

Pertussis toxin enhanced IgG1 and IgE responses to primary tetanus immunization are mediated by interleukin-4 and persist during secondary responses to tetanus alone

Matthew H. Samore*†‡ and George R. Siber*†§

Pertussis toxin (Ptx), the major toxin product of Bordetella pertussis, has potent immunologic effects including adjuvant effects on antibody responses and sensitization for anaphylaxis. In order to further define the effect of Ptx on the class and subclass of murine antibody response, we measured total and antigen specific IgG subclasses and IgE in Balb/c mice after primary and secondary immunization with tetanus toxoid (TT). Low doses of Ptx (100 ng) given intravenously at the time of primary immunization increased primary IgG1 and IgE anti-TT antibodies as well as total IgG1 and IgE concentrations compared to controls. The increase in IgG1 subclass and IgE response when Ptx was present during primary immunization was even more pronounced after secondary immunization with TT alone 3 weeks or 3 months later. Similar effects were noted after diphtheria toxoid immunization in the presence of Ptx. Administration of the anti IL-4 monoclonal antibody (11B11) suppressed the enhanced total and TT-specific IgE responses but not the enhanced IgG1 responses. The presence of low concentrations of Ptx during primary immunization primes for induction of IL-4 producing T-cell help which enhances IgG1 and IgE responses to the primary exposure as well as to subsequent exposures of the antigen in the absence of Ptx. This phenomenon may have significance for the adjuvant activity of vaccines containing Ptx as well as for the immune response to natural pertussis. Copyright © 1996 Elsevier Science Ltd.

Keywords: Pertussis toxin; immune responses; interleukin-4; anti-tetanus toxoid antibodies

Pertussis toxin (Ptx) is the major toxin product of *Bordetella pertussis*¹. Like other bacterial toxins, Ptx consists of an enzymatically active A subunit and a binding moiety, called the B oligomer². The B-oligomer adheres to mammalian cells and delivers the A subunit to its targets. The A subunit catalyzes the transfer of ADP-ribose from NAD to members of the family of guanine nucleotide binding regulatory proteins (G-proteins) in target cells. By this mechanism, Ptx exerts diverse biologic effects, which include sensitization of mice to histamine, enhancement of insulin secretion in response to regulatory signals and both suppressive and stimulatory immunologic effects^{1,3}. Ptx also causes the marked

lymphocytosis associated with typical pertussis by inhibiting the migration of circulating lymphocytes into lymphoid organs^{4,5}, suppresses chemotaxis and phagocytosis by neutrophils and macrophages^{6,7} and increases susceptibility of infant rats to *Haemophilus influenzae* type b infection⁸. Conversely, Ptx stimulates delayed-type hypersensitivity responses⁹, and augments antibody responses to concurrently administered antigens³. In mice, nanogram quantities of Ptx administered concurrently with egg albumin markedly enhances serum activities mediating passive cutaneous anaphylaxis which are believed to be due to specific IgE antibody¹⁰. In high concentration, Ptx is directly mitogenic for lymphocytes^{11–14}. This effect occurs with enzymatically inactive Ptx mutants or with the B-oligomer alone^{15,16} and thus differs from the other immunomodulatory effects which depend on enzymatic activity¹⁷.

In order to further examine the immunomodulating properties of Ptx, we investigated the effect of a low subimmunogenic dose of Ptx on antibody class and subclass responses to tetanus toxoid (TT) and diphtheria toxoid (DT). These antigens were chosen because they

*Laboratory of Infectious Diseases, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA. †The Massachusetts Public Health Biologic Laboratories, State Laboratory Institute, Jamaica Plain, MA, USA. ‡Current address: Division of Infectious Diseases, New England Deaconess Hospital, One Deaconess Road, Boston, MA 02215, USA. §To whom correspondence should be addressed. (Received 24 April 1995; revised 18 August 1995; accepted 12 September 1995)

are clinically relevant protein antigens which are usually given together with pertussis vaccines. Booster doses of TT or DT were given to animals at different intervals after primary immunization in order to compare the effect of Ptx on primary and secondary antibody responses. Finally, the role of interleukin-4 (IL-4) in mediating the adjuvant activity of Ptx was examined with a neutralizing monoclonal anti-IL-4 antibody (11B11).

MATERIALS AND METHODS

Animals

Female Balb/c mice 8–12 weeks of age obtained from Jackson Laboratories were used in all experiments.

Monoclonal antibodies/hybridomas

The following hybridomas were obtained: rat IgG1 monoclonal anti-mouse IL-4 (11B11)¹⁸ (kindly provided by Dr Richard Hom, DFCI), rat IgG1 anti-p21 protein product of the ras oncogene (Y13-259)¹⁹ (American Type Culture Collection, No. CRL 1742), and rat IgG2a anti-mouse IgE (EM95)²⁰ (kindly provided by Dr Abul Abbas). Nude mice were induced to produce ascites by i.p. inoculation with hybridoma cells. Monoclonal antibody (MAb) was partially purified and concentrated by ammonium sulfate precipitation of ascitic fluid and rat IgG was quantitated by ELISA.

Immunizations/animal experiments

Female Balb/c mice, 8–12 weeks of age, were randomly separated into groups of 3–5 animals, individually marked with an ear hole puncher. Primary immunization consisted of 2 Lf (9.4 μ g protein) TT (Massachusetts Public Health Biologic Laboratories, Jamaica Plain, MA, lot LP462PP) or saline administered ip concurrently with 0 or 100 ng of Ptx, diluted in 0.25% human albumin (HSA)/phosphate-buffered saline (PBS) (kindly provided by Dr Larry Winberry at the Massachusetts Public Health Biologic Laboratories) administered i.v. into the tail vein. Booster doses of TT (secondary immunization) were administered 3 weeks to 3 months after primary immunization. Ptx was not given with 2° immunizations. In otherwise identical experiments, 2 Lf (6.47 μ g protein) DT (Massachusetts Public Health Biologic Laboratories, lot PT238P) was used in place of TT as the immunizing antigen.

In other experiments, 1 mg of rat monoclonal anti-IL-4 (11B11) or rat monoclonal anti-p21 protein (Y13-259) was administered to mice i.p. simultaneously with primary or secondary immunization.

Sera were collected by retroorbital bleed on the day of primary and secondary immunizations, 16 days after primary immunization and 10 days after secondary immunization. At the conclusion of the experiments, animals were killed by cervical dislocation and blood was collected by intracardiac puncture.

Assays for immunoglobulins and antibodies

ELISA for total murine IgG1, IgG2a, IgG2b, IgG3 and IgM. Microtiter plates (Flow Laboratories, McLean, VA) were coated by incubation at 37°C for 2 h with 100 μ l well⁻¹ of unlabeled goat anti-mouse IgG1, IgG2a, IgG2b, IgG3 (Southern Biotechnology, Birmingham, AL) or goat anti-mouse IgM (Tago, Burlingame, CA),

diluted to 2.5 μ g ml⁻¹ in PBS, pH 7.2, for measurement of the respective IgG subclass or IgM. Plates were washed with PBS-Tween between all steps. Serial dilutions of sera in PBS-Tween were added to the plates for overnight incubation at room temperature. For measurement of the respective isotype, the alkaline phosphatase conjugate of goat anti-mouse IgG1, IgG2a, IgG2b, IgG3 (0.8 μ g ml⁻¹) (Southern Biotechnology) or IgM (0.8 μ g ml⁻¹) (Tago) was added for a 3 h incubation at 37°C. Substrate (Sigma 104 phosphatase substrate) was added and the reaction was stopped after 30 min with 75 μ l well⁻¹ of 1 M NaOH. The O.D. was read at 405 nm on an automated ELISA reader and converted to immunoglobulin concentrations by extrapolation from standard curves determined with myeloma proteins of known concentration (Organon Teknika, West Chester, PA). The absence of significant cross-reactivity or interference from other isotypes was verified for each assay.

ELISA for total murine IgE. A double sandwich ELISA was used. The microtiter plates were coated with a 1/2000 dilution of rat monoclonal anti-mouse IgE (EM95) at 37°C for 2 h. Serial dilutions of sera or standard were added for overnight incubation at room temperature. Rabbit anti-mouse IgE (Nordic Laboratories, Tilburg, Netherlands), that was pre-absorbed against mouse IgG immobilized on agarose (Sigma, St. Louis, MO), was added at 1/2000 dilution for 3 h incubation at 37°C. After washing, a 1/1000 dilution of alkaline phosphatase conjugated goat anti-rabbit IgG (Caltag Labs, South San Francisco, CA) was added for another 3 h incubation at 37°C. The plates were developed with substrate and read at 405 nm on the automated ELISA reader. IgE concentration was determined by extrapolation from a standard curve using monoclonal mouse anti-DNP IgE (ICN Immuno-Biologicals, Lisle, IL). The sensitivity of this assay for detection of IgE was ca 1 ng ml⁻¹.

ELISA for IgG subclass and IgM antibodies to TT. Microtiter plates were coated with TT at 37°C for 2 h (2.0 μ g ml⁻¹ for IgG1; 5.0 μ g ml⁻¹ for other IgG subclasses). Serial dilutions of sample sera or pooled standard sera were added for overnight incubation at room temperature. Alkaline phosphatase conjugates of goat anti-mouse IgG1, IgG2a, IgG2b and IgG3 (0.8 μ g ml⁻¹) were added for the determination of the respective IgG subclass anti-TT antibody. Substrate was added and O.D. determined on the automated ELISA reader. The O.D. was converted to μ g ml⁻¹ by extrapolation from the standard curve of a mouse reference serum pool. The method of Zollinger and Boslego²¹ was employed to estimate the concentrations of specific antibody in the reference serum. Antibodies to DT were assayed using the same method and reagents, except that plates were coated with DT instead of TT.

Measurement of IgM-anti-TT antibodies was attempted but found to be unreliable because of high levels of background activity in pre-immunization sera. Pre-immunization sera for IgG subclass TT antibodies were uniformly negative at the lowest dilution tested.

ELISA for IgE antibodies to TT. Plates were coated with TT (5.0 μ g ml⁻¹) for 2 h at 37°C. In order to reduce

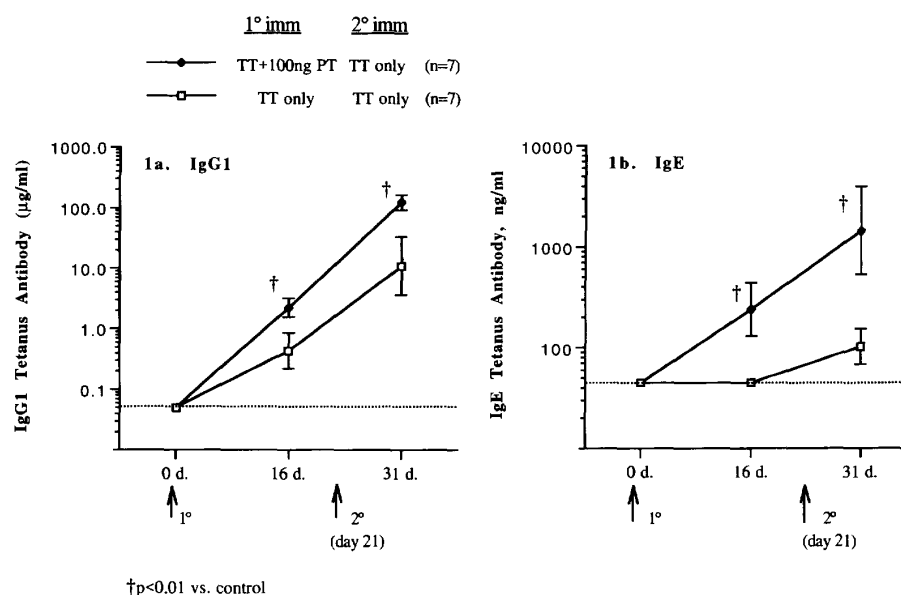


Figure 1 (a) and (b) Geometric mean IgG1- and IgE-anti-tetanus toxoid antibody ($\pm$ S.E.M.) after primary immunization (TT plus Ptx or saline control) and secondary immunization (TT alone) on day 21

the concentrations of IgG antibodies which compete with IgE antibodies for binding to TT, the sera or pooled standard were diluted 1/10 with protein G coupled to sepharose beads (Gammagone®, Genex, Gaithersburg, MD), vortexed 4 times over 5 min and centrifuged at 3000g for 10 min. In separate experiments this procedure was shown to remove >90% of IgG-anti-TT antibody. The supernatants were collected, diluted further in PBS-Tween and added to the plates for overnight incubation at room temperature. A 1/1000 dilution of rabbit anti-mouse IgE (Nordic) (absorbed against mouse IgG agarose) was added for a 3 h incubation at 37°C and followed by a 3 h incubation with a 1/1000 dilution of goat anti-rabbit IgG alkaline phosphatase. Substrate was added and the plates were read at 405 nm on the automated ELISA reader. The O.D. was converted to ng ml⁻¹ of antibody by extrapolation from the standard curve of a pooled reference serum.

Statistical analysis. In experiments consisting of two groups, *t*-tests were used to compare geometric mean TT antibody concentrations and mean total immunoglobulin class and subclass concentrations. In experiments consisting of more than two groups, pairwise comparisons of geometric mean antibody concentrations and mean total immunoglobulin concentrations were performed using Tukey's HSD test (Systat for Dos, Evanston, IL). For purposes of statistical analysis, sera with undetectable antibodies were assigned values equal to one half of the lowest detectable level of antibody.

RESULTS

Effect of Ptx on primary and secondary anti-TT antibody responses

The primary IgG1-anti-TT antibody response was enhanced 5.1-fold in animals that received a 100 ng Ptx concurrently with TT immunization (geometric mean:

Ptx/TT=2.21 μ g ml⁻¹; saline/TT=0.43 μ g ml⁻¹; $P<0.01$) (Figure 1a). In contrast, primary IgG2a responses were similar in the 2 groups (geometric mean: Ptx/TT=151 ng ml⁻¹; saline/TT=108 ng ml⁻¹). After secondary immunization with TT alone at 3 weeks, IgG1-anti-TT antibodies were further increased in animals that had been primed in the presence of Ptx (geometric mean: Ptx/TT=123.6 μ g ml⁻¹; saline/TT=10.8 μ g ml⁻¹; $P<0.01$, an 11.4-fold difference). To a lesser degree, secondary immunization also boosted IgG2a-anti-TT concentrations to a greater extent in Ptx exposed animals than in control animals (Ptx/TT=9.5 μ g ml⁻¹; saline/TT=1.6 μ g ml⁻¹; $P=0.04$). IgG2b- and IgG3-anti-TT antibodies were not detectable at the lowest dilution tested (1/50) even after secondary immunization. Control animals who received Ptx alone did not mount an anti-TT antibody response.

Similar to IgG1 antibody responses, IgE-anti-TT antibody concentrations following both primary and secondary immunization were significantly enhanced in animals that were primed in the presence of Ptx. After primary immunization, IgE-anti-TT antibodies were undetectable in a 1/50 dilution (<50 ng ml⁻¹) in animals receiving only TT compared to a geometric mean of 198 ng ml⁻¹ in animals that received Ptx (Figure 1b). Following secondary immunization with TT alone, IgE-anti-TT antibody levels were 14-fold higher in animals primed in the presence of Ptx (1435 ng ml⁻¹) compared to controls (102 ng ml⁻¹) ($P<0.01$).

The interval between primary and secondary immunizations was extended to 3 months to determine whether the effect of Ptx on the class and subclass distribution of the secondary antibody responses would be maintained. A tenfold enhancement of IgG1- and IgE-anti-TT antibodies was still observed in animals primed in the presence of Ptx compared to controls (Figure 2a,b) but there was no difference in IgG2a-anti-TT antibody concentrations between groups.

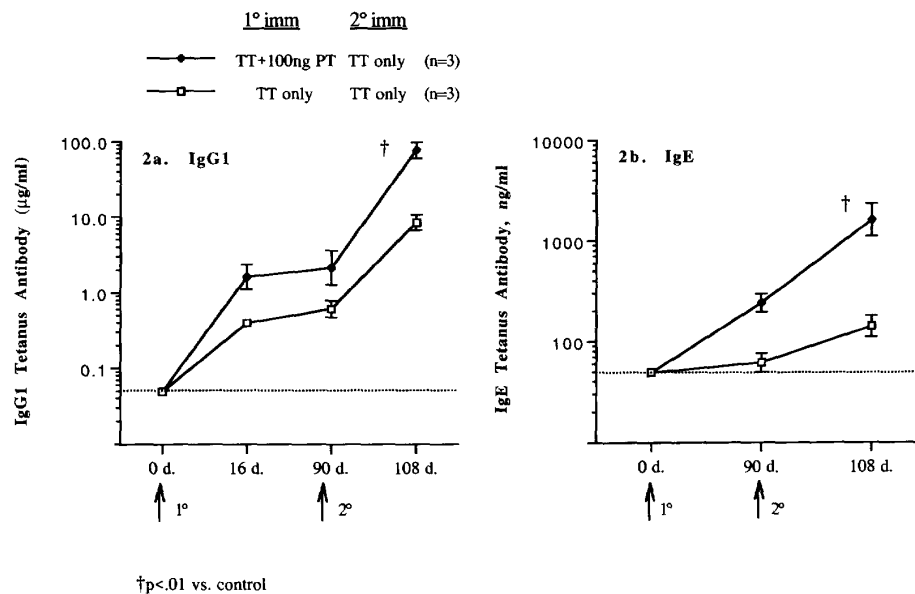


Figure 2 (a) and (b) Geometric mean IgG1- and IgE-anti-tetanus toxoid antibody ($\pm$ S.E.M.) after primary immunization (with TT plus Ptx or saline control) and secondary immunization (TT alone) on day 90. Error bars which are not visible are too narrow to be shown

Table 1 Diphtheria toxoid (DT)-IgG1 antibody ($\times/\pm$ S.E.M.) after secondary immunization on day 21

Primary immunization	Secondary immunization	No. animals	Geometric mean IgG1-DT antibody (ng ml ⁻¹) $\times/\pm$ S.E.M.
DT	DT	10	761 $\times/\pm$ 1.52
DT/Ptx	DT	9	9173 $\times/\pm$ 2.29*

* $P<0.05$ vs control

Effect of Ptx on antibody responses to DT

In order to verify that the effect of Ptx on antibody responses was not specific to TT, similar experiments were performed using DT as the immunizing antigen. IgG1-anti-DT antibodies were increased 12-fold after secondary immunization with DT alone in animals given Ptx with primary immunization ($P<0.05$ vs control) (Table 1). Accurate measurement of IgE-anti-DT antibodies was not possible because of nonspecific binding.

Effect of Ptx on total immunoglobulins

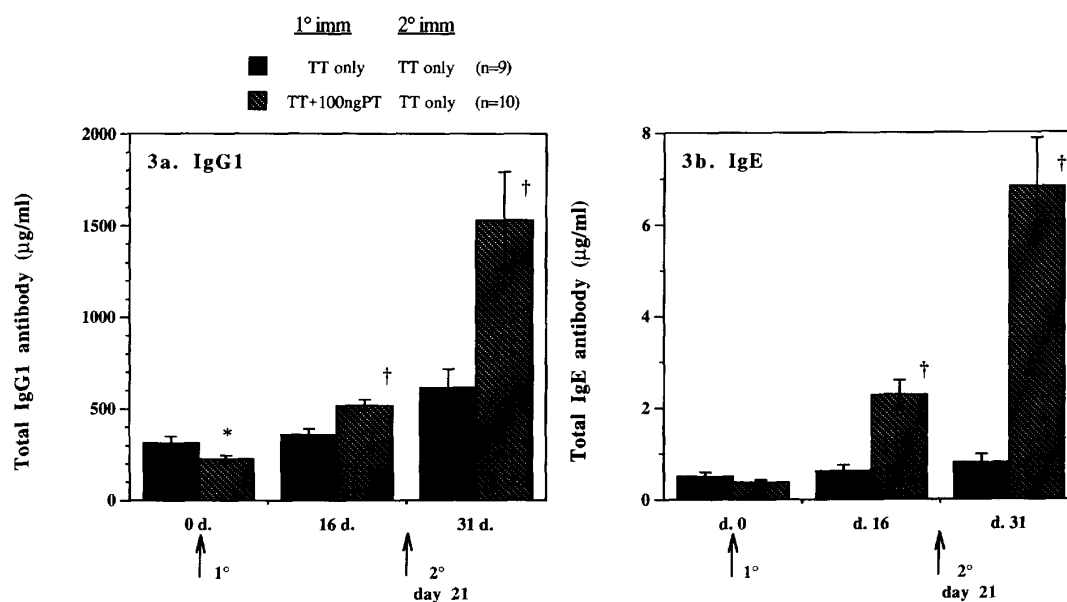
Administration of Ptx with TT increased the concentration of total IgG1 and IgE 1.5 and 3.4-fold relative to control animals at 16 days after 1° immunization ($P<0.01$ vs control) (Figure 3a,b). Total IgG1 and IgE concentrations were increased further after secondary immunization in animals primed in the presence of Ptx (2.5-fold and 10.3-fold greater than controls, respectively; $P<0.01$). The enhanced IgG1 and IgE response was seen whether the booster dose of TT alone was given 3 weeks or 3 months after 1° immunization (Figure 4a,b). Concentrations of other IgG subclasses and IgM were not significantly different between groups after primary or secondary immunization.

Animals given a 100 ng dose of Ptx, without TT, did not have significant changes of any immunoglobulin class or subclass including IgG1 and IgE (mean IgE concentration: Ptx=599 ng ml⁻¹; saline control=432 ng ml⁻¹; $P>0.25$; mean IgG1 concentration: Ptx=282 µg ml⁻¹; saline control=336 µg ml⁻¹; $P>0.25$).

Blocking of Ptx induced IgE responses by anti-IL-4

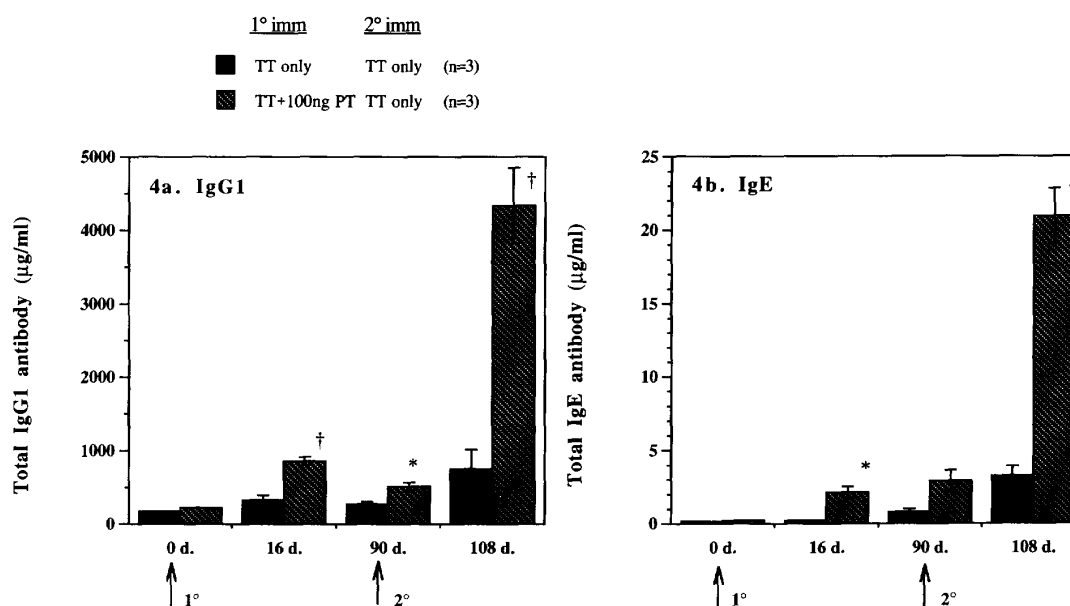
The increase in total IgE concentration after primary immunization with TT and Ptx was completely blocked by simultaneous administration of 1 mg of anti-IL-4 ($P<0.01$), but not by administration of the unrelated rat IgG1 MAb, Y13-259 (anti-p21 protein product of the ras oncogene) (Figure 5). Concentrations of IgE-anti-TT antibody after primary immunization were too low to examine the effect of anti-IL-4 on primary IgE-anti-TT responses. Increases in total IgG1 and IgG1-anti-TT after 1° immunization were not inhibited by anti-IL-4. In fact, animals that received either anti-IL-4 or Y13-259 simultaneously with Ptx and TT had higher total IgG1 concentrations than animals that received Ptx and TT without a MAb or animals that received TT and a MAb alone (mean total IgG1 concentration: Ptx/TT-anti-IL-4=754 µg ml⁻¹; Ptx/TT=461 µg ml⁻¹; $P<0.01$).

Primary immunization in the presence of anti-IL-4 also affected responses to a booster dose of TT given 60 days later. Anti-IL-4 inhibited the secondary IgE responses of animals primed with Ptx and TT. A single dose of anti-IL-4 given with primary immunization inhibited the fold rise after secondary immunization of total IgE by 64% and of IgE-anti-TT antibodies by 84% (Figure 6a,b). A second dose of anti-IL-4 given at the time of the secondary immunization inhibited these IgE responses to a greater extent (Figure 6a,b). Administration of the unrelated rat IgG1 MAb (Y13-259) did not inhibit the induction of IgE-anti-TT antibody by Ptx.



*p<.05 vs control, †p<.01 vs control

Figure 3 (a) and (b) Mean total IgG1 and IgE concentrations after primary immunization (TT plus Ptx or saline control) and secondary immunization (TT alone) on day 21



*p<.05 vs control, †p<.01 vs control

Figure 4 (a) and (b) Mean total IgG1 and IgE concentrations after primary immunization (TT plus Ptx or saline control) and secondary immunization (TT alone) on day 90. Error bars which are not visible are too narrow to be shown

DISCUSSION

The major findings of this study are that:

1 very low, sub-immunogenic doses of Ptx enhances the specific antibody responses as well as total

immunoglobulin levels after simultaneous immunization with protein antigens such as TT and DT;

2 the enhanced response consists mainly of the IgG1 subclass and IgE; and

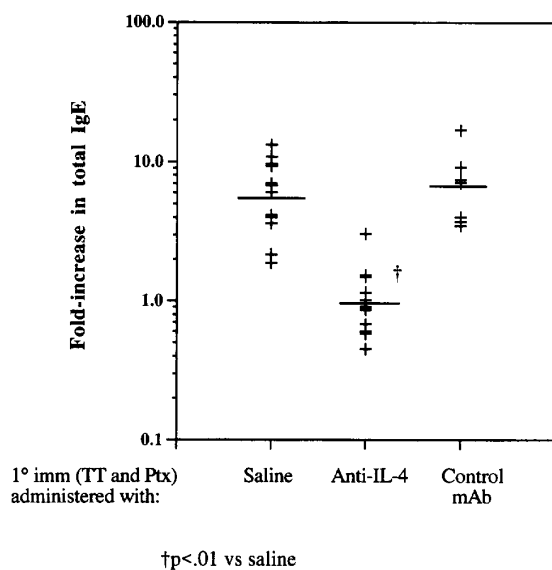


Figure 5 Effect of anti-IL-4 on fold-increase in total IgE after primary immunization with TT and Ptx

3 neutralization of IL-4 with the anti-IL-4 MAb, 11B11, abrogates the enhanced IgE response but not the IgG1 response.

The administration of Ptx concurrently with a primary dose of protein antigen produced an enhancement in antibody production that is both antigen-specific and polyclonal, involving mainly IgG1 and IgE. Secondary TT antibody responses, particularly of IgG1 and IgE-class antibodies, were further enhanced in animals that had been primed in the presence of Ptx. The enhancement of secondary antibody responses was seen whether the booster dose of TT alone was given 3 weeks or 3 months after primary immunization. Total IgE concentrations and, to a lesser extent, total IgG1 concentrations were also increased following primary and secondary immunization in animals exposed to Ptx compared to controls. As with antibody responses, it did not matter whether the booster dose was given 3 weeks or 3 months after primary immunization. The sub-immunogenic dose of Ptx alone failed to increase total IgG1 and IgE. Therefore, co-administration of a protein antigen, in this case TT, is required for stimulation of the polyclonal response, which presumably represents a nonspecific bystander effect.

Class and subclass-specific immunoglobulin production have been associated with specific lymphokines derived from distinct subsets of T helper lymphocytes, Th₁ and Th₂²². IL-4, which is secreted by Th₂ lymphocytes^{23,24}, enhances secretion of IgG1 and IgE by B lymphocytes and acts by inducing B lymphocytes to switch isotypic antibody expression. In contrast, the secretion of IgG2a is preferentially enhanced by Th₁ lymphocytes, which secrete γ -interferon and interleukin-2 (IL-2)^{22,24}. IL-4 is an essential cytokine for *in vivo* induction of IgE in various murine models, such as infection with the parasite *Nippostrongylus brasiliensis*, and has been convincingly linked with *in vivo* production of IgE in humans²⁵⁻²⁹. *In vitro*, IL-4

induces human B cell production of both IgE and IgG4 if cognate interactions with T-lymphocytes are permitted³⁰.

The enhancement of IgG1 and IgE responses suggests that Ptx may act on T helper lymphocyte subsets to promote IL-4 secretion. In support of this hypothesis, 1 mg of anti-IL-4 effectively blocked the induction of IgE by Ptx, inhibiting total and antigen specific IgE by the same amount. An unrelated rat IgG1 MAb failed to inhibit IgE responses, indicating that the effect is attributable to neutralization of IL-4. These results are consistent with a recent study by Mu and Sewell³¹, in which ovalbumin was used as the antigenic stimulus. In their study, Ptx enhanced IL-4 secretion and IL-4 mRNA levels in spleen cells and lymph nodes and anti-IL-4 inhibited the inductive effects on ovalbumin-specific and total IgE.

We found that a single dose of anti-IL-4 with 1° immunization also inhibited the IgE response to booster doses of antigen given 2 months later, suggesting that presence of IL-4 itself during the primary response mediates the induction of T cells which produce IL-4 during subsequent secondary responses and stimulate IgE antibodies. Other studies also support the concept that the T-helper response becomes committed to a particular phenotypic pathway at the time of initial antigenic exposure and that IL-4 is an important mediator in this process³². In contrast to its effects on IgE, anti-IL-4 did not inhibit total or antigen specific IgG1 responses. In fact, both anti-IL-4 and the unrelated rat antibody enhanced total IgG1 levels in animals that received Ptx and TT, perhaps because the rat MAb represented an additional antigenic stimulus. Others, using much higher doses of anti-IL-4 MAb, also noted that IgE antibodies could be suppressed but that IgG1 could not^{22,25}. The reason for the lack of inhibition of IgG1 is not clear. One possibility is that pathways not dependent on IL-4 may be important in the stimulation of IgG1 responses²³. Another possibility is that stimulation of IgE production requires high IL-4 concentrations which are readily inhibited by MAb, whereas stimulation of IgG1 requires only very low IL-4 concentrations which are difficult to neutralize by MAb³³. In support of the role of IL-4 in IgG1 production is the observation that IL-4 deficient mice have a tenfold reduction in their IgG1 levels³⁴.

Our studies and those of others³¹ do not resolve whether Ptx acts directly to promote IL-4 secretion or indirectly via other cytokines and cellular targets. Sewell *et al.* have shown that Ptx stimulates IL-2 which may in turn augment IL-4 production³⁵. Alternatively, Ptx induced alterations in lymphocyte migration may result in enhanced IL-4 production. Sewell and colleagues suggest that Ptx does not selectively stimulate Th₂ cells because it does not induce IL-5 secretion, a Th₂ cell product, but does induce γ -interferon, a Th₁ cell product^{35,36}. The induction of γ -interferon may explain why the secondary IgG2a response to TT was enhanced in Ptx primed animals in one of our experiments. Of note, increased γ -interferon levels have been detected in serum and bronchoalveolar lavage fluid of *B. pertussis* infected mice³⁷.

Evidence that Ptx exposure of humans is associated with enhanced IgE production is provided by the observation that children convalescing from natural pertussis have elevated IgE concentrations³⁸. The

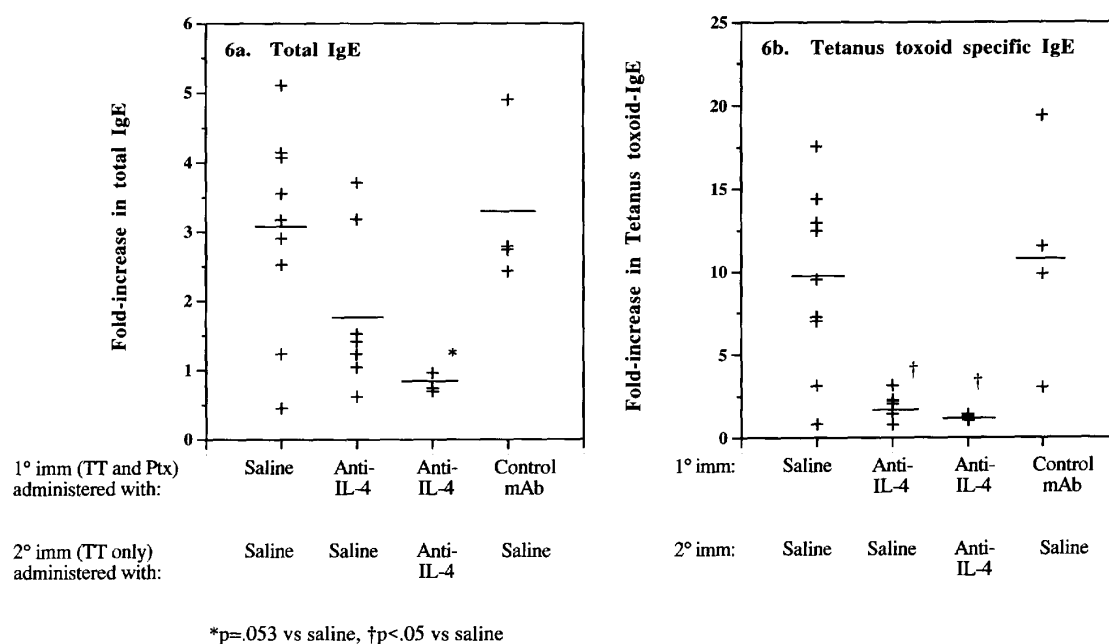


Figure 6 (a) and (b) Effect of anti-IL-4 on fold increase in total and IgE-anti-TT antibody after secondary immunization with TT on day 60

immunologic effects of Ptx on the class and subclass composition of the antibody response to Ptx itself and to concurrently administered antigens may also have significance for active immunization. Currently used whole cell pertussis vaccines typically contain low concentrations of active Ptx, which may be responsible for the enhanced antibody response to concurrently administered DT and TT in animals and in humans³⁹. Diphtheria-pertussis-tetanus vaccination also has been associated with increased tetanus-specific IgE responses⁴⁰. Anaphylactic reactions occur rarely after DTP immunization⁴¹. Some of the acellular pertussis vaccines, such as JN1H6 and JN1H7 used during efficacy studies in Sweden, also contain residual active Ptx^{42,43}. Seven of 10 children who received primary and secondary immunization with JN1H6 developed detectable IgE antibodies to Ptx⁴⁴. Some acellular pertussis vaccines which contain Ptx detoxified by genetic means⁴⁵ or by chemical methods such as tetranitromethane inactivation contain little or no residual Ptx activity⁴⁶. It would be of interest to determine whether children immunized with such vaccines have lower levels of IgE antibodies to Ptx and to concurrently administered antigens.

In summary, our results suggest that low concentrations of active Ptx induce primary and secondary IgG1 and IgE antibody responses to clinically relevant antigens. The presence of the lymphokine, IL-4, at the time of priming appears to be necessary for the primary and secondary IgE responses. Further research into the mechanism of these effects will contribute to our understanding of immunologic responses to pertussis.

ACKNOWLEDGEMENTS

We thank Claudette Thompson for assistance in preparation of the manuscript. Presented in part at the *Interscience Conference on Antimicrobial Agents and Chemotherapy*, 17-20 September, 1989, Abstract 445.

Supported by Grants 5T32AI07061-13 and AI18125 from the National Institutes of Health.

REFERENCES

- Pittman, M. The concept of pertussis as a toxin-mediated disease. *Ped. Infect. Dis.* 1984, **3**, 467-486
- Tamura, M., Yogimori, K., Murai, S. *et al.* Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. *Biochemistry* 1982, **21**, 5516-5522
- Munoz, J.J. *Action of Pertussigen (Pertussis Toxin) on the Host Immune System in Pathogenesis and Immunity in Pertussis* (Eds Wardlaw, A.C. and Parton, R.). Wiley and Sons, 1988, pp. 173-192
- Morse, S.I. and Morse, J.H. Isolation and properties of the leukocytosis and lymphocytosis-promoting factors of *Bordetella pertussis*. *J. Exp. Med.* 1976, **143**, 1483-1502
- Morse, S.I. and Barron, B.A. Studies on the leukocytosis and lymphocytosis induced by *Bordetella pertussis*—III. The distribution of transfused lymphocytes in pertussis-treated and normal mice. *J. Exp. Med.* 1970, **132**, 663-672
- Spangrude, G.J., Sacchi, F., Hill, H.R., Van Epps, D.E. and Daynes, R.A. Inhibition of lymphocyte and neutrophil chemotaxis by pertussis toxin. *J. Immunol.* 1985, **135**, 4135-4143
- Meade, B.D., Kind, P.D., Ewell, J.B., McGrath, P.P. and Manclard, C.R. *In vitro* inhibition of murine macrophage migration by *Bordetella pertussis* lymphocytosis-promoting factor. *Infect. Immun.* 1984, **45**, 718-725
- Samore, M.H. and Siber, G.R. Effect of pertussis toxin on susceptibility of infant rats to *Haemophilus influenzae* type b. *J. Infect. Dis.* 1992
- Sewell, W.A., Munoz, J.J. and Vadas, M.A. Enhancement of the intensity, persistence, and passive transfer of delayed-type hypersensitivity lesions by pertussigen in mice. *J. Exp. Med.* 1983, **157**, 2087-2096
- Munoz, J.J., Arai, H., Bergman, R.K. and Sadowski, P.L. Biological activities of crystalline pertussigen from *bordetella pertussis*. *Infect. Immun.* 1981, **33**, 820-826
- Andersen, V., Hertz, J.B., Sorensen, S.F. *et al.* *In vitro* stimulation of human lymphocytes by *Bordetella pertussis*. *Acta Path. Microbiol. Scand.* 1977, **85** Sect C, 65-72
- Kashiwa, H., Hyodo, S., Kishi, T. *et al.* The mitogenic effect of the lymphocytosis promoting factor from *Bordetella pertussis* on human T_H and non-T_H cells. *Clin. Exp. Immunol.* 1984, **58**, 436-443

- 13 MacIntyre, E.A., Tatham, P.E.R., Abdul-Gaffar, R. and Linch, D.C. The effects of pertussis toxin on human T lymphocytes. *Immunology*. 1988, **64**, 427-432
- 14 Kong, A.S. and Morse, S.I. The *in vitro* effects of *Bordetella pertussis* lymphocytosis-promoting factor on murine lymphocytes. *J. Exp. Med.* 1977, **145**, 151-162
- 15 Tamura, M., Nogimori, K., Yajima, M., Ase, K. and Ui, M. A role of the B-oligomer moiety of islet-activating protein, pertussis toxin, in development of the biological effects on intact cells. *J. Biol. Chem.* 1983, **258**, 6756-6761
- 16 Nencioni, L., Pizza, M., Volpini, G., De Magistris, M.T., Giovannoni, F. and Rappuoli, R. Properties of the B oligomer of pertussis toxin. *Infect. Immun.* 1991, **59**, 4732-4734
- 17 Black, W.J., Munoz, J.J., Peacock, M.G. *et al.* ADP-ribosyltransferase activity of pertussis toxin and immunomodulation by *Bordetella pertussis*. *Science* 1988, **240**, 656-659
- 18 O'Hara, J. and Paul, W.E. Production of a monoclonal antibody to and molecular characterization of B-cell stimulator factor-1. *Nature* 1983, **315**, 333-336
- 19 Furth, M.E., Davis, L.J., Fleurdelys, B. and Scolnick, E.M. Monoclonal antibodies to the p21 products of the transforming gene of harvey murine sarcoma virus and of the cellular *ras* gene family. *J. Virol.* 1982, **43**, 294-304
- 20 Baniyash, M. and Eshhar, Z. Inhibition of IgE binding to mast cells and basophils by monoclonal antibodies to murine IgE. *Eur. J. Immunol.* 1984, **14**, 799-807
- 21 Zollinger, W.D. and Boslego, J.W. A general approach to standardization of the solid-phase radioimmunoassay for quantitation of class specific antibodies. *J. Immunol. Meth.* 1981, **46**, 129-140
- 22 Finkelman, F.D., Holmes, J., Katona, I.M. *et al.* Lymphokine control of *in vivo* immunoglobulin isotype selection. *A. Rev. Immunol.* 1990, **8**, 303-333
- 23 Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.H. and Coffman, R.L. Two types of murine helper T cell clones 1. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986, **136**, 2348-2357
- 24 Mosmann, T.R. and Coffman, R.L. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *A. Rev. Immunol.* 1989, **7**, 145-173
- 25 Finkelman, F.D., Katona, I.M., Urban, J.F. Jr, Snapper, C.M., O'Hara, J. and Paul, W.E. Suppression of *in vivo* polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. *Proc. Natl Acad. Sci. USA* 1986, **83**, 9675-9678
- 26 King, C.L. and Low, C.C., Nutman, T.B. IgE production in human helminth infection. Reciprocal interrelationship between IL-4 and IgG-gamma. *J. Immunol.* 1993, **150**, 1873-1880
- 27 Geha, R.S. Regulation of IgE synthesis in humans. *J. Allergy Clin. Immunol.* 1992, **90**, 143-150
- 28 Kimata, H., Yoshida, A., Ishioka, C., Lindley, I. and Mikawa, H. Interleukin 8 (IL-8) selectively inhibits immunoglobulin E production induced by IL-4 in human B cells. *J. Exp. Med.* 1992, **176**, 1227-1231
- 29 Finkelman, F.D., Katona, I.M., Urban, J.F. *et al.* IL-4 is required to generate and sustain *in vivo* IgE responses. *J. Immunol.* 1988, **141**, 2335-2341
- 30 Vercelli, D., Jabara, H.H., Arai, K. and Geha, R.S. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. *J. Exp. Med.* 1989, **169**, 1294-1307
- 31 Mu, H.H. and Sewell, W.A. Enhancement of interleukin-4 production by pertussis toxin. *Infect. Immun.* 1993, **61**, 2834-2840
- 32 Gross, A., Ben-Sasson, S.Z. and Paul, W.E. Anti-IL-4 diminishes *in vivo* priming for antigen-specific IL-4 production by T cells. *J. Immunol.* 1993, **150**, 2112-2120
- 33 Snapper, C.M., Finkelman, F.D. and Paul, W.E. Differential regulation of IgG1 and IgE synthesis by interleukin-4. *J. Exp. Med.* 1988, **167**, 183-196
- 34 Kuhn, R., Rajewsky, K. and Muller, W. Generation and analysis of interleukin-4 deficient mice. *Science* 1991, **254**, 707-710
- 35 Sewell, W.A., DeMoerloose, P.A., Hamilton, J.A., Schrader, J.W., Mackay, I.R. and Vadas, M.A. Potentiation of delayed-type hypersensitivity by pertussigen or cyclophosphamide with release of different lymphokines. *Immunology* 1987, **61**, 483-488
- 36 Mu, H.H., Penny, R. and Sewell, W.A. Interleukin-5 is necessary for eosinophilia induced by cyclophosphamide in immunized mice. *Immunology* 1993, **79**, 452-458
- 37 Torre, D., Pugliese, A., Speranza, F. *et al.* Interferon- γ levels in serum and bronchoalveolar lavage fluid of mice infected with *Bordetella pertussis*. *J. Infect. Dis.* 1993, **167**, 762-765
- 38 Torre, D., Issi, M., Giovanni, C., Fiori, G.P. and Sampietro, C. Total serum IgE levels in children with pertussis. *Am. J. Dis. Child* 1990, **144**, 290-291
- 39 Munoz, J.J. and Bergman, R.K. *Bordetella pertussis: Immunological and Other Biological Activities*. Marcel Dekker, Inc., New York, 1977
- 40 Matuhasi, T. and Hakuo, I. Elevation of levels of IgE antibody to tetanus toxin in individuals vaccinated with diphtheria-pertussis-tetanus vaccine. *J. Infect. Dis.* 1982, **146**, 290
- 41 Howson, C.P. and Fineberg, H.V. Adverse events following pertussis and rubella vaccines. Summary of a report of the Institute of Medicine. *J. Am. Med. Assoc.* 1992, **267**, 392-396
- 42 Iwasa, S., Ishida, S., Asakawa, S. and Akama, K. A curious histamine-sensitizing activity shown by the newly developed Japanese acellular pertussis vaccine. *Dev. Biol. Stand.* 1985, **61**, 453-460
- 43 Quentin-Millet, M.J., Arminjon, F., Danve, B., Cadoz, M. and Armand, J. Acellular pertussis vaccines: evaluation of reversion in a nude mouse model. *J. Biol. Stand.* 1988, **16**, 99-108
- 44 Hedenskop, S., Bjorksten, B., Blennow, M., Granstrom, G. and Granstrom, M. Immunoglobulin E response to pertussis toxin in whooping cough and after immunization with a whole-cell and an acellular pertussis vaccine. *Int. Arch. Allergy Appl. Immunol.* 1989, **89**, 156-161
- 45 Pizza, M., Covacci, A., Bartoloni, A. *et al.* Mutants of pertussis toxin suitable for vaccine development. *Science* 1989, **246**, 497-500
- 46 Siber, G., Wkinberry, L., Todd, C., Samore, M., Sentissi, A. and Cohen, N. Safety and immunogenicity in adults of pertussis toxoid inactivated with tetranitromethane. Abstracts of the international Workshop on *Bordetella pertussis*, Rocky Mountain Laboratories, 18-20 August, 1988