

particular, in the case of a family history of febrile episodes, the possibility of TRAPS should be considered if an aetiology of autoimmune or infectious nature is excluded. The discovery of three different mutations in a population as small as the Finnish, reinforces the notion that the extracellular domain of *TNFRSF1A* is prone to be mutated at the entire stretch of its cysteine-rich domains and not only at a limited number of sites, suggesting the absence of a founder effect in TRAPS. This study also demonstrates the challenges of clinical work in differentiating the symptoms of rare genetic disorders from those of other pathological conditions, and presents the possibility of an auto-inflammatory disorder as being the underlying cause of severe clinical complications. Furthermore, functional studies of fresh blood leukocytes show that TRAPS is often associated with a low NF- $\kappa$ B and p38 phosphorylation profile, although low phosphorylation levels are not a requirement for the development of TRAPS. The aberrant signalling profile would suggest that the hyperinflammatory phenotype of TRAPS is the result of compensatory NF- $\kappa$ B-mediated regulatory mechanisms triggered by a deficiency of the innate immune response.

### List of original publications

The thesis is based on the following original communications, referred to in the text as Roman numerals:

- I. Nevala H, Karenko L, Stjernberg S, Raatikainen M, Suomalainen H, Lagerstedt A, et al. A novel mutation in the third extracellular domain of the tumor necrosis factor receptor 1 in a Finnish family with autosomal-dominant recurrent fever. *Arthritis Rheum* 2002; 46: 1061-1066.
- II. Stjernberg-Salmela S, Pettersson T, Karenko L, Blazevic V, Nevala H, Pitkänen S, Peterson P, Ranki A. A novel tumour necrosis factor receptor mutation in a Finnish family with periodic fever syndrome. *Scand J Rheumatol* 2004; 33: 140-144.
- III. Stjernberg-Salmela S, Kivisaari A, Puolakkainen P, Färkkilä M, Kemppainen E, Ranki A, Pettersson T. Intra-abdominal abscess in a patient with tumour necrosis factor receptor-associated periodic fever syndrome. *J Intern Med* 2006; 259: 209-213.
- IV. Stjernberg-Salmela S, Ranki A, Karenko L, Siitonen S, Mustonen H, Puolakkainen P, Sarna S, Pettersson T, Repo H. Low TNF-induced NF- $\kappa$ B and p38 phosphorylation levels in leukocytes in tumour necrosis factor receptor-associated periodic syndrome (TRAPS). Submitted.

In addition this thesis contains some previously unpublished data.

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## Topical Tacrolimus in Atopic Dermatitis: Effects of Long-term Treatment on Skin and Respiratory Symptoms

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**Dr Hannele Virtanen from the Department of Dermatology, Allergology, and Venereology, University of Helsinki, Finland, defended her PhD thesis on 25 February 2008 in Helsinki. The opponent was Professor Thomas Ruzicka from the Ludwig-Maximilians University, Munich, Germany. The thesis was supervised by Docent Sakari Reitamo. The thesis publication is available at: <http://urn.fi/URN:ISBN:978-952-10-4520-2>.**

Atopic dermatitis (AD) is a chronic pruritic skin disease often complicated by *Staphylococcus aureus* colonization and superinfection. In patients with AD, concomitant occurrence of other atopic diseases, such as asthma and allergic rhinoconjunctivitis, is common. Skin atrophy is the major problem in conventional corticosteroid-based treatment of AD, which limits treatment to short periods, especially in areas of thin skin. After the treatment period, AD often relapses. In practice, mainly the patients with mild disease achieve a longer remission with periodic short-term use of topical corticosteroids. Patients with moderate or severe AD are usually poorly controlled with short-term treatments and experience continuous skin inflammation of varying degrees. The second-line treatments, such as ultraviolet light therapy, cyclosporine, and azathioprine, diminish the need for topical

corticosteroids but do not replace them. The barrier function of the inflamed skin is weakened, possibly enabling an epicutaneous sensitization to occur, which may lead to atopic respiratory disease via systemic T-cell responses and airway hyper-responsiveness. The need has therefore been great for a non-atrophogenic treatment option, especially for patients with moderate-to-severe AD requiring long-term treatment.

Although AD is a very common and chronic disease, efficacy in placebo-controlled studies has been shown only for topical corticosteroids, oral cyclosporine, and azathioprine. Furthermore, only a few long-term studies involve topical corticosteroids. Tacrolimus ointment belongs to the group of topical immunomodulators, which have a more specific mode

of action on the skin than the broad-scale effects of topical corticosteroids. It is the first topical compound suitable for long-term treatment. Based on a short-term study tacrolimus has shown no atrophogenic properties. This thesis elucidates the background of AD and its treatments in general, and concentrates on the effects of long-term topical tacrolimus monotherapy on major problem areas in the treatment of AD, namely *S. aureus* colonization, collagen synthesis of the skin, and treatment of areas of thin and sensitive skin, such as the eyelids; and finally, it examines the interaction between AD and the other atopic diseases asthma and allergic rhinitis.

Patients with moderate-to-severe AD were treated with intermittent 0.1% tacrolimus ointment in prospective, open, long-term studies lasting 6–48 months. In Study I, staphylococcal colonization of their skin was followed by bacterial cultures for 6–12 months. In Study II, skin thickness and collagen synthesis were followed by skin ultrasound and procollagen I and III propeptide concentrations of the suction blister fluid samples for 12–24 months and compared with a group of AD patients treated with topical corticosteroids and with a group of healthy subjects. Study III was a cross-sectional study including patients with AD and healthy controls. The occurrence of respiratory symptoms was determined with a questionnaire, bronchial hyper-responsiveness by bronchial histamine challenge, and sputum eosinophilia by induced sputum test. Atopy status was assessed by skin prick test reactivity and serum immunoglobulin E concentration. In Study V, the same parameters as in Study III were assessed in patients with AD before and after 12–48 months of topical tacrolimus treatment. Study IV was a retrospective follow-up of the effect of tacrolimus 0.03% ointment on severe atopic blepharoconjunctivitis and conjunctival cytology samples.

The clinical response of AD to topical tacrolimus was very good in all studies ( $p \leq 0.008$ ). Staphylococcal colonization decreased significantly, and the effect was sustained throughout the study period ( $p = 0.01$ ). Skin thickness ( $p < 0.001$ ) and markers of collagen synthesis ( $p < 0.001$ ) increased significantly in the tacrolimus-treated patients, whereas they decreased or remained unchanged in the corticosteroid-treated controls. Symptoms of asthma and allergic rhinitis ( $p < 0.0001$ ), bronchial hyper-responsiveness ( $p < 0.0001$ ), and sputum eosinophilia ( $p < 0.0001$ ) were significantly more common in patients with AD than in healthy controls, especially in subjects with positive skin prick tests or elevated serum immunoglobulin E (IgE). During topical tacrolimus treatment the asthma and rhinitis ( $p = 0.005$  and  $p = 0.002$ , respectively) symptoms and bronchial hyper-responsiveness ( $p = 0.02$ ) decreased significantly in patients with AD, and serum IgE and sputum eosinophils showed a decreasing trend in patients with AD with a continuously good treatment response. Treatment of eyelid dermatitis with tacrolimus ointment resulted in a marked clinical response and a significant decrease in eosinophils, lymphocytes, and neutrophils in the conjunctival



Fig. 1. Hannele Virtanen with the opponent Thomas Ruzicka from Munich, Germany (middle), and the supervisor of the thesis, Docent Sakari Reitamo (right).

cytology samples. No significant adverse effects or increase in skin infections occurred in any study.

The results suggest that replacing topical corticosteroid treatment with long-term intermittent topical tacrolimus treatment for AD is not associated with the adverse events common during corticosteroid treatment, such as skin atrophy, and has no marked safety issues, even after several years of intermittent monotherapy. Studies I, II, and V were conducted in the context of multicentre open-label long-term trials, the primary endpoint being the safety of topical tacrolimus in long-term treatment of AD. Although they included no randomized control groups, these studies are the first long-term studies to show significant improvement in staphylococcal colonization, suppression of skin collagen synthesis, and atopic respiratory symptoms, which are typical problems complicating AD and its treatment.

Tacrolimus ointment efficiently suppresses the T-cell-induced inflammation of AD. It has a normalizing effect on the function of the skin measured by the decrease in staphylococcal colonization. It does not cause skin atrophy as do corticosteroids but restores the skin collagen synthesis in patients who have used corticosteroids. The effects of tacrolimus ointment are not restricted to the skin, because improvement in respiratory atopy may also be achieved. However, tacrolimus ointment has no marked systemic effect, as the absorption of the drug is minimal and decreases along with skin improvement. The effects on the airways – decrease in bronchial hyper-responsiveness and respiratory symptoms – can be speculated to be caused by the decrease in T-cell trafficking from the skin to the respiratory tissues as the skin inflammation resolves, as well as inhibition of epicutaneous invasion of various antigens causing systemic sensitization when the skin barrier is disrupted. These results have special importance to patients with moderate-to-severe AD – the patient group most likely to suffer from the adverse events of topical corticosteroids, undertreatment of AD, or respiratory atopy.

### List of original publications

- I. Remitz A, Kyllönen H, Granlund H, Reitamo S: Tacrolimus ointment reduces staphylococcal colonization of atopic dermatitis lesions. *J Allergy Clin Immunol* 2001; 107: 196–197.
- II. Kyllönen H, Remitz A, Mandelin JM, Elg P, Reitamo S. Effect of one-year intermittent treatment with topical tacrolimus monotherapy on skin collagen synthesis in patients with atopic dermatitis. *Br J Dermatol* 2004; 150: 1174–1181.
- III. Kyllönen H, Malmberg P, Remitz A, Ryttilä P, Metso T, Helenius I, Haahtela T, Reitamo S. Respiratory symptoms, bronchial hyper-responsiveness, and eosinophilic airway inflammation in patients with moderate-to-severe atopic dermatitis. *Clin Exp Allergy* 2006; 36:192–197.
- IV. Virtanen H, Kari O, Kari M, Reitamo S. Tacrolimus ointment in the treatment of atopic blepharoconjunctivitis. A retrospective study. *Acta Ophth Scand* 2006; 84: 693–695.
- V. Virtanen H, Remitz A, Malmberg P, et al.. Topical tacrolimus for atopic dermatitis. Does it benefit the airways? A four-year follow-up. *J Allergy Clin Immunol* 2007; 120: 1464–1466.

## Antimicrobial Activities of Histidine-rich Glycoprotein and Cationic Peptides

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**Dr Victoria Rydengård from the Department of Dermatology and Venereology, Lund University, Sweden, defended her PhD thesis on 11 May, 2008 in Lund. The thesis was supervised by Associate Professor Artur Schmidtchen from the Department of Clinical Sciences, Lund University. The opponent was Professor Birgitta Agerberth from the Karolinska Institut in Stockholm.**



In an environment full of potential pathogens it is important for organisms to mount a fast and effective defence. Antimicrobial peptides are ancient and integral effector molecules of the innate immune system. They are found in all kinds of species, from bacteria to plants and animals, indicating their importance during evolution. They possess a broad-spectrum antimicrobial activity and some peptides can also participate in wound healing and connect the innate and adaptive immune systems.

Results presented in this thesis show that structural motifs connected with heparin-binding may confer antimicrobial activity on a given peptide. Peptides from various heparin-binding endogenous proteins exerted antimicrobial activity against Gram-positive and Gram-negative bacteria, and similar results were obtained with consensus sequences for heparin-binding. Furthermore, we demonstrated that replacement of lysine and arginine by histidine in the consensus motifs abrogated the antibacterial effects of these peptides. Antibacterial effects of the histidine-rich consensus peptides were restored by the addition of zinc ions ( $Zn^{2+}$ ) or low pH. Similar results were obtained with histidine-rich peptides derived from domain 5 of kininogen and histidine-rich glycoprotein (HRGP).

HRGP, an abundant heparin-binding plasma protein, exerted antimicrobial effects against Gram-positive and Gram-nega-

tive bacteria and fungi. The antibacterial activity of HRGP was dependent on  $Zn^{2+}$  ions or low pH, and the antifungal activity was increased under low pH conditions.

Electron microscopy demonstrated that HRGP induced lysis of bacteria and fungi. Truncated HRGP, devoid of the heparin-binding and histidine-rich domain, was not antimicrobial. In addition, HRGP was found to have antifungal effects *ex vivo* when bound to fibrin clots.

### List of original publications

1. Andersson E, Rydengård V, Sonesson A, Mörgelin M, Björck L, Schmidtchen A. Antimicrobial activities of heparin-binding peptides. *Eur J Biochem* 2004; 271: 1219–1226.
2. Rydengård V, Andersson Nordahl E, Schmidtchen A. Zinc potentiates the antibacterial effects of histidine-rich peptides against *Enterococcus Faecalis* *FEBS J* 2006; 273: 2399–2406.
3. Kacprzyk L, Rydengård V, Malmsten M, Schmidtchen A. Antimicrobial activity of histidine-rich peptides is dependent A of acidic conditions (manuscript).
4. Rydengård V, Olsson A-K, Mörgelin M, Schmidtchen A. Histidine-rich glycoprotein exerts antibacterial activity *FEBS J* 2007; 274: 377–389.
5. Rydengård V, Kacprzyk L, Olsson A-K, Mörgelin M, Malmsten M, Schmidtchen A. Antifungal activity of histidine-rich glycoprotein (manuscript).