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X-Linked Immunodeficient Mice as a Model for Testing the Protective Efficacy of Monoclonal Antibodies against *Pseudomonas aeruginosa*

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(DBA/N[♀] × CBA/2[♂])F_1 males have been reported to be deficient in producing antibodies against a number of antigens, including carbohydrates (I. Scher, Adv. Immunol. 35:1–71, 1982). We show that F_1 male mice, in contrast to females, made less lipopolysaccharide (LPS)-specific antibodies after immunization with heat-inactivated *Pseudomonas aeruginosa* and had significantly less naturally occurring LPS-specific antibodies. Furthermore, neutropenic males were 50 to 1,000 times more sensitive to challenge with representative isolates belonging to the seven Fisher immunotypes. Administration to neutropenic F_1 males of a human monoclonal antibody specific for the O carbohydrates of *P. aeruginosa* immunotype 2 LPS or administration of serum from rabbits immunized with heat-inactivated *P. aeruginosa* immunotype 1 raised the level of resistance to bacterial challenge close to that of females. The results show that the X-linked immunodeficient mouse is an excellent model with which to test the protective efficacy of *P. aeruginosa*-specific monoclonal antibodies.

Mice are generally resistant to challenge with a wide variety of gram-negative bacteria such that lethal challenge doses in excess of 10^7 bacteria per mouse may be required. This is due mostly to an efficient phagocytic system and to the presence of naturally occurring antibodies against O-serotypic determinants on lipopolysaccharides (LPS) (6, 7, 13, 15, 21, 25). These antibodies may have arisen because of previous exposure to gram-negative bacteria or to other determinants that resemble LPS-associated carbohydrates. To test the protective efficacy of monoclonal antibodies, animal models that employ challenge doses much lower than 10^7 organisms are preferred. This can be accomplished in the mouse model by using cytotoxic drugs such as cyclophosphamide (5), trauma-inducing treatment such as local burns (7, 23), or enhancing the virulence of the infecting organisms by coadministration of ferric ions (11). However, these treatments do not always increase sensitivity sufficiently, and the presence of variable levels of circulating antibodies may complicate the interpretation of the experimental results.

We have generated human monoclonal antibodies against *Pseudomonas aeruginosa* (H. J. Zweerink, M. C. Gammon, C. F. Hutchison, J. J. Jackson, D. L. Lombardo, K. M. Miner, J. M. Puckett, T. J. Sewell, and N. H. Sigal, submitted for publication). Most of these antibodies react with the O-specific carbohydrates on the LPS of the seven Fisher Devlin immunotypes of *P. aeruginosa* (4, 10). To test whether these antibodies are protective, we investigated the utility of the X-linked immunodeficient (xid) mouse as an in vivo protection model. Xid mice are defective in generating an antibody response to a variety of antigens including high-molecular-weight carbohydrates (1, 2, 17, 18, 20, 22). The gene that is responsible for this defect is X linked and is expressed in CBA/N mice and F_1 males derived from the female CBA/N parents. We hypothesized that F_1 males, in contrast to female littermates, lack protective antibodies against the O-specific carbohydrate determinants of LPS, and therefore are more sensitive to *P. aeruginosa* infections than females; therefore, the administration of protective antibodies to male xid mice should reconstitute their level of resistance close to that of females.

In this report we describe the results of a series of experiments that are consistent with these predictions, and we demonstrate the utility of the xid mouse model for the evaluation of monoclonal antibodies against *P. aeruginosa* for protective efficacy.

MATERIALS AND METHODS

Solutions and reagents. The following solutions and reagents were used: 0.15 M NaCl and 0.01 M phosphate buffer (PBS; pH 7.2); PBS containing 0.02% sodium azide (PBS-Az); coating buffer consisting of 0.05 M Na_2CO_3 (pH 9.6); Tris washing buffer consisting of 0.15 M NaCl, 0.01 M Tris hydrochloride, and 0.02% sodium azide (pH 7.4); substrate buffer consisting of 0.05 M Na_2CO_3 and 0.001 M $MgCl_2$ (pH 9.8); and substrate solution consisting of 10 mg of 4-methylumbelliferyl-phosphate (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per 100 ml of substrate buffer that was prepared and filtered (pore size, 0.22 μm) immediately before use.

LPS from *P. aeruginosa* immunotypes 1 and 4 was obtained from List Biological Laboratories (Campbell, Calif.), and immunotype 2-specific LPS was purified from *P. aeruginosa* 4702 as described previously (8). LPS was dissolved at 1 mg/ml in a 1% solution of triethylamine in water and stored at 4°C. Solutions were made at least 24 h prior to use.

Purified recombinant hepatitis B antigen (12) was obtained from D. Lehman (Merck Sharp & Dohme Research Laboratories, West Point, Pa.).

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Bacterial strains. A representative isolate of each of the seven Fisher immunotypes of *P. aeruginosa* was obtained from the American Type Culture Collection, Rockville, Md. (catalogue numbers 27312 through 27318; our isolate numbers 4701 through 4707, respectively). Additional isolates that had been serotyped with standard sera from Difco Laboratories (Detroit, Mich.) were made available by Daniel Shungu from the Culture Collection, Merck Clinical Microbiological Service.

For immunization experiments, bacteria were grown in Trypticase soy broth (Difco) to a concentration of approximately 10^9/ml and harvested by centrifugation (6,500 × *g* for 20 min). They were washed two times with PBS, suspended in PBS–0.01% thimerosal (Sigma Chemical Co., St. Louis, Mo.) at a concentration to yield 12 to 16% transmission, and placed for 2.5 h in a boiling water bath. These suspensions were stored at 4°C.

For protection experiments bacteria were grown and washed as described above and frozen at −70°C in PBS with 1% glycerol. Bacterial suspensions were thawed immediately before use, and the number of viable bacteria was determined by plating the suspensions on Trypticase soy agar.

Mouse strains. The F_1 progeny from a cross between CBA/N (♀) and DBA/2 (♂) and CBA/J and C57B1/6 mice were obtained from Jackson Laboratories (Bar Harbor, Maine). All mice were housed and handled under specific pathogen-free conditions in microisolator cages in laminar flow racks, with the feed, water, cages, and bedding autoclaved prior to use.

Immunizations. (CBA/N × DBA/2)F_1 males and females were immunized subcutaneously with 0.25 ml of heat-inactivated *P. aeruginosa* immunotypes 1, 2, or 4 (percent transmission of suspension was 15) on days 0, 14, and 21. Sera were collected by cardiac puncture from groups of five mice on days 0, 14, 21, and 28 and were pooled.

Immunization of New Zealand White rabbits with Formalin-treated *P. aeruginosa* immunotype 1 has been described previously (19).

Detection of LPS-specific antibodies in sera. LPS in 1% triethylamine was diluted 100-fold in coating buffer, and 0.1 ml was added to flat-bottom, 96-well plates (Flow Laboratories, Inc., McLean, Va.). Plates were kept overnight at 4°C, and unreacted sites were blocked for 2 h with PBS-Az containing 1% bovine serum albumin (Miles Laboratories, Inc., Naperville, Ill.). Serum samples of 50 μl diluted in PBS-Az plus 0.1% bovine serum albumin were added in duplicate wells, and plates were incubated at room temperature for at least 3 h. Antibody binding was measured by adding 100 μl of affinity-purified goat anti-human or anti-mouse immunoglobulin A (IgA), IgG, and IgM conjugated to alkaline phosphatase (1 μg/ml in PBS-Az containing 1% bovine serum albumin; Kirkegaard and Perry Laboratories, Gaithersburg, Md.).

After overnight incubation at 4°C, plates were washed three times with PBS-Az and once with Tris washing buffer, and 100 μl of substrate was added. Plates were incubated in the dark for 1 h at room temperature and fluorescence determined at an excitation wavelength of 355 nm and an emitted wavelength of 480 nm in a Titertek Fluoroskan (Flow Laboratories, Inc., Santa Barbara, Calif.). Under the experimental conditions the emission of umbelliferone at 1 μg/0.1 ml was 4,440 units.

Antibodies against gelatin and recombinant hepatitis B antigens were measured in similar assays. Plates were coated with antigen solutions at 10 μg/ml.

Mouse challenge and protection experiments. Mice (age, 10 to 14 weeks) were made neutropenic by treating them with cyclophosphamide (250 mg/kg; Bristol Laboratories, Syracuse, N.Y.). Four days later animals were challenged intraperitoneally, in groups of 5 to 20 mice, with different doses of *P. aeruginosa* (diluted in PBS). Mice were observed for 5 days, and the 50% lethal doses (LD_{50}s) and their 95% fiducial limits were calculated by Probit analysis (9). Significant differences were determined by the procedure of Mantel and Haenszel (14).

To determine the protective efficacy of monoclonal antibodies, 0.2 ml of antibiotic-free supernatant from the lymphoblastoid cell lines RM5 and FDD7 (sources of human monoclonal antibodies reactive with LPS from *P. aeruginosa* immunotypes 2 and 4, respectively; H. J. Zweerink, M. C. Gammon, C. F. Hutchison, J. J. Jackson, D. L. Lombardo, K. M. Miner, J. M. Puckett, T. J. Sewell, and N. H. Sigal, manuscript in preparation) or control antibiotic-free growth medium (RPMI) was injected into the tail vein 2 h prior to bacterial challenge. The protective efficacy of immune rabbit serum was evaluated by injecting 0.2 ml of serum that was diluted 1:5 in PBS.

RESULTS

Generation of LPS-specific antibodies in (CBA/N × DBA/2)F_1 male and female mice. Male and female F_1 progeny from a cross between CBA/N females and DBA/2 males were immunized with heat-inactivated *P. aeruginosa* immunotypes 1, 2, or 4 on days 0, 14, and 21; and sera were collected on days 0, 14, 21, and 28 and were titrated in solid-phase fluorescent assays against LPS from the same *P. aeruginosa* immunotypes. Results in Fig. 1 with sera from mice immunized with *P. aeruginosa* immunotype 1 indicate that (i) preimmune serum from females bound slightly to

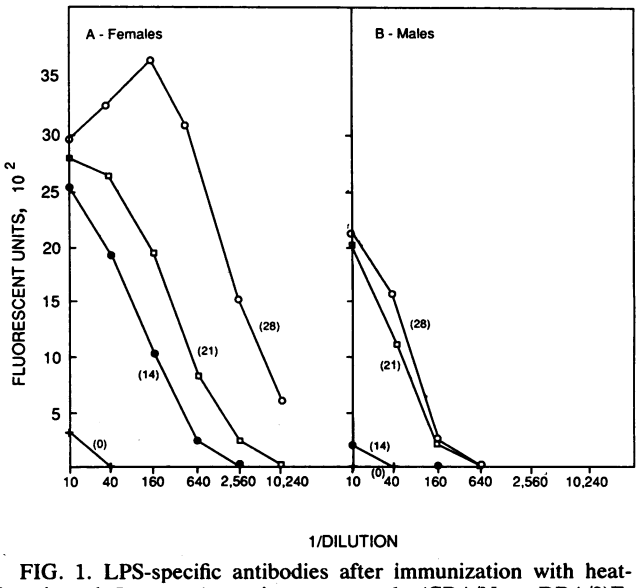

FIG. 1. LPS-specific antibodies after immunization with heat-inactivated *P. aeruginosa* immunotype 1. (CBA/N × DBA/2)F_1 male and female mice were immunized with heat-inactivated *P. aeruginosa* immunotype 1 on days 0, 14, and 21. Sera from five mice were pooled on days 0, 14, 21, and 28; and antibodies reactive with LPS from *P. aeruginosa* immunotype 1 were detected by titration in solid-phase fluorescent immunoassays. (A) Sera from female mice; (B) sera from male mice. Sera were collected on days 0 (+), 14 (●), 21 (□), and 28 (○).

immunotype 1 LPS at a 1:10 dilution, (ii) LPS-specific binding increased significantly after immunization, and (iii) sera from F_1 female mice contained 10- to greater than 100-fold more LPS-specific antibodies than did sera from F_1 males. It should also be noted that the antibody response was largely immunotype specific. When the 28-day serum samples (Fig. 1) were reacted with LPS from *P. aeruginosa* immunotypes 2 and 4, only 700 and 180 fluorescent units (for female- and male-derived sera, respectively) bound at a 1:10 dilution. This indicates that the majority of the antibodies were against the O carbohydrates.

Results were similar with sera that were obtained after immunization with heat-inactivated *P. aeruginosa* immunotypes 2 and 4 (data not shown).

Naturally occurring LPS-specific antibodies in (CBA/N × DBA/2)F_1 male and female mice. The data in Fig. 1 indicate that serum from nonimmunized F_1 females contained low levels of antibodies that recognized immunotype 1-specific LPS. This analysis was extended to determine whether serum from F_1 male and female mice contained antibodies that reacted with LPS from all seven *P. aeruginosa* immunotypes and with two control antigens (gelatin and hepatitis B). Sera were pooled from 10 (CBA/N × DBA/2)F_1 male and female mice, and these were reacted in a solid-phase fluorescent assay with the various antigens. Wells that received coating buffer only served as controls. Antibody levels are expressed in Table 1 as fluorescent units generated at a serum dilution of 1:20. Whereas binding of serum from F_1 males to wells coated with LPS from any of the immunotypes was not significantly above that of the control, it did bind to gelatin and hepatitis B antigens. Binding of F_1 female serum was significant with LPS from all immunotypes and with gelatin and hepatitis B antigens.

Since levels of LPS-specific antibodies in F_1 females were low (compare fluorescent units in Table 1 with those in Fig. 1), the following experiment was carried out to establish their significance relative to the antibody levels required for protection. A human monoclonal antibody specific for LPS from *P. aeruginosa* immunotype 2 was administered to neutropenic male mice at a concentration (1 μg per mouse) that significantly increased resistance to bacterial challenge (see below). Serum was collected 2 h after antibody administration, and the level of the monoclonal antibody was determined as described in the footnotes to Table 1 with LPS from *P. aeruginosa* immunotype 2. Bound fluorescent units (1,145) were in the same range as those in the sera of female mice. Thus, it appears that (CBA/N × DBA/2)F_1 female mice have LPS-specific antibodies at levels that are sufficient to make them more resistant to *Pseudomonas* infection.

TABLE 1. LPS-specific antibodies in (CBA/N × DBA/2)F_1 male and female mouse sera[a]

Antigen	Fluorescent units at a serum dilution of 1:20 for:		
	Male	Female	Male + RM5[b]
LPS IT 1	171.2 ± 6.0	819.8 ± 46.6[c]	
LPS IT 2	134.7 ± 5.6	1,125.2 ± 45.5[c]	1,145.1 ± 19.5[c]
LPS IT 3	154.3 ± 6.8	1,815.5 ± 43.7[c]	
LPS IT 4	99.3 ± 7.1	1,590.8 ± 61.7[c]	
LPS IT 5	105.8 ± 12.1	1,670.2 ± 69.5[c]	
LPS IT 6	98.0 ± 8.5	2,888.3 ± 33.7[c]	
LPS IT 7	120.2 ± 6.3	1,472.2 ± 49.8[c]	
Gelatin	285.7 ± 22.8[c]	920.5 ± 26.6[c]	
Hepatitis B	268.8 ± 12.1[c]	1,196.0 ± 18.2[c]	
Control	162.7 ± 13.1	492.2 ± 21.2	

[a] Sera from 10 neutropenic male and female mice were pooled and assayed (6 replicate samples) at dilutions of 1:20 in a solid-phase fluorescent immunoassay against LPS from *P. aeruginosa* immunotypes (IT) 1 through 7 and against gelatin and hepatitis B antigens. Average fluorescent units and standard errors of the mean are shown. Control values were determined on wells with coating buffer only.

[b] Human monoclonal antibody RM5 was administered intravenously to neutropenic male mice at 1 μg per mouse, and blood was collected 2 h later. Sera from five mice were pooled and titrated against LPS from immunotype 2.

[c] $P < 0.001$, compared with controls.

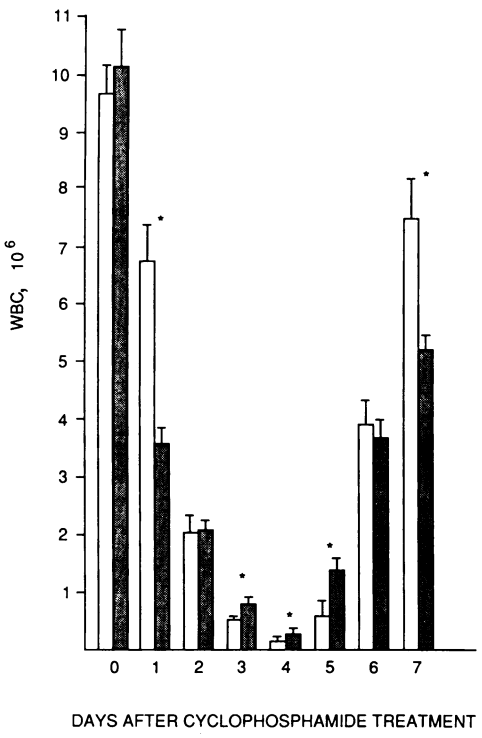

FIG. 2. Leukocytes (WBC) in (CBA/N × DBA/2)F_1 male and female mice after administration of cyclophosphamide. Mice received cyclophosphamide (250 mg/kg), and leukocyte counts were determined at daily intervals by using blood from five mice (at least 200 cells were counted for each mouse). These results are plotted as averages with standard errors of the mean. Asterisks indicate significant differences ($P < 0.05$) between samples from males and females at that particular day. Symbols: ▭, males; ▬, females.

Susceptibility of neutropenic (CBA/N × DBA/2) F_1 male and female mice to challenge with *P. aeruginosa*. Male and female F_1 progeny from a cross between CBA/N females and DBA/2 males were treated with cyclophosphamide at 250 mg/kg, and 4 days later, when the leukocyte counts were at their lowest (Fig. 2), animals were challenged with representative isolates belonging to each of the seven Fisher Devlin *P. aeruginosa* immunotypes. Neutropenic males were more susceptible to infection with *P. aeruginosa* than were neutropenic female controls (Table 2). Different immunotypes and isolates within each immunotype differed with regard to the dose required to kill 50% of the animals, but the LD_{50}s for males were always 50 to 1,000 times lower than those for females.

Leukocyte counts in (CBA/N × DBA/2)F_1 males and females after cyclophosphamide treatment. LD_{50}s for all seven *P. aeruginosa* immunotypes were 10^7 and above when males and females were infected without prior cyclophosphamide treatment (data not shown). This confirms earlier observations (25) that phagocytic leukocytes are important determi-

TABLE 2. Susceptibilities of neutropenic (CBA/N × DBA/2)F_1 male and female mice to infection with *P. aeruginosa*[a]

Immunotype	Isolate	Log LD_{50} for:	
		Males	Females
1	4701	2.6	4.3
	5559	4.08	5.7
	15925	0.49	2.36
	14254	2.38	4.04
	4500	2.91	5.87
2	4702	1.97	5.49
	2490	4.79	6.79
3	4703	2.77	4.11
4	4704	2.6	6.08
5	4705	3.6	6.04
6	4706	1.36	3.18
7	4707	0.56	2.56
	2405	0.25	2.62
	2952	1.87	3.30

[a] Mice were made neutropenic by treating them with cyclophosphamide (250 mg/kg) 4 days prior to bacterial challenge and divided into groups of five. They were challenged intraperitoneally with different doses of the various isolates and observed for 5 days, and LD_{50}s were calculated.

nants in protection against challenge with *P. aeruginosa*. Since differences in bacterial and susceptibility between (CBA/N × DBA/2)F_1 males and females were apparent only in neutropenic animals, it is important to establish that they respond in the same manner to cyclophosphamide. Leukocytes decreased in animals of both sexes to their lowest number on days 4 and 5 after cyclophosphamide administration, and cellular recovery thereafter proceeded in a similar fashion (Fig. 2). In addition, on days 3, 4, and 5, leukocyte counts, although low, were higher in females than in males (Fig. 2). Differential cell counts established that neutrophils constituted 7% of the total leukocytes on day 0 in males and females and that neutrophils could not be detected in either sex on days 4 and 5.

Small differences in total leukocyte counts were also observed when male and female CBA/J and C57Bl/6 mice were treated with cyclophosphamide. However, neutropenic male and female CBA/J and male and female C57BL/6 mice did not differ in their susceptibilities to infection with *P. aeruginosa* (data not shown). These results suggest that the increased susceptibility of the (CBA/N × DBA/2)F_1 males is not due to the slightly lower leukocyte counts at the time of bacterial challenge (day 4).

Protection with LPS-specific antibodies in (CBA/N × DBA/2)F_1 males. Two experiments were carried out to determine whether resistance to infection with *P. aeruginosa* could be increased in neutropenic F_1 males by administration of LPS-specific antibodies. First, neutropenic F_1 male mice were challenged with *P. aeruginosa* immunotype 1 (strain 14254) after they received serum from rabbits that had been immunized with heat-inactivated *P. aeruginosa* immunotype 1. The LD_{50} for control (PBS-treated) neutropenic males was 1.5×10^2, whereas that for control neutropenic females was 1.7×10^4 (Table 3). Pretreatment of males with type 1-specific serum raised the LD_{50} approximately 70-fold, to 1.1×10^4, whereas preimmune serum increased the LD_{50} 5-fold to 9×10^2.

In a second experiment, neutropenic male mice were treated prior to bacterial challenge (*P. aeruginosa* immunotype 2, strain 4702) with supernatant from a lymphoblastoid cell line that secretes human IgM monoclonal antibody (RM5) specific for the O-specific determinants on LPS of *P. aeruginosa* immunotype 2. Control animals were treated with tissue culture medium (RPMI–15% fetal bovine serum) or supernatant from a lymphoblastoid cell line (FDD7) that secretes IgM-specific LPS from *P. aeruginosa* immunotype 4 (a manuscript describing the properties of these monoclonal antibodies is in preparation [Zweerink et al., in preparation]). LD_{50}s for neutropenic males and females that received tissue culture medium prior to bacterial challenge were 8.7×10^1 and 1.9×10^5, respectively (Table 4). Administration of control human IgM monoclonal antibody (FDD7) to males had little effect (LD_{50}, 1.3×10^2). However, administration of type 2-specific monoclonal antibody RM5 resulted in significant protection of the male mice; it increased the LD_{50} to 6.1×10^4, a value close to that for females and nearly 700-fold greater than that for control males that received RPMI.

DISCUSSION

It has been reported that, in contrast to female littermates, the male progeny from a cross between female CBA/N and male DBA/2 mice (or any other males) are deficient in producing antibodies against high-molecular-weight carbohydrates (1, 18, 22). We extended these observations to show that (CBA/N × DBA/2)F_1 males are deficient in raising antibodies against the O-specific determinants on the LPS molecule after immunization with heat-inactivated *P. aeruginosa* (Fig. 1). We also established that females contain low but significant levels of naturally occurring LPS-specific antibodies, whereas males do not (Table 1). Presumably, these antibodies develop naturally in females because of exposure to gram-negative organisms or to antigens that resemble LPS-associated carbohydrates. Antibodies against the O-specific carbohydrates are more important mediators in protection against bacterial challenge than antibodies against other LPS-associated carbohydrates or lipid A (16,

TABLE 3. Protection of neutropenic (CBA/N × DBA/2)F_1 male mice with immune rabbit serum against challenge with *P. aeruginosa* immunotype 1[a]

Sex	Treatment[b]	No. of survivors/no. challenged for challenge doses of[c]:					LD_{50}
		10^5	10^4	10^3	10^2	10^1	
Female	PBS	0/10	12/15	14/15	14/14		1.7×10^4
Male	PBS		0/14	4/14	8/14	12/14	1.5×10^2
Male	Preimmune serum		1/14	11/15	11/15	13/15	9×10^2[d]
Female	Immune serum		8/15	14/15	15/15	15/15	1.1×10^4[d]

[a] Cyclophosphamide (250 mg/kg) was administered 4 days prior to bacterial challenge.
[b] Mice received intravenously 0.2 ml of PBS or rabbit sera diluted 1:5 in PBS. Immune serum was from a rabbit immunized with heat-inactivated *P. aeruginosa* immunotype 1.
[c] Values are the number of survivors per number of challenged animals. Mice were challenged intraperitoneally with *P. aeruginosa* immunotype 1, strain 14254.
[d] Significantly different ($P < 0.05$) from control (PBS-treated) males.

TABLE 4. Protection of neutropenic (CBA/N × DBA/2)F_1 male mice with monoclonal antibody RM5 against challenge with *P. aeruginosa* immunotype 2[a]

Sex	Treatment[b]	No. of survivors/no. challenged for challenge doses of[c]:						LD_{50}
		10^6	10^5	10^4	10^3	10^2	10^1	
Female	RPMI	2/20	17/20	17/20				1.9×10^5
Male	RPMI			0/5	1.10	3/10	10/10	8.7×10^1
Male	FDD7			0/5	1/10	5/10	10/10	1.3×10^2
Female	RM5	0/10	4/10	9/10	10/10	10/10		6.1×10^4[d]

[a] Cyclophosphamide (250 mg/kg) was administered 4 days prior to bacterial challenge.
[b] Mice received intravenously 0.2 ml of tissue culture supernatant from lymphoblastoid cells that secreted immunotype 2-specific IgM monoclonal antibody (RM5) or supernatant from lymphoblastoid cells that secreted control IgM monoclonal antibody FDD7. Control mice received tissue culture medium (RPMI). Supernatants were diluted in RPMI to contain 1 μg of IgM per ml.
[c] Values are the number of survivors per number of challenged animals. Mice were challenged intraperitoneally with *P. aeruginosa* immunotype 2, strain 4702.
[d] Significantly different ($P < 0.05$) from control (RPMI-treated) males.

25). Since the LPS-reactive antibodies in female mice (Table 1) were not characterized for their epitope specificities, it can be suggested but not proven that they are involved in the protection of neutropenic females against bacterial challenge. Reconstitution experiments with a protective monoclonal antibody demonstrated that levels of LPS-reactive antibody in females are sufficient for protection.

Neutropenic (CBA/N × DBA/2)F_1 males were significantly more susceptible to infection with all seven immunotypes of *P. aeruginosa* than female controls (Table 2), whereas neutrophil-replete (not treated with cyclophosphamide) males and females were equally resistant to infection. These findings support results of earlier reports (25), in which it was found that the major determinants in resistance are phagocytic neutrophils; but they also suggest that naturally occurring antibodies become important when neutrophils are depleted. Neutropenic females had slightly elevated total leukocyte counts relative to those of males (Fig. 2); however, neutrophils could not be detected in either sex.

Administration of LPS-specific antibodies to (CBA/N × DBA/2)F_1 males increased their resistance, which became close to that of females (Tables 3 and 4). Serum from rabbits immunized with heat-inactivated *P. aeruginosa* immunotype 1 or monoclonal antibody against the O-specific side chain of LPS from immunotype 2 significantly raised the resistance of neutropenic males to challenge with *P. aeruginosa* immunotypes 1 and 2, respectively. Results with the monoclonal antibody were more dramatic, partially because neutropenic males and females differed much more in their sensitivities to infection with *P. aeruginosa* immunotype 2 (strain 4702) than to infection with immunotype 1-specific strain 14254. Administration of normal rabbit serum resulted in a small but significant increase in protection (Table 3). No significant increase was observed with control medium or irrelevant monoclonal antibody FDD7. This reflects the fact that monoclonal antibody preparations are less likely to contain nonspecific enhancers of host resistance that can change sensitivity to bacterial infection independent of antibody.

The low background levels of naturally occurring LPS-specific antibodies in F_1 males allows the more definitive evaluation of the role of exogenously administered antibodies in the protection of neutropenic animals. Furthermore, a comparison of the sensitivity of neutropenic F_1 males and females to the various strains of *P. aeruginosa* within a particular immunotype defines more precisely the role of antibodies versus those of other determinants of sensitivity to infection. These determinants could include differences in accessibility of LPS on the bacterial surface to antibody or differences in bacterial virulence factors. From the results of this study, we can predict that it is difficult with the xid model to show protective efficacy of an immunotype 7 LPS-specific monoclonal antibody if isolate 4707 is used, since both neutropenic males and females were very sensitive to this particular isolate.

In this report we have extended the work of Briles and colleagues (3, 24), who showed that (CBA/N × DBA/2)F_1 males lack antibodies against phosphocholine and are more susceptible to infection with *Streptococcus pneumoniae* and that the administration of phosphocholine-specific antibodies to F_1 males increases their resistance to pneumococcal infection significantly.

The major question of the relevance of the xid mouse model to the human disease situation cannot be addressed at this point. Answers will be provided once antibodies against the most common immunotypes are available in sufficient quantities for clinical trials.

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