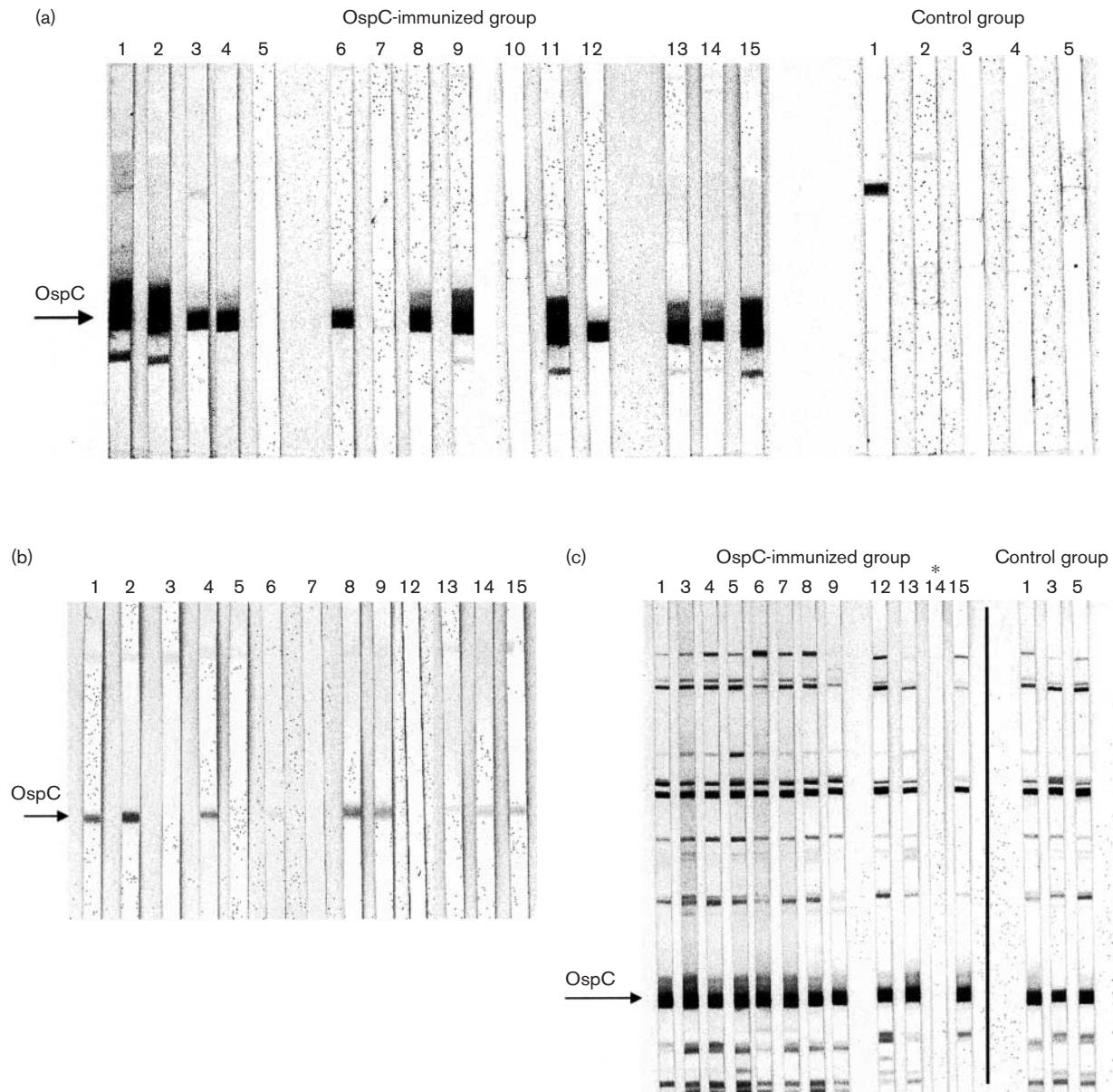


Fig. 1. Immunoblots showing serological reactivity throughout the experiment of 15 individual OspC-vaccinated mice and five control mice that were inoculated with *E. coli* protein lysate containing plasmid vector only. Bands representing OspC on the immunoblots are indicated by arrows. All serum samples were assayed at 1 : 100 dilution. (a) Seroconversion against OspC approximately 8 weeks post-immunization. Mice nos 5, 7 and 10 did not show measurable seroconversion. The five control mice demonstrated no seroreactivity against OspC. (b) Seroconversion against OspC approximately 1 year post-immunization. Mice nos 3, 6, 12 and 13 have lost seroreactivity, and mice nos 1, 2, 4, 8, 9, 14 and 15 show diminished reactivity. Mice nos 10 and 11 died prior to this time period and are not shown. Mice were challenged by tick-bite 3 weeks following this bleeding. (c) Serological profiles of the OspC-immunized mice and the control group 18 days after *B. burgdorferi* challenge by tick-bite. The broad spectrum of reactive antigens seen in the immunized mice is identical to that of the control mice and is indicative of infection, which was confirmed by culture. Mouse no. 14 (asterisk) shows no immunoreactivity against *B. burgdorferi* antigens. There is a weak OspC band present, but it did not reproduce well in this figure. Mouse no. 14 was protected from infection. Immunized mouse no. 2 and control mice nos 2 and 4 died prior to challenge and are not shown.

time of challenge, 1 year following the primary inoculation, three mice had died, leaving 12 OspC-immunized mice. Additionally, two of the five control mice died during this period. Mice were challenged with an infectious dose of

B. burgdorferi strain B31 by tick-borne transmission. The mice were bled 18 days post-challenge and assayed serologically for evidence of infection. Immunoblots were performed and are shown in Fig. 1c. Eleven of the 12 OspC-primed mice

displayed seroconversion against several *B. burgdorferi* proteins; this was indicative of infection, as seen with the control mice. At 4 weeks post-challenge, ear biopsies were taken from each mouse and cultured for *B. burgdorferi*. Confirming the serological results, 11 of the 12 OspC-primed mice were ear-explant culture-positive; all three of the control mice were also positive. These results demonstrated that OspC-primed mice, when the circulating antibody against OspC had dropped to low or undetectable levels, did not develop a secondary protective immune response against the challenge with tick-transmitted *B. burgdorferi*.

Due to the advanced age of the mice and recent stress from the tick challenge, it was decided not to subject the mice to further stress by bleeding just a few days after challenge. With only one post-challenge bleed at 18 days, it was not possible to ascertain whether there was an earlier, immediate secondary anti-OspC response prior to the elicitation of primary responses against other borrelial antigens. OspC-specific antibodies are normally generated during the course of infection, as can be seen by the serological profiles of the control mice (Fig. 1c). If an anamnestic anti-OspC response was indeed produced, (i) it was not protective, and (ii) it was not of a level that was measurably higher than the primary anti-OspC polyclonal response of the non-immunized control mice at 18 days post-infection.

However, one of the OspC-primed mice was protected (Fig. 1c, no. 14). Ticks collected from this mouse harboured spirochaetes (as observed by fluorescent antibody microscopy), indicating that the mouse had resisted infection. Whether this protection was due to (i) the mouse possessing a circulating blood anti-OspC antibody level of sufficient strength to prevent transmission from the tick, (ii) elicitation of a protective anamnestic response by the invading borreliae or (iii) chance, is not known. The protected mouse had detectable anti-OspC antibody prior to challenge, as seen by immunoblotting; this was similar to other mice that eventually became infected (Fig. 1b, nos 1, 2, 4, 8, 9 and 15). ELISA and immunoblots showed that the protected mouse had approximately the same pre-challenge anti-OspC antibody titre as mice that were susceptible to the infectious challenge (data not shown). Additionally, ELISA results showed that the 18-day post-challenge anti-OspC antibody titre of the protected mouse was equivalent to its titre prior to challenge, suggesting that this mouse did not generate a secondary anti-OspC immune response in reply to the infectious challenge.

***B. burgdorferi* OspC phenotypes in ticks during and following challenge**

To observe OspC phenotypes of the infective borreliae, the salivary glands and midguts of ticks removed from the mice at 72 h post-attachment were subjected to a double-staining indirect immunofluorescence assay (IFA), as described previously (Gilmore & Piesman, 2000). The fluorescein-labelled anti-*B. burgdorferi* antibody enabled the observation of all borrelial cells in the sample (green fluorescence),

whereas the Alexa Fluor 594-labelled antibody bound to the anti-OspC antibody provided a view of those cells in the population that expressed OspC (red fluorescence).

Qualitative observation of individual tick salivary glands at 72 h post-attachment (a timepoint when borreliae are being transmitted from the tick to the host) taken from mice that eventually became infected (including both primed mice and non-immunized controls), showed *B. burgdorferi* cells numbering approximately < 10 to > 100 , depending on the tick. OspC-positive borreliae were seen in the salivary glands and were estimated to comprise 10–20% of the total bacteria, which is in agreement with a previous description by Ohnishi *et al.* (2001). Midgut smears from these ticks revealed large numbers of *B. burgdorferi*, with a significant proportion (approx. 25–40%) of the population observed to be OspC-positive, which also agrees with a previous study (Schwan & Piesman, 2000). By considering observations from our laboratory and other researchers, we have made a qualitative assessment that a subpopulation of midgut-localized borreliae expresses OspC at this time-point during the mammalian transmission stage (Schwan *et al.*, 1995; Schwan & Piesman, 2000; Gilmore & Piesman, 2000; Ohnishi *et al.*, 2001). Our results show that the OspC expression pattern within ticks is the same in primed mice that became infected as that in non-immunized control mice.

B. burgdorferi was observed in the salivary glands of a tick taken at 72 h post-attachment from the mouse (no. 14) that was protected from infection (Fig. 2a, available as supplementary data in JMM Online). Although surprising, this result is consistent with data from an earlier study from our laboratory, which demonstrated the diminishing of *B. burgdorferi* in the salivary glands of ticks that fed on OspC-immunized mice (Gilmore & Piesman, 2000). Evaluation of the data from that study indicated that even though the mice were protected from infection, *B. burgdorferi* could be isolated from tick salivary glands in a few instances.

Additionally, within the tick from mouse no. 14, the midgut contained large numbers of borreliae; however, unlike the ticks that fed on the unprotected mice, only a scant number of OspC-positive cells could be observed (estimated at $< 1\%$; data not shown). This result is consistent with a previous study, where ticks that fed on OspC-immunized mice harboured non-OspC-expressing organisms (Gilmore & Piesman, 2000). Upon close inspection, a borrelial cell that expressed OspC was found in the salivary gland of this tick (Fig. 2b, available as supplementary data in JMM Online). This result is presented only because it demonstrates that it was possible to find a salivary gland-localized, OspC-expressing organism within a tick that fed on a mouse that was protected from infection. Because such a limited number of ticks ($n = 1$) was available to be assayed from the feeding stage of what ultimately became the only mouse that was resistant to infection in this experiment, these results can only be evaluated as a snapshot of the OspC-expression phenotype that occurs in salivary gland migration during tick engorgement on OspC-immunized, challenge-protected

animals. Thus, *B. burgdorferi* and OspC surface presentation in salivary glands are not absolute indicators of eventual infectious transmission to a host, which is undoubtedly also influenced by other factors.

Hypothetical models

In a non-vaccinated host, the antibody response against OspC is usually observed shortly after *B. burgdorferi* infection, indicating the efficient presentation of this antigen by the immune system. Because of this phenomenon, we hypothesized that OspC from an invading spirochaete could trigger the classical secondary humoral response in a primed host. In this manner, OspC immunization could function by a prototypical vaccine mechanism, whereby memory cells would be activated in response to an active infection and protection would not be dependent on circulating antibodies provided by booster immunizations.

The results of this study demonstrated, however, that OspC-primed mice were not protected from a tick-borne *B. burgdorferi* infection following a period in which the antibody level had waned. As shown by the post-challenge immunoblots, all infected mice elicited an anti-OspC response (as is normally seen during an infection), indicating that OspC was present on the invading borreliae. This response, however, did not appear to be the expansive anamnestic response one would have expected, given the previous priming of these mice. We offer two hypotheses to explain these observations.

In the first model, the protective epitope from the priming antigen (the recombinant OspC) was not present on the surface-exposed OspC from the invading borreliae, thus resulting in no memory response to this epitope. This explanation requires a dynamic fluidity for the surface structure of OspC during the spirochaete's migration from tick to host; a model for OspC surface exposure has indeed been proposed, as an architecture to explain the organism's persistence in infection (Cox *et al.*, 1996). For example, *B. burgdorferi* OspC synthesis during the feeding phase of the tick is generated in response to signals that stimulate the borreliae to migrate from the tick midgut to the next host. The newly synthesized OspC molecules are formed on the surface in a conformation-dependent manner, to function in the migration of the infectious borreliae. Following transmission from the tick into a mammalian host, the bacteria find themselves in a new environment and surface-protein composition may change in response. In this model, the conformation of the OspC molecules may differentiate structurally, and although processed by the host's immune system, do not elicit antibodies to the protective epitope that was existent in the previous tick stage. Our laboratory has shown that the OspC protective epitope for *B. burgdorferi* strain B31 is conformational (Gilmore & Mbow, 1999). A similar proposal has been made by Zhong *et al.* (1999) to explain their results from a study where passive immunization with anti-recombinant OspC polyclonal serum cured *B. burgdorferi* infection in mice, but active immunization of

infected mice with the recombinant OspC, which resulted in generation of the same anti-OspC antibody, did not produce the same therapeutic results. Their finding led them to postulate that protection is achieved only upon vaccination of naive, but not primed (i.e. infected), mice; this suggests that the protective epitope is either not expressed or not immunogenic upon the secondary boost. Zhong *et al.* (1999) refer to the latter instance as the phenomenon of priming-induced deflection of antibody responses, whereby the antibody response against the infecting organisms in OspC-primed mice would be expected to be directed against cross-reactive epitopes, expressed on both the native and recombinant OspC proteins, and not against new ones.

In the second model, it is possible that a short-lived secondary anti-OspC response was generated following tick-bite infection, but failed to protect for the following reason. A recent study by Liang *et al.* (2002) demonstrated that anti-OspC antibodies would select preferentially for non-OspC-expressing *B. burgdorferi in vivo*, thereby allowing OspC-negative spirochaetes to remain viable and to survive in the infected host. These authors proposed that, by this mechanism, the organisms are able to evade the immune system and persist in Lyme disease patients. Moreover, a separate study by Ohnishi *et al.* (2001) reported that infectious *B. burgdorferi* transmitted from ticks has both OspC⁺ and OspC⁻ phenotypes, an observation that was corroborated during the current study by observation of immunofluorescent-stained organisms in the salivary glands. Therefore, the evidence suggests that the humoral response directed against OspC following infection, whether primary or secondary, selected for the borreliae that were not expressing OspC, and this is the population of bacteria that established the infection.

Conclusions

In conclusion, this report describes the absence of a protective anamnestic immune response within OspC-primed mice against *B. burgdorferi* transmitted by a tick-borne inoculum approximately 1 year following immunization. Putative mechanisms for the lack of protection include the reconfiguration of OspC surface epitopes on the borrelial cell surface, priming-induced deflection of the secondary immune response or the selection *in vivo* of non-OspC-expressing *B. burgdorferi*. The results presented here have important implications for the design and implementation of an OspC-based Lyme disease vaccine. In view of the OspC-expression heterogeneity of infective *B. burgdorferi*, prophylactically vaccinated individuals may not counterattack infection successfully by activation of the anamnestic OspC immune response. Therefore, the traditional vaccine strategy that relies on the induction of the secondary antibody response to an infection may not be applicable to an OspC-based vaccine. Furthermore, these observations open new avenues for deliberation regarding the mechanisms of OspC expression and surface composition on *B. burgdorferi* during infection of non-immunized and immunized hosts.

REFERENCES

- Cox, D. L., Akins, D. R., Bourell, K. W., Lahdenne, P., Norgard, M. V. & Radolf, J. D. (1996). Limited surface exposure of *Borrelia burgdorferi* outer surface lipoproteins. *Proc Natl Acad Sci U S A* **93**, 7973–7978.
- de Silva, A. M., Telford, S. R., III, Brunet, L. R., Barthold, S. W. & Fikrig, E. (1996). *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. *J Exp Med* **183**, 271–275.
- Dolan, M. C., Maupin, G. O., Panella, N. A., Golde, W. T. & Piesman, J. (1997). Vector competence of *Ixodes scapularis*, *I. spinipalpis*, and *Dermacentor andersoni* (Acari: Ixodidae) in transmitting *Borrelia burgdorferi*, the etiologic agent of Lyme disease. *J Med Entomol* **34**, 128–135.
- Dressler, F., Whalen, J. A., Reinhardt, B. N. & Steere, A. C. (1993). Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* **167**, 392–400.
- Exner, M. M., Wu, X., Blanco, D. R., Miller, J. N. & Lovett, M. A. (2000). Protection elicited by native outer membrane protein Oms66 (p66) against host-adapted *Borrelia burgdorferi*: conformational nature of bactericidal epitopes. *Infect Immun* **68**, 2647–2654.
- Fikrig, E., Barthold, S. W., Kantor, F. S. & Flavell, R. A. (1990). Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. *Science* **250**, 553–556.
- Fikrig, E., Feng, W., Barthold, S. W., Telford, S. R., III & Flavell, R. A. (2000). Arthropod- and host-specific *Borrelia burgdorferi* *bbk32* expression and the inhibition of spirochete transmission. *J Immunol* **164**, 5344–5351.
- Gilmore, R. D., Jr & Mbow, M. L. (1999). Conformational nature of the *Borrelia burgdorferi* B31 outer surface protein C protective epitope. *Infect Immun* **67**, 5463–5469.
- Gilmore, R. D., Jr & Piesman, J. (2000). Inhibition of *Borrelia burgdorferi* migration from the midgut to the salivary glands following feeding by ticks on OspC-immunized mice. *Infect Immun* **68**, 411–414.
- Gilmore, R. D., Jr, Kappel, K. J., Dolan, M. C., Burkot, T. R. & Johnson, B. J. (1996). Outer surface protein C (OspC), but not P39, is a protective immunogen against a tick-transmitted *Borrelia burgdorferi* challenge: evidence for a conformational protective epitope in OspC. *Infect Immun* **64**, 2234–2239.
- Hagman, K. E., Lahdenne, P., Popova, T. G., Porcella, S. F., Akins, D. R., Radolf, J. D. & Norgard, M. V. (1998). Decorin-binding protein of *Borrelia burgdorferi* is encoded within a two-gene operon and is protective in the murine model of Lyme borreliosis. *Infect Immun* **66**, 2674–2683.
- Hanson, M. S., Cassatt, D. R., Guo, B. P., Patel, N. K., McCarthy, M. P., Dorward, D. W. & Hook, M. (1998). Active and passive immunity against *Borrelia burgdorferi* decorin binding protein A (DbpA) protects against infection. *Infect Immun* **66**, 2143–2153.
- Leuba-Garcia, S., Martinez, R. & Gern, L. (1998). Expression of outer surface proteins A and C of *Borrelia afzelii* in *Ixodes ricinus* ticks and in the skin of mice. *Zentbl Bakteriol* **287**, 475–484.
- Liang, F. T., Jacobs, M. B., Bowers, L. C. & Philipp, M. T. (2002). An immune evasion mechanism for spirochetal persistence in Lyme borreliosis. *J Exp Med* **195**, 415–422.
- Mbow, M. L., Gilmore, R. D., Jr & Titus, R. G. (1999). An OspC-specific monoclonal antibody passively protects mice from tick-transmitted infection by *Borrelia burgdorferi* B31. *Infect Immun* **67**, 5470–5472.
- Ohnishi, J., Piesman, J. & de Silva, A. M. (2001). Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. *Proc Natl Acad Sci U S A* **98**, 670–675.
- Padula, S. J., Dias, F., Sampieri, A., Craven, R. B. & Ryan, R. W. (1994). Use of recombinant OspC from *Borrelia burgdorferi* for serodiagnosis of early Lyme disease. *J Clin Microbiol* **32**, 1733–1738.
- Preac-Mursic, V., Wilske, B., Patsouris, E. & 7 other authors (1992). Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *B. burgdorferi* infection. *Infection* **20**, 342–349.
- Probert, W. S., Crawford, M. & LeFebvre, R. B. (1997). Antibodies to OspB prevent infection of C3H mice challenged with *Borrelia burgdorferi* isolates expressing truncated OspB antigens. *Vaccine* **15**, 15–19.
- Schaible, U. E., Kramer, M. D., Eichmann, K., Modolell, M., Museteanu, C. & Simon, M. M. (1990). Monoclonal antibodies specific for the outer surface protein A (OspA) of *Borrelia burgdorferi* prevent Lyme borreliosis in severe combined immunodeficiency (scid) mice. *Proc Natl Acad Sci U S A* **87**, 3768–3772.
- Schwan, T. G. & Piesman, J. (2000). Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. *J Clin Microbiol* **38**, 382–388.
- Schwan, T. G., Piesman, J., Golde, W. T., Dolan, M. C. & Rosa, P. A. (1995). Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad Sci U S A* **92**, 2909–2913.
- Sigal, L. H., Zahradnik, J. M., Lavin, P. & 16 other authors (1998). A vaccine consisting of recombinant *Borrelia burgdorferi* outer-surface protein A to prevent Lyme disease. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. *N Engl J Med* **339**, 216–222.
- Simon, M. M., Schaible, U. E., Kramer, M. D., Eckerskorn, C., Museteanu, C., Muller-Hermelink, H. K. & Wallich, R. (1991). Recombinant outer surface protein A from *Borrelia burgdorferi* induces antibodies protective against spirochetal infection in mice. *J Infect Dis* **164**, 123–132.
- Sinsky, R. J. & Piesman, J. (1989). Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. *J Clin Microbiol* **27**, 1723–1727.
- Steere, A. C., Sikand, V. K., Meurice, F. & 8 other authors (1998). Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. *N Engl J Med* **339**, 209–215.
- Zhong, W., Gern, L., Stehle, T., Museteanu, C., Kramer, M., Wallich, R. & Simon, M. M. (1999). Resolution of experimental and tick-borne *Borrelia burgdorferi* infection in mice by passive, but not active immunization using recombinant OspC. *Eur J Immunol* **29**, 946–957.
- Zhong, W., Stehle, T., Museteanu, C., Siebers, A., Gern, L., Kramer, M., Wallich, R. & Simon, M. M. (1997). Therapeutic passive vaccination against chronic Lyme disease in mice. *Proc Natl Acad Sci U S A* **94**, 12533–12538.