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# Monoclonal Antibodies as Probes of Tetanus Toxin Structure and Function

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Monoclonal antibodies specific for fragment B, fragment C, and light chain of tetanus toxin were prepared by fusion of P3X63Ag8 BALB/c myeloma cells with spleen cells from BALB/c mice immunized with tetanus toxoid or fragment B. Hybridoma colonies were assayed for antibody production by an enzyme-linked immunosorbent assay. Fourteen positive clones were identified, cloned by limiting dilution, and injected intraperitoneally into mice to obtain ascites fluids. Thirteen of the monoclonal antibodies were of the immunoglobulin G1 subclass and one was immunoglobulin G2. Two of the antibodies were directed against sites on fragment C, nine were directed against the light chain, and three were directed against the portion of fragment B which does not comprise the light chain of tetanus toxin. At least one antibody in each group exhibited significant toxin neutralization activity. However, only one of these neutralizing antibodies strongly inhibited the binding of $^{125}$I-tetanus toxin to ganglioside-coated plates. These data indicate that interference with receptor recognition is not the only means of neutralizing tetanus toxin. Monoclonal antitoxins as potential therapeutic and prophylactic reagents are discussed.

Tetanus toxin is composed of two nonidentical polypeptide chains connected by a disulfide linkage: a heavy chain of ca. 100,000 molecular weight and a light chain of ca. 50,000 molecular weight (7, 15, 21). Papain digestion of the holotoxin cleaves the heavy chain to yield a fragment of ca. 47,000 molecular weight (fragment C) and a larger fragment (fragment B) which is composed of the remainder of the heavy chain with the attached light chain (15) (Fig. 1). Tetanus toxin is thought to act by inhibition of neurotransmitter release (11); however, the mechanism of this inhibition is not fully understood.

Fragment C has been shown to be nearly as effective as the holotoxin with respect to binding to neural or thyroid membranes or to certain gangliosides. In contrast, fragment B shows no retention of these binding activities (23). Fragment C alone is atoxic (14, 15); fragment B alone does not cause the typical signs of tetanus, but at very high doses it can induce a neurological disorder in mice (14).

One of the initial aspects of the pharmacokinetics of tetanus toxin is its internalization at nerve endings and its subsequent retrograde axonal transport to the neuronal cell body (3, 11). Fragment C, but not fragment B, is also taken up and transported by this mechanism (12).

These observations suggest that fragment C is responsible for both the binding of tetanus toxin to neuronal cells and its subsequent uptake by neuronal axons and that the active site for the toxicity of the molecules resides in one or more determinants on fragment B. To further investigate the mechanism of action of tetanus toxin, we have produced monoclonal antibodies against various determinants on the molecule. This paper describes these antibodies in terms of their ability to neutralize toxin and to alter toxin binding to receptors.

## MATERIALS AND METHODS

**Materials.** Peroxidase-conjugated antibodies to mouse immunoglobulin A (IgA), IgG, and IgM; purified mouse IgM, IgA, IgG2a, and IgG3; and peroxidase-conjugated goat antibody to rabbit IgG (H + L) were from Litton Bionetics (Kensington, Md.). Rabbit anti-mouse (IgG + IgA + IgM) and 2,2' azino-di(3-ethylbenzthiazoline sulfonic acid) were from Zymed Laboratories (Burlingame, Calif.). Fetal bovine serum, nonessential amino acids (100×), sodium pyruvate (100 mM), L-glutamine (200 mM), and NCTC-109 medium were from M.A. Bioproducts (Walkersville, Md.). Dulbecco modified Eagle medium, penicillin-streptomycin, hypoxanthine, and thymidine were from GIBCO Laboratories (Grand Island, N.Y.). Protein A-peroxidase, aminopterin, oxaloacetic acid, bovine serum albumin (BSA), and bovine insulin were from

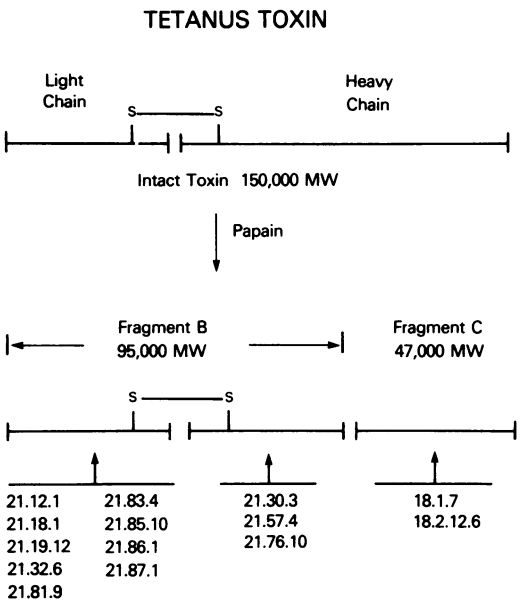


FIG. 1. The structure of tetanus toxin and fragments obtained by papain treatment of the holotoxin (adapted from reference 15). The binding domains of the monoclonal antibodies described in this paper are indicated.

Sigma Chemical Co. (St. Louis, Mo.). Ganglioside $GT_{1b}$ was generously donated by Ron Schnaar, Johns Hopkins University School of Medicine, Baltimore, Md.

Tetanus toxin, purchased from Massachusetts Public Health Biologic Laboratories, Boston, Mass., was further purified on a hydroxylapatite column; the resultant toxin was homogeneous when examined by gel electrophoresis and contained about $2 \times 10^7$ mouse minimal lethal doses per mg of protein. The light chain of tetanus toxin was obtained as a by-product during this further purification. $^{125}$I-labeled tetanus toxin was prepared by labeling with Bolton-Hunter reagent (New England Nuclear Corp., Boston, Mass.) to a specific activity of ca. 2 mCi/mg (5). Toxin fragments B and C were a generous gift of Behringwerke AG, Marburg, Federal Republic of Germany. Tetanus toxoid for use in the hemagglutination assay and enzyme-linked immunosorbent assay (ELISA) was purchased from Connaught Laboratories, Ltd., Willowdale, Ontario, Canada, and contained 1,900 flocculation units per mg of nitrogen.

**Immunization.** The monoclonal antibodies are the products of two separate immunization protocols and fusions. Fragment C-specific antibodies were obtained from a fusion (number 18) utilizing the spleen cells of a BALB/c mouse injected initially with 0.5 ml of diphtheria toxoid-tetanus toxoid-pertussis vaccine, adsorbed (Connaught Laboratories, Inc., Swiftwater, Pa.; lot 2360KE), subcutaneously in the nape of the neck, followed by four 0.5-ml intraperitoneal injections of tetanus toxoid, adsorbed (Connaught Laboratories, Inc.; lot 2046HA) at 7-day intervals. Fusion was performed 3 days after the last injection. Fragment B-specific antibodies were obtained from a fusion (number 21) utilizing the spleen cells of a BALB/c mouse injected subcutaneously with 0.5 ml of a solution of 50 μg of fragment B in complete Freund adjuvant on day 1. On days 9, 21, and 28, 50 μg of fragment B in incomplete Freund adjuvant was given intraperitoneally. On day 45, 50 μg of fragment B in phosphate-buffered saline (PBS) was administered intravenously, and the fusion was performed 3 days later.

A positive control serum pool (BALB/c-immunized) was obtained 10 weeks after intraperitoneal injection of 0.5 ml of tetanus toxoid-diphtheria toxoid, adsorbed (Connaught Laboratories, Inc.; lot 2295KA).

**Production of hybrids.** BALB/c myeloma cells (P3X63Ag8) were obtained from Marshall Nirenberg, Laboratory of Biochemical Genetics, National Institutes of Health, Bethesda, Md., and were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. Fusion was performed by an adaptation of the method of Galfre et al. (8). Washed spleen cells (approximately $10^8$ mononuclear cells) were combined with approximately $10^7$ P3X63Ag8 myeloma cells and centrifuged for 5 min at $800 \times g$. The cell pellet was gently disrupted, and 1 ml of 50% polyethylene glycol 1000 solution was added gradually over 1 min. The mixture was allowed to sit for an additional minute, and then 20 ml of Dulbecco modified Eagle medium was added over the next 6 min with gentle shaking of the tube. After centrifugation at $800 \times g$ for 5 min, the cell pellet was resuspended in 125 ml of medium S/HAT (medium S/HAT is identical to selective medium D20 SHAT of Berzofsky et al. [2], except that it contains aminopterin at 1 μM and bovine insulin at 40 mU/ml). The cell suspension was distributed into eight 96-well microplates (Linbro 76-003-05) with 150 μl added per well.

Media from wells with visible colonies were assayed for antibody by ELISA methods as described below. Selected hybridomas were expanded and injected intraperitoneally into BALB/c mice primed 2 weeks previously with pristane. Ascites fluids were collected and stored frozen.

**Anti-tetanus toxin antibodies.** Hybridoma culture media and ascites fluids were assayed for their ability to bind to tetanus toxin, tetanus toxoid, fragment B, fragment C, or light chain by an ELISA in which peroxidase-labeled reagents were used. Individual wells of 96-well MICROELISA plates (Immulon II; Dynatech Laboratories, Alexandria, Va.) were incubated with 60 μl of a 4-μg/ml solution of the appropriate ligand in a 1:5 dilution of Dulbecco PBS for at least 2 h at room temperature or overnight at 4°C. The coating solutions were aspirated and 0.5% BSA in PBS (BSA-PBS) was added for 1 h to saturate all remaining protein-binding sites. The wells were then washed twice with 200 μl of 0.1% BSA-PBS with a MINIWASH plate washer (Dynatech). Sixty-microliter samples of hybridoma culture or ascites fluids diluted in 0.1% BSA-PBS were added to the appropriate wells and allowed to incubate at room temperature for at least 2.5 h, and the wells then were washed twice with 200 μl of 0.1% BSA-PBS. Sixty microliters of rabbit anti-mouse (IgG + IgA + IgM) diluted in 0.1% BSA-PBS was added to all wells. After incubation for 2 h at room temperature, the wells were washed twice with 0.1% BSA-PBS. Sixty microliters of protein A-peroxidase, ca. 10 ng/ml in 0.1% BSA-PBS, was then

added to all wells and incubated at room temperature for 1 h, and the wells then were washed three times with 0.1% BSA-PBS. One hundred microliters of 2,2'-azino-di(3-ethylbenzthiazoline sulfonic acid), diluted in 0.1 M citrate buffer (pH 4.2) with 0.03% hydrogen peroxide, was added to each well and incubated at room temperature. One hundred microliters of 2 mM sodium azide was added to stop the reaction, and the optical density at 410 nm was determined in each well with a MICROELISA MINIREADER MR590 (Dynatech).

In experiments designed to determine immunoglobulin class, antigen-coated plates were incubated first with culture medium or ascites fluid, washed, and then incubated with peroxidase-conjugated specific antiserum to mouse IgA, IgG, or IgM. Wells coated with purified mouse IgA, IgG, or IgM were used as positive controls. For determination of IgG subclass specificity, antigen-coated wells were incubated with culture media or ascites fluid, washed, and then incubated with rabbit antisera to mouse IgG1, IgG2, or IgG3. Bound rabbit anti-mouse antibody was detected by an additional incubation with peroxidase-conjugated goat anti-rabbit IgG (H + L). Wells coated with purified IgG1, IgG2A, or IgG3 served as positive controls.

The toxin neutralization titer of these antibodies was determined by the ability of hybridoma supernatants or ascites fluids to neutralize the lethal effects of tetanus toxin with a modification of the method of Barile et al. (1a). Each mixture of sample and toxin was inoculated into at least two mice. The limit of sensitivity of the test was 0.001 U/ml.

Antitoxin titers were also evaluated by passive hemagglutination with a modified procedure adapted from Levine et al. as previously described (13). Fresh sheep cells were sensitized with tetanus toxoid for these assays. Fluids were inactivated at 56°C but not adsorbed with erythrocytes. The units in both the hemagglutination and neutralization tests were determined using U.S. standard tetanus antitoxin (lot no. E 124) as a reference.

The ability of the monoclonal anti-tetanus toxin antibodies to influence the binding of tetanus toxin to ganglioside $GT_{1b}$ was determined using an adaptation of the polystyrene-adsorbed ganglioside assay described by Holmgren et al. (17). Fifty microliters of 0.4 μM ganglioside $GT_{1b}$ in a 1:5 dilution of PBS was added to individual wells of Immulon II Removawell strips (Dynatech) and incubated overnight at 4°C. Unoccupied binding sites on the plastic were covered by incubating the wells with 0.2 ml of 0.5% crystallized BSA (Sigma A7638) in PBS for 1 h at room temperature. Ascites preparations were diluted in 0.1% BSA-PBS and incubated for 1 h at room temperature with ca. 50,000 cpm of $^{125}$I-tetanus toxin before the mixtures were transferred to the ganglioside-coated wells for incubation overnight at 4°C. The wells were then washed three times with 200 μl of 0.1% BSA-PBS and counted in a gamma counter.

## RESULTS

**Hybridoma cultures.** Colonies were visible within 2 to 3 weeks in 116 of 768 wells in fusion 18 and in 598 of 768 wells in fusion 21. Initial screening of these colonies with tetanus toxoid or tetanus toxin detected 5 positive cultures from fusion 18 and 88 positive cultures from fusion 21. Culture medium from the positive wells was further assayed using plates coated with tetanus toxin, tetanus toxoid, fragment B, fragment C, or light chain. Based on these assays, 2 colonies from fusion 18 and 12 colonies from fusion 21 were selected and were cloned by limiting dilution.

**Antibody characterization.** The titers of 12 of the ascites preparations determined by ELISA with microplates coated with tetanus toxoid are shown in Fig. 2. Also shown are the serum titers from a pool of BALB/c mice immunized with tetanus toxoid (BALB/c-imm) and from control BALB/c mice (BALB/c-cont). Dilutions of ascites which were required to produce one-half of the maximal color development in the ELISA (optical density at 410 nm = 1.0) ranged from approximately 1:20,000 to 1:200,000. Ascites preparations were assayed for their ability to bind to tetanus toxin, tetanus toxoid, fragment B, fragment C, or light chain. Table 1 shows the reaction profile of a 1:5,000 dilution of each ascites preparation and of BALB/c-imm and BALB/c-cont sera. There was virtually no reactivity in the BALB/c-cont serum, whereas BALB/c-imm serum showed reactivity with all ligands. The ascites preparations fell into four groups (Table 1): (i) two preparations which showed reactivity against tetanus toxin, tetanus toxoid, and fragment C but did not react with fragment B or light chain (antibodies 18.1.7 and 18.2.12.6); (ii) eight preparations which showed reactivity against tetanus toxin, tetanus toxoid, fragment B, and light chain but did not react with fragment C (antibodies 21.12.1, 21.18.1, 21.19.12, 21.32.6, 21.83.4, 21.85.10, 21.86.1, and 21.87.1); (iii) three preparations which reacted with tetanus toxin, tetanus toxoid, and fragment B but did not react with light chain or fragment C (antibodies 21.30.3, 21.57.4, and 21.76.10); and (iv) one preparation which reacted with tetanus toxin, tetanus toxoid, and light chain but did not react with fragments B or C (antibody 21.81.9).

Two of the preparations (21.18.1 and 21.57.4) showed low reactivity against tetanus toxoid, and therefore their titers against toxoid are not included in Fig. 2. However, when their titers against tetanus toxin were determined, these two preparations produced an optical density of 1.0 at dilutions of ca. 1:50,000 (data not shown).

Table 1 also shows the antitoxin titers of the preparations as determined by toxin neutralization in mice and by hemagglutination. Both neutralizing and nonneutralizing antibodies were detected.

Table 2 shows the effect of each of the ascites preparations on the binding of $^{125}$I-tetanus toxin to $GT_{1b}$-coated microwells. The most dramatic

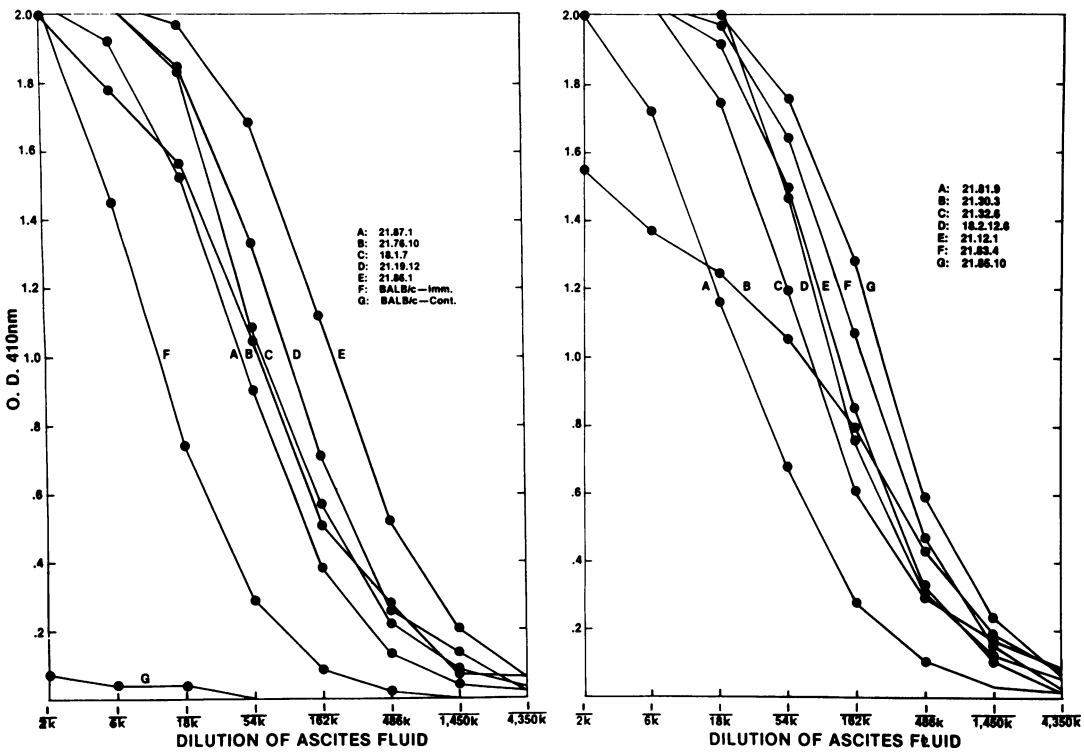


FIG. 2. Titration of ascites fluids using tetanus toxoid-sensitized ELISA plates. Controls consisted of mouse polyclonal anti-tetanus serum and serum from nonimmunized mice.

TABLE 1. Characterization of anti-tetanus toxin antibodies

| Antibody (1:5,000) | ELISA reactivity ($OD_{420}$)[a] in wells coated with: | | | | | Neutralization titer (U/ml) | Hemagglutination titer (U/ml) |
|---|---|---|---|---|---|---|---|
| | Tetanus toxin | Tetanus toxoid | Fragment C | Fragment B | Light chain | | |
| 18.1.7 | >2 | >2 | 1.35 | 0.01 | 0.00 | 0.3 | 320 |
| 18.2.12.6 | >2 | >2 | 0.55 | 0.00 | 0.00 | <0.001 | 10 |
| 21.12.1[b] | 0.92 | 1.99 | 0.03 | 1.12 | 1.82 | 0.003 | 160 |
| 21.18.1 | 0.86 | 0.29 | 0.01 | 0.57 | 1.59 | <0.001 | NV[c] |
| 21.19.12[d] | 0.90 | 1.90 | 0.00 | 1.23 | 1.83 | <0.001 | NV |
| 21.32.6[d] | 0.83 | >2 | 0.01 | 1.03 | 1.82 | 0.003 | 160 |
| 21.81.9 | 0.97 | 1.94 | 0.00 | 0.02 | 0.44 | 0.03 | 80 |
| 21.83.4[b] | 1.29 | >2 | 0.06 | 1.51 | >2 | 0.03 | 320 |
| 21.85.10[b] | 1.00 | 1.95 | 0.00 | 1.37 | 1.91 | 0.03 | 320 |
| 21.86.1[b] | 0.80 | 1.83 | 0.00 | 0.93 | 1.72 | 0.03 | NV |
| 21.87.1[b] | 0.65 | 1.74 | 0.01 | 0.78 | 1.53 | 0.003 | NV |
| 21.30.3 | 0.28 | 1.22 | 0.00 | 0.96 | 0.00 | 0.03 | 1,280 |
| 21.57.4 | 0.72 | 0.28 | 0.13 | 1.38 | 0.19 | <0.001 | 2.5 |
| 21.76.10 | 0.41 | 1.86 | 0.01 | 0.92 | 0.00 | 3.0 | >160 |
| BALB/c-imm | 1.50 | >2 | 1.23 | 0.40 | 0.64 | 3.0 | 40 |
| BALB/c-cont | 0.11 | 0.09 | 0.13 | 0.11 | 0.15 | <0.001 | <0.0024 |

[a] $OD_{420}$, Optical density at 420 nm.
[b] These clones appeared to be identical when examined by gel electrofocusing. See the text for details.
[c] NV, Nonvalid. Nonsensitized cells agglutinated at the same dilution as sensitized cells.
[d] See footnote *b*.

TABLE 2. Effect of anti-tetanus toxin antibodies on binding of $^{125}$I-tetanus toxin to $GT_{1b}$-coated microtiter plate

| Antibody[b] | Antibody dilution (mean cpm ± SD)[a] | | |
|---|---|---|---|
| | 1:400 | 1:2,000 | 1:10,000 |
| None[c] | | | |
| 18.1.7 | 1,533 ± 83 | 1,709 ± 85 | 2,105 ± 63 |
| 18.2.12.6 | 13,512 ± 763 | 13,093 ± 927 | 15,211 ± 820 |
| 21.12.1 | 3,192 ± 264 | 3,753 ± 176 | 4,606 ± 173 |
| 21.18.1 | 5,588 ± 349 | 4,004 ± 330 | 4,234 ± 472 |
| 21.19.12 | 3,192 ± 350 | 3,531 ± 394 | 4,424 ± 50 |
| 21.32.6 | 3,197 ± 172 | 3,630 ± 307 | 4,869 ± 346 |
| 21.81.9 | 3,616 ± 126 | 3,907 ± 598 | 5,817 ± 155 |
| 21.83.4 | 3,349 ± 300 | 4,101 ± 116 | 4,308 ± 443 |
| 21.85.10 | 3,348 ± 188 | 3,642 ± 83 | 4,198 ± 117 |
| 21.86.1 | 3,892 ± 589 | 3,745 ± 94 | 4,524 ± 343 |
| 21.87.1 | 3,745 ± 224 | 4,293 ± 224 | 5,153 ± 461 |
| 21.30.3 | 3,734 ± 179 | 3,409 ± 301 | 4,136 ± 348 |
| 21.57.4 | 4,279 ± 109 | 4,295 ± 143 | 4,565 ± 372 |
| 21.76.10 | 4,784 ± 469 | 4,298 ± 156 | 5,304 ± 213 |
| BALB/c-imm | 1,425 ± 84 | 2,245 ± 317 | 4,727 ± 140 |

[a] $n = 3$.

[b] Uncoated plates bound about 300 cpm in the presence or absence of added antibodies.

[c] cpm ± standard deviation, 5,277 ± 439.

effects were seen with the two antibodies specific for fragment C of the toxin molecule, 18.1.7 and 18.2.12.6. Binding was inhibited by preincubation with 18.1.7 to the same extent as was seen with the highest concentration of BALB/c-imm serum, whereas it was greatly enhanced by preincubation with 18.2.12.6. Varying degrees of inhibition were seen with the other preparations.

All ascites preparations were assayed for their immunoglobulin class and subclass specificity by ELISA. Antibody 18.2.12.6 was shown to be IgG2; all other antibodies were IgG1.

## DISCUSSION

Two of our monoclonal antibodies showed specificity for fragment C of tetanus toxin. One of these, antibody 18.1.7, had high neutralization and hemagglutination titers. The other, antibody 18.2.12.6, had no detectable neutralization titer and a low hemagglutination value. However, ELISA and other tests demonstrated that this antibody does indeed bind to toxin. We have previously shown that antibody 18.2.12.6, while bound to tetanus toxin, is internalized by rat sciatic nerve axons and transported retrogradely toward the cell body (11a). In contrast, antibody 18.1.7 completely blocks the uptake and retrograde transport of toxin. Antibody 18.1.7 inhibited binding, whereas antibody 18.2.12.6 enhanced binding of $^{125}$I-tetanus toxin to the ganglioside $GT_{1b}$ (Table 2). These results are consistent with the transport experiments and suggest that these two antibodies bind to different determinants on fragment C and that antibody 18.1.7 binds to, or near, the portion of fragment C which is responsible for the binding of tetanus toxin to its initial receptor.

Eight other antibodies reacted with the light chain of tetanus toxin and with fragment B (which contains the light chain portion of the molecule). None of these reacted with fragment C. These antibodies exhibited a wide range of neutralization titers (Table 1). Although differences in antibody titers can reflect differences in antibody concentrations, gel electrofocusing analyses of our ascites fluids indicated similar levels of antibody production in most cases. Hence, we attribute the major differences in neutralizing activity primarily to differences in affinity and epitope specificity. However, a definitive explanation will require the study of affinity-purified antibodies. The observed binding specificity and examination of the electrophoretic mobility of toxin-antibody complexes (11a) indicate only one binding site for each of the antibodies tested.

One other antibody, 21.81.9, has an intermediate neutralization titer and consistently shows specificity toward light chain without any cross-reactivity with fragment B. This seemingly anomalous observation is similar to the recently reported binding of a human monoclonal antibody to fragment B but not to either the light or heavy subunits of toxin (9). A simple explanation is that the proper epitope is either absent or inaccessible despite the notion that appropriate pairs of these subunits or fragments should represent the entire toxin molecule when added together.

Three antibodies react with fragment B but not with light chain, suggesting that their binding determinants are on the portion of fragment B which does not contain the light chain. One of these antibodies, 21.76.10, has a high neutralization titer; another, 21.30.3, has an intermediate titer; and the other, 21.57.4, has a low titer.

Some perspective on the neutralization titers can be obtained by comparing them with the generally accepted protective titer in humans, which is $\geq$0.01 U/ml (20). It is of interest that there is no obligatory correlation among the neutralization, hemagglutination, or ELISA titers; as expected, mere binding of antibody to the toxin is not sufficient for inactivation. Of particular interest is the fact that both neutralizing and nonneutralizing antibodies are directed against each portion of the toxin molecule investigated (Fig. 1; Table 1). Of these antibodies, only the fragment C-specific antibody, 18.1.7, shows extensive inhibition of $^{125}$I-tetanus toxin binding to ganglioside $GT_{1b}$ (Table 2). Although

these observations do not definitely establish that these other antibodies fail to prevent binding of native tetanus toxin to its receptor, they do suggest the possibility that the neutralizing ability may be due to inhibition of some event which occurs subsequent to the initial toxin-receptor complex formation. For example, if the heavy-chain portion of fragment B is involved in insertion of the toxin in a membrane, as was recently suggested by Boquet and Duflot (6), then antibody to fragment B might be expected to effect neutralization. One of our antibodies (21.76.10) with this specificity is neutralizing. However, antibody 21.57.4, which also reacts with fragment B, does not neutralize tetanus toxin.

Spectrotype analysis of ascites fluids was performed using gel electrofocusing. These experiments strongly suggested that several of the clones produced identical antibodies (Table 1). The similarity of the results of the other assays for these clones supports this conclusion. We may, therefore, have only nine different monoclonal antibodies in this study. The results obtained with the additional clones are included, however, to demonstrate the reproducibility and reliability of our growth conditions and analytical methods.

Monoclonal antibodies can be simply considered as isolated components of a polyclonal antibody response. For certain applications they have obvious advantages, e.g., uniformity and unlimited supply. However their superiority as reagents for the treatment or prophylaxis of tetanus has not yet been established. Reported toxin neutralization titers for affinity-purified monoclonal antibodies (22), including 0.4 U/mg for 18.1.7 (data not shown), are far below those reported for purified polyclonal preparations (65 and 300 U/mg for human [24] and equine [4] antitoxins, respectively).

Mizuguchi et al. (22) have prepared several mouse hybridomas which secrete antibody to tetanus toxin. When two of their monoclonal antibodies were combined, the toxin neutralization titers increased up to 50-fold over the sum of the individual titers, suggesting an advantage of such mixtures as medically useful reagents. The explanation for the apparent superiority of polyclonal antibodies may lie in their ability to form stable dimers and larger complexes (16). Such complexes could utilize both nonneutralizing and neutralizing antibodies in inactivating the toxin.

Two additional mouse monoclonal antibodies of the IgG1 isotype with similar affinities for tetanus toxin have been described (10; V. R. Zurawski, J. G. R. Hurrell, W. C. Latham, P. H. Black, and E. Haber, Fed. Proc. **39**:1204, 1980). Only one of these antibodies was considered protective, producing about 0.6 antitoxin units per ml of ascites fluid. The protective effect was attributed to an ability of this antibody to interfere with the binding of toxin to target cells (10). Our results suggest that this may not be the only mechanism by which monoclonal antibodies can neutralize toxin.

Human monoclonal anti-tetanus toxoid antibodies of the IgM class have been obtained from virally transformed B cells (18). These cells were subsequently fused with mouse myeloma cells to increase antibody yields (19). Unfortunately, the antibody produced by either system was not characterized with respect to reactivity with tetanus toxin.

We have previously shown that the nonneutralizing antibody 18.2.12.6 can, when combined with toxin, be taken up by neuronal axons (11a). We are now examining the possibility that some of our neutralizing antibodies may, when complexed with tetanus toxin, be taken up by neuronal axons and that their neutralizing ability results from the inhibition of some action of tetanus toxin within the cell. The observation that some antibody-toxin complexes can be internalized suggests delivering antibodies or other drugs to the central nervous system by use of antibodies which bind to less toxic carrier substances such as fragment C of tetanus toxin or the B subunit of cholera toxin, both of which are known to undergo axonal transport. For example, this method of delivery could be accomplished by chemically coupling the drug with the carrier or with an antibody which undergoes carrier-dependent uptake or by developing bifunctional antibodies which react with both the carrier and the desired drug.

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### ADDENDUM IN PROOF

The properties of 12 additional monoclonal antibodies to tetanus toxin and toxoid have recently been described (1).

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