Recurrent Aphthous Stomatitis

A study, with emphasis on host genetics, oral microbiota composition, and immunoregulatory networks

Akademisk avhandling

som för avläggande av odontologie doktorsexamen vid Sahlgrenska Akademin, Göteborgs Universitet kommer att offentligen försvaras i Hörsal Arvid Carlsson, Akademicum, Medicinaregatan 3, Göteborg

Fredagen den 3 februari 2017, kl.9.00

av

Maria Bankvall
legitimerad tandläkare

Fakultetsopponent:
Martin H. Thornhill
Professor of Translational Research in Dentistry
Academic Unit of Oral and Maxillofacial Medicine and Surgery
University of Sheffield School of Clinical Dentistry
Sheffield, United Kingdom

Avhandlingen baseras på följande delarbeten

I. Bankvall M, Östman S, Jontell M, Torinsson-Naluai Å. A genome-wide association study of recurrent aphthous stomatitis. In manuscript


III. Bankvall M, Jontell M, Wold A, Östman S. Tissue-specific differences in immune cell subsets located in the oral-associated lymphoid tissues. In manuscript

Abstract

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal lesions. The aetiology is unknown and currently there is no consensus regarding suitable treatment regimens. RAS is recognised as a multi-factorial condition in which both endogenous and exogenous factors contribute to the recurrent oral ulcerations characteristic of this oral mucosal disease.

The overall aim of this thesis was to study the aetiological factors associated with RAS. Previously, it has been suggested that genetic factors, a microbiological component, and the abrogation of tolerance to specific food antigens are of importance in RAS. Hence, two clinical studies were conducted to explore the roles in RAS of host genetics and the composition of the oral microbiota. To reveal the actions of food components as exogenous triggering factors for RAS, it is necessary to understand the immuneregulatory networks involved in the induction of tolerance in the oral cavity. Extensive pre-clinical studies of these mechanisms are required before translating the acquired knowledge to the clinical setting. Therefore, two pre-clinical studies in mice were performed to explore the roles of the oral cavity and associated lymphoid tissues in comparison to those of the mesenteric lymph nodes (MLN), which are known to be of importance for oral tolerance induction.

The specific aims of the clinical studies were to: (i) identify patterns of association and segregation regarding genetic variants passed down to the off-spring within families with RAS and to identify the genes and signalling pathways that determine the risk of developing this condition; and (ii) compare the oral microbiota profiles of patients with RAS and healthy control subjects, so as to define microbiota changes in relation to disease activity. The specific aims of the pre-clinical studies were to: (i) identify differences between the murine APC and T-cell populations of the oral-associated lymphoid tissues [i.e., the nose-associated lymphoid tissues (NALT) and the cervical lymph nodes (CLN)] and the MLN; and (ii) determine whether the passage of an antigen through the oral cavity contributes to the overall immunological response and the degree of tolerance induced, as compared to gastric administration of the same antigen.

Buccal swabs were obtained from non-ulcerative areas of the mouths of patients with RAS (N=60) and healthy age- and gender-matched controls (N=60), with some of the patients (N=42) presenting with lesions upon sampling. Additional swabs from members of 16 families with RAS (N=91) were also included. The human DNA was analysed in a Genome-wide association study (GWAS), using a CoreExome array, and the bacterial DNA was analysed by Terminal-restriction fragment length polymorphism (T-RFLP). Flow cytometry and in vitro proliferation were used to analyse the APC and T-cell subsets at the different sites in the BALB/c mice. To compare oral and gastric administration of the antigen (ovalbumin, OVA), a DO11.10 TCR transfer model and an oral tolerance model, using BALB/c mice, were applied.

No pattern of association or segregation for genetic variants being passed down to the off-spring within these families was detected. The most significant pathways implicated in RAS were the Ras signalling pathway, the PI3K-Akt signalling pathway, pathways in cancer, circadian entrainment, and the Rap 1 signalling pathway. The oral microbiota profiles differed between patients and controls, especially regarding the profiles of patients who presented with lesions during sampling, which clustered furthest from the profiles of the controls. The NALT contained a higher proportion of APCs and a lower proportion of T cells than the CLN and MLN. The APCs of the NALT displayed few signs of activation, instead showing high-level expression of markers associated with effector and tolerogenic functions. Furthermore, the T cells in the NALT more often showed a memory/effector phenotype, whereas those in the CLN and MLN had a naïve phenotype. In general, the cells of the NALT did not proliferate upon in vitro stimulation with concanavalin A, in contrast to the cells from the CLN and MLN. A similar activation pattern and degree of tolerance induction emerged when the two administration routes were compared.

In summary, understanding the genetic basis of RAS may allow the identification of individuals who are at risk of acquiring this condition. Changes to the oral microbiota may trigger the development of lesions or vice versa. The NALT displayed effector and tolerogenic functions as opposed to the other sites that demonstrated a strong capacity for primary immune activation. The contribution of the mucosal immune system, besides the intestine, for induction of oral tolerance remains to be further investigated. Suitable and efficient treatment strategies for RAS can be developed only when the aetiology of this condition is fully understood.

Keywords: Aphthous stomatitis, oral mucosa, oral medicine, genome-wide association study, genetic linkage, association, genetic polymorphism, microbiota, restriction fragment length polymorphism, antigen-presenting cell, T-lymphocyte, lymph node, flow cytometry

ISBN: 978-91-628-9994-3 (Print)  http://hdl.handle.net/2077/48668
ISBN: 978-91-628-9993-6 (PDF)