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Phase I Study of Antilipopolysaccharide Human Monoclonal Antibody MAB-T88

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Monoclonal antibody MAB-T88 is a human monoclonal immunoglobulin M antibody directed at the lipopolysaccharide of gram-negative bacteria. In this study, nine patients who were expected to become neutropenic from antineoplastic chemotherapy received an infusion of MAB-T88, three patients at each of three doses: 1, 4, and 8 mg/kg of body weight. MAB-T88 was shown to be safe, with an effective half-life in plasma of 25.4 h, and no patient developed immunoglobulin G antibody to MAB-T88.

Bacterial endotoxins play a key role in septic shock by increasing the levels of interleukin-1 and tumor necrosis factor and by the humoral activation of at least four systems involved in the physiologic alterations that occur in patients with septic shock, i.e., complement, coagulation, fibrinolysis, and bradykinin (4, 17). Bacterial lipopolysaccharides (LPSs) are composed of three portions: the oligosaccharide side chains, the core LPS, and lipid A, which is considered to be the toxic moiety. Since there is much less variation in the core LPS-lipid A complex than in the side chains of different species of gram-negative bacteria, antibodies to a J5 mutant of *Escherichia coli* deficient in side chains have been produced, with the expectation that they would confer protection by cross-reacting with a wide variety of gram-negative bacteria (19).

There have been three approaches to the development of passive immunization to endotoxin: (i) use of pooled blood bank plasma rich in anti-LPS (6, 13, 14), (ii) use of pooled immunoglobulin from volunteers immunized with an *E. coli* J5 boiled cell vaccine (2, 19), and (iii) use of mouse (8, 9) or human (5, 12, 18) monoclonal antibodies to *E. coli* J5.

In this report, we discuss a phase I study of monoclonal antibody MAB-T88 in neutropenic patients. MAB-T88 is a novel human monoclonal immunoglobulin M (IgM) antibody selected for its affinity for *E. coli* J5, *Salmonella minnesota* R595 (Re rough mutant), and wild-type (smooth) LPS and for its ability to opsonize and lyse bacteria in the presence of human complement (16).

The patients entered in the study had to have a histologically confirmed malignancy and be under therapy with nonbiological antineoplastic agents. Patients had to be expected to become neutropenic in the near future by a documented neutropenic episode on a prior cycle of chemotherapy or have at least 2 days with less than 2,500 leukocytes per mm^3. Three patients in each of three groups received MAB-T88 antibody at doses of 1, 4, and 8 mg/kg of body weight. Each dose was administered by a single intravenous infusion over 30 min. All patients gave informed consent before entry into the study. The study protocol was approved by the institutional review board of the University of California at Davis Medical School. A physical examination was performed on all patients. A chest roentgenogram, an electrocardiogram, and blood for culture were obtained before antibody treatment. The clinical status of the patients was monitored throughout the 30-min antibody infusion. Subsequent evaluations consisted of physical examinations at 1, 2, and 4 weeks. Laboratory studies were performed on study days 1, 2, 4, 7, 14, and 28 and included a complete blood count with differential, platelet count, prothrombin time, partial thromboplastin time, fibrinogen, fibrin split products, urinalysis, and chemistry panel, including liver function tests. Serum samples for pharmacokinetic analysis were obtained at the baseline; at the completion of the infusion; at 2, 4, 6, 24, 48, and 96 h after the start of the infusion; and on study day 7. Sera were obtained for analysis of the immune response to MAB-T88 before treatment and on study days 3, 7, 14, and 28.

Human MAB-T88 was developed from a mouse-human heteromyeloma, as described previously (15). Briefly, human lymphocytes from a blood bank donor expressing 6-thioguanine resistance were fused with a mouse myeloma cell. The human-mouse nonsecreting hybrid (F3B6) was selected for with 6-thioguanine and ouabain, and B cells from a human spleen were fused with the human-mouse hybrid partner. The spleen donor was not immunized with *E. coli* J5 vaccine prior to splenectomy. The resultant trioma was obtained from a screening process designed to detect antibodies with a high affinity for antigens shared by rough *E. coli* J5 and *S. minnesota* R595 (Re rough mutant). Binding and blocking studies have demonstrated high affinity for wild-type LPS (16). Immunochemical analyses show that MAB-T88 is an IgM with a molecular mass of approximately 10^6 Da and comprises human heavy and light chains in equal stoichiometries.

An enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of MAB-T88 for pharmacokinetic analysis. A microdilution plate was coated with a mouse anti-MAB-T88 idiotype monoclonal antibody (2H6) at 5 μg/ml, incubated overnight, and washed in the morning. Monoclonal antibody 2H6 is highly specific for MAB-T88. Two hundred microliters of blocking buffer was added to each well, the plate was incubated for 2 h at ambient temperature, and the plate was washed and used immediately. The serum sample was diluted in phosphate-buffered saline to fall between 0.25 and 5.0 ng/ml for the assay. One hundred microliters of standards, controls, and samples was added to the appropriate wells of a microtiter plate. The

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plate was incubated for 2 h at 21°C, the solution was aspirated, and the plate was washed three times. MAB-T88 bound to the solid-phase capture antibody was detected by means of peroxidase-conjugated monoclonal antibody 2H6 in blocking buffer with 0.3% goat gamma globulin (Sigma); this was followed by the addition of *o*-phenylenediamine substrate. The optical density of each well was read at 490 nm. The sensitivity of the assay in serum was 2.5 ng/ml.

Concentration in serum data sets from each patient were fit to a two-compartment model with zero-order input (0.5 h) and first-order output from the central compartment by using RSTRIP (version 5.0) software obtained from MicroMath Scientific Software, Salt Lake City, Utah. Data were weighted as the reciprocals of the concentrations squared, and the best fits were determined by least-squares analysis. The parameters obtained from the curve fits included the volume of the central compartment (V_c) and the half-lives at the distribution and elimination phases ($t_{1/2\alpha}$ and $t_{1/2\beta}$, respectively). The area under the serum concentration-versus-time curve (AUC) and the area under the serum first moment curve (AUMC) were calculated by using the trapezoidal rule and the model parameters. The AUCs were corrected for the infusion time and were extrapolated to infinity. The following constants were then calculated: the clearance rate (CL), by CL = dose/AUC; the mean residence time (MRT), by MRT = AUMC/AUC; the volume of distribution at a steady state (V_{ss}), by V_{ss} = dose · AUMC/AUC^2; and the effective $t_{1/2}$, by $t_{1/2}$ = 0.693 · MRT (7). Because the group sizes were small, the nonparametric Kruskal-Wallis test was applied to the ranks of the values, and the level of significance was set at $P = 0.05$.

IgG antibodies to MAB-T88 were detected and quantitated by an indirect ELISA. MAB-T88 at a concentration of 5 μg/ml was coated onto microtiter plates, after which human serum was applied to each well. The plates were incubated for 2 h to allow antigen-antibody binding to approach equilibrium. The wells were washed and reacted with a goat anti-human IgG antibody conjugated with horseradish peroxidase. After a 2-h incubation, the wells were washed and *o*-phenylenediamine was added. The optical density of each well was read at 490 nm. The sensitivity of the assay in serum was less than 15 ng/ml, but it was dependent on the affinity of the IgG antibody for MAB-T88.

Three patients had acute leukemia, two patients had lymphoma, and four patients had solid tumors. The ages of the patients ranged from 24 to 71 years. While all patients ultimately had negative blood cultures, two patients were suspected of having an infection at the time of MAB-T88 infusion and were being treated with antibiotics.

No clinical or laboratory evidence of toxicity that was considered to be related to the antibody administration was observed at any dose level. Furthermore, no fever or hypotension was associated with drug administration. No IgG response to MAB-T88 was detected in the dose range tested in all nine patients monitored out to 28 days.

Concentrations of MAB-T88 in the sera of the nine patients are shown in Fig. 1. Each line represents the best fit to each data set. The two-compartment model gave better curve fits for all nine data sets than did the one-compartment model, on the basis of the model selection criterion, a modification of the Akaike Information Criterion, calculated by the software. For the two-compartment model, all correlation coefficients were greater than 0.99. Concentrations in serum increased approximately in proportion to the administered dose and were detectable for a minimum of 7 days. Parameter values derived from the curve fits shown in Fig. 1

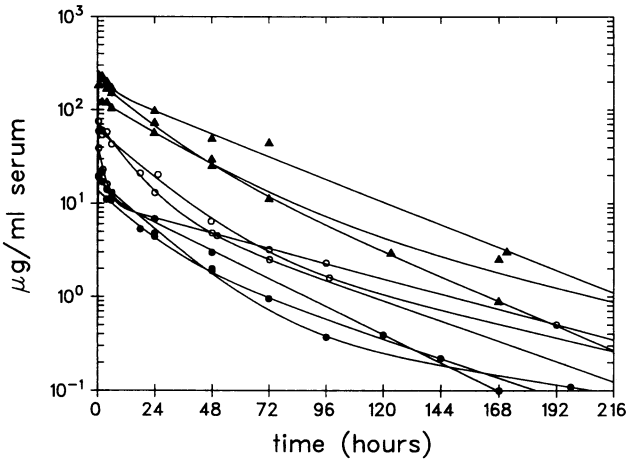

FIG. 1. Log values of the concentration of MAB-T88 in serum versus time. The concentration of MAB-T88 was determined by ELISA and was quantitated on a standard curve. Patients 1 to 3 (●) received a single intravenous infusion of 1 mg of MAB-T88 per kg, patients 4 to 6 (○) received 4 mg of MAB-T88 per kg, and patients 7 to 9 (▲) received 8 mg of MAB-T88 per kg.

are presented in Table 1. The AUCs obtained by the trapezoidal rule and the model parameters were nearly identical, and hence, the model parameters were used to calculate the derived constants. CL ranged from 1.05 to 5.50 ml/h/kg and appeared to be lower for the 8-mg/kg dose level ($P < 0.05$). The mean effective $t_{1/2}$ over all nine patients enrolled in the study was 25.4 h.

The initial distribution (V_c) of this large protein approximated the reported plasma volume of 45 ml/kg (11). Only 76% of IgM has been reported to be located in the intravascular compartment (1), and this might account for the two-compartment model following MAB-T88 infusion. However, the mean effective $t_{1/2}$ of 25.4 h for MAB-T88 is shorter than the 5.1-day $t_{1/2}$ reported for normal human IgM in normal recipients (1). A mean $t_{1/2}$ of 15.9 h in serum in septic patients has been reported for HA-1A, a human monoclonal IgM antibody directed against the lipid A of endotoxin (5). E5, a murine monoclonal IgM antibody with reactivity to lipid A, has a reported $t_{1/2}$ of 10 h in patients with suspected gram-negative sepsis (8). The pharmacokinetics of both of these monoclonal antibodies have been described by a

TABLE 1. MAB-T88 pharmacokinetic data[a]

Dose (mg/kg)	CL (ml/h/kg)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	MRT (h)	V_c (ml/kg)	V_{ss} (ml/kg)
1	2.99	10.3	35.4	35.2	72.0	105.1
	2.16	2.4	24.1	32.7	44.2	70.6
	2.54	13.0	76.4	35.8	55.5	90.8
4	3.33	7.6	33.6	27.4	53.8	91.2
	5.50	1.7	44.3	57.4	85.6	315.8
	2.83	12.9	50.9	32.9	64.3	93.1
8	1.05	2.8	29.7	41.0	28.7	43.0
	1.68	11.7	27.7	28.5	39.1	47.9
	1.94	18.2	51.1	39.8	61.5	77.2

[a] CL, clearance rate; $t_{1/2\alpha}$, α half-life; $t_{1/2\beta}$, β half-life; MRT, mean residence time; V_c, volume of the central compartment; V_{ss}, volume of distribution at a steady state.

single-compartment model (5, 8), but this may be an artifact of the spacing and the duration of sampling.

The pharmacokinetics of MAB-T88 in patients with gram-negative septic shock may well be different from those in cancer patients receiving chemotherapy. Antineoplastic drugs can suppress humoral immunity (10), and patients with chronic lymphocytic leukemia are reported to have hypercatabolism of immunoglobulins, as are patients in septic shock (3, 5). Consequently, having demonstrated the safety of MAB-T88 in the patient population described here, we are planning to perform pharmacokinetic studies with patients with presumed gram-negative sepsis.

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