SENSING OF PATHOGENS BY TOLL-LIKE RECEPTORS IN SARCOIDOSIS

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Sarcoidosis is a systemic disorder of unknown aetiology characterized by the formation of non-caseating granulomas, most often affecting the lungs, lymph nodes and skin. Sarcoidosis is characterized by a strong Thelper-1 driven immune reaction, making viruses or intracellular pathogens, such as Mycobacterium tuberculosis and Propionibacterium acnes, leading suspects as causative agents. The innate immune system is important in the first recognition of pathogens, mainly via recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs).The family of TLRs consists of 11 different receptors, each recognizing a distinct repertoire of microbial products. Innate immune responses can dictate the magnitude and direction of the subsequent adaptive immune response. The hypothesis of this thesis is that genetic and functional defects in TLRs are involved in the innate immune recognition of intracellular bacteria in sarcoidosis, and play a role in disease susceptibility and clinical outcome.

Furthermore, it is hypothesized that mycobacteria and propionibacteria are possible antigenic triggers in sarcoidosis patients in The Netherlands. Genetic variation of 21 single nucleotide polymorphisms (SNPs) located in 7 genes were tested in sarcoidosis patients and healthy controls. The selected genes were TLR-1, TLR-2, TLR-4, TLR-6, TLR-9, TLR-10 and CD14 based on their capacity to recognize bacterial proteins. In vitro experiments on TLR-2 and TLR-9 function in sarcoidosis were also performed. Finally, using Interferon-Gamma Release Assays (IGRA), T-cell responses against mycobacteria and propionibacteria in sarcoidosis patients was assessed. The 4 genes associated with sarcoidosis are all involved in TLR-2 expression and/or function. A genetic association was found for TLR-2 as well as for

3 different co-receptors of TLR-2, to be exact TLR-1, TLR-10 and CD14. Subjects carrying the TLR-2 promoter genotype -16934 AA, which is suggested to be more prevalent in patients with chronic sarcoidosis, produce significantly more Tumor Necrosis Factor- alpha (TNF-α) upon stimulation with TLR-2 agonists compared to subjects without the AA-genotype. TNF-α is important in sarcoidosis inflammation and various clinical trials demonstrated that blocking TNF-α using antagonists such as infliximab or eternacept results in clinical improvement.
In addition, in vitro experiments revealed that Peripheral Blood Mononuclear Cells (PBMCs) of sarcoidosis patients produce significantly less IL-23, important in the Thelper-17 pathway, upon stimulation with a TLR-9 agonist compared to PBMCs from healthy controls. Further studies are needed to clarify this observation.

Moreover, latent tuberculosis infection was found in 5.3% of the sarcoidosis patients tested, which does not provide a strong links between previous M. tuberculosis infection and sarcoidosis in Dutch patients. Identification of a distinct subgroup of patients with propionibacteria as causative agents was not found based on the observation that these bacteria demonstrate mitogenic activity.

In conclusion, genetic differences in TLR-2 or its co-receptors are found in patients with a chronic form of sarcoidosis. This suggests that an aberrant TLR-2 function can predispose for developing chronic disease in patients already affected by sarcoidosis. Our in vitro data support this hypothesis. It seems that M. tuberculosis is not an important cause for sarcoidosis in Dutch patients. Further studies are needed to answer this question for propionibacteria.