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Short communication

Evidence for adjuvanticity of anthrax edema toxin

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Abstract

Bacillus anthracis edema factor (EF) is an adenylate cyclase that increases intracellular cAMP concentrations. Since EF is present as a contaminant in the licensed protective antigen(PA)-based vaccines, we investigated its effect on anti-PA humoral immune response in BALB/c mice. We observed a significant increase of anti-PA IgG response in mice immunised with PA in association with EF as compared to PA alone. These results clearly show an adjuvant effect of EF, which is consistent with the data concerning the cellular effects of EF on antigen-presenting cells.
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Keywords: Anthrax; Edema factor; Adjuvant

1. Introduction

Anthrax is a life-threatening disease of broad concern, especially since it was exploited for bioterrorism. Its causative agent is *Bacillus anthracis*, a spore-forming bacterium which major known virulence factors include two toxins: edema toxin (ET) and lethal toxin (LT) formed by a protective antigen (PA) component associated with edema factor (EF) or lethal factor (LF), respectively. PA binds to at least two independent receptors on target cells. Upon heptamerization on the cell surface, it facilitates the translocation of the LF/EF moieties into the cytosol where they exert their toxic activities [1]. EF is a calcium- and calmodulin-dependent adenylate cyclase that increases intracellular cAMP concentrations [2]. LF is a zinc-dependent metalloprotease that cleaves mitogen-activated protein kinase (MAPK) kinases [3,4].

We recently demonstrated that ET impairs host dendritic cell (DC) functions disrupting the secretion of cytokines, especially interleukin (IL)-12p70 and tumor necrosis factor (TNF)-α but not IL-10 [5]. The effects of ET on cytokine secretion are very similar to the effects of other bacterial toxins: cholera toxin (CT), pertussis toxin (PT) and *Escherichia coli* heat-labile enterotoxin which have been used as mucosal adjuvant.

Because of its pivotal role in toxin penetration into the cells, PA is the main target of anthrax vaccines. The protective immunity obtained with acellular PA-based vaccines has been demonstrated in several animal models [6]. The current licensed PA-vaccines consist of filtered supernatants of an avirulent toxinogenic *B. anthracis* culture. The U.K. vaccine contains ET and LT as contaminants [7]. As a result, a strong anti-LF response was observed in vaccinated volunteers [8].

To clarify the potential effects of anthrax toxins in vaccines, we measured the anti-PA antibody response after immunisation with either PA, or PA and/or EF and/or LF. We observed that EF induced a significant increase of anti-PA antibody titres, showing clearly an adjuvant effect of EF.

2. Materials and methods

2.1. Mice and reagents

Six 8-week-old male BALB/c mice (CERJ, Mayenne, France) were housed under standard conditions. All

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experiments were approved by the institutional ethical committee for animal experiments.

PA, LF and EF were all from List Biological Laboratories, Campbell, CA. CpG oligonucleotide 1826 (sequence TCC ATG ACG TTC CTG ACG TT) was synthesised with a nuclease-resistant phosphorothioate backbone (MWG biotech, Germany) [9].

2.2. Mouse immunisation

Mice were injected subcutaneously (200 μl per mice) with PA alone or PA + LF or PA + EF or PA + LF or PA + LF + EF (10 μg of each per mice, all three components from List Biological Laboratories, Campbell, CA). CpG 1826 was added as adjuvant (10 μg/mice). Control mice received CpG only. Animals were immunised on days 0 and 8. Mice were bled 1 week later and serum were kept at −80 °C until use.

2.3. Anti-PA titre measurements by ELISA

96-well plates (Nunc) were coated with purified PA, or EF, or LF (100 ng/well) overnight at 4 °C. Plates were saturated with bovine serum albumin (BSA) 5% in phosphate buffered saline (PBS) at 37 °C for 1 h. After washing, serial dilutions of serum were added for 1 h at 37 °C. Plates were then washed and peroxidase labelled anti-mouse IgG or anti-IgG1/IgG2a antibodies (Southern Biotechnology Associates, Birmingham, AL) were added for 1 h at 37 °C. Plates were washed and revelation was performed using tetramethylbenzidine substrate. End point titres were expressed as the $\log_{10}$ of the reciprocal of the last dilution of sample which gave an OD value $\geq 2 \times$ OD of a pool of control serum.

2.4. Statistical analysis

Statistical analysis was performed using the Sigmastat™ software. Statistical differences between the groups were determined by a factorial analysis of variance (one way ANOVA, Holm-Sidak method).

3. Results and discussion

We addressed the effects of anthrax toxins (EF and/or LF associated to PA) on the response against PA, the toxin component currently used in licensed vaccines. After the first injection, serum anti-PA IgG titres increased in the PA + EF group as compared to PA alone but not significantly (Fig. 1A). After one boost, the difference in anti-PA IgG titres became highly significant (one way analysis of variance test, Holm-Sidak method; $P < 0.0001$) (Fig. 1B). The effects of EF on humoral anti-PA immune response favour the idea that EF acts as an adjuvant when given with PA. We also observed a significant increase of anti-PA antibodies in PA + LF and PA + EF + LF groups ($P < 0.0001$).

Next, we measured the anti-EF and anti-LF antibody titres in serum of mice in the relevant PA + EF versus PA + EF + LF, or PA + LF versus PA + EF + LF groups. We observed a slight increase of anti-LF titres in PA + LF + EF groups as compared to PA + LF only (Fig. 2), whereas anti-EF titres did not significantly differ in PA + EF and PA + EF + LF groups (data not shown). Taken together, these results confirmed that EF increase the anti-PA and anti-LF antibody responses, when co-administered.

We have previously shown that ET impairs DC cytokine secretion after infection of cells by spores [5]. We observed that ET specifically inhibited DC TNF-α (a pleiotropic cytokine with a diverse range of biological activity in inflammation) and IL-12p70 (the main cytokine that drives T-helper lymphocyte type 1 polarization), while IL-10 secretion was not affected. In a vaccine context, an imbalance toward high IL-10 and low IL-12p70 secretions triggers clearly a Th2 shift for the lymphocyte bearing antigen-specific T cell receptor. We measured the anti-PA IgG1/IgG2a isotype secretions in PA and PA + EF groups. Although we saw a

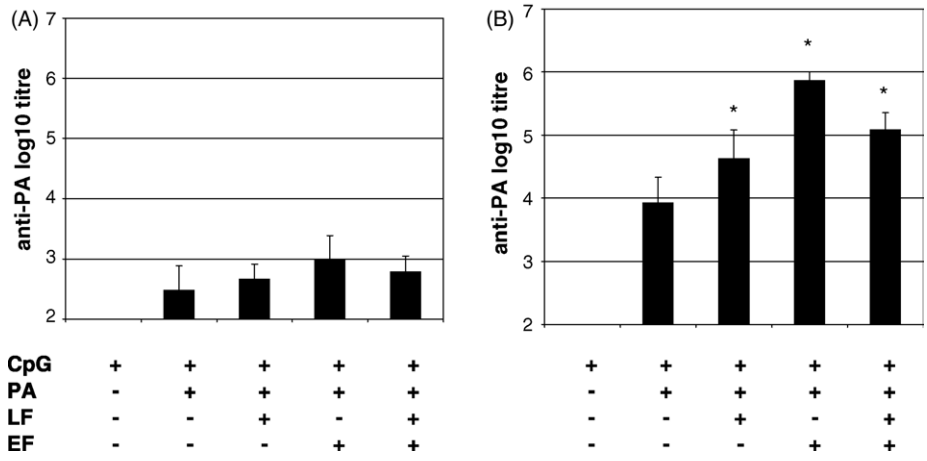

Fig. 1. Anti-PA IgG titres after one injection (A), and after one injection and one boost (B). Mice were immunised and bled as described in Section 2. Results shown are representative of three separate experiments (mean ± S.D.); one way analysis of variance test, Holm-Sidak method; *$p < 0.0001$ compared to PA group.

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