Mechanistic studies of the adjuvant effects of CTA1-DD and the native cholera toxin: Impact of cell targeting and tissue localization

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs Universitet, kommer att offentligen försvaras i hörsal Ivan Östholm, Medicinaregatan 13, Göteborg.

Fredagen den 1:a juni 2012 kl. 9.00

av

Johan Mattsson

Fakultetsopponent
Professor Bjarne Bogen
CIR – Center för Immunreglering, Avdelningen för Immunologi, Oslo Universitet, Oslo, Norge.

Avhandlingen baseras på följande delarbeten:

I. Complement activation and complement receptors on follicular dendritic cells are critical for the function of a targeted adjuvant

II. The adjuvant function of cholera toxin is independent of IL-12 and mediated by CD11b+CD11c+ dendritic cells inducing not only Th2- but also Th1 and Th17 responses
Mattsson J, Schön K, Yrlid U, Lycke NY. Manuscript

III. CTA1-DD adjuvant targets follicular dendritic cells and up-regulates the expression of germinal center-promoting genes

UNIVERSITY OF GOTHENBURG
Mechanistic studies of the adjuvant effects of CTA1-DD and the native cholera toxin: Impact of cell targeting and tissue localization

Johan Mattsson
Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Sweden, 2012.

Abstract

Vaccines are the most effective means of preventing infectious diseases and improving global health. However, few vaccines have successfully been developed for protection at mucosal surfaces where most pathogens gain access. The reason for this poor outcome has been the lack of immunoenhancers, or adjuvants, that allow for efficient mucosal immunizations. Empirical data has identified cholera toxin (CT) as one of the most effective adjuvant molecules known today. Because of its inherent toxicity, clinical use of CT is precluded. The closely related CTA1-DD adjuvant share the same dependence on the ADP-ribosylating enzymatic activity of the A1 subunit, however the differential binding properties of CTA1-DD renders the molecule safe and non-toxic. The aim of this thesis work has been to increase the knowledge about how adjuvants function by studying CT and the CTA1-DD adjuvant. To delineate key elements required for the adjuvant effects, we explored their in vivo distribution in tissues and the dependence on specific components of the immune system.

We found that both CT and CTA1-DD localizes to the marginal zone macrophages (MZMs) of the spleen after iv. injection. To investigate the importance of this finding we treated mice with clodronate liposomes, depleting the MZMs, and found that immunizations with CT or CTA1-DD generated unperturbed immune responses in the treated mice, suggesting that this cell subset is dispensable for their adjuvant effect.

Following initial accumulation in MZMs, CTA1-DD localized to the follicular dendritic cell (FDC) network. This correlated with the ability of CTA1-DD to activate complement primarily via the alternative pathway, allowing the adjuvant to bind to the complement receptors 1 and 2 (CR1/CR2) on FDCs. We found that adjuvanticity was dramatically reduced in Cr2 knockout mice, where this localization is absent. This prompted us to isolate FDCs from mice immunized with CTA1-DD and assess their activation status using RT-PCR. We found that a number of genes important for the ability of FDCs to support germinal center (GC) formation were up-regulated. Whereas FDCs are highly involved in orchestrating the GC reaction it was feasible that a direct effect of CTA1-DD on FDC functions promoted GC formations.

Conventional dendritic cells (DCs) are believed to be essential for generating follicular helper T (Tfh) cells, but it is unknown to what extent CTA1-DD affects this process. Unexpectedly, when using the CD11c-DTR mouse model to deplete DCs, we found Tfh cell priming appeared to be normal in terms of expansion and phenotype, however a significant reduction in the expression of the Tfh cell transcription factor Bcl-6 was recorded following immunization. Despite potentially reduced Tfh function, we observed that the ability of CTA1-DD to promote antibody production and GC formations was still significant. We speculate that this was the result of a compensatory mechanism employed by the CTA1-DD adjuvant, possibly via the activation of FDCs.

Finally, we examined the immunomodulatory properties of CT. CT is generally considered a Th2 adjuvant and has been reported to inhibit Th1 responses by down-regulating IL-12 production. Here we demonstrated that CT rather induces a mixed Th1/Th2/Th17 response, independently of IL-12. Interestingly, iv immunization with CT completely blocked the ability to respond to a subsequent immunization, and both Th1 and Th2 responses were inhibited, arguing that an early event in the priming process was impaired. This correlated well with the observation that CD11b+ DCs were activated, thus compromising their ability to process additional antigens. In addition we found that the CD8α+ DC population was depleted following CT-administration and could therefore not be involved in the adjuvant effect of CT. Finally, reconstituting CT-treated mice with DCs re-established their ability to respond to a subsequent immunization.

In conclusion, we have demonstrated that the differential binding properties of the related adjuvants, CT and CTA1-DD, critically affects the mechanisms by which they modulate immune responses. This underpins the importance of targeting adjuvants to specific components of the immune system in order to efficiently deliver stimulation and avoiding toxic side effects, an important insight when designing future vaccines.

Keywords: adjuvants, vaccines, CTA1-DD, cholera toxin, follicular dendritic cells, dendritic cells, Th1, Th2, Th17, Tfh, germinal centers, complement.