CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 2002, p. 919–920 Vol. 9, No. 4
1071-412X/02/$04.00+0 DOI: 10.1128/CDLI.9.4.919–920.2002

NOTES

In Vitro Assessment of Antiborrelial Activity of OspA Vaccine Sera

Paul T. Fawcett,[1,2]* Carlos D. Rose,[2,3] and Kathleen M. Gibney[1]

Immunology Laboratory, Department of Research,[1] and Division of Rheumatology, Department of Pediatrics,[3] A. I. duPont Hospital for Children, Wilmington, Delaware, and Department of Pediatrics, Thomas Jefferson University, Philadelphia, Pennsylvania[2]

Received 29 June 2001/Returned for modification 7 September 2001/Accepted 6 May 2002

Prevention of Lyme disease by the recombinant OspA-based vaccine reportedly works by preventing transmission of spirochetes from ticks to humans. We report on an in vitro microculture assay, which can be used to provide an indicator of the need for booster doses of vaccine.

To date, there is no commercially available assay for assessing whether a recipient of the recombinant OspA-based vaccine for Lyme disease possesses adequate antiborrelial activity to prevent transmission of *Borrelia burgdorferi* from ticks (7). Efficacy trials and marketing of the vaccine were recently suspended by the manufacturer; however, interest in sublicensing of the product and patents covering potential use of variants of the OspA lipoprotein suggest that a need for monitoring antiborrelial status of vaccine recipients will continue to be clinically relevant. We describe an in vitro microculture assay that can be easily established in and performed by any laboratory equipped to grow *B. burgdorferi* in culture. This method is similar to the borreliacidal and other in vitro assays for detecting antibodies with antiborrelial activity that have been previously reported (1, 8, 13). Unlike those assays, this method is intended solely as a means of determining the need for booster doses of vaccine to maintain efficacy. The design of our method was directed by the novel mechanism of intratick killing of *B. burgdorferi* by which this vaccine works (6). The implications of this mechanism on assay design include the following. (i) Use of high serum dilutions to provide an index of the titer is probably unnecessary, since the volume of blood entering a feeding tick greatly exceeds the volume of the tick's fluids in which the blood is "diluted." (ii) It has been reported that both the tick and the spirochete itself possess anticomplement activity (9, 14); therefore, an assay to assess antiborrelial factors resulting from vaccination with OspA should be capable of detecting complement-independent antiborrelial activity.

The in vitro assessment of antiborrelial activity was performed using a microculture system. The bacteria (*B. burgdorferi* ATCC strain B31) were grown to log phase in modified BSK-H medium (Sigma) at 29°C. Aliquots (150 μl) of the borrelia were then transferred to microculture wells in 48-well plates. An equal volume of test sera was added, and cultures were then incubated overnight at 29°C. Following incubation, samples from each culture were prepared as thin-film wet preps and examined microscopically using a 40× phase-contrast objective on a Zeiss axioplan photomicroscope.

Antiborrelial effects of serum were determined by scoring each culture for motility, aggregation, bleb formation, and lysis of spirochetes using a scale of 0 to 4, where 0 corresponds to uninfected, healthy appearance and 4 corresponds to extensive evidence of lysis, bleb formation, aggregation, or loss of motility. The scoring was assessed by comparison of blind readings by two individuals. Justification for the scoring system was based on results obtained for healthy, uninfected individuals and from testing of serial samples from patients with documented reinfection with *B. burgdorferi*. In a parallel study, aliquots of serum specimens from healthy, uninfected individuals and vaccine recipients were incubated at 56°C for 1 h to inactivate endogenous complement. Results showed that heat inactivation of complement decreased the degree of lysis observed in some samples but had no effect on aggregation, bleb formation, loss of motility, or overall score. Furthermore, inactivation of complement did not eliminate antiborrelial activity of any sample, a finding consistent with previous reports of non-complement-dependent borreliacidal antibodies (2, 10, 12, 13).

Serum samples obtained from 20 recipients of the recombinant OspA-based vaccine were tested by in vitro incubation with viable spirochetes (4). Specimens included serum samples obtained at time zero (before any doses of vaccine were given) (baseline values), 30 days following the first dose of vaccine, 30 days following the second dose of vaccine, 6 months following the second dose, 1 year following the first dose, and 30 days following the third dose of the vaccine. Results depicted in Table 1 show no samples positive at time zero and 17 of 20 samples positive at 30 days following the second dose. Antiborrelial activity was reduced in 6-month and 1-year follow-up samples, returning to high levels after the third dose of vaccine. Of particular note was the observation that although antiborrelial activity showed a tendency to increase with increasing titer of antibody (measured by a standard enzyme-linked immunosorbent assay [ELISA]), there was not a direct correspondence. Indeed, subject 4, who never seroconverted according to the results of ELISA and Western blot tests showed

* Corresponding author. Mailing address: Immunology Laboratory, A. I. duPont Hospital for Children, 1600 Rockland Rd., Wilmington, DE 19803. Phone: (302) 651-6776. Fax: (302) 651-6881. E-mail: pfawcett@nemours.org.

TABLE 1. In vitro antiborrelial activity of vaccine recipient sera

Subject	Antiborrelial score/ELISA titer at:		
	30 days after dose 2	6 mo after dose 2	30 days after dose 3
1	2/320	1/<80	3/5,120
2	3/2,560	2/160	3/10,240
3	3/1,280	2/160	4/10,240
4[a]	3/<80	0/<80	1/<80
5	3/640	0/80	4/10,240
6	0/320	0/<80	3/5,120
7	2/320	1/80	4/1,280
8	2/320	1/<80	3/1,280
9	1/320	2/80	3/2,560
10	3/320	0/<80	3/2,560
11	3/320	0/<80	3/5,120
12	1/160	2/<80	3/5,120
13	3/320	2/<80	3/2,560
14	3/160	1/<80	3/1,280
15	2/80	0/<80	2/640
16	2/160	0/80	3/1,280
17	2/160	1/<80	3/1,280
18	2/160	1/<80	3/2,560
19	2/160	2/80	2/1,280
20	3/160	2/<80	3/2,560

[a] All samples from this subject tested negative for antibodies to *B. burgdorferi.* The antiborrelial score at time zero (baseline) was 0.

significant antiborrelial activity in vitro (extensive agglutination) following the second dose of vaccine (3, 5).

Data obtained by testing serum samples from patients with documented reinfection with *B. burgdorferi* (11) are shown in Table 2. Seventeen serum specimens obtained from five patients were assessed for in vitro antiborrelial activity. All five patients had samples available from the time of first infection at follow-up (3 to 7 months after infection) and a sample acquired at the time when they were diagnosed as reinfected with *B. burgdorferi.* In addition, two patients had follow-up samples (1 and 16 months) obtained after resolution of their second infection. Results obtained showed that these patients had no or low levels of antiborrelial activity (score of 0 [three patients], 1 [one patient], and 2 [one patient]), and their levels of antiborrelial activity remained unchanged through follow-up (3 to 7 months). At the time of reinfection (8 months to 5 years), all of these patients had low levels of antiborrelial activity (for one patient, the original score of 2 had decreased to 0 at the time of reinfection).

TABLE 2. Antiborrelial activity of serial samples from patients reinfected with *B. burgdorferi*

Patient	Antiborrelial score/ELISA titer at:			
	Primary infection	Primary infection follow-up	Reinfection	Reinfection follow-up
1	2/640	2/160 (3 mo)	0/80 (8 mo)	
2	1/160	1/160 (3 mo)	2/640 (11 mo)	
3	0/640	0/160 (6 mo)	0/1,280 (5 yr)	
4	0/320	0/<80 (7 mo)	1/1,280 (2 yr 3 mo)	3/2,560[a] (1 mo)
5	0/80	0/80 (6 mo)	0/80 (9 mo)	0/80 (16 mo)

[a] This specimen was obtained immediately following treatment with antibiotics and may have contained residual levels of the antibiotics which contributed to the antiborrelial score.

Results obtained from this in vitro assessment of antiborrelial activity indicate that the assay may be clinically useful (if the LYMErix or another OspA-based vaccine is released for use). It is particularly noteworthy that this assay demonstrated a rapid decline in antiborrelial activity (by 6 months after the second dose) when tested on a small population, whereas the phase III trails of the vaccine required several years and thousands of patients to determine a need for decreasing the time between the second and third doses of vaccine.

REFERENCES

1. **Callister, S. M., R. F. Schell, L. C. L. Lim, D. A. Jobe, K. L. Case, G. L. Bryant, and P. E. Molling.** 1994. Detection of borreliacidal antibodies by flow cytometry. Arch. Intern. Med. **154:**1625–1632.
2. **Coleman, J. L., R. C. Rogers, and J. L. Benach.** 1992. Selection of an escape variant of *Borrelia burgdorferi* by use of bactericidal monoclonal antibodies to OspB. Infect. Immun. **60:**3098–3104.
3. **Fawcett, P. T., C. D. Rose, and K. M. Gibney.** 1995. Comparative evaluation of adsorption with *E. coli* on ELISA tests for Lyme borreliosis. J. Rheumatol. **22:**684–688.
4. **Fawcett, P. T., C. D. Rose, S. M. Budd, and K. M. Gibney.** 2001. Effect of immunization with recombinant OspA on serologic tests for Lyme borreliosis. Clin. Diagn. Lab. Immunol. **8:**79–84.
5. **Fawcett, P. T., K. M. Gibney, C. D. Rose, S. B. Dubbs, and R. A. Doughty.** 1998. Frequency and specificity of antibodies that cross-react with *Borrelia burgdorferi* antigens. J. Rheumatol. **19:**582–587.
6. **Fikrig, E., S. R. Telford, S. W. Barthold, et al.** 1992. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. Proc. Natl. Acad. Sci. USA **89:**5418–5421.
7. **Golde, W. T., J. Piesman, M. C. Dolan, M. Kramer, P. Hauser, Y. Lobet, C. Capiau, P. Desmons, P. Voet, D. Dearwester, and J. C. Frantz.** 1997. Reactivity with a specific epitope of outer surface protein A predicts protection from infection with the Lyme disease spirochete, *Borrelia burgdorferi*. Infect. Immun. **65:**882–889.
8. **Kraiczy, P., K.-P. Hunfeld, S. Peters, R. Wurzner, G. Acker, B. Wilske, and V. Brade.** 2000. Borreliacidal activity of early Lyme disease sera against complement-resistant *Borrelia afzelii* FEM1 wild-type and an OspC-lacking FEM1 variant. J. Med. Microbiol. **49:**917–928.
9. **Nowling, J. M., and M. T. Philipp.** 1999. Killing of *Borrelia burgdorferi* by antibody elicited by OspA vaccine is inefficient in the absence of complement. Infect. Immun. **67:**443–445.
10. **Pavia, C. S., V. Kissel, S. Bittker, F. Cabello, and S. Levine.** 1991. Antiborrelial activity of serum from rats injected with Lyme disease spirochete. J. Infect. Dis. **163:**656–659.
11. **Rose, C. D., P. T. Fawcett, J. D. Klein, S. C. Eppes, G. M. Caputo, and R. A. Doughty.** 1993. Reinfection in pediatric Lyme borreliosis. Ann. Rheum. Dis. **163:**695–696.
12. **Sadziene, A., M. Jonsson, S. Bergstrom, R. K. Bright, R. C. Kennedy, and A. G. Barbour.** 1994. A bactericidal antibody to *Borrelia burgdorferi* is directed against a variable region of the OspB protein. Infect. Immun. **62:**2037–2045.
13. **Sadziene, A., P. A. Thompson, and A. G. Barbour.** 1993. In vitro inhibition of *Borrelia burgdorferi* growth by antibodies. J. Infect. Dis. **167:**165–172.
14. **Stevenson, B., N. El-Hage, M. A. Hines, J. C. Miller, and K. Babb.** 2002. Differential binding of host complement inhibitor factor H by *Borrelia burgdorferi* Erp surface proteins: a possible mechanism underlying the expansive host range of Lyme disease spirochetes. Infect. Immun. **70:**491–497.