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IMMUNE RESPONSES TO LIPOPOLYSACCHARIDE
IN RELATION TO ALLERGIC DISEASE,
A TLR4 GENE POLYMORPHISM AND ENDOTOXIN EXPOSURE

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ABSTRACT

Background: Allergic diseases have increased during the last decades, particularly in affluent countries, possibly due to a reduced and/or altered microbial exposure during infancy. Activation of the immune system by microbes early in life is probably required for accurate maturation of the immune system and tolerance development. It is not fully understood how microbial exposure is associated with the development of allergic diseases, however. Genetic factors may influence microbial induced immune responses. A certain polymorphism, in the gene coding for the Toll-like receptor 4, *i.e.* (*TLR4* Asp299Gly), has been suggested to alter the immunological responsiveness to bacterial lipopolysaccharide (LPS).

Aim: The aim of this thesis was to study the interplay between LPS induced immune responses, LPS signalling related genetic polymorphisms, allergic disease and endotoxin exposure.

Subjects: The thesis is based on the results obtained from individuals in three different study groups, *i.e.* Estonian and Swedish children followed prospectively from birth up to five years of age, Swedish school-children eight and 14 years of age and young adults.

Methods: The study subjects were clinically evaluated regarding allergic diseases with skin prick tests, circulating IgE levels, validated questionnaires and clinical examinations by paediatricians or research nurses. The gene polymorphisms *TLR4* Asp299Gly and *CD14*-159 were analysed. Peripheral blood mononuclear cells were isolated from blood and cultured with LPS from two Gram negative bacterial strains, *i.e.* *Salmonella enterica* serotype Typhimurium (Serotype Typhimurium) and *Escherichia coli* (*E. coli*). Cytokine and chemokine secretions were analysed with Luminex or ELISA technique. Receptor expression of circulating peripheral blood monocytes was analysed with flow cytometry. The phosphorylation of intracellular proteins involved in LPS signalling pathways was analysed with Luminex technique. mRNA expression of proteins involved in LPS signalling pathways and of markers for T regulatory cells were analysed with realtime-PCR.

Results: In school-children and young adults, the *TLR4* Asp299Gly gene polymorphism was associated with reduced LPS induced I κ B α phosphorylation, IL-10 and IL-12 cytokine secretion. Interestingly, these findings were observed only when the cells were cultured with LPS from Serotype Typhimurium but not with LPS from *E. coli*. The polymorphism was positively associated with asthma, especially atopic asthma.

Several differences in immunological responses to LPS were observed between allergic and non-allergic individuals. Asthma in school-children was associated with reduced LPS induced cytokine production of IL-10 and IL-12. The phosphorylation of I κ B α was lower in adult allergic compared to non-allergic individuals. Swedish children who had developed allergic disease at five years of age had lower TLR2 mRNA expression at birth compared to children who remained healthy.

Estonian children displayed generally lower LPS induced cytokine and chemokine production as compared to Swedish children both at birth and at 3 and 6 months of age. The mRNA expression of the T regulatory associated markers Foxp3 and Ebi3 were higher in the Estonian compared to the Swedish children at birth.

Conclusion: Polymorphisms in genes coding for pattern recognition receptors can alter the immune responsiveness of the host to microbial components and may be of importance for the development of asthma. Lower LPS induced cytokine response and higher expression of T regulatory associated markers were seen in children from Estonia as compared to Sweden, suggesting an increased capacity for early immune regulation among infants from a country with a low prevalence of allergic disease.