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Protection against Gram-negative bacteremia and endotoxemia with human monoclonal IgM antibodies

(heteromyeloma/J5 mutant *Escherichia coli*/antitoxin/lipopolysaccharide/lipid A)

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ABSTRACT **Hybridomas producing human monoclonal IgM antibodies (mAbs) against bacterial lipopolysaccharide (LPS) were generated by fusion of B lymphocytes from sensitized human spleen with heteromyeloma cells. The splenocytes were from patients undergoing splenectomy during staging for Hodgkin disease after vaccination with the J5 mutant of *Escherichia coli*, which is deficient in O antigenic side chains. This deficiency exposes the core oligosaccharide, common to LPS of all Gram-negative bacteria. The mAbs cross-reacted strongly with endotoxins from a wide range of unrelated species of Gram-negative bacteria. The mAbs also gave strong protection against LPS in the dermal Shwartzman reaction and against lethal Gram-negative bacteremia in mice. These findings indicate that monoclonal IgM against LPS endotoxin can neutralize its toxicity *in vivo* and might be valuable for treatment of patients with Gram-negative bacteremia. Analysis of one of the hybridoma clones, A6(H4C5), showed that the IgM mAb is directed against the covalently bound lipid A, which represents the most conservative and least variable structural element of LPS.**

Overwhelming infections of the blood with Gram-negative bacteria have become a leading cause of death from infection in the hospital (1, 2). The high mortality rate from Gram-negative bacteremia can be explained partly by the lowered resistance of patients who lose their immunity as a result of disease or immunosuppressive drugs and partly by the failure of antibiotics against these bacteria. An important factor in the failure of antibiotics is the toxic lipopolysaccharide (LPS) or endotoxin in all Gram-negative bacteria (3). This toxin can produce lethal shock and is not affected by antibiotics. To protect against endotoxin we developed an antitoxin against endotoxin and produced a human antiserum that halved the death rate from Gram-negative bacteremia in patients (4). This antiserum was prepared by vaccinating healthy young men with a vaccine composed of killed cells of a mutant *Escherichia coli* O111:B_4 known as J5 (5). This mutant is deficient in the enzyme uridine 5′-diphosphate-galactose 4-epimerase so that it cannot attach the O side chains that create marked antigenic diversity among Gram-negative bacteria. Without the side chains, the core oligosaccharide with its covalently bound lipid A, which has a similar structure in various Gram-negative bacteria, becomes exposed and can stimulate antibody response. Such antibody might react with the glycolipid in the core region or lipid A of all endotoxins, regardless of bacterial species.

In view of the success of human antiserum against Gram-negative bacteremia (4) and the improvement of human monoclonal antibody (mAb) technology (6), we decided to develop a human mAb against the J5 mutant. An anti-J5 mAb could be produced in the amount needed for large-scale medical distribution without the inconveniences of human vaccination and antiserum collection. A hybridoma-produced human monoclonal immunoglobulin, in contrast with xenoantibodies, would have the advantage of not stimulating immune responses in patients. In addition, mAbs will have reproducible specificity and affinity.

We have taken advantage of the recently constructed heteromyeloma cell lines which were established by fusing a hypoxanthine/aminopterin/thymidine (HAT)-sensitive nonproducer mouse myeloma line with a HAT-sensitive human myeloma cell line. The resultant hybrids were shown to be efficient fusion partners and stable immunoglobulin producers (6, 7). The following report describes the production and effectiveness of human monoclonal antibodies against glycolipids of the J5 mutant *E. coli*.

MATERIALS AND METHODS

Vaccinated Human Spleen Lymphoid Cells. Two patients with Hodgkin disease who were to undergo staging laparotomy with splenectomy were given injections of J5 vaccine after they had given written informed consent. This study was approved by the Committee for the Protection of Human Subjects of the Stanford University School of Medicine. Two separate sites were selected for simultaneous subcutaneous injection of 1 ml of J5 vaccine, and similar injections were repeated 48 hr later. Each milliliter of the vaccine contained 5×10^9 heat-inactivated cells of the J5 mutant of *E. coli* O111. The method for preparing the vaccine is described elsewhere (4). Our previous experience with over 600 volunteers showed that vaccinations given in this fashion produced minimal side effects (4), and none were reported by the recipients in this study. They underwent staging laparotomies and splenectomies 1 week after immunization. At the time of surgery, both patients' sera showed antibodies against J5 LPS by hemagglutination and ELISA testing, and the spleens were not involved with Hodgkin disease by histologic examinations. A single-cell suspension was prepared from the spleen tissue by mincing and filtering through a nylon mesh (Tetko, Monterey Park, CA). Erythrocytes and granulocytes were removed by Ficoll/Hypaque (Pharmacia) gradient centrifugation, and the viable mononuclear cells were then suspended in Iscove's medium with 15% fetal calf serum.

Heteromyeloma Cell Lines. The myeloma fusion partners were different heteromyelomas derived from a series of fusions between human myeloma FU266-E1 and mouse myeloma (X63-Ag8.653) described previously (6). FU266-E1 is

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Abbreviations: mAb, monoclonal antibody; LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay; EBV, Epstein–Barr virus; HAT, hypoxanthine/aminopterin/thymidine.
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¶Deceased, Dec. 5, 1984.

a HAT-sensitive mutant human myeloma cell line derived from the original myeloma U266 (8) and transfected by protoplast fusion with DNA of the recombinant plasmid vector pSV2-neo^r to introduce a neomycin analogue, G418, drug resistance marker. X63-Ag8.653 is a nonproducer mouse myeloma sensitive to G418 and HAT but naturally resistant to ouabain.

Transformation by Epstein–Barr Virus (EBV). The B lymphocytes were separated from the T lymphocytes by sheep erythrocyte rosetting and Ficoll/Hypaque gradient centrifugation. The enriched B-lymphocyte population was infected with EBV derived from marmoset cell line B95-8 as described (9). Transformed lymphoblastoid cells appeared 10–14 days after infection and the numbers of cells were expanded. The lymphoblastoid supernatant contained human immunoglobulins, but specific reactivity against J5 LPS was too low to detect.

Fusion Procedure. The cell fusions were carried out with 40% polyethylene glycol 1300–1600 (American Type Culture Collection) in calcium- and magnesium-free phosphate-buffered saline (P_i/NaCl) as previously described (6). The hybrids were selected in HAT medium (100 μM hypoxanthine/0.4 μM aminopterin/16 μM thymidine) containing 0.5 μM ouabain and G418 antibiotic at 400 μg/ml, except when X63-Ag8.653 was used as a fusion partner, in which case only HAT medium was used. Feeder layers, prepared from BALB/c mice thymocytes, significantly increased the number of viable hybrids, which appeared in culture about 2 weeks after fusion. Supernatants were harvested to test for immunoglobulin specificity against J5 LPS. Producer clones were immediately subcloned by the limiting dilution method.

Producer hybridomas were conditioned to grow in low-serum medium by culturing the cells in progressively lower concentrations of fetal calf serum. The cells were passed into lower-serum medium every 2 weeks or after they had passed the lag phase and started exponential growth.

Preparation of LPS Endotoxins and Lipid A. LPSs of smooth bacteria were isolated by a modification of the phenol/water method of Westphal *et al.* (10). Cultures grown for 18 hr in trypticase soy broth were killed by a terminal concentration of 1% formaldehyde. The middle layer between the phenol and water was discarded and the endotoxin was harvested from the upper water layer by ultracentrifugation (105,000 × *g* for 4 hr). The pellet was resuspended in sterile pyrogen-free water, precipitated in 5 vol of cold 95% (vol/vol) ethanol, and washed three times in ethanol and then with diethyl ether. It was brought to constant weight at 37°C.

LPSs of rough bacteria (J5 mutant of *E. coli* O111:B_4 and RE mutant of *Salmonella minnesota* 595) were extracted by the method of Galanos *et al.* (11). Lipid A was extracted from the J5 mutant of *E. coli* O111:B_4 by hydrolysis of endotoxin in 1% acetic acid at 100°C for 2 hr (12). The water-insoluble free lipid A was washed once with distilled water and twice with 95% ethanol and was brought to constant texture with ether. Lipid A was prepared for antibody determinations by alkaline hydrolysis as previously described (13).

Antibody Determinations. Serum antibody in the vaccinated subjects was measured by agglutination of human group O erythrocytes sensitized with alkali-hydrolyzed LPS (14). Antibody to LPS in hybridoma fluid was measured by the enzyme-linked immunosorbent assay (ELISA) (12) and by immunoblotting on strips of nitrocellulose paper (0.45-μm pore; Schleicher & Schuell). Ten-microliter samples of LPS (2–5 mg/ml) were applied at different points on the paper and allowed to dry. The strips of nitrocellulose paper inoculated with LPS were immersed in P_i/NaCl, pH 7.0, containing 0.05% Tween 20 and were gently rotated for 30 min before the buffer was decanted. Hybridoma supernatant was diluted 1:1 in Dulbecco's medium and added to dishes containing the nitrocellulose strips. The strips immersed in hybridoma supernatant were gently rotated for 1–2 hr and then washed three times in P_i/NaCl/Tween 20 with 10-min shaking during each wash. A horseradish peroxidase conjugate of goat antibodies to human IgM (Tago, Burlingame, CA) was diluted 1:1000 in P_i/NaCl/Tween 20, and 20 ml was poured over the nitrocellulose strips for a 90-min incubation with shaking. The strips were washed as before with P_i/NaCl/Tween 20 and then covered with substrate solution containing 0.5 mg of 5-chloro-1-naphthol per ml of P_i/NaCl, methyl alcohol, and H_2O_2 (100:20:1, vol/vol). Positive reactions between human monoclonal IgM and LPS were identified colorimetrically.

Quantitation of Immunoglobulins. IgG and IgM were measured quantitatively by ELISA with standardized IgG and IgM (Tago).

Production of Lethal Bacteremia. Female CF1 mice (Charles River Breeding Laboratories), 6–8 weeks old, were inoculated with graded doses of an 18-hr culture of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* O111:B_4, *Escherichia coli* O17, or *Streptococcus pneumoniae*. All bacterial strains were isolated from the blood of patients except *E. coli* O111:B_4, the standard laboratory strain from which the J5 mutant was derived. The strain of *E. coli* O17 is resistant to multiple antibiotics. Four graded doses were prepared by serial 1:10 dilutions of the 18-hr cultures of Gram-negative bacilli in trypticase soy broth (Baltimore Biological Laboratory), and each dilution was mixed with equal volumes of 14% hog gastric mucin (Sigma) before 0.5 ml of the bacterial suspension was injected into the mouse peritoneum. Cultures of *Strep. pneumoniae* were diluted similarly and 0.5 ml was inoculated intraperitoneally without mucin. Each graded dose of bacteria was inoculated into 12 mice, 6 of which received anti-J5 monoclonal antibody and 6 of which received control injections: Iscove's medium with 15% fetal calf serum or human hybridoma supernatants containing no antibody activity against LPS. Thus 48 mice were used for each experiment.

Production of the Dermal Shwartzman Reaction. New Zealand White rabbits, weighing 1–1.5 kg, were inoculated intradermally with endotoxin and 18 hr later intravenously with the same endotoxin. Thirty-four rabbits received *E. coli* O111 endotoxin; another 10 received *K. pneumoniae* (Caroli strain) endotoxin. The intradermal dose of both endotoxins was 0.125 mg. The intravenous dose of *E. coli* O111 endotoxin was 0.03 mg, and that of *K. pneumoniae* endotoxin was 0.06 mg.

Immunoprotection Against Bacteremia with mAb. Protection studies were done with mAbs in hybridoma fluid from clone A6(H4C5). Each mouse received a subcutaneous injection of 1.0 ml of a 1:4 dilution of hybridoma supernatant, equivalent to 2–5 μg of mAb IgM, 18 hr before bacterial challenge. Control mice received either 1.0 ml supernatant from a human hybridoma containing the same amount of IgM but without anti-J5 activity, or 1.0 ml of Iscove's medium with 15% fetal calf serum. The LD_{50} was calculated after 72 hr by the method of Reed and Muench (15) and the significance was determined by χ^2 analysis.

Immunoprotection Against the Dermal Shwartzman Reaction with mAb. A 5.0-ml portion of human mAb was injected into the ear vein of the rabbits 2 hr before the intravenous (provocative) dose of endotoxin. Fifteen rabbits received *E. coli* O111 endotoxin and 10 received *K. pneumoniae* endotoxin. Control rabbits each received 5.0 ml of Iscove's medium. Protection was determined by the complete absence of hemorrhagic necrosis at the site of injection of endotoxin.

RESULTS

Production of Human mAbs. Two patients with Hodgkin disease were vaccinated with *E. coli* J5 vaccine before surgi-

cal staging laparotomy and splenectomy. The first patient received two immunizations as described previously, which produced a serum anti-J5 antibody titer of 512 by the ELISA method and 64 by the hemagglutination assay at the time of staging laparotomy. The second patient received one inoculation and responded with a serum anti-J5 titer of 64 by ELISA and 16 by hemagglutination. Mononuclear cells from the spleens were separated by Ficoll/Hypaque gradient centrifugation and enriched for B lymphocytes by mass rosetting with neuraminidase-treated sheep erythrocytes. Portions of the B-lymphocyte population were infected with EBV and the remaining population was fused with different heteromyeloma cell lines to preserve the antibody production permanently.

The fusions of the enriched B lymphocytes resulted in numerous viable clones, and many of them produced immunoglobulins, some of which were specific to J5 LPS. In another set of cellular fusions, the lymphoblastoid cell line generated by EBV infection of the sensitized lymphocytes was fused with several heteromyelomas, including a nonproducer mouse myeloma X63-Ag8.653-NP. The fusion results are summarized in Table 1. Hybridoma clones usually appeared in the culture plates within 10–14 days after cell fusion. The initial growth of the hybridomas varied from 0% to 65% of the microwells seeded. Each microwell contained 1–2 $\times 10^5$ lymphocytes initially. Four of the fusions with myeloma parents SHMD33 (G3), D36, A6(H4), and mouse myeloma X63-Ag8.653 produced human antibodies specific to J5 LPS as measured by ELISA. These were subcloned and expanded, and their supernatants were collected for analysis. Ninety percent of the hybrid clones produced IgM with either κ or λ light chains. These clones produced 2–30 μg of IgM per 10^6 cells per 24 hr. The growth rates of the hybridomas were similar to those of their parental myeloma lines, with a doubling time of 22–40 hr. The metaphase chromosome spreads of the hybridomas showed a mixture of mouse and human chromosomes similar to those we had encountered in previous fusions. Fig. 1 gives an example of one of the producer clones, A6(H4C5), with a modal number of 77 mouse and 8 human chromosomes. After the fusions, some of the hybrids lost production of immunoglobulin within the first 2 months. This is especially evident in the hybrids derived from mouse myeloma X63-Ag8.653, probably caused by the preferential loss of human chromosomes during somatic segregation, not infrequently seen in human–mouse hybrids.

Table 1. Fusions of various heteromyeloma clones

Heteromyeloma	Wells with viable hybrids No.	%	% Ig producers	No. of J5-specific clones	Ig class
Fusions with splenocytes					
SHMD33	9/180	5	100	1	IgM
	7/60	12	50	0	0
	5/57	9			
SHMD36	184/600	30	85	5	IgM
	11/180	6	80	1	IgG(λ)
Fusions with EBV-transformed splenocytes					
SHMD39	0/120	0	0	0	0
SHMD33(G3)	39/112	34	100	1	IgM(κ)
SHMD49	0/120	0	0	0	0
SHMD36(D7)	5/120	4.2	100	1	NT
SHMA6(H4)	58/120	48	78	3	IgM(κ)
SHMD42	2/120	1.6	50	0	0
SHMD29	2/120	1.6	100	0	0
SHMD70	4/120	3.3	100	0	0
X63-Ag8.653-NP	78/120	65	92	2	IgM(κ, λ)

NT, not tested.

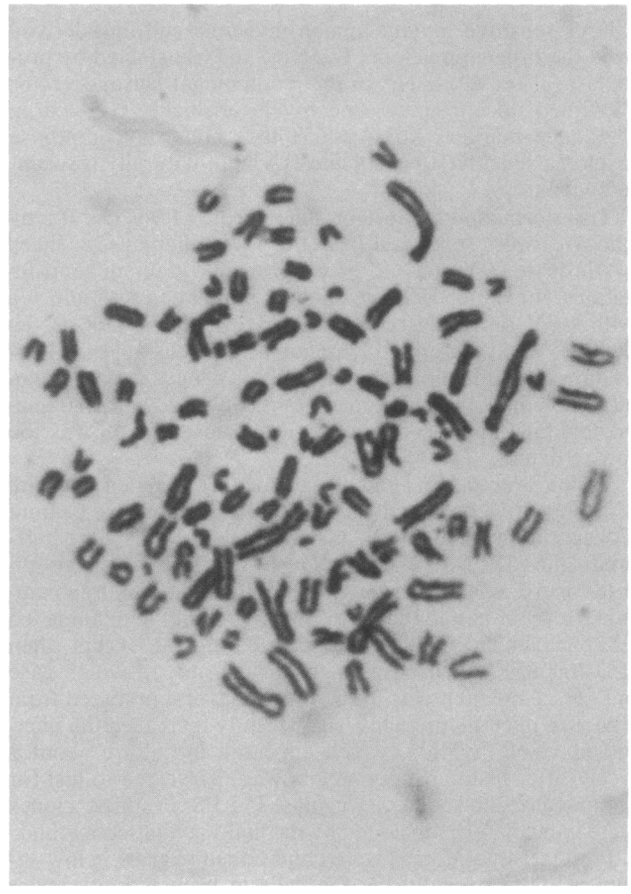

FIG. 1. Metaphase chromosome spread of hybrid clone A6(H4C5) producing human monoclonal IgM specific to J5 endotoxin.

Low-Serum Growth Condition. It is desirable for both large-scale production and therapeutic usage to have mAbs free of foreign protein contamination. One major source of xenogenic proteins is the fetal calf serum in all hybridoma growth media. In an attempt to circumvent this problem, one of the stable producer hybridomas was adapted for serum-free growth. After successful conditioning to low-serum growth (1% fetal calf serum), the hybridoma cells were tested for their growth characteristics and immunoglobulin production. We detected no difference in growth behavior or change in production level of immunoglobulins (Fig. 2). Furthermore, the supernatants from the low-serum grower showed no reduction in protection against lethal bacteremia in mice and the Shwartzman reaction in experimental rabbits (Tables 2 and 3). The hybridoma also can be cultured in growth medium completely free of fetal calf serum, with some reduction in the growth rate but not production (data not shown).

Cross-reactivity of Endotoxin LPS. Hybridoma supernatants from different clones that contained IgM mAbs usually reacted in ELISA to J5 LPS with a reciprocal titer of 8 to 64. One of the clones, A6(H4C5), which reacted at 16 to 32, was selected for detailed study. This mAb reacted broadly against either LPS or whole bacterial cells isolated from blood cultures of bacteremic patients, whereas the control IgM mAbs from nonspecific sister clones or the hybridoma growth medium showed no reactivity. As shown in Table 4, the bacterial cells of each strain of Gram-negative bacteria isolated from the blood of hospitalized patients with bacteremia reacted with J5 human mAb when measured by ELISA. The reciprocal titer was as high as 1024 for one of the Gram-negative bacilli, *Proteus vulgaris*.

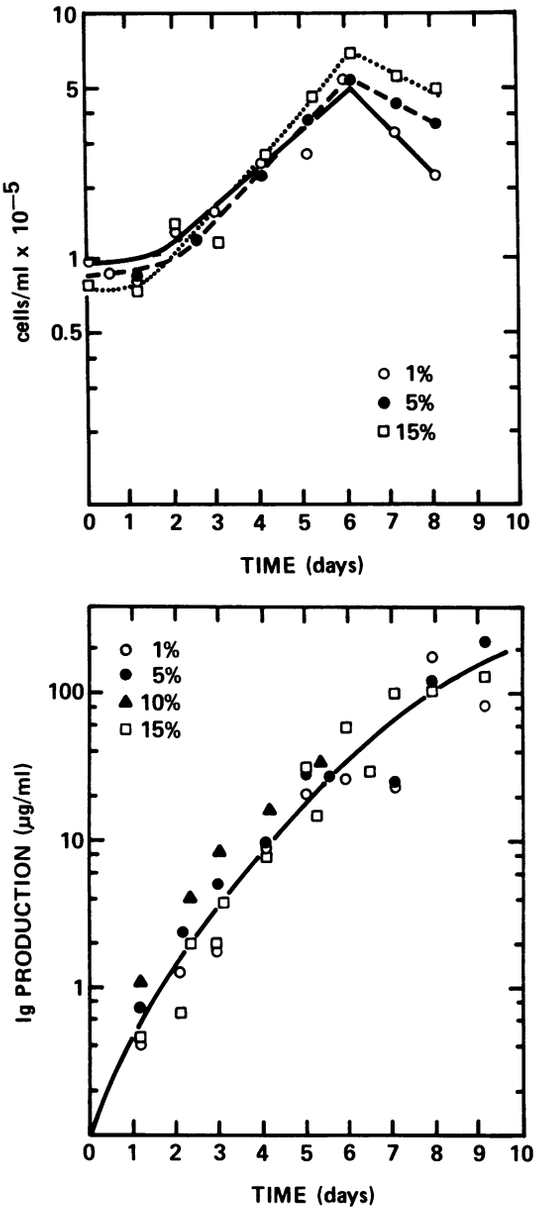

FIG. 2. Characteristics of hybrid clone A6(H4C5) with different concentrations of fetal calf serum in the growth medium. (*Upper*) Growth. (*Lower*) Immunoglobulin production measured in the spent growth media.

A6(H4C5) mAbs also reacted strongly against purified LPS from a broad range of antigenically different Gram-negative bacteria in immunoblots (Table 5). Most significantly, the commonly shared structural element of LPS, lipid A, elicited the strongest reactions with mAb supernatants (ELISA reciprocal titer, 256) and also competitively inhibited the mAbs binding to different Gram-negative bacteria. The mAb also bound to chitin (Sigma), a pure polymer of *N*-acetylglucosamine (data not shown).

Table 2. Protection against lethal bacteremia in mice with human anti-J5 IgM mAb

Organism	LD_{50}, no. of bacteria		Protection ratio*	*P*
	mAb	Control		
Ps. aeruginosa	1900	48	40/1	<0.001
E. coli O111:B_4	190	11	17/1	<0.01
K. pneumoniae	1300	73	18/1	<0.001
E. coli O17	60	4	15/1	<0.02
Strep. pneumoniae	3	3	1/1	NS
Ps. aeruginosa†	2500	16	156/1	<0.001

NS, no significant difference.
*LD_{50} (mAb treatment group)/LD_{50} (control).
†Experiment conducted with 1.0 ml of undiluted supernatant containing 1% fetal calf serum.

Table 3. Prevention of dermal Shwartzman reactions with human anti-J5 IgM mAb

% fetal calf serum in growth medium	Source of endotoxin	Incidence of positive reactions*	
		mAb	Controls
15	*E. coli* O111:B_4	2/15	19/19
	K. pneumoniae	1/10	9/10
	Total	3/25 (12%)	28/29 (96.6%)
			$P < 0.001$
1	*E. coli* O111:B_4	0/5	5/5

*Number of positive reactions per total rabbits tested.

Immunoprotection Against Gram-Negative Bacteremia. Human mAbs with ELISA reciprocal titers of 16 to 32 against J5 LPS and 256 against lipid A showed significant immunoprotection against a broad range of Gram-negative bacteria, including *E. coli, K. pneumoniae*, and *Ps. aeruginosa*, but not against the Gram-positive bacteria *Strep. pneumoniae* (Table 2). The protection ratio of induced lethal bacteremia in mice varied from 15 to 156. The protection ratio is defined as the ratio of LD_{50} between the mAb-treated group and the control group. The specificity of the mAbs against endotoxins is further substantiated by the absence of protection against pneumococcus (*Strep. pneumoniae*), a Gram-positive organism containing no endotoxin LPS. Pneumococcal septicemia was highly lethal in mice, with no survivors in either treatment or control group challenged with an inoculum of only 3 pneumococci.

Prevention of Dermal Shwartzman Reaction. Table 3 shows an overall reduction in the incidence of hemorrhagic skin necrosis from nearly 100% in the controls to 12% ($P < 0.001$) in those animals that received anti-J5 IgM mAb. This experiment again illustrates the specific antitoxic effect of mAbs, since only endotoxin rather than live bacteria was used to evoke the reaction. It also demonstrates the broad range of protection against antigenically different *Klebsiella* and *E. coli*. Table 3 also shows that the protection was not reduced by mAb supernatants from hybridomas adapted to growth in lower concentrations of fetal calf serum.

Table 4. Cross-reactivities of human anti-J5 IgM mAb to Gram-negative bacilli causing human bacteremia

Strain	Reciprocal titer against whole cells
Escherichia coli	16
Enterobacter cloacae	32
Enterobacter cloacae	32
Flavobacterium	128
Klebsiella pneumoniae	64
Klebsiella pneumoniae	128
Klebsiella I and II	64
Proteus vulgaris	1024
Pseudomonas aeruginosa	64
Salmonella group B	64
Serratia marcescens	64
Serratia marcescens	128

Bacteria were isolated in consecutive samples from hospitalized patients with Gram-negative bacteremia. Titer was determined by ELISA.

Table 5. LPSs reacting with anti-J5 IgM mAb in immunoblots

Smooth bacteria	Rough bacteria
Escherichia coli O4	*Salmonella minnesota* R60 (Ra)
Escherichia coli O111:B_4	*Salmonella minnesota* R345 (Rb)
Serratia marcescens	*Salmonella minnesota* R5 (Rc)
Klebsiella pneumoniae	*Salmonella minnesota* R7 (Rd)
Neisseria meningitidis	*Salmonella minnesota* R595 (Re)
Neisseria gonorrhoeae	*Escherichia coli* O111:B_4 J5 mutant
Pseudomonas aeruginosa	Lipid A
Salmonella typhimurium	*Salmonella minnesota* R595
Salmonella minnesota	*Escherichia coli* J5

DISCUSSION

The biological activity of the human mAbs described in this study has both theoretical and practical significance. This work demonstrates the ability of the heteromyeloma system to yield stable production of biologically active human immunoglobulin molecules. The broad *in vitro* and *in vivo* reactivities of A6(H4C5) IgM mAbs to LPS from antigenically distinct species, the J5 mutant, the parent *E. coli* O111, *K. pneumoniae*, *Ps. aeruginosa*, and *E. coli* O17 lead to the following conclusions.

First, the general cross-reactivity of mAbs demonstrates that a commonly shared antigenic determinant is present in the LPS of all these unrelated species. Furthermore, a single class of mAb to this antigenic site confers protection both by neutralizing the toxic activity of LPS and by preventing death from Gram-negative bacteremia. The specificity of protection against LPS is further indicated by the failure of anti-J5 mAb to protect against the pneumococcus, a Gram-positive organism without LPS.

Second, because of the broad range of anti-J5 mAb activity against LP5 from the J5 mutant *E. coli* O111:B_4 and against each of the chemotype strains of rough mutants of *Salmonella*, among which only lipid A and 2-keto-3-deoxyoctonate are shared determinants, it is likely that lipid A is the common reactive component. This hypothesis is strengthened by the fact that lipid A reacted much more strongly with mAb in ELISA than did any more complete LPS preparation and competitively inhibited the binding of mAb to Gram-negative bacteria. It is of interest to note that the presence of O antigen side chains does not prevent access of the anti-J5 IgM mAb to the common determinant with core LPS, in view of older impressions that the antigenically active region of lipid A was cryptic in LPS and not available for immune response (16).

Third, reaction of antibody with only one antigenic site can neutralize the toxic activity and prevent death from bacteremia. The antigenic site is probably in the vicinity of the lipid A backbone, the disaccharide linkage of the D-glucosamine, as further evidenced by the strong ELISA binding of mAbs to chitin, which is a polymer of *N*-acetylglucosamine.

The advantages of utilizing human mAb rather than polyclonal antiserum from volunteer donors have been mentioned earlier. In addition to the need for frequent immunization and phlebotomies, potential transmission of hepatitis, cytomegalovirus, and the agent responsible for the acquired immunodeficiency syndrome (AIDS) are serious disadvantages associated with the use of polyclonal antiserum. One of the concerns about lymphoblastoid cellular products is the possible contamination by EBV. In our system, the fused hybrids between heteromyeloma and lymphoblastoid cell lines frequently lost some of the human chromosomes (Fig. 1) and resulted in the elimination of the EBV-carrying genome. This is confirmed by the negative immunofluorescence staining for Epstein–Barr nuclear antigen (EBNA) in all the hybridoma clones tested. EBV nucleic acid hybridizations further confirmed this hypothesis in our previous study (17). In addition, reverse transcriptase assays revealed no retroviral activity, suggesting that the cells were not contaminated with murine retroviruses.

Mouse and human mAbs have been reported to prevent lethal infection in animals (16–18); however, they are usually serotype-specific antibodies that do not offer cross-protection, and large amounts of mAb are required for the prevention of LPS toxicity. In contrast, the human IgM mAb described in this paper has broad reactivity to all Gram-negative bacteria tested, without exception, and has been demonstrated to provide potent protection of lethal bacteremia with a dose as low as 2 μg, which is considerably lower than the doses previously reported. Such human mAb should hold great promise in both analysis and treatment of Gram-negative bacteremia and endotoxemia.

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