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Paradoxical Effect of Pertussis Toxin on the Delayed Hypersensitivity Response to Autoantigens in Mice

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Abstract

Background: Pertussis toxin (PTX), an exotoxin of *Bordetella pertussis*, enhances the development of experimental autoimmune diseases such as experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis (EAE) in rodent models. The mechanisms of the promotion of experimental autoimmune diseases by PTX may be based upon PTX-induced disruption of the blood eye/brain barriers facilitating the infiltration of inflammatory cells, the modulation of inflammatory cell migration and the enhancement of the activation of inflammatory cells. We hypothesized that the facilitation of experimental autoimmunity by PTX suggests that its influence on the *in vivo* immune response to auto-antigen may differ from its influence on non-self antigens.

Methodology/Principal Findings: We have evaluated the effect of PTX on the simultaneous generation of delayed type hypersensitivity (DTH) responses and autoimmune responses to uveitogenic interphotoreceptor retinoid binding protein peptide ($IRBP_{161–180}$), encephalitogenic myelin oligodendrocyte glycoprotein peptide ($MOG_{35–55}$) or ovalbumin (OVA). PTX injection of mice immunized to IRBP $peptide_{161–180}$ led to (i) the development of EAU as shown by histopathology of the retina, (ii) pro-inflammatory cytokine production by splenocytes in response to IRBP peptide $_{161–180}$, and (iii) symptomatic EAE in mice immunized with encephalitogenic MOG $peptide_{35–55}$. However, mice that received PTX had a reduced DTH response to $IRBP_{161–180}$ peptide or MOG $peptide_{35–55}$ when challenged distal to the site affected by autoreactive T cells. Moreover, footpad challenge with $MOG_{35–55}$ peptide reduced EAE in mice immunized with MOG peptide. In contrast, the use of PTX when immunizing with OVA protein or an OVA immunogenic peptide did not affect the DTH response to OVA.

Conclusions/Significance: The results suggest that that the reduced DTH response in mice receiving PTX may be specific for autoantigens and autoantigen-reactive T cells are diverted away from ectopic sites that received the autoantigen and towards the tissue site of the autoantigen.

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Introduction

Autoimmune uveitis, a disease that targets neural retina, is characterized by massive uveal inflammation, vasculitis, and the destruction of photoreceptor cells. Experimental autoimmune uveitis (EAU) is induced in mice by active immunization with evolutionarily conserved retinal proteins such as interphotoreceptor retinoid binding protein (IRBP), Complete Freunds' Adjuvant (CFA) and pertussis toxin [1–4]. Pertussis toxin (PTX), derived from *Bordatella perttussis* is usually required to induce experimental autoimmune disease. In addition to amplifying the activation of immunocompetent cells and proinflammatory cytokine production [5,6]; PTX may play a role in opening the blood/brain barrier, and influences the migratory patterns of inflammatory cells [7–11]. In aggregate, PTX induces changes in vascular permeability, thus facilitating the breakdown of blood–tissue barriers and thereby facilitates the infiltration of inflammatory cells into the target organ.

Despite the use of PTX for over two decades to enhance the induction of experimental autoimmune disease, the mechanism of its action is still not yet understood. Although some of the enhancing effects of PTX on the induction of experimental autoimmunity are thought to be due to changes in vascular permeability when administered at the time of immunization, PTX also promotes the production of Th1 cytokines and low doses of PTX promote a delayed-type hypersensitivity response [10,12]. The effects of PTX on antigen-presenting cells may underlie this phenomenon [13,14]. Pertussis toxin also modulates the immune response to neural antigens injected with Incomplete Freund's Adjuvant: inducing Th1 cells and experimental autoimmune encephalomyelitis (EAE) in the presence of high frequencies of Th2 cells [14]. However, high doses of the toxin can result in reduced disease or DTH [5,10]. The mechanism of this strict dose dependency of the effect of PTX is unknown.

Because PTX enhances proinflammatory cytokine production and Th1-based autoimmune diseases, we investigated the role of PTX in the induction of delayed-type hypersensitivity (DTH) to self-peptides in conjunction with the induction of autoimmune disease models. We observed that although PTX is critical for development of EAU in mice immunized with CFA, PTX and the uveitogenic peptide $IRBP_{161–180}$, PTX reduced or retarded DTH

responses to autoantigens in an antigen-specific manner by reducing cellular infiltration at the site of challenge even though PTX enhanced the production of proinflammatory cytokines. This inhibitory effect of PTX on DTH is limited to self antigens suggesting that the apparent inhibition of DTH to the autoantigens may be due to differential a migration of antigen specific lymphocytes and retention of effector T cells at a site expressing the self antigen.

Materials and Methods

Animals

Female C57BLB10.RIII-H2rH2-T18b (C57BLB10.RIII) and C57BL6 mice 6–8 weeks old were purchased from Jackson Laboratories (Bar Harbor ME and Charles River Laboratories (Wilmington, MA, USA) respectively. All animals were maintained by the Center for Laboratory Animal Care at the University of Connecticut Health Center. The use of animals adhered to the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in ophthalmic and vision research. All work with animals has been reviewed and approved by the University of Connecticut Health Center Animal Care Committee (ACC 2004-380).

Antigens and reagents

Human IRBP peptide$_{161–180}$ (SGIPYIISYLHPGNTILHVD) that constitutes a major pathogenic epitope for C57BL10.RIII mice [4,12] was prepared by Anaspec Laboratories, San Diego, CA. Myelin oligodendrocyte glycoprotein peptide$_{35–55}$ (MOG$_{35–55}$) was prepared by the Yale University W.M. Keck Facility. Ovalbumin (OVA) peptide; (TEWTSSNVMEERKIKV amino acids 265–280) for CD4 T cells purchased from Invitrogen Life Sciences (Carlsbad, CA) was the generous gift of Drs. A. Vella and A. Menoret, Department of Immunology, University of Connecticut Health Center. Ovalbumin protein was purchased from Sigma (St. Louis, MO). Pertussis toxin (PTX) was purchased from List Biological Laboratories, Campbell,CA. Complete Freund's adjuvant (CFA),Incomplete Freund's Adjuvant (IFA) and *Mycobacterium tuberculosis* strain H37RA were purchased from Difco (Detroit, MI).

Induction and scoring of EAU [15]

Mice were immunized on the dorsal surface of their back intradermally with a 0.2 ml of the emulsion of 50–100 μg IRBP$_{161–180}$ peptide in CFA (1:1, v/v), containing 2.5 mg/ml of M. tuberculosis H37RA. Mice were injected with PTX, 1 μg in phosphate-buffered saline (PBS, pH 7.2) in a total volume of 0.1 ml i.p. 24 h after immunization with IRBP and CFA. Freshly enucleated eyes were collected for histopathology on specific days after immunization, and were fixed in 4% paraformaldehyde (PFA) solution. The incidence and severity of EAU was scored on an arbitrary scale of 0–4 on hematoxylin and eosin-stained sections, according to a semi quantitative system described [15]. Two independent observers did the scoring in blinded manner and results compared. The clinical scale measured was as follows: 0 = normal, 1 = mild distortion of layers of retina, 2 = mild to moderate distortion of layers of retina and minimal inflammatory cell infiltrate, 3 = moderate distortion of layers of retina and massive inflammatory cell infiltrate and 4 = severe distortion of retinal layers with total disruption of retinal architecture and massive cellular infiltrate.

Induction and scoring of Experimental Allergic Encephalomyelitis

For the induction of active EAE, C57BL/6 mice were injected intradermally (ID) with 200 μg of emulsion containing 200 μg MOG$_{35–55}$ in Incomplete Freund's Adjuvant (IFA) supplemented with 500 μg of *M. tuberculosis*. Mice were injected i.p with 200 ng PTX in 100 μl of PBS shortly after and 48 h after the first immunization. Following immunization, animals were kept under observation to score the disease. The study was done in a blinded fashion. The clinical scale measured was as follows: 0 = normal, 1 = limp tail, 2 = paraparesis with a clumsy gait, 3 = hind limb paralysis, 4 = quadriplegia, 5 = death.

Induction of Delayed-Type Hypersensitivity

To induce a maximum DTH response mice were immunized with a subcutaneous (sc) injection of 50 μl CFA containing 100 μg emulsified IRBP peptide$_{161–180}$, or 200μg MOG peptide$_{35–55}$, 50 μg OVA$_{265-280}$ peptide or 200 μg OVA protein into a flank. The control group did not receive any immunization or in some cases CFA without IRBP, MOG peptide or OVA. Seven days after immunization the mice were anesthetized with ketamine (75 mg/kg) and xylazine (15 mg/kg) and footpad thickness of both hind footpads measured with a digital micrometer (Mitatoyo, Tokyo, Japan). One footpad was challenged with an intradermal (ID) injection of 50 μg IRBP$_{161-180}$ peptide, MOG$_{35-55}$ peptide, OVA or OVA$_{265-280}$ peptide in PBS and the other footpad was challenged with PBS only. Approximately 24, 48 and sometimes 72 h later, the mice were anesthetized with ketamine/xylazine and footpad thickness re-measured. Swelling was computed as the difference in thickness (in μm) of the challenged footpad at 24 h minus the difference in thickness (in μm) of the vehicle-challenged

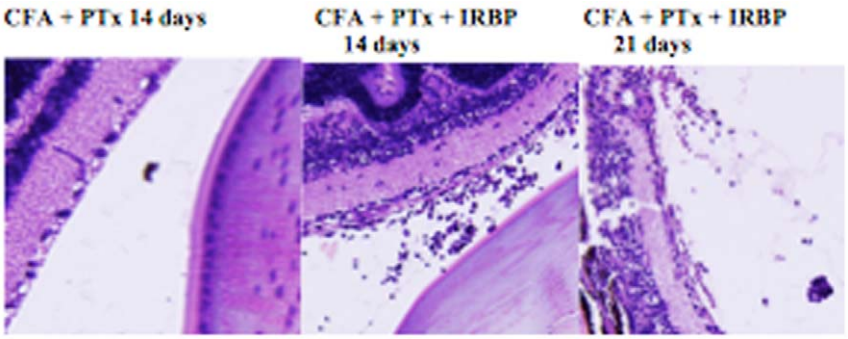

Figure 1. Induction of EAU with IRBP peptide. C57BL10.RIII mice received sc IRBP peptide$_{161–180}$+ CFA. Twenty-four hr later the mice received PTX i.p. Fourteen or 21 days P.I. the mice were euthanized, eyes enucleated, sectioned and stained with H&E. Figure is representative of sections from 5 individual mice.
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footpad at 24 h. Some mice were euthanized and footpads were collected and fixed in 4% paraformaldehyde (PFA). Histopathology sections were taken and stained with hematoxylin and eosin (H&E). Sections were analyzed for leukocyte infiltration and inflammatory cell accumulation.

In Vitro Stimulation of T cells with IRBP peptide

Splenocytes were obtained from immunized mice on day 7-post immunization. Splenocytes were cultured at 37°C in flat bottom 12 well plates at a concentration of 1×10^7 cells/well and stimulated with 100 ng IRBP peptide or medium only in a volume of 0.5 ml. Supernatants were harvested 24 h post in-vitro culture and stored at −20°C. until assayed for cytokines.

Cytokine assays

Supernatants that were collected after 24 h from in-vitro cultures were assayed for presence of cytokines (IL-1ß, TNF-α, IFN-γ and IL-10) by ELISA using kits from R & D Laboratories (Minneapolis,MN) as described by the manufacturer.

Statistics

Footpad swelling and cytokine assays were compared between groups and a paired t-test was used to do statistical analysis. In all comparisons, $P<0.05$ was used to determine statistical significance.

Results

Induction of EAU by IRBP peptide$_{161-180}$

C57BL10.RIII mice were immunized with IRBP peptide$_{161-180}$, CFA and PTX and euthanized on days 14 or 21 PI. The eyes were enucleated; sections obtained and stained with H&E. Sections were analyzed for integrity, architecture of the retinal layer and mononuclear cell infiltrate. The histopathology of the eyes of mice receiving CFA + PTX only, CFA or PTX only, or CFA + IRBP peptide but no PTX were no different from mice receiving PBS only or no injection. Mice that received 50–100 μg IRBP peptide, CFA and 1 μg PTX attained a loss of architecture of the retinal layers and extensive mononuclear cell infiltration 14 days P.I. (Fig 1). The histopathology of the retinal layer was similar on day 14 P.I. in mice immunized with 50 or 100 μg IRBP peptide. Extensive mononuclear cell infiltration and a marked disorder of the retinal outer nuclear layer (EAU score 3+) was observed by day 14 or 21 in mice receiving 50 or 100 μg IRBP peptide and 0.5–1 μg PTX. EAU was diminished significantly by day 21 P.I. in mice immunized with 25 or 50 mg IRBP peptide but was maintained in mice receiving 100 μg IRBP peptide (Fig. 2). Although damage to the outer nuclear layer of the retina was extensive by day 21 P.I., the infiltrate of mononuclear cells was diminished.

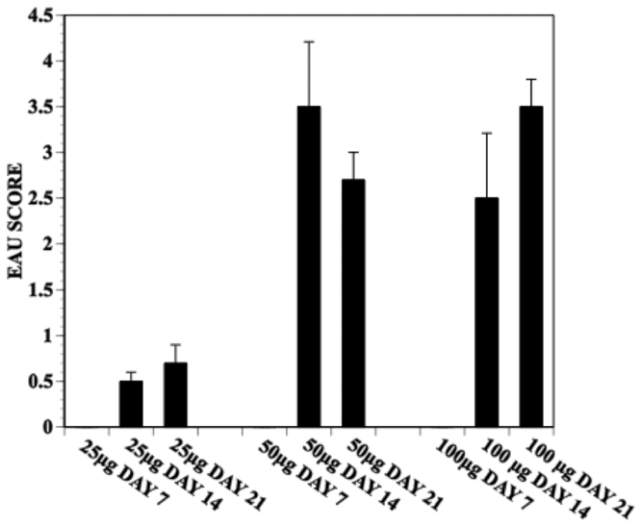

Figure 2. Dose/response and kinetics of the development of EAU by immunization with IRBP peptide. Mice were immunized with IRBP peptide$_{161-180}$, CFA and PTX. Mice were euthanized 7,14 or 21 days P.I., eyes enucleated and sectioned and stained with H&E. EAU is scored as described in Materials and Methods and the data represent the average score +/−S.E.M. of 5–6 mice/group.
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PTX enhances Th1 cytokine production but diminishes the DTH response to IRBP peptide$_{161-180}$

Cytokine production. Fourteen days after C57BL10.RIII mice were immunized with IRBP peptide$_{161-180}$, spleens were recovered and spleen cells cultured +/− the IRBP peptide. Splenocytes recovered from mice immunized with CFA, IRBP and PTX produced measurable amounts of TNF-α and IFN-γ when cultured *in vitro* without IRBP peptide (Figure 3A,B). However, mice receiving PTX produced 5-35-fold more TNF-α or IFN-γ. More cytokines were produced when the splenocytes were cultured with lipopolysaccharide (LPS) and IRBP peptide (data not shown).

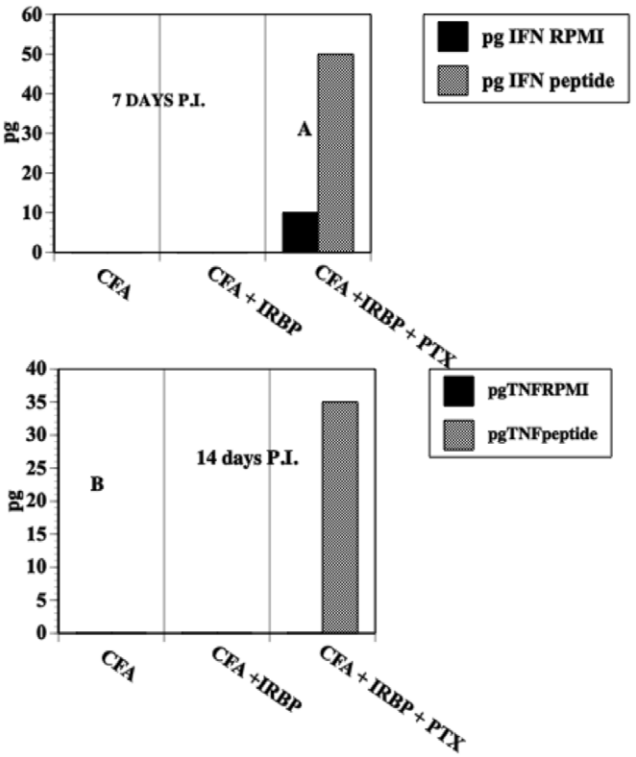

Figure 3. PTX potentiates the production of Th1 cytokines by splenocytes. C57BL10.RIII mice were immunized with IRBP peptide$_{161-180}$, CFA +/− PTX. Splenocytes were obtained from immunized mice on day 7 or 14 days P.I. Splenocytes were cultured at 37°C. in flat bottom 12 well plates at a concentration of 1×10^7 cells/well and stimulated with 100 μg IRBP peptide or medium only in a volume of 0.5 ml. Supernatants from 3 cultures were harvested 24 h post in-vitro culture and stored at −20°C. Supernatants were assayed by ELISA for TNF-α or IFN-γ̃.
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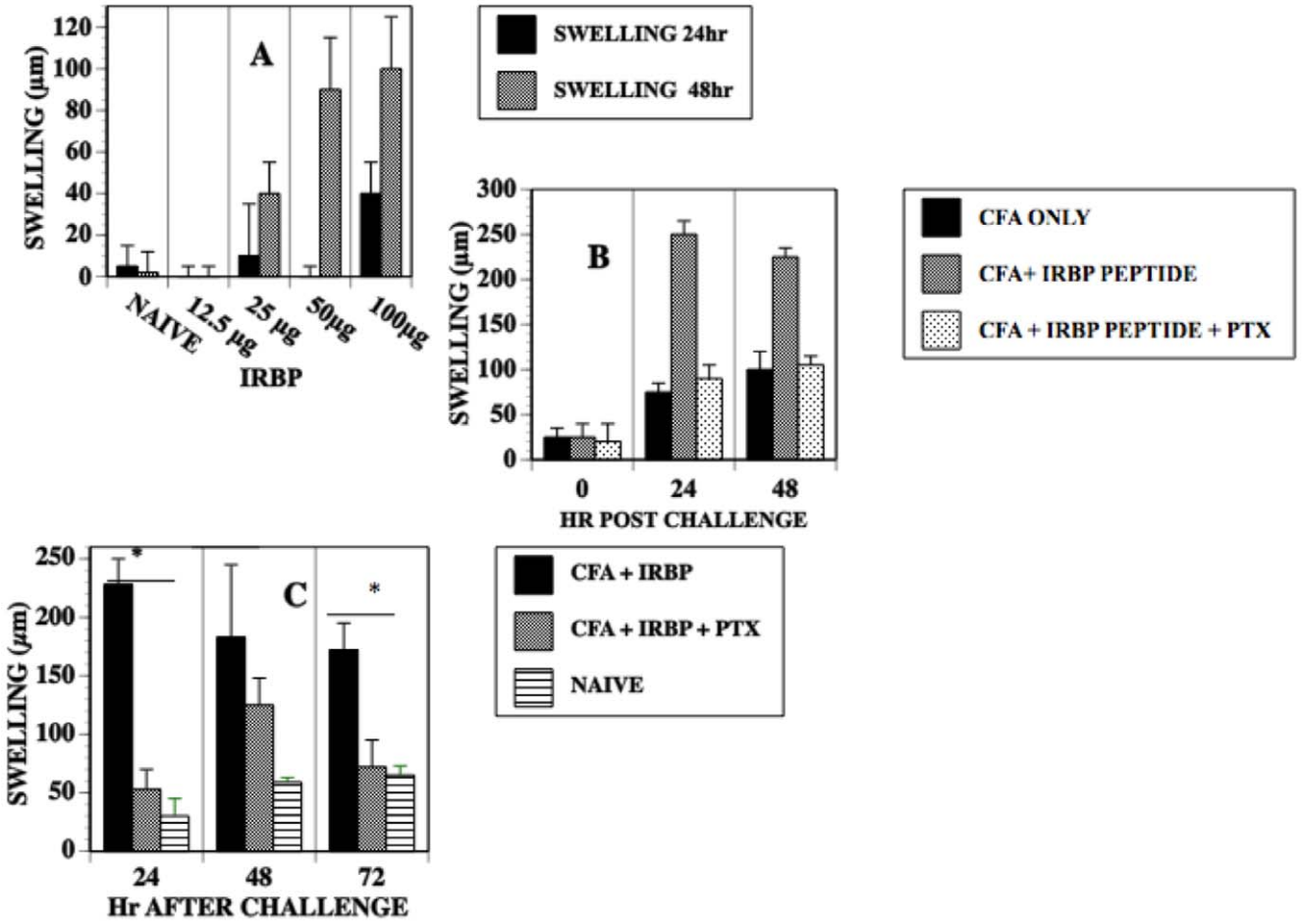

Figure 4. DTH to IRBP peptide$_{161-180}$ is diminished in mice receiving PTX. C57BL10.RIII mice were immunized with IRBP peptide, CFA +/− PTX. Seven days P.I. a footpad was challenged with an intradermal injection of IRBP peptide and swelling measured 24 and 48 hr after challenge. The data represents the footpad swelling +/− S.E.M. of 6–8 mice/group in two experiments.
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Delayed-type Hypersensitivity. Because PTX augments the generation of EAU and the production of inflammatory cytokines, we reasoned that PTX would also enhance a DTH response to IRBP peptide. To test this hypothesis, mice were immunized with IRBP peptide, CFA and varying doses of PTX. Seven days P.I. a footpad of the immunized mice was challenged with an intradermal injection of IRBP peptide. Footpad thickness was measured pre, 24, and 48 hr post challenge. Although mice immunized with 12.5, 25 μg IRBP peptide and CFA produced footpad swelling approximately 50% greater than naïve mice when challenged with IRBP peptide (data not shown) maximum swelling was achieved in mice immunized with 100 μg IRBP peptide and CFA (Figure 4). However mice receiving 1 and 5 μg PTX post immunization with IRBP peptide and CFA had markedly reduced swelling after challenge. In many instances this reduction in swelling in mice receiving PTX was noted at 24 hours post challenge but sometimes was less profound at 48 hr post challenge. Seventy-two hr post challenge the increment in swelling in mice

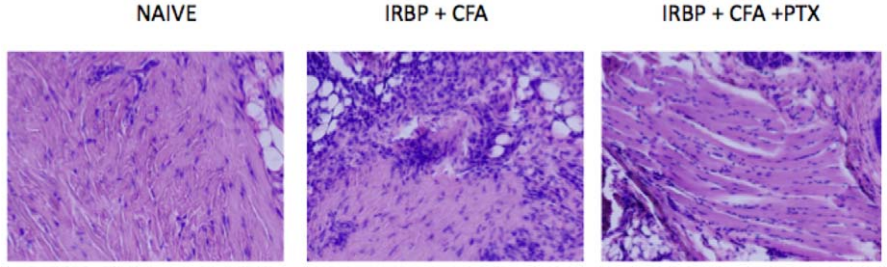

Figure 5. Pertussis toxin reduces the cellular infiltrate in the DTH response to IRBP peptide$_{161-180}$. C57BL10.RIII mice were immunized with IRBP peptide $_{161-180}$, CFA +/− PTX. Seven days P.I. a footpad was challenged with intradermal IRBP peptide$_{161-180}$. Twenty four hr after challenge footpads were harvested, sectioned and stained with H&E. Section is 10X and represents footpad of one of three mice.
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treated with PTX was no greater than that of naïve mice. There was no effect on DTH swelling in mice injected with less than 250 ng PTX (data not shown). Thus, there is a dichotomy in proinflammatory cytokine production by splenocytes and DTH responses in the periphery. H & E sections of the challenged footpads (Fig 5) revealed a reduced mononuclear cell infiltrate in the challenged footpad of immunized mice that received PTX.

PTX fails to diminish the DTH response to OVA but reduces the DTH response to $MOG_{35–55}$ peptide

We investigated the effect of PTX on immunization for a local DTH response to OVA protein, $OVA_{265–280}$ peptide or the encephalitogenic MOG $peptide_{35–55}$ by immunizing C57BL/6 mice with MOG peptide, OVA protein, $OVA_{265–280}$ peptide, CFA +/− PTX. Seven days after immunization, a footpad of the immunized mice was challenged with intradermal $OVA_{265–280}$ peptide or $MOG_{35–55}$ peptide. Footpad swelling to a challenge with OVA protein or OVA peptide was not affected by the administration of PTX during immunization (Fig 6A,B). In contrast, 7 days P.I. in mice immunized with encephalitogenic $MOG_{35–55}$ peptide CFA and PTX, the local DTH response to $MOG_{35–55}$ peptide was reduced significantly as compared to mice immunized with $MOG_{35–55}$ peptide and CFA only. However, mice receiving PTX with $MOG_{35–55}$ peptide PTX and CFA had robust EAE 14 days P.I. (Fig 7). However, EAE was reduced at day 14 in $MOG_{35–55}$ peptide-immunized mice whose footpad received intradermal MOG peptide on day 7 P.I. EAE was not affected in MOG peptide immunized whose footpad was challenged with IRBP peptide.

Discussion

The induction of EAU in C57BL10.RIII mice by immunization with IRBP $peptide_{161–180}$, CFA and PTX is well documented [1–5,12,15]. Consistent with those reports we observed that EAU was not induced unless the mice received PTX post- immunization with IRBP peptide and CFA. EAU was characterized by a strong infiltration of mononuclear cells to the retina and extensive damage to the retinal outer nuclear layer. By day 21, damage to the outer nuclear layer was equal to or greater than that observed 14 days P.I. but more than 21 days P.I., the number of infiltrated mononuclear cells was reduced markedly or not detected. Additionally, 21+ days P.I. there appeared to be some resolution of damage to the outer nuclear layer of the retina (data not shown).

PTX enhanced the production of the Th1 cytokines IFN-γ and TNF-α7 days P.I. consistent with the effect of PTX on the enhancement of the induction of cell-mediated immunity [5,6,10,12]. In fact, Th17 cells, essential to autoimmunity and DTH [16–18] are promoted by PTX [19]. Accordingly, we reasoned that PTX would also enhance a DTH response in the C57BL10.RIII mice immunized with CFA and IRBP. To our surprise, we found that the DTH-induced swelling of footpads of

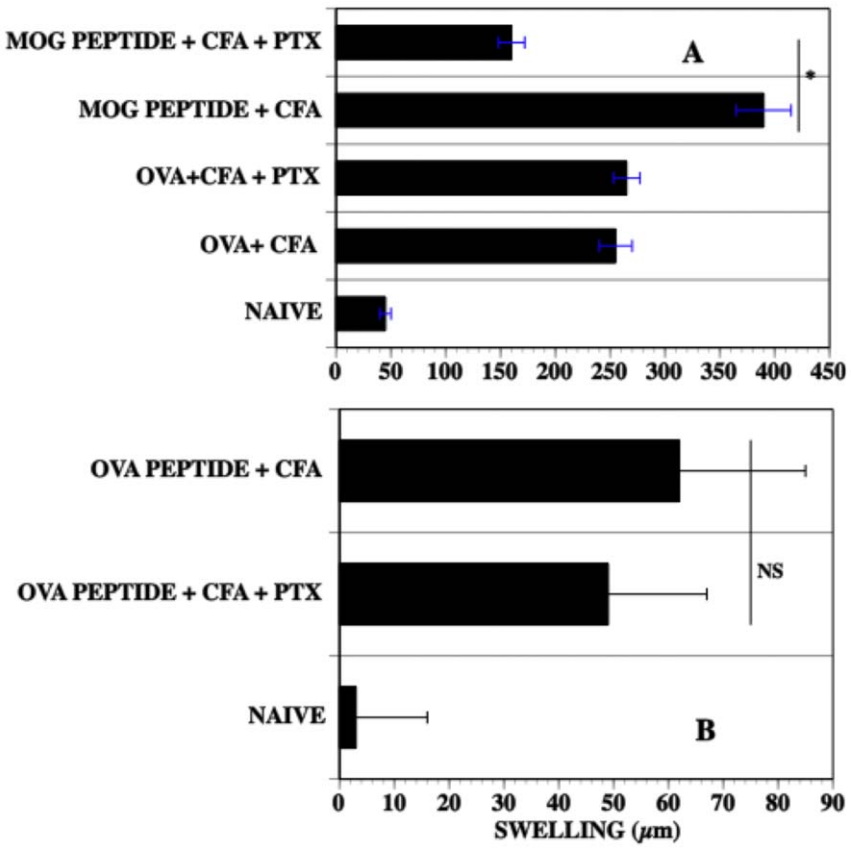

Figure 6. PTX reduces DTH to $MOG_{35–55}$ peptide but not to OVA. (A) C57BL/6 mice were immunized with MOG $_{35–55}$ peptide or OVA, CFA +/− PTX. Seven days P.I. footpads of the mice were challenged with intradermal MOG $peptide_{35–55}$ or OVA respectively. Swelling was measured 24 and 48 hr later. Data represents the mean swelling (μm) +/−S.E.M. of five mice/group. The experiment was done twice. (B) C57BL10.RIII mice were immunized with $OVA_{265–280}$ peptide, CFA +/− pertussis toxin. Seven days P.I, footpads were challenged with 50 μg $OVA_{35–55}$ peptide and swelling measured 24 hr post-challenge.Data represents the mean +/−S.E.M. of 4 mice. NS: not significant.
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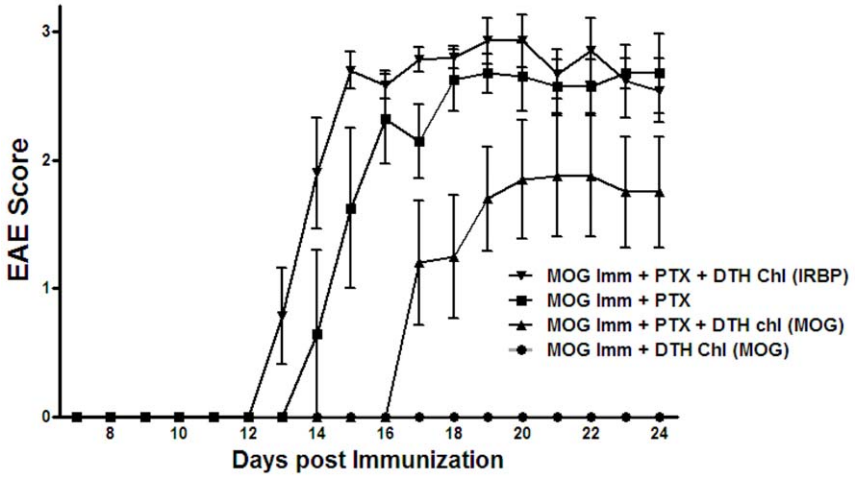

Figure 7. EAE is reduced by a challenge to the footpad with $MOG_{35–55}$ peptide. EAE was assessed fourteen days after C57BL/6 mice were immunized with MOG $peptide_{35–55}$, CFA and PTX or immunized and a footpad challenged with intradermal MOG $peptide_{35–55}$ or IRBP $peptide_{161–180}$ 7 days P.I. Following immunization, animals were kept under observation to score the disease. The study was done in a blinded fashion. The EAE scale was as follows: 0 = normal, 1 = limp tail, 2 = paraparesis with a clumsy gait, 3 = hind limb paralysis, 4 = quadriplegia, 5 = death. Data represents the mean score +/− S.E.M. of five mice/point. The experiment was done twice. DTH: delayed type hypersensitivity, chl: challenge
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IRBP immunized mice challenged with intradermal IRBP was absent or reduced markedly in mice receiving PTX. This swelling correlated with a reduced number of monocytic cells that infiltrated the site challenged with IRBP $peptide_{161–180}$. Our results with IRBP peptide differ from those of Silver *et al* [12] who demonstrated an enhancement of DTH to IRBP protein in C57BL10.RIII mice receiving PTX. However, Silver *et al* measured DTH to IRBP -induced DTH on day 21 P.I. rather than day 7 P.I. using significantly lower amounts of PTX. Although we observed lower DTH on day 21 P.I., 24 hr after challenge, there was a modest reduction in swelling in the footpads of mice immunized with IRBP peptide and CFA only and some increase in swelling in mice receiving PTX (Fig 4). However, the increase in swelling in the group receiving PTX was transient and not apparent 72 hr after challenge. Although we observed extensive damage to the outer nuclear layer by day 21 P.I., monocytic infiltration to the retina was reduced markedly. Silver *et al* did not get robust DTH without PTX although in our hands mice immunized with $MOG_{161–180}$ peptide and CFA (without PTX) did develop robust DTH. Additionally, the enhancement of DTH by PTX is due to the B subunit while the A subunit may induce the inhibition of an autoimmune response, or, at the doses of PTX we used, the inhibition of DTH [5].

Although the DTH response of C57BL/6 mice to OVA or OVA peptide was not influenced by PTX, the DTH response of C57BL/6 mice to encephalitogenic $MOG_{35–55}$ peptide was diminished in mice immunized with $MOG_{35–55}$ peptide, CFA and PTX even though these mice exhibited robust EAE. However, EAE was significantly reduced in these mice after their footpad was challenged with MOG peptide but not with IRBP $peptide_{161–180}$.

Thus, mice immunized with IRBP or MOG (self) peptide, CFA and a PTX dose that facilitates the induction of autoimmunity had a strong immune response to these autoantigens yet exhibited a diminished or absent DTH reaction when challenged with the autopeptides. In contrast, immunized mice that did not receive PTX (that did not develop autoimmunity) have a strong DTH response to the self-peptides. Moreover, the DTH response to OVA was not affected by PTX. In fact, PTX can amplify the DTH response to bacterial antigens [20]. Although PTX is thought to modulate lymphocyte migration [12,21], the administration of PTX before sensitized mice are challenged with the cognate antigen does not inhibit the DTH reaction [21]. Therefore, it is unlikely that PTX administered 6–10 days before the challenge affected the DTH response by activated T cells that includes the recruitment of monocytic cells. In aggregate, because the suppression of DTH by PTX is localized to self antigens, it is tempting to speculate that the lack of a local DTH response in mice with a localized, ongoing autoimmune response is due to a "diversion" of sensitized T cells to the site(s) containing the autoantigen, eg the retina (IRBP) or neurons (MOG). In that regard, the apparent reduction in EAE in MOG-immunized mice that received a challenge to the footpad with MOG peptide could be due to a diversion of antigen-reactive T cells to the challenge site. These possibilities are under investigation.

Author Contributions

Conceived and designed the experiments: RY REC. Performed the experiments: RY SB PG. Analyzed the data: RY SB PG JO REC. Wrote the paper: RY JO REC.

References

1. Forrester JV (2007) Intermediate and posterior uveitis. Chemical Immunology &Allergy 92: 228–43.
2. Caspi RR (2006) Ocular autoimmunity: the price of privilege? Immunological Reviews 213: 23–35.
3. Gery I, Mochizuki M, Nussenblatt RB (1986) Retinal specific antigens and immunopathogenic processes they provoke. Prog Retinal Res. pp 75–105.
4. Caspi R (2003) Experimental autoimmune uveoretinitis in the rat and mouse. Current protocols in immunology 15.6.1: 1–15.6.20.
5. Su Shao Bo, Silver B, WangPeng, ChanCh--Chao, Caspi RR (2003) Dissociating the enhancing and inhibitory effect of pertussis toxin on autoimmune disease. J. Immunol 171: 2314–2319.
6. Ryan M, McCarthy R, Rapppuoli B, McMahon P, Mills KH (1998) Pertussis toxin potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and the expression of the co-stimulatory molecules B7-1, B7-2 and CD28. International Immunology 10: 651–662.

7. Kugler S, Bocker K, Heusipp G, Greune L, Kim KSetal (2007) Pertussis toxin transiently affects barrier integrity, organelle organization and transmigration of monocytes in a human brain microvascular endothelial cell barrier model. Cellular Microbiology 9: 619–32.
8. Clifford PM, Zarrabi S, Siu G, Kinsler KJ, Kosciuk MC, et al. (2007) A beta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons. Brain Research 1142: 223–36.
9. Millward JM, Caruso M, Campbell IL, Gauldie J, Owens T (2007) IFN-gamma-induced chemokines synergize with pertussis toxin to promote T cell entry to the central nervous system. Journal of Immunology 178: 8175–82.
10. Agarwal RK, SunShu Shi, SuBo Shao, ChanChi-Chao, et al. (2002) Pertussis toxin alters the innate and the adaptive immune responses in a pertussis-dependent model of autoimmunity. Journal of Neuroimmunology 129: 133–140.
11. Linthicum DS, Munoz JJ, Blaskett A (1982) Acute experimental autoimmune encephalomyelitis in mice I. Adjuvant action of *Bordetella pertussis* is due to vasoactive amine sensitization and increased vascular permeability of the central nervous system. Cell Immunol 73: 299–310.
12. Silver PB, ChanChi-Chao, Wiggert B, Caspi RR (1999) The requirement for pertussis to induce EAU is strain-dependent. B10.RIII, but not B10.A mice, develop EAU and Th1 responses to IRBP without pertussis treatment.Invest. Ophthalmol Vis Sci 40: 2898–2905.
13. Denkinger CM, Denkinger MD, Forsthuber TG (2007) Pertussis toxin-induced cytokine differentiation and clonal expansion of T cells is mediated predominantly via costimulation. Cellular Immunology 246: 46–54.
14. Hofstetter HH, Shive CL, Forsthuber TG (2002) Pertussis toxin modulates the immune response to neuroantigens injected in incomplete Freund's adjuvant: induction of Th1 cells and experimental autoimmune encephalomyelitis in the presence of high frequencies of Th2 cells. Journal of Immunology 169: 117–25.
15. Caspi RR (2003) Experimental Autoimmune Uveoretinitis in the rat and mouse. Current Protocols in Immunology Supplement 53. pp 15.6.1–15.6.20.
16. Kunz M, Ibrahim SM (2009) Cytokines and cytokine profiles in human autoimmune diseases and animal models of autoimmunity. Mediators of Inflammation. 979258:979258.
17. Damsker JM, Hansen AM, Caspi RR (2010) Th1 and Th17 cells: adversaries and collaborators. Annals of the New York Academy of Sciences 1183: 211–21.
18. Nakae S, Korniyama Y, Nambu A, Sudo K, Iwase M, et al. (2002) Antigen-specific T cell sensitzation is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 17: 375–387.
19. Chen X, Howard OM, Oppenheim JJ (2007) Pertussis toxin by inducing IL-6 promotes the generation of IL-17- producing CD4 cells. Journal of Immunology 178: 6123–9.
20. McCarthy RM, Rappuoli l, Mahon R, Mills KH (1998) Pertussis toxin potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28. International Immunology 10: 651–662.
21. SuShao Bo, Silver PB, Zhang M, Chan C-C, Caspi RR (2001) Pertussis toxin inhibits induction of tissue-specific autoimmune disease by disrupting G Protein-coupled signal. Journal of Immunology 167: 250–256.