Pharmacokinetic and Pharmacodynamic Analysis of a Human Immunoglobulin M Monoclonal Antibody in Neonatal *Macaca fascicularis*

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ABSTRACT. We have developed a human MAb that opsonizes group B streptococci, the major cause of gram-positive bacterial sepsis in newborns. It is an IgM class human MAb that possesses unique protective activity against experimental infections caused by the predominant group B capsule serotypes III and I. Preliminary preclinical studies with the IgM human MAb were designed to provide initial information useful for predicting its safety and pharmacokinetic properties. Two neonatal *Macaca fascicularis* monkeys were infused with the human MAb at either 17.8 or 230 mg/kg. Safety was evaluated by visually monitoring postinfusion clinical status and by standard clinical chemistry analyses and quantitative hematology on blood samples collected for 30 d. The serum antibody levels were determined by ELISA and antibody functional activity in serum samples by opsonophagocytic assays. The IgM human MAb appeared safe (normal laboratory values and clinical status) with a half-life of 2.5 d, a period compatible with the 5-d half-life reported for human IgM in adult serum. In addition, the human MAb retained functional opsonic activity for at least 30 d. Human MAb may offer a safe alternative for treating severe bacterial infections. (*Pediatr Res* 29: 310-314, 1991)

Abbreviations

GBS, group B streptococci
PK, pharmacokinetic
PD, pharmacodynamics
anti-Id, antiidiotype

Passive immunotherapy with MAb may provide a superior alternative or adjunctive treatment modality to standard antibiotic treatment of bacterial infections. Although several murine antitumor MAb have been administered to humans (1), only one human and one murine MAb, both against the core region of gram-negative bacterial endotoxin, have been similarly tested (2). Despite the recent development and clinical testing of mouse MAb, it is apparent that human MAb offer particular advantages (3), and may be preferred. For example, human MAb should elicit, at most, a minimal host response and possess extended half-lives. Before MAb of any origin can be considered for clinical use, their safety must be established. The likelihood that human MAb will be safe is supported by extensive clinical experience with i.v. administered human Ig-based formulations. In adults and neonates, these products have not elicited significant negative side effects and therefore appear safe for general use (4, 5).

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Neonatal infections caused by GBS and *Escherichia coli* K1 still have unacceptably high mortality and morbidity rates (6). To supplement current antibiotic therapy, we have developed human MAb with immunotherapeutic potential. Human IgM MAb produced against the group carbohydrate of GBS or the *E. coli* K1 capsule were found to protect against lethal infections in animal models (7, 8). Although administration of human MAb does not have obvious risks, it is still necessary to insure the safety of individual MAb and the methods used for their preparation. Moreover, because these MAb are intended for treatment of infections in neonates, exploring their safety is of paramount importance.

Before initiating formal preclinical safety and PK studies, we performed a preliminary study using two nonhuman primate neonates. The data indicate the GBS MAb is safe without apparent toxicity for the major organ systems or impairment of bone marrow function. Further, in neonatal monkeys the IgM MAb has a half-life of approximately 2.5 d and maintains commensurate opsonic activity.

MATERIALS AND METHODS

Animals. Less than 7-d-old male and female monkeys (*M. fascicularis*) were obtained from and were housed by the Regional Primate Research Center at the University of Washington, Seattle. The animals were housed in individual isolettes in a room designated for newborn monkeys and separated from all other animals. One male (T89001) weighing 550 g and one female monkey (T89008) weighing 416 g were administered antibody at 5 d of age. These particular animals had been rejected by their mothers and were therefore bottle fed before and throughout the study. The Primate Center conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Human MAb. A human IgM MAb (4B9) against the group B polysaccharide of GBS was derived by Epstein Barr virus transformation of normal B cells (9). Antibody was purified from nutrient-exhausted culture supernatant by affinity chromatography on a murine antihuman IgM column (7). Concentrated culture supernatant was passed over a Sepharose 4B-murine MAb (Genetic Systems Corp., Seattle, WA) antihuman IgM column (CNBr-activated Sepharose). Bound antibody was eluted with 2 M MgCl₂ in PBS. Purified antibody preparations were dialyzed against 10 mM phosphate in physiologic saline, filter sterilized, and assayed for pyrogen using the Limulus Amebocyte assay QCL-100 (M. A. Bioproducts, Walkersville, MD). Purity was examined by SDS-PAGE of reduced samples followed by silver nitrate staining (10, 11). Antibody activity and integrity were determined using an antigen-binding ELISA (8).

Protocol. The antibody was administered via an i.v. catheter placed into an arm vein by percutaneous puncture. The catheter

was attached to an infusion pump calibrated to deliver the antibody over 15 min (0.67 mg/min for monkey T89001 and 6.67 mg/min for monkey T89008). To achieve doses of 20 and 200 mg/kg, monkey T89001 received approximately 10 mL of MAb at 1.0 mg/mL, and monkey T89008 approximately 13 mL of MAb at 7.7 mg/mL, respectively. Based on their weights shortly before injection, the actual antibody doses administered to the monkeys were 17.8 mg/kg (monkey T89001) and 230 mg/kg (monkey T89008). Blood samples drawn from the femoral artery provided serum for PK and functional opsonic assays (0.5 mL, no anticoagulant), and plasma and white blood cells for safety studies (1.0 mL in citrate). Samples for PK determinations were obtained according to the following schedule: before injection, at 5, 15, 30, and 60 min, at 6, and 24 h, and then at 2, 3, 4, 7, 11, 15, 20, 25, and 30 d. These samples were clotted on ice for 2–3 h and the serum collected after centrifugation, divided into three aliquots, and frozen at -70°C . Blood for safety determinations was obtained before injection and at approximately 7, 15, 25, and 30 d for clinical chemistry and at 15 and 30 d for hematology analyses. Quantitative hematology (total white blood cells and differentials) was performed at the Regional Primate Center and clinical chemistry analyses at the Children's Hospital and Medical Center (Seattle, WA) clinical chemistry laboratory.

PK assay. A PK assay was developed using a MAb anti-Id ELISA-based capture format. An antihuman MAb 4B9 idiotype-specific murine MAb was produced by fusing spleen cells from Balb/C mice immunized with the human MAb to murine myeloma cells (P3X63Ag8, 12). The anti-Id used in the PK assays reacted only with the immunizing IgM antibody, and not other human IgM MAb or the IgM fraction of pooled normal human serum (ICN ImmunoBiologicals, Lisle, IL), suggesting that this anti-Id did not detect a cross-reactive idiotype determinant present in other human antibodies. The multivalency of the IgM MAb was exploited by using one anti-Id MAb as both the plastic-bound capture antibody and the fluid-phase biotinylated signal source. For the assay, microtiter plate wells were coated overnight at 4°C with 100 μL of anti-Id (8F2, IgG_{2a}) at 2.0 $\mu\text{g}/\text{mL}$ in carbonate-bicarbonate buffer. After washing and blocking (5% nonfat dry milk solution in 0.02% Tween/PBS) diluted control and serum samples were added to the wells followed by incubation at room temperature for 60 min. After an additional wash step, microtiter wells received biotinylated anti-Id (100 $\mu\text{L}/\text{well}$) for 45 min at room temperature, were washed, and were treated with Vectastain (Vector Labs, Burlingame, CA) for an additional 20 min (13). After washing and substrate addition, the assay was completed as previously described. Antibody half-life was calculated from the PK assay data using a computer software program (PKCALC, 14).

Opsonophagocytic assay. The functional activity or "pharmacodynamics" of the antibody in serum samples was quantitated

Fig. 1. PK activity of a human MAb (17.8 mg/kg) administered to a neonatal monkey. Insert depicts antibody PK during first 6 h postinjection. Human MAb concentrations were determined using an anti-Id MAb capture ELISA.

following a protocol described previously (8). MAb-mediated opsonic activity in monkey T89008 serum samples was assayed against two GBS (capsule serotype III) clinical isolates (COH 1 and type IIIR, provided by Drs. C. Rubens and H. Hill, University of Utah School of Medicine, Salt Lake City, UT, respectively, (15, 16). Serum samples were diluted 1:1000 in PBS to dilute out opsonic activity detected in prebleed sample. For the assay, duplicate tubes were prepared containing in 500 μL 250 μL of diluted serum or MAb, 50 μL of freshly isolated human neutrophils ($5 \times 10^7/\text{mL}$), 75 μL human serum as a complement source, 100 μL of the GBS strains ($4 \times 10^2 \text{ CFU}/\text{mL}$), and 25 μL of Hanks' Balanced salt solution containing 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid and 0.1% gelatin. The bacteria were first incubated with antibody or serum for 30 min at 37°C , followed by the addition of the other assay components. The entire mixture was rotated for 60 min at 37°C . Negative controls were prebleed serum samples for postinjection serum samples and reaction mixtures lacking complement (heat-inactivated FCS substituted for complement) for MAb experiments. In other experiments (data not shown), substituting a negative control human MAb, omitting the neutrophils, or omitting the complement all gave very similar negative control values. Therefore, in these experiments, only the complement control was used. For the serum samples and the MAb, the data are reported as follows: $100 \times [1 - (\text{CFU remaining after incubation with neutrophils, complement, and postinjection serum sam-})]$

Fig. 2. PK activity of a human MAb (230 mg/kg) administered to a neonatal monkey. Insert depicts antibody PK during first 6 h postinjection. Human MAb concentrations were determined as described in Figure 1.

Fig. 3. PK activity of human MAb administered to neonatal monkey. Sera from T89008 monkey (230 mg/kg) were tested for complement-dependent opsonic activity against two capsule serotypes III human neonatal clinical isolates (COH 1, open circles; type IIIR, open squares). All serum samples were diluted 1:1000 for the assay and combined with human neutrophils and complement.

Fig. 4. Opsonophagocytic activity of human MAb administered to neonatal monkeys. Human MAb 4B9 was titrated in an opsonophagocytic assay against two clinical isolates (COH 1, open circles; type IIIR, open squares).

ples)/(CFU remaining after incubation with neutrophils, complement, and prebleed serum)] or $100 \times [1 - (\text{CFU remaining after incubation with neutrophils, complement, and MAb}) / (\text{CFU remaining after incubation with neutrophils, FCS, and MAb})]$.

RESULTS

PK. Affinity column purified antibody was shown by SDS-PAGE to be essentially free of protein contaminants (data not shown) and to contain 0.26 ng endotoxin/8.5 mg MAb (30 pg/mg). Five-d-old cynomolgous (*M. fascicularis*) monkeys were injected i.v. with either 17.8 (T89001) or 230 mg/kg (T89008) of MAb 4B9. Blood samples were drawn starting 5 min after infusion for 30 d. The MAb concentration in serum was quantitated using an anti-Id-based capture ELISA. Because IgM antibodies are multivalent, it was found that the same murine anti-Id MAb could function as both the capture antibody and the signal antibody (biotinylated). By limiting the capture antibody, the idiotopes on each 4B9 IgM molecule were not saturated, thereby allowing signal anti-Id MAb to bind the same IgM molecules. The assay is sensitive to 1 ng MAb/mL and is not affected by <10% monkey or human serum.

Using this assay, it appeared that in high and low dose monkeys

the human MAb was affected by an initial 6-h clearance and equilibration phase where the antibody concentration fell by 30–40% (Figs. 1 and 2, insets) followed by a more prolonged, gradual decrease. From these studies, it was shown that the human MAb had a 2.5-d half-life in monkey T89001 (Fig. 1) and a 2.4 d half-life in monkey T89008 (Fig. 2).

Opsonic activity. Although the physical presence of antibody was detected in serum by the PK assay, an equally important question is whether the antibody retains opsonic or functional activity during this time. Antibody opsonic activity was quantitated in the serum samples obtained from monkey T89008. Serum from each time point was diluted 1:1000 and used in opsonic assays against two serotype III GBS clinical isolates (COH 1 and type IIIR). The data show that the pharmacodynamics of the MAb differ depending on the clinical isolate (Fig. 3). Strain COH 1 appears to require less antibody to be effectively opsonized than does strain type IIIR. This observation is reflected in control opsonization experiments when the same antibody administered to the monkeys was tested against these strains (Fig. 4). Approximately four times more MAb was necessary to provide 50% of the maximal opsonic activity (80% killing) against strain type IIIR than was required against strain COH 1 (80 versus 20 ng/mL, respectively). Because the relative opsonic activity of control and *in vivo* circulating MAb was the same, these data suggest that the physical detection of the MAb (PK) is reflected by its functional opsonic activity.

Safety parameters. The safety of the MAb was assessed by observing clinical signs after injection and by monitoring a number of clinical chemistry and hematology parameters. During and after MAb administration the monkeys did not demonstrate any behavioral changes that would suggest discomfort, pain, or trauma. Their general activity and feeding levels remained unchanged throughout the study. Blood samples for these studies were obtained before injection and then at approximately 7- or 15-d intervals. Values for kidney function (creatinine and blood urea nitrogen) and liver enzymes (serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase) were within normal limits throughout the study, except for the prebleed and 7-d bleed for monkey T89001, which had slightly elevated SGOT levels due to hemolysis of the sample (Table 1). Extensive evaluation of hematology parameters also shows that there was little fluctuation from the prebleed values, suggesting the MAb did not adversely affect circulating cells or bone marrow function (Table 2).

Table 1. Serum chemistries of newborn monkeys receiving human MAb*

Monkey	Days postinfusion	Assays			
		BUN (mg/dL) (8–26)†	Creatinine (mg/dL) (0.7–1.5)†	SGOT (IU/L) (8–74)†	SGPT (IU/L) (5–60)†
T89001‡	Preinjection	4	0.7	128	13
	7§	4	0.6	99	<3
	15	6	0.4	47	11
	25	6	0.3	38	8
	30	7	0.4	38	19
T89008	Preinjection	<2	0.6	50	25
	11	2	0.4	49	3
	15	4	0.3	40	6
	25	5	0.3	19	12
	29	13	0.3	33	19

* BUN, blood urea nitrogen; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

† Normal range for newborn monkey.

‡ Low-dose monkey (20 mg/kg).

§ Hemolyzed sample.

|| High-dose monkey (200 mg/kg).

Table 2. Quantitative hematologic evaluation of newborn monkeys receiving human MAb

Test	T89001 (d post-infusion)			T89008 (d post-infusion)		
	Pre	15	30	Pre	15	30
Hb (g%)	15.4	12.2	14.6	12.0	12.6	12.0
Hematocrit (vol%)	46.8	38.7	45.0	37.5	40.2	43.5
Red blood cell count ($\times 10^6/\text{mm}^3$)	6.4	5.6	5.7	5.0	5.4	5.2
Mean corpuscular Hb (μg)	24.1	21.9	23.0	24.0	23.2	24.0
Mean corpuscular vol (μ^3)	74	71	71	76	75	72
Platelet count	Nm*	Nm	Nm	Nm	Nm	Nm
Capillary clot time	Nm	Nm	Nm	Nm	Nm	Nm
White blood cell count ($\times 10^3/\text{mm}^3$)	8.2	7.9	8.1	8.3	6.8	7.3
Neutrophils	33	20	27	33	38	35
Lymphocytes	67	78	73	62	59	65
Monocytes	0	2	0	2	2	0
Eosinophils	0	0	0	3	1	0
Red blood cell morphology	Nm	Nm	Nm	Nm	Nm	Nm

* Nm, within normal limits.

DISCUSSION

These studies report the first safety and half-life data in which neonatal monkeys were administered a human IgM MAb. Two 5-d-old cynomolgous (*M. fascicularis*) monkeys were infused with either 17.8 or 230 mg/kg of an anti-GBS HuMAb. Both doses were well tolerated based on clinical observation and safety parameters. No apparent changes from prebleed values occurred in serum levels of liver transaminases, in serum creatinine clearance by the kidneys, or in hematologic parameters. The circulating half-life of the MAb was directly quantified using an anti-Id capture ELISA was found to be approximately 2.5 d regardless of the antibody dosage. The integrity and activity of antibody Fc regions was not determined by the anti-Id assay. To explore whether the IgM MAb retained functional activity, the same monkey serum samples were tested for opsonic activity in an *in vitro* assay. Our results show that after 30 d of *in vivo* circulation the antibody still maintains opsonic activity. Moreover, in control experiments comparing the same MAb administered to the monkeys with MAb in the serum samples, it was shown that they both had similar relative activities against the two GBS clinical isolates, further suggesting the antibody was stable after long-term *in vivo* passage.

One particular focus of these studies was the half-life of an IgM MAb in neonatal monkeys. At birth there is little or no IgM present in the neonate because IgM antibodies are not transplacentally passed to the fetus. Therefore, the only data on the half-life of human IgM antibodies are from studies in juveniles and adults, indicating that the average half-life of IgM antibodies is approximately 5 d (17). The finding that the GBS MAb has a half-life of approximately 2.5 d in cynomolgous monkeys is encouraging, and leads us to anticipate that the half-life will be no less in human neonates. A 2- to 3-d half-life should avoid the need for a particularly high antibody dosage to maintain a clinically efficacious concentration of circulating antibody.

The half-life directly relates to predicting the dosage necessary to manifest clinical efficacy. An average anticipated dosage of 20 mg/kg in human neonates is based on body weight extrapolations from studies in a neonatal rat infection model (8). This dosage was administered to monkey T89001 (17.8 mg/kg) and approximately 10 times this amount to monkey T89008 (230 mg/kg). Based on data obtained from opsonophagocytic experiments performed with serum from the high-dose monkey and MAb from the same lot, it seems likely that even in the monkey

receiving 17.8 mg/kg, an adequate concentration of MAb remained after 25 d to opsonize even the more resistant GBS strain (type IIIR).

Because human MAb have not previously been administered to neonatal monkeys or humans, the only relevant safety studies are with i.v. Ig products intended for use in preventing and/or treating neonatal bacterial infections. Studies in human neonates have shown that doses up to 750 mg/kg are safe in this patient population, and preliminary studies indicate that these products may possess antiinfective potential (5, 18). The safety in neonates of a formulation containing 12% IgM Ig by weight has also been shown (19). However, the use of i.v. Ig formulations may not be without its risks. To maintain elevated serum levels of anti-GBS antibodies, administration of 500 mg/kg i.v. Ig was required (5). At this dosage, the possibility arises of increasing the catabolic rate of IgG and perhaps reducing IgG titers of specific opsonic antibodies (20). In addition, in experimental GBS infection studies, high doses of i.v. Ig administered alone (21) or in conjunction with antibiotics resulted in less therapeutic efficacy compared with control treatments (22). These studies suggest that high doses of i.v. Ig may cause phagocytic cell receptor blockade or other immunoinhibitory activity. By using substantially smaller doses of human MAb, we hope to avoid these potential problems and gain the advantage of administering standardized dosages of opsonic antibodies.

We began this effort by administering one human IgM MAb to healthy, neonatal nonhuman primates. The apparent safety of this human IgM in neonatal monkeys encouraged us to proceed with developing the anti-GBS MAb for additional pre-clinical safety and PK studies.

REFERENCES

- Wettenhorff M, Iliopoulos D, Tempero M, Kay D, DeFreitas E, Koprowski H, Herlyn D 1989 Idiotype cascades in cancer patients treated with monoclonal antibody CO17-1A. *Proc Natl Acad Sci USA* 86:3787-3791
- Harkonen S, Scannon P, Mischak R, Spitler L, Foxall C, Kennedy D, Greenberg R 1988 Phase I study of a murine monoclonal anti-lipid A antibody in bacteremic and nonbacteremic patients. *Antimicrob Agents Chemother* 32:710-716
- Glassy M, Dillman R 1988 Molecular biotherapy with human monoclonal antibodies. *Mol Biother* 1:7-12
- Fischer G, Weisman L, Hemming B, London W, Hunter D, Bosworth J 1984 Intravenous immunoglobulin in neonatal group b streptococcal disease. Pharmacokinetic and safety studies in monkeys and humans. *Am J Med* 75:117-123
- Chirico G, Rondini G, Plebani A, Chiara A, Massa M, Ugazio A 1987 Intravenous gamma globulin therapy for prophylaxis of infection in high-risk neonates. *J Peds* 110:437-442
- Baker C 1986 Group B streptococcal infections in newborns. *N Engl J Med* 314:1702-1705
- Raff H, Devereux D, Shuford W, Abbott-Brown D, Maloney G 1988 Human monoclonal antibody with protective activity against *E. coli* K1 and *Neisseria meningitidis* group B infection. *J Infect Dis* 157:118-125
- Raff H, Siscoe P, Wolff E, Maloney G, Shuford W 1988 Human monoclonal antibodies to group B *Streptococcus*: reactivity and *in vivo* protection against multiple serotypes. *J Exptl Med* 1988:905-917
- Siadak A, Lostrom M 1985 Cell driven viral transformation. In: EG Engleman, SKH Fount, J Larrick, A Raubitschek (eds) *Human Hybridomas and Monoclonal Antibodies*. Plenum Publishing, New York, pp 167-185
- Haekeshoven J, Dernick R 1985 Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. *Electrophoresis* 6:103-108
- Laemmli U 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-683
- Kolher G, Milstein C 1975 Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495-497
- Shively J, Wagener C, Clark B 1986 Solution-phase RIA and solid-phase EIA using avidin-biotin systems for analysis of monoclonal antibody epitopes and affinity constants. *Meths Enzymol* 121:459-472
- Shumaker R 1986 PKCALC: a BASIC interactive computer program for statistical and pharmacokinetic analysis of data. *Drug Metab Rev* 17:331-348
- Bohnsack J, Hawley M, Pritchard D, Egan M, Shigeoka A, Hill H 1989 An

- IgA monoclonal antibody directed against type III antigen on group b Streptococci acts as an opsonin. *J Immunol* 143:3338-3342
16. Martin J, Rubens C, Wilson C 1988 Lung anti-bacterial defense mechanisms in infants and adult rats: implications for group B streptococcal infections of neonatal lungs. *J Infect Dis* 157:91-97
 17. Jeske D, Capra J 1984 Immunoglobulins: structure and function. In: W Paul (ed) *Fundamental Immunology*, Raven Press, New York, pp 131-166
 18. Hill H, Shigeoka A, Gonzales L, Christensen R 1989 Intravenous immune globulin use in newborns. *J Allergy Clin Immunol* 84:617-624
 19. Haque K, Zaidi M, Bahakim H 1988 IgM-enriched intravenous immunoglobulin therapy in neonatal sepsis. *Am J Dis Child* 142:1293-1296
 20. Christensen K, Christensen P 1986 Intravenous gamma-globulin in the treatment of neonatal sepsis with special reference to group B Streptococci and pharmacokinetics. *Pediatr Infect Dis* 5:S189-S192
 21. Weisman L, Lorenzetti P 1989 High intravenous doses of human immune globulin suppress neonatal group B Streptococcal immunity in rats. *J Pediatr* 115:445-450
 22. Kim K 1989 Hi-dose intravenous immune globulin impairs antibacterial activity of antibiotics. *J Allergy Clin Immunol* 84:579-588

Announcement

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