SUPPRESSION OF CYTOTOXICITY OF DIPHTHERIA TOXIN BY MONOCLONAL ANTIBODIES AGAINST PHOSPHATIDYLINOSITOL PHOSPHATE

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The structure and chemical class of the cellular receptor for diphtheria toxin (DT) has not yet been determined. The receptor has been proposed to be a protein (1), a carbohydrate (2), or a glycoprotein (3). Recently it has been demonstrated that DT is a phosphate-binding protein (4–6). We have shown that DT binds specifically to the phosphate portion of some, but not all, phospholipids in liposomes (4). Based on these findings we proposed that the receptor for DT includes a minor membrane phospholipid such as phosphatidylinositol phosphate (PIP). To test this hypothesis we have examined the ability of monoclonal antibodies directed against liposomal phospholipids to inhibit cytotoxicity of DT.

RESULTS AND DISCUSSION

Hybridoma monoclonal "anti-liposome" antibodies were produced after injecting a mouse with liposomes containing dimyristoyl phosphatidylcholine (DMPC), cholesterol, lipid A, and PIP (7). The specificity of a typical monoclonal anti-PIP antibody is shown in Table I. Immune damage to test liposomes was inhibited by inositol hexaphosphate, phosphocholine, and nucleotides (ATP > AMP), but not by choline or inositol. The specificity of the anti-PIP antibody apparently was similar to that of DT in that both proteins had the ability to bind both phosphate and PIP, and both were inhibited by nucleotides (ATP > AMP) (4, 9). We reasoned that if PIP were the cellular receptor for DT, or even if the phosphate (i.e., PIP) binding site of DT were important only in the internalization of DT, then the toxicity of DT might be blocked by a monoclonal anti-PIP antibody. Such blocking did occur, as shown below.

The cytotoxicity assay consisted of incubating 0.01 ml of undiluted ascites fluid containing or lacking anti-PIP monoclonal antibody for 30 min at 37°C with 2×10^4 Chinese hamster ovary (CHO) cells previously attached (overnight) to microtiter wells. Various concentrations of DT were then added to the cells and the incubation was continued for an additional 30 min. The cells were washed to remove unbound toxin, fresh media were added, and toxicities were monitored microscopically for four days. The minimum cytotoxic dose (MCD) of toxin is defined as that amount required to kill 100% of the CHO cells. Preincubation of the CHO cells with control ascites fluid lacking anti-PIP antibody did not alter the MCD of DT (Table II). Preincubation with each of four different anti-PIP monoclonal antibodies increased the MCD for DT from 600 ng to at least 10,000 ng (Table II). Each of the anti-PIP monoclonal antibodies protected CHO cells from subsequent DT toxicity, provided the antibody was preincubated with CHO cells. Suppression of cytotoxicity was not observed when anti-PIP antibodies and DT were mixed and added simultaneously to CHO cells (data not shown). These data indicate that the monoclonal anti-PIP antibody inhibits DT cytotoxicity by interacting with the CHO cell rather than with the toxin molecule.

Pseudomonas toxin A, has an intracellular mechanism

TABLE I
SPECIFICITY OF ANTI-PIP ANTIBODIES

Inhibitor	Percent of trapped glucose released* at inhibitor concentration of	
	8 mM	30 mM
None	46	46
Inositol	45	47
Inositol hexaphosphate	0	1
Choline	48	42
Phosphocholine	3	
AMP	40	27
ATP	22	12

*Complement-dependent immune damage resulting in release of trapped glucose from liposomes was assayed as previously described (8) The liposomes consisted of DMPC, cholesterol, dicetyl phosphate, and PIP in molar ratios of 1/0.75/0.11/0.1. Each 0.005 ml aliquot of liposomes was incubated with 10^{-5} ml of ascites fluid containing anti-PIP antibodies and with 0.08 ml of guinea pig complement.

TABLE II

ABILITY OF ANTI-PIP MONOCLONAL ANTIBODIES TO INHIBIT CHO CELL CYTOTOXITY INDUCED BY BACTERIAL TOXINS

Antibody*	MCD	
	DT	*Pseudomonas toxin A*
	(ng/2 × 10^4 cells)	
None (control)	600	200
anti-PIP-1	>10,000	200
anti-PIP-2	10,000	200
anti-PIP-3	10,000	200
anti-PIP-4	10,000	200

*Each anti-PIP antibody consisted of ascites fluid derived from a separate clone after a single immunization and fusion. The original immunized mouse was BALB/c, and spleen cells were fused with myeloma line P3-X63-Ag8 (10). The control consisted of ascites fluid derived from fusion of cells from an unimmunized mouse

of action identical to that of DT, but the two toxins utilize different cell surface receptors (11). Indeed, Table II shows that the anti-PIP antibodies did not influence the toxicity of *Pseudomonas* toxin A.

Our data are compatible with our previous suggestion (4) that the cellular receptor for DT is a membrane phospholipid, and that PIP is a promising candidate as the receptor molecule. Even if PIP is not the initial receptor, it may have an important role during internalization of the toxin, or during channel formation through the lipid bilayer (12).

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