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Murine Responses to Immunization with Pertussis Toxin and Bovine Serum Albumin: I. Mortality Observed after Bovine Albumin Challenge is Due To an Anaphylactic Reaction

SUSAN E. WIEDMEIER, HUN-TAEG CHUNG, BAIK HWAN CHO, UH-HYUN KIM, AND RAYMOND A. DAYNES

Department of Pathology, University of Utah Medical Center, Salt Lake City, Utah 84132

ABSTRACT. It has been suggested that pertussis toxin (Ptx) is involved in the pathogenesis of the adverse neurologic reactions that can occur in infants and children after pertussis immunization. One group of investigators has recently reported that a clinical syndrome with pathological features very similar to post-pertussis vaccination encephalopathy can be induced in specific strains of mice after their immunization with bovine serum albumin (BSA) and Ptx. The aim of this investigation was to further characterize the immunologic mechanisms operative in this murine model. Studies were undertaken to determine whether the role played by Ptx in this condition required the A-protomer of the toxin to enter a cell and ADP-ribosylate a nucleotide binding protein (a Class I activity) or was dependent upon the binding of the B-oligomer of the toxin to the surface of target cells (a Class II activity). The results of our experiments have established that the disease induced by coimmunizing mice with Ptx and BSA is due to an immediate type hypersensitivity reaction rather than an encephalopathy and that the mechanism of action of Ptx in this system seems to be dependent upon a Class II activity of the toxin and independent of its ADP-ribosyl transferase activity. (*Pediatr Res* 22: 262–267, 1987)

Abbreviations

Ptx, pertussis toxin
Ni, subunit of the guanine nucleotide regulatory protein
BSA, bovine serum albumin
mPtx, methylated pertussis toxin
IV, intravenous
IP, intraperitoneal
^{3}[H]NAD, Tritiated nicotinamide adenine dinucleotide
IAP, islet-activating protein
LPF, lymphocytosis-promoting factor
HSF, histamine-sensitizing factor
MEM, minimal essential medium
FCS, fetal calf serum

Convulsions, infantile spasms, hypotonic hypotensive episodes, encephalopathy, and even death have been reported following pertussis vaccination in young children (1–8). It has been suggested but not proven that at least a portion of these sequelae may be caused by the Ptx contained within vaccine preparations (9, 10). Ptx, also referred to as IAP, LPF, or HSF is one of the exotoxins produced by *Bordetella pertussis*. It is an oligomeric protein (Mr = 117,000) consisting of an A (active) protomer and a B (binding) oligomer (11) and is known to have a wide variety of biological activities (12). The diversity of these activities *in vivo* is related to the multiplicity of target cells that are susceptible to the toxin's effects (13–15). Many of these effects have been ascribed to the capacity of Ptx to modify intracellular cyclic nucleotide metabolism (14, 16). The A-protomer of Ptx is capable of altering the regulation of cAMP by ADP-ribosylating nucleotide-binding proteins. Ni, a protein capable of inhibiting the activity of adenylate cyclase (17), transducin, a protein capable of enhancing the activity of cyclic GMP phosphodiesterase (18), and N_0, a protein whose function and relationship to Ni is currently unknown (19, 20), have all been reported to be ribosylated by enzymatic activity of Ptx. Recent evidence also suggests that Ptx may be capable of modulating transduction systems other than adenylate cyclase (21–23). In addition, it is now appreciated that a number of the biological activities of this toxin, especially those involved in immunomodulation, are unrelated to its ADP-ribosyl transferase activity (21). Such evidence emanated from the finding that methylation or acetamidination of Ptx resulted in a loss of a number of its reported bioactivities, without modifying its ADP-ribosyl transferase activity (24, 25).

The objective of this study was to determine whether the mechanism(s) of action of Ptx in the murine postpertussis "encephalopathy" model recently described by Steinman *et al.* (26, 17) is dependent on its ability to ADP-ribosylate nucleotide binding proteins. These investigators reported that a clinical syndrome with pathological features closely resembling human postpertussis vaccination encephalopathy can be reproduced in certain strains of mice by immunization with purified preparations of Ptx and BSA (26). Our results demonstrate that the disease process invoked in mice employing this experimental model system is not a true encephalopathy. Rather, this condition represents hypovolemic shock induced by an acute hypersensitivity reaction (anaphylaxis) and not a specific neurological insult. Furthermore, the development of this immediate hypersensitivity response was established to be dependent on a class II activity of Ptx and unrelated to the capacity of toxin to alter intracellular cyclic nucleotide metabolism.

MATERIALS AND METHODS

Animals. BALB/cAnNCr and CR:SW mice were originally obtained from the animal production facility of the National Cancer Institute (Bethesda, MD) and bred in our colony. All mice were housed at a maximum density of six animals per 7 ×

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Correspondence and requests for reprints Raymond A. Daynes, Ph.D., Department of Pathology, University of Utah Medical Center, Salt Lake City, UT 84132.
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11 inch cage and maintained on Wayne sterilizable Lab Blox and acidified water *ad libitum*. Mice used in experiments were 6–10 wk of age.

Chemicals and reagents. Purified pertussis toxin was purchased from List Biological Laboratories (Campbell, CA), nicotinamide adenine dinucleotide, guanosine triphosphate, adenosine triphosphate, and thymidine from Sigma Chemical Co. (St. Louis, MO), borane-pyridine complex from Aldrich Chemical Co., Inc. (Milwaukee, WI), formaldehyde (37%) from J. T. Baker Chemical Co. (Phillipsburg, NJ), methyseride maleate from Sandoz Inc. (East Hanover, NJ), and cyproheptadine HCl from Merck Sharp and Dohme (West Point, PA).

Methylation of Ptx. Methylated Ptx was prepared as described by Ui *et al.* (25). Briefly, 250 μl of a stock solution of Ptx (100 μg/ml) in 0.01 M phosphate buffer (pH 7.0)/0.05 M NaCl was mixed with an equal volume of 20 mM formaldehyde and 30 mM borane-pyridine complex (pH 7.0) in the phosphate buffer. This reaction mixture was incubated for 2 h in an atmosphere of nitrogen at room temperature followed by exhaustive dialysis against phosphate-buffered saline. As a control, Ptx was treated in an identical manner with the omission of the incubation step in the presence of formaldehyde and borane-pyridine complex.

Isolation of peritoneal macrophages. Resident macrophages were obtained by lavaging the peritoneal cavity of mice with 5.0 ml of cold heparinized (2 U/ml) MEM (Flow Laboratories, Inc., McLean, VA). Cells in the peritoneal fluid were washed and resuspended in heparin free MEM containing 10% FCS (Hy-Clone Sterile System, Inc., Logan, UT) and incubated in a glass Petri dish at 37° C within a humidified incubator (5% CO_2) for 90 min. The nonadherent cells were discarded and the macrophage monolayer was collected by treatment with lidocaine.

ADP-ribosylation of Ni by Ptx and mPtx. The capacity of Ptx and mPtx to ADP-ribosylate membrane proteins was evaluated using a slight modification of the procedure as described by Backlund *et al.* (28). Briefly, murine peritoneal macrophages (5×10^6 cells/ml) were suspended in MEM containing 10% FCS and either Ptx or mPtx (20 ng/ml) for 2 h at 37° C. Crude membrane homogenates were then prepared from both the control and treated macrophages and the membrane preparations were incubated with ^{3}[H]NAD and fresh Ptx. The standard reaction was carried out in 0.1 M potassium phosphate (pH 7.0), containing 10 mM thymidine, 1 mM ATP, 0.1 mM GTP, 1.8 μM ^{3}[H]NAD (sp act: 27.1 Ci/mmol), activated Ptx (500 ng) and cell membranes for 1 h at 37° C in a total volume of 50 μl. Ptx was activated by incubation of the toxin for 30 min at room temperature in 50 mM potassium phosphate buffer, pH 7.0 containing 25 mM dithiothreitol. Membranes were washed free of unreacted NAD and the amount of incorporated radioactivity was counted with a scintillation counter (Packard Instrument Co., Inc., Dower Grove, Il).

Effect of Ptx and mPtx on epinephrine-induced hyperglycemia. The ability of Ptx and mPtx to inhibit epinephrine-induced hyperglycemia was evaluated using a slight modification of the method described by Yajima *et al.* (29). Briefly, the mice were given an IV injection of either Ptx or mPtx (130 ng). Control animals were given an injection of normal saline. Seventy-two h later the mice (fasted for 6 h) were subcutaneously injected with epinephrine (200 μg/kg of body weight). The blood glucose concentration of each animal was determined immediately before and 60 min after the epinephrine injection. Blood specimens used for blood glucose determinations were obtained via the retroorbital sinus. The glucose concentration of each sample was determined by the glucose oxidase method (30).

Lymphocytosis-promoting activity of Ptx and mPtx. The lymphocytosis-promoting capacity of Ptx and mPtx was assessed by quantitating the number of lymphocytes in the peripheral circulation of mice that had received an IV injection (250 ng) of either Ptx or mPtx 72 h previously. Control animals received an IV infusion of normal saline. Fifty μl of blood were obtained from each experimental and control mouse in heparinized capillary tubes. Peripheral leukocyte counts were determined with a hemocytometer and differential analysis of leukocytes was performed on Wrights stained blood smears. The mean values obtained from three similarly treated mice were used as an index of lymphocytosis.

Induction of the BSA-Ptx disease. Mice were immunized according to the protocol as previously described by Steinman *et al.* (26). On days −1 and +1 the mice (BALB/cAnNCr) were given 1 mg of BSA in 0.2 ml of phosphate-buffered saline IP, and on days 0 and 2 they were given 100 ng of Ptx or mPtx IV. On day 6 the animals were given a 1 mg BSA injection and observed closely for the development of lethargy, tachypnea, cyanosis, seizures, and death.

Adoptive transfer of BSA-Ptx-induced disease. Mice (BALB/cAnNCr) to be used as donors in adoptive transfer experiments were given 1 mg of BSA in 0.2 ml of phosphate-buffered saline IP on days −1 and +1, and 100 ng of Ptx or mPtx by intravenous infusion on days 0 and 2. Peripheral blood was collected from each animal on day 6 and following sacrifice their peripheral lymph nodes were surgically excised. The peripheral blood samples were allowed to clot and then centrifuged. The serum was collected and transferred IV (0.25 ml/animal) into normal BALB/cAnNCr mice or mice that had been injected IV with Ptx 24 h previously. Donor lymph nodes were gently dissociated in RPMI 1640 (Dutchland Laboratory, Inc., Denver, PA) supplemented with 5% FCS and the resulting single cell suspension was transferred IV (10^7 cells/recipient) into normal mice or mice that had received an IV infusion of Ptx or mPtx 24 h previously. One h following the infusion of serum or lymphoid cells all recipients were given 1.0 mg of BSA intraperitoneally. The animals were then observed closely for the next 2 h for lethargy, tachypnea, cyanosis, seizures, and death.

RESULTS

Ptx and methylated Ptx can ribosylate nucleotide binding proteins. The biological activities of Ptx on susceptible target cells have recently been divided into two general categories. Those that are dependent on the ability of the A-protomer of the toxin to enter a cell and ADP-ribosylate nucleotide binding proteins such as Ni, a 41kDa membrane protein involved in cyclic nucleotide regulation, have been termed class I activities (24). Those activities which are secondary to the binding of the B-oligomer of Ptx to the cell surface and do not involve the modification of nucleotide binding proteins by ribosylation have been termed class II activities (24). Ui *et al.* (25) have previously shown that reductive methylation of Ptx, a procedure which results in over 90% of the amino groups of the lysine residues being dimethylated, markedly diminishes the capacity of the toxin to elicit class II activities while leaving the class I activities relatively intact.

Our first series of experiments were designed to confirm that both Ptx and mPtx were capable of ADP-ribosylating nucleotide binding proteins *in vivo* and *in vitro*. As shown in Table 1, the preincubation of intact macrophages with either Ptx or mPtx resulted in an equivalent reduction of the available endogenous membrane proteins susceptible to ribosylation with activated Ptx subsequent to membrane preparation.

Experiments were also conducted where preparations of macrophage membranes were incubated with ^{3}[H]NAD in the presence of either Ptx or mPtx. The radiolabeled membranes then were washed and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Only one band of radioactivity was found, corresponding to a molecular weight of 41 kDa. Scintillation counting of the isolated 41 kDa band determined that the ability of mPtx and Ptx to catalyze the ADP-ribosylation of the membrane protein was equivalent (data not shown). These results indicate that both Ptx and mPtx were capable of endogenously ribosylating a 41 kDa membrane protein, a class I Ptx activity.

Table 1. *ADP-ribosylation of macrophage membranes by Ptx or mPtx*

Treatment of macrophages*	Percent of Ni ribosylated†
None	0
Ptx (20 ng/ml)	57.0 ± 1.2
mPtx (20 ng/ml)	62.6 ± 1.6

* Murine peritoneal macrophages were suspended at 5×10^6 cells per ml and incubated for 2 h at 37° C with either Ptx or mPtx. Crude membrane homogenates were then prepared from both the control and treated macrophages and the membrane preparations were incubated with [^{3}H]NAD and fresh Ptx. Incorporated radioactivity was then measured by scintillation counter. Maximal incorporated radioactivity ranged from 4,500–11,600 cpm for control membranes depending on the experiment.

† The amount of radioactivity incorporated into control membranes was considered to be 100%. Results are expressed as the percentage of endogenous Ni no longer available for ribosylation with radiolabeled NAD. (Percentage of Ni ribosylated during initial incubation with Ptx or mPtx.) The ADP-ribosylation reactions were performed in duplicate and the experiment was repeated numerous times with equivalent results.

Ptx and mPtx block catecholamine-induced hyperglycemia. To question whether Ptx and mPtx could also function *in vivo* to mediate class I Ptx activities, experiments were designed to compare the effects of Ptx and mPtx on catecholamine-induced hyperglycemia. Katada and Ui (31) have previously demonstrated that the failure of epinephrine to induce hyperglycemia in pertussis vaccine-treated animals resulted from a paradoxical epinephrine-induced hyperinsulinemia. These investigators also presented data which established that the exaggerated insulin secretion was a consequence of the ability of Ptx to abolish the normal α-adrenergic secretion of pancreatic cells to epinephrine (32), a class I effect.

It was determined (Table 2) that epinephrine-induced hyperglycemia could not be elicited in animals who had received either Ptx or mPtx. These findings indicate that mPtx was able to block the α-adrenergic response of pancreatic islet cells to epinephrine to the same degree as native Ptx, a consequence previously established to be dependent upon the modification of cAMP responses in the pancreatic islet cells (33).

The methylation of Ptx attenuates the toxin's ability to mediate class II activities. We next questioned what effect methylation would have on the lymphocytosis-promoting activity of Ptx. The lymphocytosis-promoting action of Ptx has been shown to represent a class II effect of the toxin (24). As shown in Table 3, the number of lymphocytes in the peripheral circulation of animals who had been treated with native Ptx was greatly elevated over the number found in control animals. However, peripheral lymphocyte counts of animals who had received mPtx were only slightly elevated over control values. These results indicate that modification of Ptx by methylation is capable of attenuating its lymphocytosis-promoting activity and are in agreement with those previously reported by Ui *et al.* (25) who demonstrated that the class II biological activities of Ptx are selectively diminished following reductive methylation.

mPtx can not be substituted for Ptx in murine Ptx-BSA induced disease. Having established that mPtx preparation retains class I activities but has lost its class II activities, we addressed the question of whether the disease process induced by co-immunizing mice with Ptx and BSA is due to the stimulation of class I or class II activities of Ptx. BALB/cAnNCr mice were coimmunized with BSA and Ptx or BSA and mPtx as described in "Materials and methods." The results of this experiment (Table 4) confirm that animals coimmunized with Ptx and BSA become lethargic, cyanotic, tachypneic, and die within 2 h after receiving a challenge with BSA. In contrast, animals who had been coimmunized with methylated Ptx and BSA remained symptom free. These results suggest that the involvement of Ptx in the BSA-Ptx induced disease is not due solely to its ADP-ribosyl transferase activity. Rather, the condition must also be associated with one of the class II or immunopotentiating activities of the toxin.

Table 2. *Effect of Ptx and mPtx on epinephrine-induced hyperglycemia*

Experiment	Group	Treatment*	Blood glucose concentration (mg/dl)† Before epinephrine	After epinephrine
1	A	None	108 ± 5.7‡	146 ± 16.0
	B	Ptx	84 ± 4.1	77 ± 4.1
	C	mPtx	84 ± 3.9	74 ± 2.3
2	A	None	86 ± 0.2	130 ± 0.7
	B	Ptx	82 ± 8.3	84 ± 0.8
	C	mPtx	69 ± 1.7	82 ± 0.4

* Mice received an IV injection of either Ptx or mPtx (250 ng). Control mice received normal saline.

† Sixty-nine h after the Ptx or mPtx infusion the mice (fasted for 6 h) were subcutaneously injected with epinephrine (200 μg/kg of body weight). The blood glucose concentration of each animal was determined immediately before and 60 min after receiving the epinephrine injection.

‡ Data are expressed as the mean ± SEM of three similarly treated mice.

Table 3. *Lymphocytosis promoting activities of Ptx and mPtx*

Experiment*	Group	Treatment†	No. of lymphocytes‡ (1×10^{-6} cells/ml)
1	A	None	8.93 ± 0.27§
	B	Ptx	40.30 ± 2.06 (451%)‖
	C	mPtx	14.30 ± 1.90 (160%)
2	A	None	7.82 ± 0.84
	B	Ptx	40.30 ± 1.82 (515%)
	C	mPtx	10.60 ± 2.32 (136%)

* Mice utilized in the first experiment were CR:SW and those in the second were BALB/cAnNCr.

† Mice received an IV injection of either Ptx or mPtx (250 ng). Control mice received normal saline.

‡ Lymphocytes were quantitated from 72 h postinjection peripheral blood samples.

§ Data are expressed as the mean ± SEM of three similarly treated mice.

‖ The number in parentheses represents the percent of control.

BSA-Ptx induced disease can be adoptively transferred to normal recipients with immune serum but not sensitized lymphoid cells obtained from BSA immunized donors. Adoptive transfer experiments were conducted to determine whether the condition caused by coimmunization with BSA and Ptx could be adoptively transferred with serum or lymphoid cells to naive or toxin-treated recipients. As shown in Table 5, under no conditions were we able to adoptively transfer the clinical disease process to normal or Ptx-treated recipients with donor lymphoid cells. Further, the results demonstrate that the pathological disorder induced by immunization with BSA and Ptx could be adoptively transferred to Ptx pretreated recipients with immune sera (group H, experiment 1; and group D, experiment 2). A comparison of the results obtained from this experiment indicates that the donor of the immune serum must have received Ptx in order to generate the serum factors responsible for the adoptively transferable effect. The results also establish that recipients of the immune serum must also have been pretreated with Ptx to allow the manifestation of the condition. These findings indicate that the disease induced in animals coimmunized with BSA and Ptx is dependent on the production of a humoral substance and not on the generation of a cellular immune response. The results also suggest that Ptx treatment is essential for both the afferent and efferent phases of the BSA-Ptx induced illness.

Neither the afferent nor the efferent phase of Ptx-BSA induced disease is solely dependent on the ADP-ribosyl transferase activity

of Ptx. We next questioned whether mPtx could replace Ptx in either of the two toxin requiring phases of the disease process (afferent or efferent). To assess the toxin's role in generating the humoral component of the response, experiments were designed in which donor mice were coimmunized with either Ptx and BSA or mPtx and BSA prior to serum collection and adoptive transfer to susceptible recipients. To evaluate the role of the toxin in the efferent phase of the process, recipient animals were "primed" with either Ptx or mPtx 24 h prior to receiving immune serum and the BSA challenge. As shown in Table 6, animals that were immunized with Ptx and BSA were capable of producing a humoral substance that, when adoptively transferred to Ptx-pretreated recipients, was capable of transferring the disease process. Donor animals that were treated with mPtx and immunized with BSA did not generate the humoral components necessary to adoptively transfer the disorder to susceptible recipients (group A). Furthermore, mPtx was also incapable of rendering normal recipients susceptible to the pathological process caused by the adoptive transfer of serum from BSA and Ptx immunized donors (group D). These results indicate that the involvement of Ptx in both the afferent and efferent phases of this process are likely associated with its class II, and not class I, biological activities.

Table 4. *Murine responses to immunization with BSA and Ptx or mPtx*

Experiment	Group	Treatment*	No. affected/no. tested†
1	A	None	0/5
	B	BSA	0/5
	C	Ptx	0/5
	D	mPtx	0/5
	E	BSA + Ptx	5/5‡
	F	BSA + mPtx	0/5
2	A	None	0/5
	B	BSA + Ptx	5/5‡
	C	BSA + mPtx	0/5

* Mice received an IP injection of BSA (1 mg) on days −1 and +1, and an IV infusion of either Ptx or mPtx (100 ng) on days 0 and +2.

† On day 6 all animals were challenged with an IP injection of BSA (1 mg). Animals were then observed closely for lethargy, tachypnea, seizure activity, and death.

‡ All animals in group E (experiment 1) and group B (experiment 2) died within 2 h of BSA injection.

Symptoms of BSA-Ptx-induced disease are ameliorated by treatment of immunized mice with serotonin antagonists and can also be reversed by fluid therapy. Since we were able to adoptively transfer the BSA-Ptx induced pathologic process with immune serum we questioned whether this process was actually due to an immediate or type I hypersensitivity response. Because the primary vasoactive amine associated with immediate hypersensitivity responses in mice is serotonin (34), we designed experiments to evaluate the effect of serotonin antagonists on Ptx-BSA-induced disease. Mice were coimmunized with BSA and Ptx according to the basic protocol described in "Materials and Methods." After the challenge BSA injection on day 6, those animals that had not been pretreated with the serotonin antagonists exhibited the typical shock-like symptoms and died within 2 h. The animals that had been given a known serotonin antagonist seemed subjectively less active for a short period of time but otherwise remained symptom free (Table 7). These results strongly suggest that the murine BSA-Ptx-induced disorder is actually the product of an immediate-type hypersensitivity response.

Previous studies have shown that the death of mice from

Table 6. *mPtx is ineffective in both afferent and efferent phase of BSA-Ptx-induced disease*

Group	Donor treatment*	Recipient treatment†	No. affected‡ / No. tested
A	BSA + mPtx	Ptx + immune serum	0/10
B	BSA + mPtx	mPtx + immune serum	0/10
C	BSA + Ptx	Ptx + immune serum	8/10§
D	BSA + Ptx	mPtx + immune serum	0/10

* Donor mice (BALB/cAnNCr) received an IP injection of BSA on days −1 and +1, and an IV injection of either Ptx or mPtx (100 ng) on days 0 and +2.

† Immune serum from donor animals was prepared as described in "Materials and methods." Each recipient animal (BALB/cAnNCr) received an IV infusion of immune serum (0.25 ml) 24 h after receiving an IV injection of either Ptx or mPtx (100 ng). One h after infusion of immune serum all recipients were given an IP injection of BSA (1 mg).

‡ Animals were observed closely for lethargy, tachpnea, cyanosis, seizure activity, and death.

§ Eight of the 10 animals became lethargic and cyanotic shortly after the IP injection of BSA. Seven of the eight died within 2 h.

Table 5. *Murine BSA-Ptx-induced disease can be adoptively transferred to recipients with serum but not lymphoid cells from immunized donors*

Experiment	Group	Donor treatment*	Recipient treatment†	No. affected‡ / No. tested
1	A	BSA	Ptx + immune lymphocytes	0/5
	B	BSA	Ptx + immune serum	0/5
	C	Ptx	Ptx + immune lymphocytes	0/5
	D	Ptx	Ptx + immune serum	0/5
	E	BSA + Ptx	Immune lymphocytes	0/5
	F	BSA + Ptx	Ptx + immune lymphocytes	0/5
	G	BSA + Ptx	Immune serum	0/5
	H	BSA + Ptx	Ptx + immune serum	5/5§
2	A	BSA + Ptx	Immune lymphocytes	0/5
	B	BSA + Ptx	Ptx + immune lymphocytes	0/5
	C	BSA + Ptx	Immune serum	0/5
	D	BSA + Ptx	Ptx + immune serum	5/5§

* Donor mice (BALB/cAnNCr) received an IV injection of Ptx (100 ng) on days 0 and +2, and/or an IP injection of BSA (1 mg) on days −1 and +1. On day 6 peripheral blood was collected from each animal, they were then sacrificed and their peripheral lymph nodes excised.

† Lymphoid cells and immune serum were prepared from donor animals as described in "Material and methods." Each recipient animal received an IV infusion of 1×10^7 lymph node cells or 0.25 ml of immune serum. Selected groups of recipients had received an IV injection of Ptx 24 h prior to adoptive transfer. An IP injection of BSA was given to all recipients 1 h after receiving the infusion of immune serum or lymphoid cells.

¶ Animals were observed closely for lethargy, tachypnea, cyanosis, seizure activity, and death.

§ All recipient animals in groups H (experiment 1) and D (experiment 2) died within 2 h of receiving the IP injection of BSA.

Table 7. *Effect of serotonin antagonists on development of BSA-Ptx-induced disease*

Group*	Treatment†	No. of deaths‡ / No. tested
A	None	6/6
B	Cyproheptadine	0/6
C	Methyseride	0/6
D	IV normal saline§	0/6‖

* All mice (BALB/cAnNCr) were coimmunized with BSA and Ptx according to the protocol listed in "Material and methods."

† The animals in group B were given 0.6 mg of Cyproheptidine by gavage every 6 h beginning on day 4 of the protocol. Group C received 2.5 mg of Methsergide every 6 h. The animals in group A were gavaged with normal saline. On day 6 all animals were given an IP injection of BSA (1 mg).

‡ Animals were observed closely for lethargy, tachypnea, cyanosis, seizure activity, and death.

§ Animals were given 0.5 ml of normal saline intravenously within 10 min of becoming symptomatic (all animals in group D demonstrated lethargy, tachypnea, and mild cyanosis within 30 min of IP BSA injection).

‖ All animals in group A died within 2 h of BSA challenge.

immediate hypersensitivity reactions is due to loss of blood volume (35). With this knowledge we questioned whether volume expansion of the animals during the acute stages of their illnesses would clinically modify the disease process. One group of Ptx and BSA immunized animals was therefore given an intravenous infusion of normal saline (0.5 ml) within 10 min of becoming symptomatic. We found that the animals that received a bolus of normal saline shortly after becoming symptomatic survived (group D), while those that did not receive fluid therapy died (group A). These results lend support to the hypothesis that the clinical condition produced by coimmunizing mice with Ptx and BSA is secondary to an immediate type hypersensitivity response (anaphylaxis) and does not resemble or simulate the neurological illness temporally related to pertussis immunization in infants.

DISCUSSION

Steinman *et al.* (26) recently reported that a clinical syndrome with pathological features very similar to postpertussis vaccination encephalopathy could be induced in specific strains of mice after their immunization with BSA and Ptx. Previously, this same group established that a similar type of clinical condition could be induced in mice by immunization with BSA and pertussis vaccine (27) and suggested the possibility of an anaphylactic-type reaction being responsible.The original intention of the present investigation was to determine whether the mechanism of action of Ptx in this murine model was related to the toxin's capacity to induce a class I or class II activity. Our studies, however, have now established that Steinman's model is not one of neurologic encephalopathy but rather is the result of vascular collapse secondary to an anaphylactic reaction. Several lines of experimental evidence support this conclusion: 1) sensitivity to the BSA-Ptx-induced disease can be passively transferred with the immune serum obtained from sensitized animals into Ptx-treated recipients; 2) animals that are immunized with BSA and Ptx and then pretreated with serotonin antagonists prior to BSA challenge do not develop the typical clinical manifestations of the disorder; and 3) symptoms of the condition can possibly be alleviated by giving BSA-Ptx immunized animals fluid therapy as soon as clinical manifestations are noted following the BSA challenge.

In mice, both IgG_1 and IgE can function to induce antigen-specific release of mediators from mast cells and basophils that are capable of initiating immediate type hypersensitivity reactions (36). Antibodies belonging to the IgG_1 subclass, unlike IgE, are not inactivated by heating at 56° C or by sulfhydryl reagents (37). We have determined that heating the serum from BSA-Ptx sensitized animals for 30 min at 56° C did not diminish the ability of that immune serum to passively transfer anaphylactic sensitivity to Ptx primed recipients (data not shown). These results suggest, but do not prove, that antibodies of the IgG_1 subclass may be involved in the BSA-Ptx-induced immediate hypersensitivity reaction. Further work is in progress to test this hypothesis.

Our studies have also revealed that the development of anaphylaxis in this murine system exhibits some interesting characteristics. By employing preparations of Ptx and mPtx in parallel we were able to demonstrate that the ADP-ribosyl transferase capacity of pertussis toxin is insufficient to initiate the BSA-Ptx-induced immediate type hypersensitivity reaction. Our findings also indicate that the development of this reaction is associated with a class II or immunopotentiating activity of the toxin. In a series of adoptive transfer experiments we further demonstrated that both the donor of the humoral component and the recipients of the immune serum need to be treated with Ptx for the BSA-Ptx induced hypersensitivity response to take place. mPtx could not substitute for Ptx in either the afferent or efferent phase of this process.

While the murine model system described Steinman et al. (26) originally looked quite promising, our finding that the experimentally produced condition actually represents an immediate-type hypersensitivity reaction rather than a true encephalopathy indicates that an experimental model for human postpertussis vaccination encephalopathy is still lacking.

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