INFECTION AND IMMUNITY, Feb. 1996, p. 677–679 Vol. 64, No. 2
0019-9567/96/$04.00+0

Immunogenicity of Group B *Streptococcus* Type III Polysaccharide-Tetanus Toxoid Vaccine in Baboons

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Received 8 September 1995/Returned for modification 31 October 1995/Accepted 28 November 1995

Maternal vaccination has been proposed as a rational approach for the prevention of neonatal group B streptococcal (GBS) disease. In this study, baboons were used as a nonhuman primate model to evaluate the immunogenicity of a GBS type III glycoconjugate vaccine. Type III-specific immunoglobulin G with opsonic activity was induced after vaccination with type III polysaccharide coupled to tetanus toxoid administered with an aluminum adjuvant. This suggests that baboons could be used in evaluating maternal transfer of GBS-specific antibodies by vaccination during pregnancy.

Serious infections of peripartum women and their newborns are most often caused by organisms belonging to Lancefield's group B streptococci (GBS). Low levels of maternal antibodies to the capsular polysaccharide (CPS) of GBS have been correlated with greatly enhanced susceptibility of neonates to invasive disease (4). Since GBS is transmitted from the infected mother to the child, vaccination of pregnant women or women of childbearing age has been proposed as a way of transmitting protective antibodies to the neonate (2, 3, 5, 17). The first GBS vaccines evaluated clinically consisted of purified CPS and were variably immunogenic in adults (1) and pregnant women (7) with low preexisting levels of CPS antibodies. GBS CPS covalently coupled to a protein carrier had markedly greater immunogenicity in rabbits and mice than did uncoupled CPS (11–13, 15, 20–22). Moreover, GBS CPS-protein conjugate vaccines elicited in mice serotype-specific antibodies that cross the placenta and protect litters against GBS challenge (12, 13). In this study, we sought to establish the safety and immunogenicity of GBS CPS-protein conjugate vaccines in baboons (*Papio* spp.) as a prelude to studies of maternal antibody transfer in this nonhuman primate model.

The GBS type III CPS-tetanus toxoid conjugate vaccine (III-TT) was prepared and analyzed by published methods (20). Purified GBS type III CPS (peak M_r = 120,000) was treated with sodium periodate to create aldehydes on approximately 25% of the CPS's sialic acid residues. Oxidized type III CPS was covalently coupled to monomeric tetanus toxoid (TT) (kindly provided by AMVAX, Inc., Beltsville, Md.) by reductive amination with sodium cyanoborohydride. The purified III-TT conjugate was composed of 61% (wt/wt) protein and 39% (wt/wt) carbohydrate. Doses are given as the weight of CPS, the active component of the conjugate.

Eight groups, each with three baboons, were vaccinated intramuscularly with 0.5 ml of uncoupled type III CPS, III-TT, or uncoupled TT by using the doses and schedule shown in Table 1. Of the six groups that received III-TT, three groups received III-TT mixed 1:1 with 1.3% Alhydrogel (AlOOH; Superfos Biosector a/s, Vedbaek, Denmark) as an adjuvant. Alhydrogel was chosen for this study because aluminum adjuvants are currently the only ones included in vaccines licensed by the U.S. Food and Drug Administration for use in humans (9). In addition, this adjuvant has been used successfully with GBS glycoconjugates evaluated in mice (13, 21). Blood samples were taken from each baboon before vaccination and at 4, 8, 16, 25, 27, 29, 33, and 52 weeks after the primary dose. Serum was aliquoted and stored at −85°C.

Geometric mean antibody concentration (GMAC) specific to the type III polysaccharide was determined for each serum sample with a type III radioactive antigen binding assay (6). Type III-specific GMACs rose only in baboons that received III-TT with AlOOH (Table 1). The type III-specific GMAC in animals receiving a single 50-μg dose of III-TT with AlOOH increased over a 4-week period from a preimmunization level of 0.6 μg/ml to 5.8 μg/ml (Table 1). Although 1 year later the GMAC in this group decreased to 1.3 μg/ml, the GMAC remained over twofold greater than in baboons receiving III-TT vaccine without AlOOH (Table 1). Animals that received one or two additional doses of III-TT with AlOOH 2 and 6 months after the primary dose exhibited elevated type III antibody concentrations in response to the booster following each vaccination. Peak type III-specific GMACs in animals before vaccination and after each of the two booster doses of 50 μg as CPS of III-TT with AlOOH were 0.6, 9.4, and 29.8 μg/ml, respectively (Table 1). The magnitude and decay rate of the GMAC were similar for animals that received a 10- or a 50-μg dose of III-TT with AlOOH. Groups receiving III-TT without AlOOH, uncoupled type III CPS, or uncoupled TT failed to mount a type III-specific antibody response. These data suggest that in baboons AlOOH was necessary to elicit a response to III-TT and that primary and secondary immune responses could be increased with booster doses of vaccine. Rapid decay of type III-specific antibody is consistent with what occurs with

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TABLE 1. GBS type III-specific antibody response in baboons

Vaccine	Dose (μg)[a] (no. of doses)	Type III-specific GMAC (μg/ml) (range) at week:								
		0[b]	4	8[b]	16	25[b]	27	29	33	52
III-TT	50 (1)	0.6 (0.6–0.7)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.6 (0.6–0.7)	0.7 (0.6–0.8)	0.6 (0.6–0.6)	0.6 (0.6–0.6)
III-TT + Al[e]	50 (1)	0.6 (0.5–0.7)	5.8 (1.6–54.9)	4.6 (1.8–21.0)	2.9 (2.1–5.2)	2.3 (1.4–4.7)	2.0 (1.3–3.9)	2.0 (1.2–3.9)	1.8 (1.1–3.6)	1.3 (1.0–1.9)
III-TT	10 (3)	0.7 (0.6–0.8)	0.7 (0.7–0.7)	0.6 (0.6–0.7)	0.6 (0.5, 0.8)[d]	0.6 (0.6, 0.6)[d]	1.5 (1.3, 1.8)[d]	0.8 (0.8, 0.8)[d]	0.7 (0.6, 0.9)[d]	0.8 (0.6, 1.1)[d]
III-TT + Al[e]	10 (3)	0.7 (0.5–1.0)	3.3 (2.4–4.9)	2.9 (1.9–4.2)	13.4 (4.9–23.2)	4.0 (1.5–8.2)	23.9 (8.4–62.6)	29.8 (10.6–72.4)	19.0 (8.9–32.7)	3.2 (1.9–4.8)
III-TT	50 (3)	0.6 (0.6–0.7)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.6 (0.5–0.7)	0.6 (0.6–0.6)
III-TT + Al[e]	50 (3)	0.6 (0.5–0.9)	3.9 (1.4–15.6)	2.8 (1.1–8.0)	9.4 (3.9–17.6)	3.7 (2.6–5.4)	29.8 (15.3–60.0)	24.1 (12.5–38.2)	10.7 (6.0–15.8)	2.8 (1.5–4.8)
III CPS	50 (1)	0.6 (0.6–0.7)	0.6 (0.6–0.7)	0.6 (0.5–0.7)	0.4 (0.3–0.6)	0.5 (0.3–0.8)	0.5 (0.4–0.7)	0.6 (0.4–0.8)	0.5 (0.5–0.6)	0.6 (0.5–0.6)
TT	50[e] (3)	0.8 (0.6–1.0)	1.0 (0.6–1.7)	0.9 (0.6–1.4)	0.9 (0.5–1.3)	0.9 (0.6–1.3)	1.0 (0.6–1.5)	1.0 (0.6–1.6)	0.9 (0.5–1.4)	1.0 (0.6–1.4)

[a] Amount of CPS.
[b] Vaccination was done (0.5 ml of vaccine, intramuscularly).
[c] Al, aluminum hydroxide gel.
[d] One animal died. Values are the average for two animals and the value obtained for each animal.
[e] Amount of protein.

FIG. 1. In vitro opsonophagocytic killing of GBS strain M781. Opsonic activity of preimmunization baboon serum (Pre) and IgG and IgM separated from serum of baboons immunized with 10 μg (baboon 4626) and 50 μg (baboon 8653) of III-TT with AlOOH was tested. Rabbit antiserum (III-TT) to III-TT vaccine served as the positive control. The average (bars) and each of duplicate determinations (closed circles) of the $\log_{10}$ reduction in GBS CFU are shown.

other antigens administered to this species in the presence of aluminum adjuvant (16).

The functional activity of baboon serum obtained before and 27 weeks after vaccination with 10 μg of III-TT with AlOOH was evaluated by an in vitro opsonophagocytic assay (8). Human peripheral blood leukocytes (0.3 ml of a mixture containing 4.3×10^7 cells per ml) were combined with 0.05 ml of 6.1×10^6 CFU of GBS strain M781 per ml, 10% human complement (normal human serum adsorbed with strain M781 cells), and 10% pooled baboon serum (diluted 1:10) in a total volume of 0.5 ml with modified Eagle's medium. Quantitative cultures were performed immediately and after 60 min of incubation at 37°C with end-over-end mixing. Immune serum obtained at week 27 mediated a >2.0 $\log_{10}$ reduction in GBS CFU, whereas preimmunization baboon serum mediated only a 0.67 $\log_{10}$ CFU reduction. In this experiment, rabbit serum (diluted 1:10) raised against III-TT vaccine (20), used as the positive control, mediated the reduction of 1.89 $\log_{10}$ CFU.

The relative opsonic activities of immunoglobulin G (IgG) and IgM fractions of the baboon serum in the presence of human complement were determined. Separation of IgG and IgM from serum (0.5 ml) obtained at week 29 from representative animals from the group receiving 50 μg of III-TT with AlOOH (baboon 4626) and the group receiving 10 μg of III-TT with AlOOH (baboon 8653) was accomplished by using a protein A-agarose affinity column (14). A GBS type III CPS enzyme-linked immunosorbent assay using two murine monoclonal IgG antibodies (each at 5 μg/ml), one for baboon IgG (γ-chain specific) (18) and the other for IgM (μ-chain specific) (19), and goat anti-mouse IgG–alkaline phosphatase conjugate (diluted 1:1,000) confirmed protein A affinity separation of baboon IgG from IgM. Fractions were adjusted to equal volumes of 3.0 ml with phosphate-buffered saline for normalization of each Ig on the basis of the original serum volume from which the fractions were purified. The relative amounts of GBS

type III-specific IgG and IgM in the serum of baboon 8653 were determined by use of a type III radioactive antigen binding assay (23). A standard curve was generated by using a pool of human serum with a known concentration of type III-specific antibody (10). The IgG fraction contained 4.1 μg of type III-specific antibody per ml, whereas there was no binding in the IgM fraction. IgG isolated from immune serum from baboons 4626 and 8653 mediated the killing of >1.2 $\log_{10}$ GBS CFU (Fig. 1); IgM was nonopsonic (<0.2 $\log_{10}$ CFU reduction). Therefore, immunization of baboons with III-TT vaccine with AlOOH induced opsonically active IgG, a result consistent with that obtained with rabbits and mice vaccinated with III-TT with an adjuvant (14, 20).

In summary, baboons produced type III CPS-specific antibody in response to vaccination with III-TT vaccine in the presence of AlOOH. The magnitude of the type III-specific antibody response increased following each of two booster doses. Immune baboon serum contained serotype-specific and opsonically active IgG. These results establish the utility of the baboon as a nonhuman primate model for evaluating maternal transfer of polysaccharide-specific antibodies induced by GBS glycoconjugate vaccines.

This work was accomplished with the technical help of April Blodgett, Julieanne Pinel, and Kenneth D. Johnson. Michael H. Shearer provided the monoclonal antibodies used in this study.

This work was supported by NIH-NIAID contracts AI-25152 and AI-35156.

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Editor: V. A. Fischetti