The immunomodulatory function of staphylococcal superantigen on oral tolerance

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin,
Göteborgs universitet kommer att offentligen försvaras i
Föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg

Fredagen den 29 oktober 2010 kl. 9.00

av
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Fakultetsopponent:
Professor William Agace
Avdelningen för experimentell medicinsk vetenskap
Lunds universitet

Avhandlingen baseras på följande delarbeten:

I. Anna Lönnqvist, Sofia Östman, Nina Almqvist, Susanne Hultkrantz, Esbjörn Telemo, Agnes E Wold, Carola Rask
Neonatal exposure to staphylococcal superantigen improves the induction of oral tolerance in a mouse model of airway allergy
European Journal of Immunology 2009, 39(2):447-54

II. Anna Stern, Agnes E Wold, Sofia Östman
Accumulation of FoxP3$^+$ Tregs in the gut of mice neonatally treated with *S. aureus* superantigen
Submitted

III. Anna Stern, Agnes E Wold, Sofia Östman
Oral tolerance improved by staphylococcal superantigen depends on functional vitamin A metabolism
In manuscript

IV. Anna Stern, Erika Lindberg, Fredrik Bäckhed, Agnes E Wold, Sofia Östman
Superantigen-producing *Staphylococcus aureus* promotes oral tolerance in mice
In manuscript

Göteborg 2010

UNIVERSITY OF GOTHENBURG

The immunomodulatory function of staphylococcal superantigen on oral tolerance

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Abstract
Exposure to soluble proteins via the gut gives rise to systemic tolerance, a phenomenon called oral tolerance. Failure of oral tolerance results in allergy, a disease that has increased during the last decades in Western industrialized countries. The cause of this rapid increase is unknown. However, according to the hygiene hypothesis and many epidemiological studies, there is a clear correlation between a hygienic lifestyle and the prevalence of allergy. The hygiene hypothesis is also supported by the results of animal studies. Specifically, germ-free mice have been found to be deficient in the development of oral tolerance and have less functional regulatory T cells. We have observed that Swedish infants have a less diverse gut microbial flora and a slower strain turnover compared to infants in developing countries, suggesting that certain microbes may have particularly strong immunoregulatory potential. Thus, neonatal colonization by *Staphylococcus aureus* (*S. aureus*) in the gut protects against food allergy development. Most *S. aureus* strains can produce one or more toxins with superantigenic function, including staphylococcal enterotoxins (SE) A, B, C, D, and E, as well as toxic shock syndrome toxin-1 (TSST-1). Superantigens are the strongest known T cell stimulants, as they stimulate 5-30% of all T cells in an antigen-independent manner by cross-linking MHC class II molecules on antigen-presenting cells with the Vβ region of the T cell receptor.

The purpose of this thesis was to study the immunomodulatory role of staphylococcal superantigen on oral tolerance in animal models of allergy. Newborn pups were exposed to SEA during the first two weeks of life. Oral tolerance was induced at 6 weeks of age by feeding the mice the model antigen ovalbumin (OVA). Oral tolerization was followed by sensitization and challenge according to an airway allergy model or a food allergy model. Neonatal SEA treatment resulted in enhanced development of oral tolerance, as evidenced by decreased sensitization in both allergy models. Further, when colonizing germ-free mice with superantigen-producing *S. aureus*, improved oral tolerance induction in the food allergy model was observed compared to mice colonized by a non toxin-producing strain. To investigate the long-term effect of SEA on the immune system, immune cells were studied at the time for oral tolerization. We found that mice neonatally treated with SEA had higher proportions of lymphocytes expressing the gut migratory markers chemokine receptor CCR9 and integrin α4β7. This was associated with higher numbers of $FoxP3^+$ regulatory T cells in the small intestinal lamina propria. In addition, neonatal SEA treatment rendered dendritic cells (DCs) more tolerogenic demonstrated by lower expression of co-stimulatory markers, higher expression of MHC class II, and reduced T cell stimulatory properties.

A subpopulation of gut DCs expressing CD103 have been suggested to be important for oral tolerance. This DC subset specifically imprints gut migratory potential on stimulated T cells and can convert naïve T cells into regulatory T cells. The unique properties of the $CD103^+$ DCs depend on their expression of retinal dehydrogenases (RALDHs), enzymes that convert vitamin A to retinoic acid (RA). By interfering with the vitamin A metabolism *in vivo* by giving mice the RALDH inhibitor Citral in their drinking water, the improvement in oral tolerance noted after neonatal SEA treatment was lost. In addition, Citral intake affected gut DCs by lowering the expression of MHC class II, suggesting that high expression of antigens via MHC class II is important for oral tolerance.

In conclusion, neonatal exposure to superantigen or colonization of germ-free mice by superantigen-producing *S. aureus* confers an increased ability for oral tolerance several weeks after treatment. This improvement is likely dependent upon an interaction between gut-residing DCs and gut-migrating lymphocytes, particularly regulatory T cells. SEA treatment affects gut DCs inducing prolonged capacity in this subset to evoke gut-homing potential to T cells. In addition, the improved oral tolerance observed following neonatal SEA treatment might also be dependent on functional vitamin A metabolism.

Keywords: staphylococcal enterotoxin, superantigen, oral tolerance, hygiene hypothesis

ISBN: 978-91-628-8155-9
E-published at http://hdl.handle.net/2077/22916