Subsets of intestinal dendritic cells and their role in orally-induced immune responses

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Avhandlingen baseras på följande delarbeten:

I. **Fahlén-Yrlid L, Gustafsson T, Westlund J, Holmberg A, Strömbeck A, Blomquist M, MacPherson G G., Holmgren J, Yrlid U**
CD11c(high) dendritic cells are essential for activation of CD4+ T cells and generation of specific antibodies following mucosal immunization
Journal of Immunology, 2009, vol. 8, Issue 183, pages: 5032-41

II. **Westlund J, Livingston M, Fahlén-Yrlid L, Oldenborg P-A, Yrlid U**
CD47-deficient mice have decreased production of intestinal IgA following oral immunization but a maintained capacity to induce oral tolerance
Immunology, 2012, vol. 3, Issue 3, pages: 236-244

III. **Westlund J, Capar S, Fahlén-Yrlid L, Livingston M, Ekman L, Lycke N Y., Yrlid U**
Oral adjuvant activity of cholera toxin is independent of classical toll-like receptor signaling but requires G_sa expression in $CD11c^+$ dendritic cells
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Subsets of intestinal dendritic cells and their role in orally-induced immune responses

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Abstract

Vaccination is 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 infectious pathogens enter our body. One major reason for this is the lack of adjuvants, immune enhancing agents, that can be administered together with the vaccine. The enterotoxin cholera toxin (CT) is a potent mucosal adjuvant but the toxicity precludes its use in humans. Derivatives of enterotoxins with reduced toxicity are today the most promising candidates for safe and efficient oral adjuvants. However, the underlying mechanisms for the adjuvant activity of enterotoxins are still not fully known.

Dendritic cells (DCs) are immune cells that sense the microenvironment and confer T cells with ability to help B cells differentiate into antibody-producing plasma cells, necessary for vaccine-induced protection. Intestinal DCs are important both for immunity and tolerance. However, intestinal DCs constitute a heterogeneous population of cells. The function of intestinal DC subsets therefore needs to be defined further to understand how these contribute to tolerance under steady state and to induce immunity during infection or following oral immunization.

In this thesis the role of intestinal DC subsets, in the induction of immune responses following oral administration of antigen, with or without CT as adjuvant, was elucidated. This was done after developing a microsurgical technique in mice that by cannulation of lymphatic vessels allows the direct collection of DCs that exit the intestine under steady state and following vaccination. This technique was combined with the use of genetically modified mice 1) in which DCs can be ablated; 2) that lack specific DC subsets; 3) that are deficient in intracellular signaling pathways in DCs or in other immune cells or 4) that lack CD47, a surface receptor known to influence cell migration.

In the thesis we demonstrate the requirement of cDCs for the activation of antigen-specific T cells and the generation of antigen-specific antibodies following oral immunization when using limiting doses of antigen and CT as an adjuvant. In addition, we show *in vivo* that intact signaling through Gsα specifically in cDCs is essential for the oral adjuvant activity of CT. Using the cannulation technique we show that four subsets of DCs migrate from the intestine under steady state and following oral immunization. Selectively the $CD11b^{-}CD8^{+}$ subset does not show signs of activation after oral CT and this subset was also found to be dispensable for the generation of antigen-specific intestinal antibodies using this adjuvant. The necessity for $CD11b^{+}CD8^{-}$ cDCs could not be establish in CD47 deficient mice, although these mice display significant reduction of this subset in intestinal tissues. Rather, expression of CD47 by non-hematopoietic cells is pivotal for intestinal antibody generation after oral immunization. Finally, signaling pathways involved in CT's adjuvanticity were addressed and shown to be independent of classical TLR-signaling. Moreover, caspase 1/11 activity was not necessary for the generation of antigen-specific serum IgG but for intestinal IgA following oral immunization with CT.

In conclusion, we have shown a requirement for cDCs and an intact signaling specifically in these cells for the oral adjuvant activity of CT. Furthermore we have identified that the generation of intestinal and systemic antibodies following oral immunization with CT are differentially regulated. These results may therefore have important implications for the development of improved oral vaccines.

Keywords: dendritic cells, oral vaccination, intestine, antibody responses, cholera toxin, gut-associated lymphoid tissue
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