NOTES

Cobra Venom Factor Abrogates Passive Humoral Resistance to Syphilitic Infection in Hamsters

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Cobra venom factor, an agent commonly used to deplete complement, lowered the resistance of hamsters to infection with *Treponema pallidum* subsp. *endemicum*, as shown by a more rapid development of cutaneous lesions in infected animals treated with cobra venom factor than in infected, untreated animals. Cobra venom factor also abrogated the passive transfer of resistance by injection of serum from syphilitic immune hamsters. These results indicate that complement influences the pathogenesis of treponemal infection.

The pathogenicity of *Treponema pallidum* can be modified by syphilitic immune serum. Serum obtained from hamsters (2, 21) or rabbits (3, 11, 18, 24, 29, 30, 31, 33) resistant to syphilitic infection can confer protection on recipients challenged with virulent treponemes. When *T. pallidum* incubated in vitro with immune serum was inoculated into animals, the recipients did not develop syphilitic lesions (2, 4). Bishop and Miller (4, 5) and Turner (30) showed that the treponemicidal activity of immune serum was dependent upon heat-labile components. Complement apparently participated in this response, since heat-treated immune serum showed reduced treponemicidal activity.

Although the role of complement in humoral immunity to bacteria and fungi is well recognized (12, 15, 28), its involvement in the defense against syphilitic infection needs to be elucidated. Cobra venom factor (CVF), a decomplementing agent, has been used frequently to define the role of complement in microbial infections (1, 14, 16, 25). CVF combines with factor B to form a complex which cleaves and activates C3, leading to its depletion and, to a lesser extent, depletion of the terminal complement proteins C5 to C9 (6, 7). We therefore administered CVF and syphilitic immune serum to hamsters and subsequently challenged them with *T. pallidum* subsp. *endemicum*.

Inbred LSH/Ss Lak hamsters were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Hamsters weighing 80 to 100 g were housed, six per cage, at an ambient temperature of 18°C, a condition which facilitates the development of cutaneous lesions (13). Before treponemal infection the hamsters were shaved, and they were maintained free of hair by clipping twice a week.

T. pallidum subsp. *endemicum* (formerly *T. pallidum* Bosnia A) was originally isolated from a dark-field-positive chancre on the shaft of the penis of a 35-year-old patient who resided in Bosnia, Yugoslavia (32). The strain has been maintained by passage in hamsters. In our laboratory the inguinal lymph nodes of the hamsters were removed aseptically 3 to 4 weeks after intradermal infection, teased apart in sterile physiological saline, and filtered through 60-mesh stainless steel screens. After centrifugation at 270 × *g* for 3

min to remove cellular debris, the number of treponemes in the supernatant was determined by dark-field microscopy.

Hamsters were infected intradermally at two sites in the inguinal region with treponemes suspended in RPMI 1640 medium. Cutaneous lesions generally developed 2 to 3 weeks after inoculation with 1×10^5 to 5×10^5 organisms. Two other pathological changes, increases in the weight of inguinal lymph nodes and in the number of treponemes in the nodes, were also used to evaluate the host response to infection, as previously described (20, 22). The same criteria were used to evaluate the immune response of hamsters adoptively immunized with serum from syphilitic immune donors.

The approximate number of treponemes per lymph node was determined by the procedure described by Miller (17). Briefly, duplicate slides of each homogenized lymph node were prepared, and 120 fields per slide were examined for treponemes by dark-field microscopy. Some lymph nodes were centrifuged at $10,000 \times g$ for 10 min, suspended in 0.02 ml of RPMI 1640 medium, and examined by dark-field microscopy.

Immune serum was prepared from 60 hamsters infected intradermally at two sites in the inguinal region with an inoculum of 10^5 *T. pallidum* subsp. *endemicum* organisms. Ten to sixteen weeks after infection the hamsters were treated with penicillin (4,000 U) to terminate infection. After another 2 weeks, five of these hamsters were reinfected intradermally with *T. pallidum* subsp. *endemicum* and did not develop further syphilitic lesions. The remaining animals were bled by intracardiac puncture to obtain sera. Concurrently normal sera were collected from 55 hamsters 14 days after treatment with penicillin. Two weeks later these animals were infected with *T. pallidum* subsp. *endemicum*, and they developed characteristic lesions.

The pooled immune and pooled normal sera were sterilized by filtration (0.45 μm; Millipore Corp., Bedford, Mass.) and stored at $-20°C$ until use. The pooled immune serum had a microhemagglutination *T. pallidum* antibody titer of 10,240, whereas normal serum had no microhemagglutination *T. pallidum* antibody titer.

Recipients were injected intravenously with immune or normal serum (0.5 ml) at 3-day intervals for 3 or 4 weeks.

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TABLE 1. Effect of CVF on the ability of syphilitic immune serum to confer protection on hamsters challenged with *T. pallidum* subsp. *endemicum*[a]

Treatment group	Lesions			Lymph nodes	
	No./total sites	Development time (days)	Diameter (mm)	Wt (mg)	Treponemes ($\times 10^3$)
CVF					
Immune serum	9/10	16 ± 1	17 ± 1	33 ± 10	868 ± 68
Normal serum	10/10	15 ± 1	21 ± 1	42 ± 3	1,148 ± 76
Control (no serum)	6/6	14 ± 1	20 ± 1	51 ± 2	1,266 ± 112
No CVF					
Immune serum	0/10			12 ± 3	1 ± 1
Normal serum	10/10	16 ± 2	14 ± 1	24 ± 3	1,219 ± 98
Control (no serum)	6/6	17 ± 2	13 ± 1	28 ± 4	2,825 ± 177

[a] *T. pallidum* subsp. *endemicum* was inoculated at two sites in each hamster. Animals were sacrificed 10 days after the controls developed lesions. All values in columns 2 to 5 are means ± standard error (*n* = 3 or 5 hamsters per group). Cobra venom was administered once a week for 4 weeks. All results were tested by analysis of variance (26).

Three days after the first injection the hamsters were challenged intradermally in the inguinal region with 10^5 *T. pallidum* subsp. *endemicum* organisms.

Lyophilized CVF from *Naja haje* (Cordis Laboratories, Inc., Miami, Fla.) was reconstituted with phosphate-buffered saline. Depletion of complement was accomplished in groups of hamsters by intraperitoneal injection of 20 U of CVF every week for 4 weeks. Treatment reduced C3 levels by 32-fold or more (control, 1:128; CVF treated, 1:2 or 1:4) for at least 6 days after administration. The levels of C3 were determined by microprecipitation and immunodiffusion as described by Garvey et al. (9).

Treatment of hamsters with CVF could prevent the passive transfer of protection with syphilitic immune serum on normal recipients challenged with *T. pallidum* subsp. *endemicum*. Two groups of 10 hamsters each were injected intravenously with immune or normal serum (0.5 ml) at 3-day intervals for 4 weeks. Five hamsters from each group were also injected with CVF at weekly intervals for 4 weeks. Three days after the first injection of serum the hamsters were challenged intradermally with an inoculum of 2 × 10^5 *T. pallidum* subsp. *endemicum* organisms. Two groups of three hamsters each, with or without CVF treatment and receiving no serum, were also infected with *T. pallidum* subsp. *endemicum*. In all hamsters receiving immune serum but not CVF, lesions failed to develop, and the lymph node weights were significantly less (P ≤ 0.1) than those of controls (Table 1). Few treponemes were detected in lymph nodes. By contrast, lesions developed after 14 ± 1 days in all other groups of hamsters. The weights and numbers of treponemes in the lymph nodes were significantly increased (P ≤ 0.01) compared with those of hamsters receiving immune serum without CVF treatment. In addition, the progression of syphilitic lesions was accelerated significantly (P ≤ 0.01) in all groups treated with CVF compared with controls (Table 1). When these experiments were repeated, similar results were observed.

CVF produced two alterations in host resistance to treponemal challenge. First, it abrogated the ability of immune serum recipients to resist syphilitic infection. In the absence of CVF treatment, immune serum conferred considerable protection on recipients challenged with *T. pallidum* subsp. *endemicum*. In animals treated with CVF, however, cutaneous lesions developed, and the weights and numbers of treponemes in the lymph nodes did not differ significantly from those of the nonimmune controls. Second, CVF enhanced the cutaneous progression of syphilis in normal

animals. These results demonstrate that complement participates in the pathogenesis of treponemal infection and is necessary for development of an effective serum-mediated treponemicidal response.

Our results suggest that complement plays a crucial role in the prevention of syphilis. CVF activates and depletes C3 (7, 8), and C3b receptors are found on granulocytes and mononuclear phagocytes (10, 19, 23, 27, 34) that actively participate in destruction of pathogens. Depletion of complement could account for the enhanced progression of syphilitic lesions in CVF-treated animals. Recognition and engulfment of antibody-coated treponemes by phagocytes utilizing the C3b receptor would be reduced.

Additional studies are needed to define the activity of individual components of complement and its receptor during elimination of the treponemes.

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