Unravelling the mechanisms for mucosal tolerance induction using the CTA1R7K-X-DD immunomodulating fusion protein

Effective treatment options for autoimmune diseases

AKADEMISK AVHANDLING

som för avläggande av medicine doktorexamen vid Sahlgrenska akademin vid
Göteborgs Universitet kommer att offentligen försvaras i hörsal
Arvid Carlson, Academicum, Medicinaregatan 3, Göteborg

Fredagen den 2 december 2016, klockan 09.00

av **Charlotta Hansson**

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Avhandlingen baseras på följande delarbeten

I. **Tolerance-induction by a mutant cholera toxin-derived fusion protein depends on migratory CD103+ DCs**
Hansson C, Schön K, Lycke NY
Manuscript

II. **IL-27R is critical for tolerance-induction by the CTA1R7K-MOG-DD fusion protein in experimental autoimmune encephalitis**
Hansson C, Verolin M, Schön K, Chandode R, Quintana F, Lycke NY
Manuscript

III. **Feeding plants that express a tolerogenic fusion protein effectively protects against arthritis**
Hansson C, Schön K, Kalbina I, Stridh Å, Andersson S, Bokarewa MI, Lycke NY
Plant biotechnol J. 2016 Apr 14(4): 1106-15. Epub 2015 Sep 25.

UNIVERSITY OF GOTHENBURG

Gothenburg, 2016

Unravelling the mechanisms for mucosal tolerance induction using the CTA1R7K-X-DD immunomodulating fusion protein:

Effective treatment options against autoimmune diseases

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Tolerance is a physiological mechanism that prevents attacks on our own cells and tissues whilst allowing immune responses directed against tumours and invading pathogens. Autoimmunity is an aggressive attack by the immune system on tissues and tissue functions that results from a loss of tolerance which can cause chronic and debilitating symptoms. Hence, reinstating tolerance to achieve physiological homeostasis is highly warranted.

In recent years significant progress has been made in our understanding of tolerance and how auto-aggression is actively controlled by multiple layers of immune regulatory mechanisms. This thesis describes a unique tolerance-inducing platform that may find clinical use in the treatment of several autoimmune diseases. The CTA1R7K-X-DD platform is a fusion protein that consists of three elements with their own respective properties; a targeting unit (DD), a disease-specific peptide (X), and an inactivated mutant of the immunomodulatory cholera toxin A1-subunit (CTA1R7K). I have exploited this platform using different peptide inserts (X) to investigate its mechanism of action and function in two experimental models of human autoimmune diseases, namely rheumatoid arthritis (RA) and multiple sclerosis (MS).

The tolerogenic CTA1R7K-COL-DD fusion protein is a therapeutically effective means to prevent collagen-induced arthritis (CIA) in mice and my aim was to understand the mechanisms by which the fusion protein reinstates tolerance. My research will contribute to a better understanding of immune regulation during autoimmunity, and provide a strategy to interfere with the progression of autoimmune diseases. By comparing the tolerogenic fusion protein with its immunoenhancing, enzymatically active, counterpart (CTA1-X-DD), I identified a yin-and-yang effect of the two fusion proteins on migratory dendritic cells (DCs), which were found to be the primary target cells *in vivo* after intranasal immunizations. While the enzymatically active CTA1-X-DD fusion protein induced the expression of co-stimulatory molecules on the DC, inactive CTA1R7K-X-DD instead promoted a set of genes associated with Tr1 induction and co-inhibition. Most importantly, the IL-27 cytokine was upregulated in both DCs and T cells, a signalling pathway known to be important in Tr1 induction and the regulation of effector responses. The differential effects observed on the DC populations were dependent on enzymatic activity and translated into differences in downstream T cell responses. I studied these two populations in detail to dissect the immunomodulating events that participated in the development of tolerance or immunity.

In the second half of my thesis work I have investigated the therapeutic effect of the fusion protein carrying disease-relevant peptides in the experimental autoimmune encephalitis (EAE) model. I used this model to further demonstrate the effects of our fusion protein on disease development, focusing on regulatory CD4 T cell subsets and the functional importance of the IL-27 pathway in tolerance induction. Finally, to meet the need for simple treatments in the clinic I have evaluated whether there would be a formulation that is cost-effective to produce, which may also secure good patient compliance. To this end, I have collaborated with a group that expressed the fusion protein in an edible plant. This allowed me to test whether oral administration of the fusion protein, when bioencapsulated, could be used to treat CIA. I found that treated mice overall exhibited significantly reduced symptoms and in fact, some mice showed no symptoms at all. Thus, this proof-of-principle study showed that a protein-based pharmaceutical administered in the form of a transgenic plant might be clinically feasible for tolerance induction.

My thesis identifies several molecular features in the early tolerization process in targeted DCs and the subsequent generation of CD4 T cells that help explain the tolerogenic functions of the CTA1R7K-X-DD fusion protein. My research also provides experimental evidence which indicate that the CTA1R7K-X-DD fusion protein could be a potential therapeutic agent for treatment of RA, MS and possibly other autoimmune diseases.

Keywords: immunological tolerance, dendritic cells, Tregs, autoimmunity, multiple sclerosis, experimental autoimmune encephalitis, rheumatoid arthritis, collagen-induced arthritis, plant vaccination
ISBN: 978-91-628-9951-6 (PDF)
ISBN: 978-91-628-9952-3 (Print)