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Immunology Letters 97 (2005) 193–198
Immunology Letters
www.elsevier.com/locate/

Review

From toxin to adjuvant: basic mechanisms for the control of mucosal IgA immunity and tolerance

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Received 18 November 2004; received in revised form 27 November 2004; accepted 30 November 2004
Available online 19 January 2005

Abstract

We provide compelling evidence that delivery of Ag in the absence of ADP-ribosylation can promote tolerance, whereas ADP-ribosyltransferase activity induces IgA immunity and prevents tolerance. By linking Ag to the ADP-ribosylating enzyme, cholera toxin subunit A1 (CTA1), we could show that the combination of targeting to antigen-presenting cells (APC) and enzymatic activity is a highly effective means of controlling the induction of tolerance or immunity. Firstly, we demonstrated that cholera toxin (CT), although potentially binding to all nucleated cells, in fact, bound preferentially to dendritic cells (DC) in vivo. Following injection of CT-conjugated Ag, we found that DC in the marginal zone (MZ) of the spleen accumulated Ag, a process that was GM1-ganglioside receptor dependent. Contrary to CTB, which also delivered Ag to the MZ DC, CT matured and activated co-stimulatory functions in the targeted DC and greatly augmented immune responses to Ag. Secondly, when Ag was incorporated into the CTA1-DD fusion protein, which equals the CT in adjuvant function but lacks GM1-ganglioside-binding ability, we greatly augmented specific responses to Ag. The DD-bound Ag was distinctly targeted to B cells and probably also to follicular dendritic cells (FDC) in vivo. Thus, in both constructs Ag was targeted to APC and associated with an ADP-ribosylating enzyme, which resulted in greatly enhanced immunogenicity. When the enzymatic activity was absent, as in CT B-subunit (CTB) or in the inactive CTA1R7K-DD mutant, Ag largely failed to stimulate an active immune response. Rather, this type of Ag exposure resulted in Ag-specific tolerance, especially when mucosal delivery of Ag was attempted. Therefore, targeting to APC in the absence or presence of the CTA1-enzyme appears to be an effective means to control tolerance and active protective IgA immunity.

Keywords: Tolerance; Mucosal; Adjuvant; Cholera toxin; ADP-ribosylation; IgA; CTA1-DD

Contents

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doi:10.1016/j.imlet.2004.12.008

1. Introduction

Activation of innate immune responses is a prerequisite for an adjuvant function and a much needed component in any vaccine. Currently, a very limited spectrum of vaccine adjuvants are used commercially, with aluminum salts still being by far the largest group [1,2]. We have exploited the cholera toxin (CT) molecule and its immunomodulating properties to better understand how a rationally designed mucosal vaccine adjuvant could be developed. An important question to answer in this context is whether mucosal tolerance and IgA immunity against a specific Ag are mutually exclusive or can co-exist and whether they represent priming of the local immune system through the same or different activation pathways [3]. We analyzed if targeting of Ag to the same antigen-presenting cell (APC) under different conditions, with CTA1-enzyme or without this ADP-ribosylating enzyme, would result in different outcomes. Two strategies were attempted: the first using CT holotoxin or the enzymatically inactive GM1-ganglioside receptor-binding B-subunit of CT (CTB) and the second using CTA1-DD or an enzymatically inactive mutant, CTA1R7K-DD, thereof. Both strategies involved the enzymatically active CTA1 subunit, which, in the case of the holotoxin, is linked to the pentamer CTB and, in the CTA1-DD, is genetically fused to a fragment-D of *Staph. aureus* proteinA [4]. Because CTB binds to the GM1-ganglioside receptor present on all nucleated cells, it is thought to be rather non-selective in its binding repertoire in vivo. Not only ADP-ribosyltransferase activity of the holotoxin has been found to be important for the adjuvant function, but also the CTB or enzymatically inactive or partly active mutations of the holotoxin have been shown to host immunoenhancing functions [5]. This is also true for the closely related *E. coli* heat-labile toxin (LT) and mutations thereof [6,7]. Because CTB and LTB bind to essentially all nucleated cells carrying the GM1-ganglioside receptor, it has been difficult to explain how the holotoxins exert adjuvant function in vivo. From a clinical point of view, discouraging findings of direct binding and accumulation of CT or LT to the nervous system following intranasal administration has been reported [8]. In fact, a commercial LT-adjuvant containing Flu-vaccine was withdrawn from the market because of suspected cases of Bell's palsy in vaccinated subjects [9]. By contrast, the CTA1-DD adjuvant targets B cells and probably FDC in vivo, thereby avoiding most other cells in the body. This is why the CTA1-DD adjuvant is safe and completely non-toxic, whereas CT is highly toxic, albeit both molecules carry equal ADP-ribosylating ability. Mice and monkeys have been given doses of more than 200 μg without any apparent side effects or signs of reactogenicity, while a similar dose of CT is known to be lethal. Noteworthy, humans can get overt diarrhea from doses as low as 10 μg of CT [10].

A majority of adjuvants are microbial products that activate innate responses through pattern recognition receptors (PRRs), which leads to the release of pro-inflammatory cytokines and up-regulates co-stimulatory molecules on the surface of antigen-presenting cells (APC) [11–13]. Although B cells and macrophages are known to act as APC, DC are considered the key APC for priming of naïve T cells [14,15]. Whether this is also the case at mucosal membranes still awaits to be proven. The difficulty in targeting DC in vivo has limited our knowledge about the priming events that determine whether Ag-stimulation will result in a tolerogenic or an immunogenic outcome [16]. Immature DC that reside in tissues are known to take up Ag and, if maturation occurs, migrate to regional lymph nodes or the spleen [17,18]. In the secondary lymphoid tissues the DC immigrants, expressing strong co-stimulation, may be inherently stimulatory, but whether resident or poorly activated immigrants are tolerogenic is currently a much debated issue. In particular, we lack in vivo information about DC at specific anatomical sites, such as the MZ of the spleen, the lamina propria of the mucosal membranes or the conduit system in the peripheral lymph nodes.

2. From toxin to adjuvant

Potent adjuvants are required for mucosal immunizations. Most commonly used adjuvants activate the innate immune system and induce local inflammatory responses. For many of these compounds there is good evidence to suggest that the degree of inflammation directly relates to their adjuvant ability and that their principal mode of action is to augment antigen processing and presentation [2,19]. The involvement of the NFκB-pathway for activation of the innate immune response appears to be central to most adjuvant active molecules. CT and LT are two of the best studied and most effective experimental adjuvants known today. The adjuvant effect is thought to involve the modulation of APC, but it is poorly understood which APC are functionally targeted in vivo. All nucleated cells, including all professional APC, can bind the toxin via the GM1-ganglioside receptor present in the cell membrane of all nucleated cells. Previous reports have documented both a pro-inflammatory and an anti-