



**Karolinska
Institutet**

Institutionen för Medicin, Solna

Immune regulation and modulation of allergy and inflammatory diseases

AKADEMISK AVHANDLING

som för avläggande av medicine doktorexamen vid Karolinska
Institutet offentligens försvaras i Rolf Luft Auditorium, L1:00
Karolinska Universitetssjukhuset Solna

Fredagen den 14 september 2012, kl. 09.00

av

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Stockholm 2012

ABSTRACT

Inflammation is evoked in defence against invading pathogens entering the body. Sometimes inflammation is started against harmless antigens, which leads to allergic diseases, or against self-antigens or commensal microbiota as in inflammatory bowel disease (IBD). This thesis addresses treatment of allergic disease and IBD and how immune cells are affected by the treatment.

To date, the only curative treatment available for allergy is allergen-specific immunotherapy (SIT), which is based on the repeated administration of disease-eliciting allergens. The aim of SIT is modification of the allergen-specific immune response so that the allergen can be tolerated, but both efficacy and safety need to be improved.

The first two papers of this thesis are focusing on different approaches to modify the allergen used in SIT in order to improve the treatment. In **paper I** we covalently coupled the immunomodulatory substance 1α , 25-dihydroxyvitamin D₃ (VD₃) to the major cat allergen rFel d 1 (rFel d 1:VD₃), to enhance the immunomodulatory effects of SIT. When tested in a mouse model of cat-allergy it was shown that SIT with the modified allergen, rFel d 1:VD₃, was effective at a lower dose than SIT with rFel d 1 alone. Airway hyperresponsiveness, cell infiltration and Th2 cytokines in bronchoalveolar lavage fluid were reduced more by rFel d 1:VD₃ than by rFel d 1, indicating that lower allergen doses may be used in SIT. Thus both efficacy and safety could be improved.

Another way of modifying the allergen is by changing the structure of the protein itself. The aim of **paper II** was to construct an altered version of rFel d 1 with reduced number of T-cell epitopes. Using error prone PCR and a phage display library, four candidate allergens were developed. They had reduced immunoglobulin (Ig) E-binding capacity and basophil reactivity compared to rFel d 1, and three of them also had lower capacity of inducing T-cell proliferation. These three allergens induced Fel d 1-specific IgG antibodies in immunised mice that had similar IgE-blocking capacity as rFel d 1. These properties suggest that the allergen-mutants will have a better safety profile, but with similar efficiency as rFel d 1, when used in SIT.

Chronic inflammatory diseases are complex and involve many different cell types and mechanisms, which are not yet completely understood. We studied patients with IBD as a model system for chronic inflammation to evaluate different cell types during resolution of inflammation. In **paper III**, IBD patients that received anti-TNF treatment were analysed during the first six weeks of therapy. There was an induction of effector T-cells in the gut mucosa of these patients at the same time as CD25+TNFR_{II}+ helper T-cells were reduced. In peripheral blood (PB), no major changes in T-cell subsets were observed, but there was an indication of changed regulatory mechanisms controlling antigen specific T-cell responses by anti-TNF treatment.

In **paper IV** we focused on the importance of monocytes in IBD. A subset of monocytes expressing high levels of HLA-DR was shown to also express the gut homing receptor CCR9. Patients with IBD had a higher percentage of HLA-DR^{hi} monocytes in PB, and higher expression of CCR9 on monocytes compared to controls. When IBD-patients were treated with granulocyte-monocyte apheresis or corticosteroids, but not with anti-TNF treatment, the percentage of HLA-DR^{hi} in PB was reduced to the same level as controls. This may be a new subset of monocytes important for inflammation in the gut and a new target for therapy.

In conclusion, this thesis presents two strategies to improve SIT by the use of modified allergens. Moreover, it supports that different mechanisms are involved in different treatments of IBD, and thus stresses the importance of therapy choice.