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Induction of polyclonal CD8+ T cell activation and effector function by Pertussis toxin ☆

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ABSTRACT

Pertussis toxin (PTX) has pronounced adjuvant activity and strongly enhances innate and adaptive immune responses, including increased antibody production and Th1/Th2 cytokine production. Adjuvant effects of PTX on Th1 and Th2 cells are primarily mediated via CD80/86 costimulation via enhanced expression of these molecules by APCs. However, it has remained unresolved whether PTX modulates the expression of costimulatory and inhibitory molecules on CD4+ and CD8+ T cells. To address this question, we determined the expression kinetics of CD28, CTLA-4, and CD40L on spleen CD4+ and CD8+ T cells after incubation with PTX. The results show that PTX upregulated the expression of CD28 by CD8+ T cells, but not by CD4+ T cells. In contrast, the expression of CTLA-4 and CD40L was not substantially altered on CD4+ or CD8+ T cells. CD28 upregulation by CD8+ T cells was paralleled by upregulation of CD69 and the induction of IFN-γ, Granzyme B (GrB), and IL-17. CD8+ T cell activation and cytokine production could be substantially blocked with anti-CD80 and CD86 antibodies, consistent with CD28 mediated signaling. Treatment of highly purified CD8+ T cells with PTX resulted in upregulation of CD28 and CD69, and production of IFN-γ. Incubation with CD28 mAb further enhanced this effect, suggesting that PTX has direct effects on CD8+ T cells which are enhanced by CD80/86-mediated costimulation provided by APCs.

1. Introduction

Pertussis toxin (PTX) is an A–B type exotoxin secreted by *Bordetella pertussis*, the causative agent of whooping cough [1,2]. PTX has a wide range of biological effects including induction of leukocytosis, lymphocyte mitogenesis, enhancement of immunoglobulin IgG and IgE synthesis and strong adjuvant effects on antigen-specific T cell immunity promoting both Th1- and Th2-type inflammatory responses [3–8]. Of note, PTX is routinely used in experimental autoimmune disease models in animals, such as experimental autoimmune encephalomyelitis (EAE) in rodents, to promote the disease [9,10]. In EAE, the disease promoting effect of PTX was initially believed to be due to an increased permeability of the blood–brain-barrier for inflammatory lymphocytes [11,12]. Recent evidence suggests that in addition, PTX may promote autoimmune pathology by furthering the expansion and effector functions of pathogenic autoimmune T cells [13,14]. Exactly how PTX functions as an immune adjuvant remains unclear. Several studies showed that it activates APCs and upregulates CD80, CD86, and MHC class II molecules, suggesting that its effects on T cells are mediated indirectly [14]. Along these lines, Wakatsuki and colleagues provided evidence that PTX bound to APCs was critical for its T cell activating properties, and Denkinger and colleagues showed that PTX-induced clonal expansion and cytokine differentiation of Th1 and Th2 cells was primarily dependent on costimulation via CD80/86 molecules [15,16]. However, some studies have suggested a direct effect of PTX on T cells [17,18], and it is conceivable that modulation of CD28 and/or CTLA-4 expression on T cells could contribute to the PTX adjuvant activity.

Interaction of CD80 and CD86 on professional APCs with CD28 on T lymphocytes is critical for providing the "second signal" for activation of CD4+ T cells. The requirement of the CD80/CD86–CD28 costimulatory pathway for CD8+ T cells is more controversial. For example, the activation of naïve and memory CD8+ T cells is not impaired in the absence of CD28-mediated costimulation in some models, while the CD28 pathway is important for the formation of primary CD8+ T cell responses under certain conditions, such as in responses against DNA and peptides, for vaccination with dendritic cells (DC), and in the induction of antitumor CD8+ T cell immunity [19–21]. Furthermore, the CD80/CD86–CD28 pathway may be

Abbreviations: PTX, Pertussis toxin; GrB, Granzyme B; APC, antigen presenting cell.

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important for controlling the magnitude of memory CD8+ T cell responses against pathogens [22,23].

Signaling via CD28 molecules is negatively regulated by CTLA-4, which is upregulated upon T cell activation and subsequently outcompetes CD28 for binding to CD80/86 due to its higher binding affinity for these molecules [24,25]. Thus, it is conceivable that modulation of CD28 or CTLA-4 on T cells by PTX could be important for its adjuvant effects.

To begin to address this issue, we determined the expression of CD28, CTLA-4, and CD40L molecules on T cells upon stimulation with PTX. Unexpectedly, PTX strongly upregulated the expression of CD28 on CD8+ T cells, but not on CD4+ T cells. CD28 upregulation on CD8+ T cells was paralleled by upregulation of CD69 and induction of IFN-γ, Granzyme B (GrB) and IL-17. CD8+ T cell activation and cytokine production could be blocked with anti-CD80 and anti-CD86 antibodies, strongly implicating costimulation via CD80/86 molecules as the mode of PTX action.

2. Materials and methods

2.1. Animals, antigens, and treatments

Female C57BL/6 mice (6–10 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained at the University of Texas at San Antonio (UTSA) under specific pathogen free conditions. All animal procedures were conducted according to the guidelines of the Institutional Animal Care and Use Committee of UTSA. Pertussis toxin was purchased from List Biological Laboratories (Campbell, CA) and used at the indicated concentrations. Blocking anti-CD80 (16-10A1) and anti-CD86 mAb (GL1) were gifts of Dr. Frederick Heinzel (Case Western Reserve University, Cleveland, OH). Anti-CD28 mAb was purchased from eBioscience (San Diego, CA). Anti-CD3 mAb was purified from hybridomas (2C11, ATCC). PMA and ionomycin was purchased from Sigma–Aldrich (St. Louis, MO). LPS (*Escherichia coli* 0111:B4) was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Cell preparations from the spleen

Single cell suspensions from spleens were prepared as described previously [8]. The cells were counted and plated with antigen in DMEM complete medium (BioWhittaker, Walkersville, MD) (containing 10% heat-inactivated FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.2 mM L-glutamine) at 5×10^6 cells per well in 24 well plates, cultured, treated and tested as indicated in the text.

2.3. Cell separations

Following preparation of single cell suspensions from spleens, CD8+ T cells were purified with a FACSAria II cell sorter (BD Biosciences, San Jose, California). Flow cytometry analysis showed 99–99.5% enrichment for CD8+ cells.

2.4. Flow cytometry analysis

Single-cell suspensions were incubated at 1×10^6 cells per sample with 0.125–0.5 μg of anti-CD4, anti-CD8, anti-CD28, anti-CD40L, or anti-CTLA-4 mAbs, (eBioscience, San Diego, CA) for 30 min at 4 °C in the dark. Cells were washed twice with PBS + 2% FCS and fixed in IC Fixation Buffer (eBioscience). For intracellular cytokine staining by flow cytometry, the cells were restimulated with anti-CD3 mAb (10 μg/ml, plate immobilized) and anti-CD28 mAb (2 μg/ml, plate bound) for 5 h in the presence of Brefeldin A solution (3.0 μg/ml, eBioscience). Staining for cell-surface antigens was performed as described above. The cells were fixed with IC Fixation Buffer for 20 min, permeabilized with Permeabilization Buffer (eBioscience) and stained with 0.125–0.5 μg of anti-IFN-γ, anti-GrB, or anti-IL-17 mAbs for 30 min at RT in the dark. Samples were analyzed on a LSR II or FACSAria using BD FACS Diva software (BD Bioscience).

2.5. Statistical analysis

Statistical analysis was performed by two-tailed Student's *t*-test using SigmaStat software (Systat Software, San Jose, CA).

3. Results

3.1. Pertussis toxin upregulates CD28 expression on CD8+ T cells

To test the effect of PTX on the expression of costimulatory molecules on T cells, we cultured spleen cells from C57BL/6 mice with medium alone, or in the presence of plate bound anti-CD3 mAb,

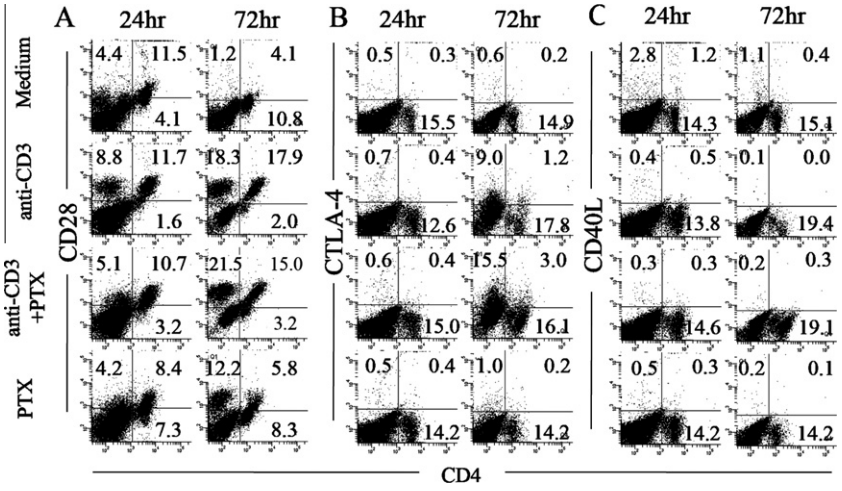

Fig. 1. PTX upregulates CD28, but not CTLA-4 or CD40L, on spleen cells. Pooled spleen cells from 3 to 5 C57BL/6 Wt mice were cultured for 24–72 h with medium, immobilized anti-CD3 mAb, immobilized anti-CD3 mAb plus PTX, or PTX alone. Expression of CD28 (A), CTLA-4 (B), and CD40L (C) was measured by flow cytometry as outline in Section 2. Shown is the percentage of cells expressing the respective surface molecules in one representative experiment of three to five separate experiments with similar results.