Functionally Distinct Monoclonal Antibodies Reactive with Enzymatically Active and Binding Domains of *Pseudomonas aeruginosa* Toxin A

JOHN K. S. CHIA,[1]* MATTHEW POLLACK,[1] DAVID AVIGAN,[1] AND SUZANNE STEINBACH[2]

Department of Medicine, Uniformed Services University of the Health Sciences, F. Edward Hébert School of Medicine, Bethesda, Maryland,[1] and Department of Microbiology and Immunology, University of Oregon Health Sciences Center, Portland, Oregon 97201[2]

Monoclonal antibodies (MAbs) are described which react with two discrete structural domains of *Pseudomonas aeruginosa* toxin A and which have two distinct functional profiles. The MAbs designated T3-1C7 and T4-1F2 reacted with a 46,000-dalton peptide similar to the putative B or binding fragment of toxin A. These antibodies neutralized the cytotoxic and lethal properties of toxin but had no effect on its ADP-ribosyl transferase activity. T4-1F2 interfered with the binding of toxin A to membrane receptors on mouse fibroblasts (L cells), although the epitope for the antibody appears to be distinct from the actual receptor binding site. The MAb designated T2-1H2 reacted with intact toxin A and with a cloned, enzymatically active carboxy-terminal polypeptide similar to the toxin A fragment. This MAb neutralized the ADP-ribosyl transferase activity of activated holotoxin and of the cloned peptide, but inhibited neither binding of toxin to membrane receptors nor its cytotoxic and lethal actions. The complementary specificity and function of these MAbs confirm the functional specialization of discrete structural domains within the toxin A molecule. Our findings suggest the greater antitoxic potential of antibodies that block binding, compared with those which inhibit the enzymatic activity of toxin A.

Pseudomonas aeruginosa is a common opportunistic human pathogen (2). The pathogenesis of *Pseudomonas* disease is complex and involves a number of bacterial virulence factors, including the extracellular protein designated toxin A (23). Produced by most clinical strains of *P. aeruginosa*, toxin A has been isolated, purified, and extensively characterized (3, 14–16, 22). It is a single-chain polypeptide with a mass of 66,000 to 71,500 daltons (Da). The toxin A gene has been cloned and sequenced, and its protein product has been identified as a 66,583-Da polypeptide with a fully defined amino acid sequence (9) and partially characterized three-dimensional structure (V. S. Allured, R. J. Collier, and D. B. McKay, Proc. Natl. Acad. Sci. USA, in press). Toxin A is an enzyme, active in eucaryotic systems, that catalyzes the transfer of the ADP-ribose moiety from NAD into covalent linkage with elongation factor 2; this reaction results in the inactivation of elongation factor 2 and the inhibition of protein biosynthesis (10). Physical and chemical manipulation of the native toxin A molecule yields 26,000- and 45,000-Da fragments, referred to as fragments A and B, which are specialized for enzymatic and binding functions, respectively (17, 33). An intact toxin A molecule comprising A and B fragments is required for expression of toxicity, while enzymatic activity may be manifested by the A fragment alone or by reduced and denatured holotoxin (33). The toxic properties of purified toxin A include cytotoxicity, necrotizing activity, and animal lethality (9, 19).

Evidence of a pathogenic role for toxin A in human *Pseudomonas* disease is indirect but compelling. The toxin is prevalent among clinical isolates and is biologically highly potent (27). In vivo production and release of toxin A during infections are indicated by detection of antibody responses in infected patients (24) and demonstration of depressed

elongation factor 2 levels in the tissues of experimental animals infected with toxinogenic strains (20, 29, 30). Moreover, toxin-deficient isogenic mutants derived from toxin-producing *P. aeruginosa* strains appear less virulent in experimental infection models (34). Immunization against toxin A produces significant, albeit incomplete, protection in some animal infection models (21), while adequate levels of naturally acquired toxin A-specific antibodies present early in *Pseudomonas* sepsis appear to reduce the mortality rate for this highly lethal disease (5, 28).

Antisera prepared against native toxin A, or a Formalin toxoid, neutralize ADP-ribosyl transferase activity and block toxicity (26). These antibody functions, however, have not been separated or identified with specific domains in the toxin A molecule. Moreover, little is known about the immunochemistry of toxin A, and no specific epitopes have been identified or characterized in this protein molecule, despite recent documentation of its complete primary structure. Although several monoclonal antibodies (MAbs) that react with toxin A have been reported (7, 12), their submolecular specificity and function are unknown. In this report, we describe functionally distinct MAbs with specificity for discrete structural regions in the toxin A molecule corresponding to the enzymatically active and binding domains, respectively.

MATERIALS AND METHODS

Purification of toxin A and preparation of toxoid. Toxin A was purified by affinity chromatography from a cell-free filtrate of an overnight culture of the nonproteolytic *P. aeruginosa* PA103 (31). Toxoid was prepared from purified toxin by treatment with 0.5% Formalin in the presence of 10^{-3} M lysine, as previously described (26).

Preparation of hybridoma clones and ascites production.

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* Corresponding author.

Six-week-old female BALB/c mice (National Cancer Institute, Bethesda, Md.) received 2.5 μg of toxoid intraperitoneally on days 0 and 7, and 4 μg intraperitoneally on days 14 and 21. Serum enzyme-linked immunosorbent assay (ELISA) titers to toxin A were checked on day 28. Immunized mice were boosted with 4 μg of toxoid 3 days before cell fusion. Fusions were performed with the non-immunoglobulin-secreting P3-X63-Ag8.653 mouse plasmacytoma cell line as the fusion partner (11). Culture supernatants were screened by ELISA for binding activity against purified toxin A (see below). Hybridomas from positive wells were cloned by limiting dilution on thymocyte feeder cells. Selected clones were adapted as mouse ascites tumors in pristane-primed BALB/c mice. Pooled ascites fluid was precipitated with 50% saturated ammonium sulfate, suspended in sterile saline, and dialyzed in the cold against phosphate-buffered saline (PBS).

Preparation of antisera. A female New Zealand White rabbit and BALB/c mice were immunized with toxoid as described previously (26). Sera from five immunized mice were pooled.

ELISA. The ELISA was performed essentially as described previously (25), except that purified toxin A was employed as the solid-phase antigen in aliquots of 100 ng per microtiter well (2 μg/ml in coating buffer) and rabbit anti-mouse K light chain (Zymed, South San Francisco, Calif.) was the second antibody used. Antibody isotyping was carried out with affinity-purified rabbit anti-mouse class- and subclass-specific reagents (Zymed) as second antibodies. The concentration of MAbs was determined by incubating appropriate dilutions of antibody at 25°C for 1 h in microtiter wells previously coated with 2 μg of affinity-purified goat anti-mouse immunoglobulin G (IgG) per ml (Cappel Scientific, Malvern, Pa.) and developing ELISA reactions as described above. Absorbance values were translated into immunoglobulin concentrations by means of a standard curve correlating optical density readings with concentrations of affinity-purified mouse IgG1.

Cytotoxicity assay. Assays employing mouse fibroblasts (L cells) were carried out in 96-well polystyrene tissue culture plates (24). A 100% cytotoxic dose was defined as the minimum amount of toxin which produced complete disruption of L-cell monolayers or >95% inhibition of [³H]thymidine uptake compared with that of unexposed cells. In neutralization assays, four times the 100% cytotoxic dose of toxin was preincubated with serial dilutions of test antibody for 1 h at 37°C, and the mixtures were then added to L-cell cultures.

ADP-ribosyl transferase assay. ADP-ribosyl transferase activity was measured by the method of Collier and Kandel (4), with minor modifications (26), using a crude wheat germ extract as a source of elongation factor 2. Toxin preparations were activated by incubation at 37°C for 24 to 72 h or by prolonged storage at −20°C. Activation was accomplished with 4 M urea and 1 mM dithiothreitol in experiments involving the C-terminal polypeptide (see below). Neutralization of ADP-ribosyl transferase activity was evaluated by preincubating 40 to 59 ng of purified toxin A in the presence of 5-μl antibody samples for 30 min at 25°C before assay.

Mouse lethality. Groups of five mice were injected intravenously with toxin A as previously described (26). The 50% lethal dose was calculated by the method of Spearman-Karber (6). Neutralization experiments were performed by preincubating 10 times the 50% lethal dose of toxin with serial twofold dilutions of the test antibody at 37°C for 1 h before injection into the mice. Saline, normal rabbit serum,

and ascites fluid containing a "nonsense" antibody were included as negative controls.

Immunofluorescence study. A total of 2 ml of L-15 medium containing approximately 2 × 10³ L-929 cells was dispensed into plastic tissue culture wells into which glass cover slips (20 × 20 mm) had been placed. After 48 h of incubation at 37°C, cover slips with adherent cells were washed in PBS, fixed for 10 min in methanol at −20°C, and rewashed with PBS. Then, 2 ml of purified toxin A was added to wells containing cover slips at a concentration of 7.5 ng/ml (about 50 times the 100% lethal dose) and was allowed to bind to adherent cells for 1 h at 4°C. After removal of toxin and further washing, the cells were incubated overnight at 4°C in the presence of MAb-containing mouse ascites fluid diluted 1:10 with PBS, or with mouse antitoxoid serum previously absorbed with L cells (positive control) or PBS alone (negative control). In parallel experiments, MAbs were preincubated with 15 ng of toxin before the mixture was added to adherent L cells. After washing, the toxin- and antibody-treated cells were incubated with 2 ml of goat anti-mouse IgG-fluorescein isothiocyanate conjugate (10 μg/ml; GIBCO Laboratories, Grand Island, N.Y.) previously absorbed with untreated L cells. The glass cover slips and adherent cells were washed with PBS followed by distilled water, dried, inverted and sealed on glass microscope slides, and evaluated with a vertical fluorescence microscope (Microstar; American Optical Corp., Buffalo, N.Y.).

Preparation of carboxy-terminal polypeptide of toxin A. The toxin A gene was kindly provided by S. Lory. Restriction analysis of the *P. aeruginosa* DNA insert demonstrated a pattern analogous to that described by Gray et al. (8) except that this gene segment originated at nucleotide base pair 333 of the Gray et al. sequence and terminated at base pair 2753 (*Eco*RI site). For expression of the C-terminal region of the toxin gene, pMS151 was propagated, and the plasmid was purified through CsCl. A *Sal*I-*Eco*RI fragment (containing toxin A gene base pairs 1743 to 2753) was inserted into *Sal*I-*Eco*RI-cleaved pUC18 and pUC19 to produce the plasmids pUC18DF101 and pUC19DF101, which were transformed into *Escherichia coli* TB1. Transformant bacteria were grown overnight at 37°C in Trypticase-digest (BBL Microbiology Systems, Cockeysville, Md.) dialysate medium with addition of monosodium glutamate and glycerol (15). The bacteria were centrifuged, washed twice with sterile PBS, suspended in 50 mM Tris (pH 7.6), and sonicated on ice with four separate 5-s bursts. The cell lysate was evaluated in the ADP-ribosyl transferase assay as described above.

SDS-polyacrylamide gel electrophoresis and immunoblots. Limited chymotrypsin digestion of denatured or undenatured toxin in the presence of NAD⁺ was performed as previously described (17). Toxin samples were solubilized by boiling for 4 min in an equal volume of sample buffer containing 200 mM Tris (pH 6.8), 4% sodium dodecyl sulfate (SDS), 20% sucrose, and 2% mercaptoethanol. The samples (5 μg) were then electrophoresed on a 10% SDS-polyacrylamide vertical slab gel, with a 4% stacking gel and a 0.1% SDS-Tris-glycine running buffer system (13). The gels were fixed overnight in 20% methanol containing 7% acetic acid, stained with 0.2% Coomassie blue (R-250; Eastman Kodak Co., Rochester, N.Y.) in 20% methanol, and destained by diffusion with 7% acetic acid in 20% ethanol. Proteins from identical companion gels were electrophoretically transferred to nitrocellulose by the method of Towbin (32), as modified by Allen et al. (1). The resulting blots were incubated sequentially at room temperature for 60 min with

antibody samples (2 to 5 µg/ml), affinity-purified rabbit anti-mouse IgG plus IgM plus IgA (1 µg/ml; Zymed), and affinity-purified goat anti-rabbit IgG-horseradish peroxidase conjugate (1 µg/ml; Bio-Rad Laboratories, Richmond, Calif.). The blots were then developed with 4-chloronaphthol substrate (Bio-Rad Laboratories).

RESULTS

We performed six fusions, screened 750 resulting hybridoma cultures, identified by ELISA 14 cultures containing antibodies reactive with purified toxin A, cloned six MAb-secreting cell lines, and further characterized the MAbs produced by three of these cloned cells lines, each derived from a separate fusion. These cell lines and their MAb products were designated T2-1H2, T3-1C7, and T4-1F2.

All three hybridomas produced MAbs of the IgG1 subclass in concentrations of 1 to 10 µg/ml in tissue culture supernatants and 1 to 8 mg/ml in mouse ascites fluid. ELISA binding curves generated with the three MAbs showed similar slopes compared with each other and with the binding curve generated with rabbit antiserum to toxoid prepared from purified toxin A (Fig. 1). ELISA reactivity of the MAbs was inhibitable with purified toxin A and with toxoid.

Purified toxin A subjected to electrophoresis in a 10% SDS polyacrylamide gel under reducing conditions migrated as a single major band corresponding to a molecular weight of approximately 71,000. Several secondary bands of slightly lower molecular weight were also present and probably represented breakdown products produced by prolonged toxin storage (Fig. 2, lane 2). Limited digestion of intact or denatured toxin in the presence of NAD$^+$ resulted in loss of the 71,000-Da band and the appearance of a new band corresponding to a molecular weight of 46,000, as well as

FIG. 2. Immunoblot analysis of MAbs reactive with *P. aeruginosa* toxin A. Lanes S, 1, and 2 contain molecular weight standards, chymotrypsin-digested toxin A, and holotoxin, respectively, subjected to electrophoresis on a 10% SDS polyacrylamide gel and stained with Coomassie blue. Lane pairs designated 3–4, 5–6, and 7–8 represent immunoblots of gels corresponding to lanes 1–2, developed with MAbs T2-1H2, T3-1C7, and T4-1F2, respectively; odd-numbered lanes contain chymotrypsin-digested toxin, and even numbered lanes contain holotoxin.
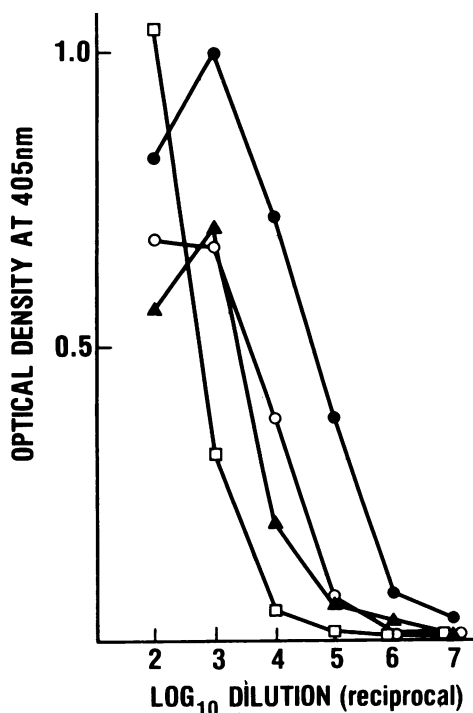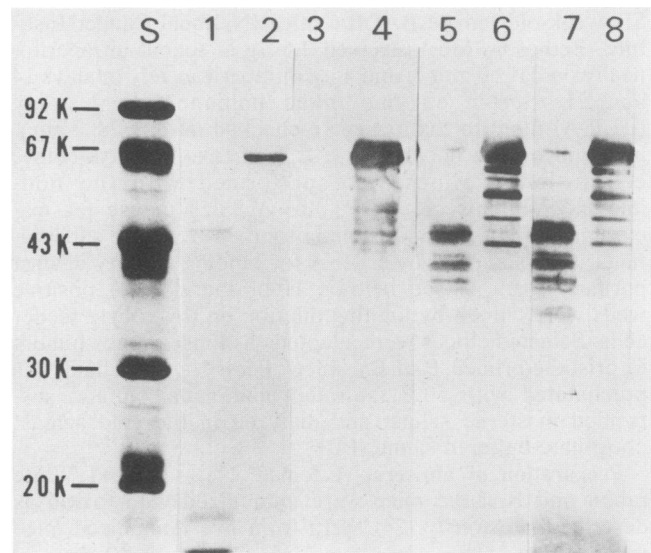

FIG. 1. Binding of MAbs to purified *P. aeruginosa* toxin A measured by ELISA. Optical density readings were obtained after 30 min of incubation with the substrate, and background levels were subtracted. Ascites fluid containing MAbs T2-1H2 (●), T3-1C7 (○), and T4-1F2 (▲) were compared with rabbit antiserum to toxin A (□).

several minor faster-migrating bands (Fig. 2, lane 1). The appearance in chymotrypsin-digested toxin of a 46,000-Da peptide consistent in size with the putative B fragment and the absence of a 25,000-Da species corresponding to the A fragment were the opposite of findings previously described by Lory and Collier (17) but similar to unreported experience from that laboratory (R. J. Collier, personal communication). Electrophoretic transfer of digested and undigested toxin from SDS polyacrylamide gels to nitrocellulose and development with MAbs (immunoblots) revealed two distinct patterns of reactivity. While all three MAbs reacted with material corresponding to a molecular weight of 71,000 (holotoxin) (Fig. 2, lanes 4, 6, and 8), only MAbs T3-1C7 and T4-1F2 demonstrated activity corresponding to the 46,000-Da peptide (Fig. 2, lanes 5, and 7). The MAb T2-1H2 produced no reactivity against digested toxin (Fig. 2, lane 3). Immunoblots developed with polyclonal rabbit antitoxin serum demonstrated a pattern of reactivity similar to that of the T3-1C7 and T4-1F2 MAbs, i.e., reactivity with holotoxin and 46,000-Da fragment but no reactivity corresponding to the putative 25,000-Da A fragment. Lack of reactivity with several nonsense MAbs (not shown) helped rule out nonspecific binding. Finally, immunoblots of the cloned, 32, 714-Da C-terminal polypeptide of toxin A, developed with MAb T2-1H2 or rabbit antiserum, demonstrated reactivity corresponding to the position of this peptide on a companion gel, while MAbs T3-1C7 and T4-1F2 produced no reactivity (data not shown).

The antitoxic activity of MAbs was examined in in vitro and in vivo assays. The MAbs T3-1C7 and T4-1F2 neutralized the cytotoxic effects of toxin A on L cells in a manner similar to that of antitoxin serum, while the T2-1H2 antibody had no activity in this assay (Fig. 3). Likewise, preincubation of 1.25 µg (10 times the 50% lethal dose) of purified toxin A with diluted T3-1C7 or T4-1F2 ascites fluid, like high-
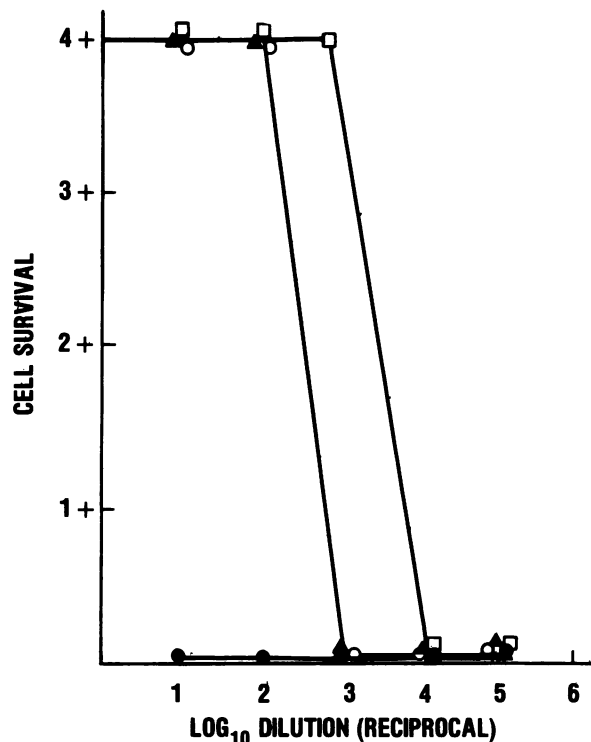
FIG. 3. Neutralization of *P. aeruginosa* toxin A-mediated cytotoxicity by MAbs. Ascites fluid containing MAb was incubated in serial 10-fold dilutions with four times the 100% cytotoxic dose of purified toxin A, and the mixture was then added to L-cell-containing microculture wells. Assays were read visually 4 days later according to the following scoring system: 4+, confluent cell growth; 3+, 50–75% confluency; 2+, 100–200 viable cells; 1+, 100 viable cells; 0, no viable cells. Ascites fluid containing MAbs T2-1H2 (●), T3-1C7 (○), and T4-1F2 (▲) were compared with rabbit antitoxin serum (□).

titered antiserum to toxin A, conferred 100% protection on mice injected intravenously with the mixture of toxin and MAb. In contrast, MAb T2-1H2, like nonimmune serum and mouse ascites fluid containing a nonsense MAb, provided no protection in this in vivo model.

The ADP-ribosyl transferase activity of toxin A originates at a yet undefined site in the A fragment of the toxin molecule. When measured in a cell-free system such as the wheat germ extract employed here, expression of ADP-ribosyl transferase activity requires functionally intact fragment A or other peptide containing the enzyme active site; the binding portion of the toxin A molecule (e.g., putative B fragment) is specifically not required for enzymatic activity in this in vitro system. It was therefore not surprising that MAbs T3-1C7 and T4-1F2, which appear to react with the binding domain or putative B fragment, had no observable effect on the expression of ADP-ribosyl transferase activity in the wheat germ system, whether this activity was generated with activated whole toxin or with the cloned C-terminal peptide (Table 1). In contrast, MAb T2-1H2 clearly neutralized the ADP-ribosyl transferase activity of holotoxin as well as that mediated by the C-terminal polypeptide (Table 1). Polyclonal rabbit antitoxin serum, previously shown to neutralize both the enzymatic activity and toxicity of toxin A, was included as a positive control; it was observed in this case to inhibit ADP-ribosyl transferase activity of intact toxin and of the C-terminal peptide.

The following experiment confirmed that MAbs T4-1F2 and T2-1H2 recognized epitopes in distinct domains of the toxin A molecule functionally related and unrelated, respectively, to cell binding. After fixation in methanol, L cells were sequentially incubated with purified toxin A, mouse antitoxoid serum, and goat anti-mouse IgG-fluorescein isothiocyanate conjugate. The majority of cells examined after this treatment demonstrated intense fluorescence (Fig. 4). This finding was interpreted as indicating that polyclonal antitoxoid antibodies reacted with free sites on toxin molecules previously bound to membrane receptors on L cells. Both MAbs T2-1H2 and T4-1F2 produced similar fluorescence when incubated with L cells previously exposed to toxin A (Fig. 4B and C). A marked diminution in fluorescence was noted, however, when toxin was incubated with MAb T4-1F2 prior to its addition to L cells (Fig. 4E); in contrast, preincubation of purified toxin with MAb T2-1H2, followed by addition of the mixture to L cells, produced fluorescence as intense as that observed when toxin and antibody were added sequentially (Fig. 4C and F). These findings indicated that both MAbs reacted with antigenic sites on toxin A, which remained free and accessible even after toxin was bound to receptors on susceptible cells, suggesting that the epitopes for both antibodies are separate from the receptor-binding site on the toxin A molecule. The fact that MAb T4-1F2 blocked toxin-binding to L cells when preincubated with toxin before cell exposure, however, suggests that the epitope for this antibody lies near the receptor-binding site in the binding domain of the toxin A molecule. In contrast, the interaction between T2-1H2 and its epitope, located in the enzymatically active domain, does not appear to block binding of toxin A to its putative membrane receptor.

DISCUSSION

Although binding by the MAbs described here to isolated A and B fragments of toxin A was not demonstrated directly, the fact that the MAbs recognized epitopes within structurally and functionally distinct domains corresponding to these fragments was amply documented on the basis of the following findings. The T3-1C7 and T4-1F2 antibodies reacted with a 46,000-Da species, suggestive of the putative B fragment, on immunoblots of chymotrypsin digests of toxin A; prevented toxin binding to L-cell receptors; blocked toxicity, presumably on the basis of their inhibitory effect on binding; but did not neutralize fragment A-mediated enzymatic activity. In contrast, MAb T2-1H2 demonstrated reactivity on immunoblots with the cloned 32,714-Da C-terminal polypep-

TABLE 1. Neutralization of ADP-ribosyl transferase activity by MAbs reactive with *P. aeruginosa* toxin A[a]

Antibody	Enzyme activity (cpm ± SE)	
	Activated toxin	C-terminal polypeptide
T2-1H2	122 ± 22	138
T3-1C7	2,842 ± 556	Not done
T4-1F2	3,020 ± 318	1,502
Rabbit antitoxoid	86 ± 3	90
None	2,863 ± 220	1,571

[a] A total of 5 μl of diluted ascites fluid containing MAb was incubated at 22°C for 1 h with 40 to 50 ng of toxin A before assay. Tris buffer (20 mM, pH 7.5) was used as diluent and negative antibody control. Also, 10 μl of cell sonicate containing the C-terminal polypeptide (see Materials and Methods) was assayed similarly.
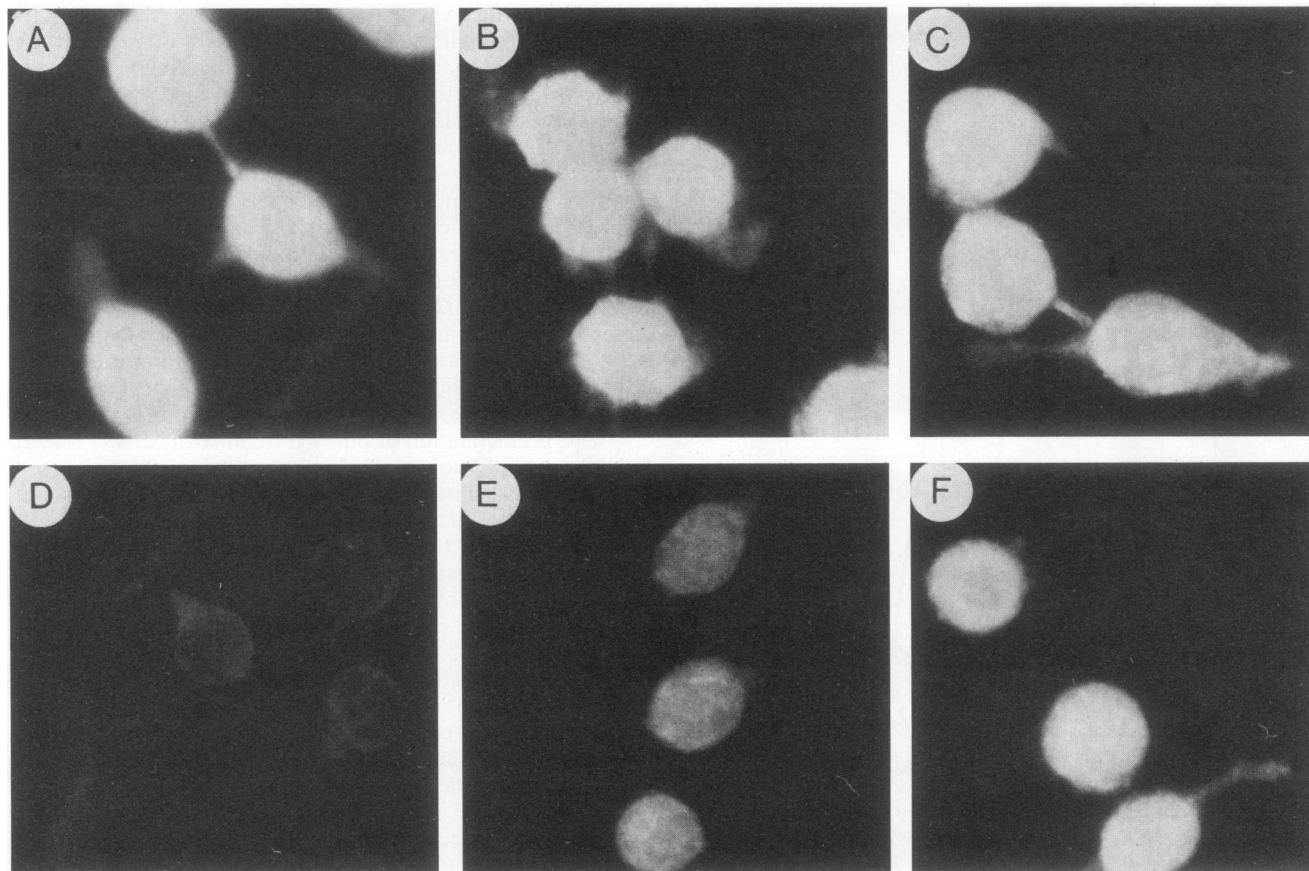
FIG. 4. Recognition by MAbs of binding sites on toxin A before and after interaction of toxin A with membrane receptors on L cells, as evaluated by immunofluorescence. (A to C) L cells exposed to purified toxin A at 4°C before incubation with mouse antitoxoid serum, MAb T4-1F2, or T2-1H2, respectively, and subsequent development with goat anti-mouse IgG-fluorescein isothiocyanate conjugate. (D) Control cells incubated with PBS following toxin exposure. (E and F) L cells exposed at 4°C to toxin which had been incubated with MAbs T4-1F2 and T2-1H2, respectively.

tide but not with the 46,000-Da fragment; did not prevent binding of toxin to membrane receptors; did not block toxicity; but did neutralize ADP-ribosyl transferase activity, including that of the cloned C-terminal peptide, which closely resembles the originally described A fragment of toxin A. We conclude on the basis of these results, which are summarized in Table 2, that MAbs T3-1C7 and T4-1F2 react with the binding domain and MAb T2-1H2 with the enzymatically active domain of toxin A.

The complementary specificity and function of these MAbs confirm the functional specialization of discrete structural domains within the toxin A molecule. The potent antitoxic properties of the T4-1F2 antibody, despite its inability to neutralize ADP-ribosyl transferase activity, underscores the functional impact of interfering with toxin-binding to host cells as a mechanism for preventing intoxication. Conversely, the failure of the T2-1H2 MAb to block toxicity, despite its clear enzyme-neutralizing properties, appears paradoxical at first. Perhaps the most likely explanation for this observation is that the antibody is dissociated from its recognition site on the toxin A molecule during the process of endocytosis or transit of the toxin-antitoxin complex to the intracellular target site of toxin A. This would result in reexposure of the enzyme-active site on the toxin

TABLE 2. Comparative immunologic and functional properties of three MAbs and rabbit cantiserum reactive with P. aeruginosa toxin A

MAb	Isotype	Binding	Cytotoxicity neutralization	Prevention of mouse lethality	Inhibition of ADPRT[b] activity	Inhibiton of toxin binding to membrane receptors
T2-1H2	IgG1	Holotoxin, A fragment	−	−	+	−
T3-1C7	IgG1	Holotoxin, B fragment	+	+	−	NT[c]
T4-1F2	IgG1	Holotoxin, B fragment	+	+	−	+
Antiserum[a]	IgG, M, A	Holotoxin, A, B fragments	+	+	+	+

[a] Rabbit antiserum to toxin A, or mouse antiserum to toxoid from toxin A.
[b] ADP-ribosyl transferase.
[c] NT, Not tested.

molecule, permitting expression of both enzymatic and toxic activities. Similar speculation has been offered to explain the inability of polyclonal antibodies reactive with the A subunit of diphtheria toxin to block the toxicity of that molecule despite the enzyme-neutralizing activity of such antibodies (18).

The structural and functional specificity of the MAbs described here make them useful reagents for elucidating the immunochemistry of toxin A. Employing these and other toxin-A-reactive MAbs in conjunction with cloned peptide fragments and synthetic peptides which reproduce short segments of the toxin molecule will permit the identification of functionally relevant epitopes. Moreover, MAbs like T3-1C7 and T4-1F2, which are capable of blocking the in vivo effects of toxin A, may be employed in animal models of *Pseudomonas* disease to help define the pathogenic role of toxin A.

Of more practical significance is the possibility that the MAbs characterized in this study, or similar human MAbs developed in the future, may represent useful immuno-prophylactic or therapeutic agents in life-threatening *Pseudomonas* disease. The encouraging relationship between high-serum-antibody titers to toxin A and survival following *Pseudomonas* septicemia in humans (5, 28) and the modest protection induced through immunization against toxin A in animal models of *Pseudomonas* disease (21) together suggest a therapeutic role for toxin-specific MAbs. This and future investigations designed to further define the functional properties of MAbs that recognize specific epitopes on the biologically active toxin A molecule should provide a solid foundation for the clinical evaluation of such antibodies, whether of murine or human origin.

ACKNOWLEDGMENTS

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