The role of innate immune cells in tissue inflammation in spondyloarthritis

Noordenbos, T.

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OBJECTIVES OF THIS THESIS
IMMUNE-MEDIATED INFLAMMATORY DISEASES

Immune mediated inflammatory diseases (IMIDs) comprise a group of clinically heterogeneous chronic disorders. Although the pathophysiology of most IMIDs remains largely unknown, these conditions are characterized by a primary mal-functioning of the immune system, rather than by secondary activation of the immune response to foreign agents as seen in infection, allergy, or transplantation [1,2].

Originally all IMIDs were considered as autoimmune disorders driven by defective tolerance of T and B cells to self-antigens [3]. However, recently it became apparent that key features of prototypic autoimmune diseases such as shared genetic risk factors, presence of disease-specific autoantibodies and response to T-cell or B-cell-targeted therapies are not recapitulated in diseases such as gout, psoriasis, Crohn’s disease and spondyloarthritis [1,4,5]. Instead, these diseases are characterized by exaggerated immune response either related to hyperactive reaction to noxious stimuli (such as in case of gout where immune response is triggered by urate crystals) or to impaired cytokine regulation (such as defective IL-10 signaling in special cases of colitis [6]. These IMIDs are now classified as autoinflammatory diseases [1,7].

Although these autoinflammatory IMIDs form a heterogeneous group, some diseases are more closely related to each other, sharing genetic, clinical and immunological features and forming a cluster, as for example spondyloarthritis, Crohn’s disease (CD), ulcerative colitis and psoriasis [4,5]. In this thesis we are mainly focused on the synovium of patients with spondyloarthritis, yet for specific questions we also examined affected tissues of spondyloarthritis-related diseases, such as psoriatic skin and CD gut.

SPONDYLOARTHRITIS

Spondyloarthritis is a prevalent form of chronic inflammatory arthritis, which affects around 1% of the Western population [8]. The onset of the disease is usually between 20 and 40 years of age and patients may have life-long functional impairment and pain. The prototypical phenotype of this disease is ankylosing spondylitis, which is characterized by inflammation and bone formation of the axial skeleton. Other manifestations include arthritis of the peripheral joints and specific extra-articular inflammatory conditions, such as gut, skin or eye inflammation [9,10]. As opposed to for example rheumatoid arthritis (RA), SpA is not characterized by disease specific antibodies or elevation of acute phase proteins and the diagnosis is mainly based on the combination of several disease characteristics. Yet, the genetic risk factor HLA-B27 may help in diagnosing patients and, although they appear late in the disease and not in all patients, radiographic changes of the sacro-iliac joint or spine are pathognomonic for the disease [11].

SpA has long been considered an autoimmune disease, however, as described above, the evidence for an important role of the acquired immune system in driving pathogenesis of SpA is limited. Thus, the genetic profile does not overlap with classical autoimmune diseases, the disease is not characterized by presence of auto-antibodies, and clinical inhibition of T cell costimulation or B cell depleting therapy are ineffective in SpA [12–14]. Therefore, we
proposed that SpA is an autoinflammatory IMID, in which the innate immune system plays a prominent role rather than an autoimmune disease driven by acquired immunity [7]. The overall objective of this thesis was to better define the phenotype and the role of innate immune cells in the pathophysiology of SpA. Since most of innate immune cells are tissue resident, we have focused our research on peripheral synovitis in SpA as this allows us to study these cells directly in the inflamed target tissue.

**OUTLINE OF THE THESIS**

In chapter 1, we reviewed the current evidence for the role of innate immune mechanisms in the pathophysiology of SpA. We discussed how the major genetic risk factor for SpA, HLA-B27, could lead to hyperactivation of the innate immune system. Furthermore we evaluated data on the role of the innate immune system in IL-1, TNF and IL-23/IL-17 cytokine pathways. Finally, we discussed contribution of innate immune cells in the target tissues to pathophysiology of SpA.

In chapter 2, we focused on macrophages as central effector cells of the innate immune system and since macrophages are a major source of cytokines in vivo we analyzed the local cytokine levels in the peripheral joint. From previous studies we know that the synovial tissue of peripheral SpA is enriched by macrophages expressing the scavenger receptor CD163, a marker for alternative activation. We questioned whether this signature of alternatively activated macrophages was associated with a specific cytokine profile in SpA. Therefore, we studied the capacity of RA and SpA synovial fluid to modulate the phenotype of peripheral blood-derived macrophages.

In chapter 3 we assessed the polarization status of macrophages in the synovial tissue based on expression of phenotypic markers. We compared SpA and RA synovitis to evaluate the distribution of specific macrophage subsets. Additionally we questioned if a difference in polarization may result from intrinsic myeloid alterations by comparing phenotypes of peripheral blood-derived macrophages from SpA and RA.

In chapter 4 we have evaluated the potential contribution of mast cells to SpA tissue inflammation. We studied the distribution of mast cells in SpA and RA synovitis and found that these innate cells are specifically enriched the inflamed SpA synovium. Since it has been described that mast cells can express IL-17A, we next investigated whether mast cells are also an important source of IL-17A in SpA joints. Additionally we assessed whether the mast cell/IL-17 axis is modulated by inhibition of TNF by analysis of pre- and posttreatment synovial tissue samples. Moreover we probed the mast cell inhibitor imatinib on synovial biopsies ex vivo and assessed its impact on general inflammation.

In chapter 5 we investigated whether IL-17A-positive mast cells are also present in the target tissues of SpA-related diseases. First of all we addressed important technical aspects such as the specificities of frequently used commercial antibodies and the confirmation that IL-17A is localized intracellularly in mast cell. Finally we applied immunostaining to examine presence of IL-17A-positive mast cells in inflamed psoriatic skin and Crohn’s disease colon.

In chapter 6 we investigated the intrinsic capacity of human tissue mast cells to produce IL-17A. As we failed to detect any signs of IL-17A production by mast cells we provided an
alternative explanation to how mast cells acquire IL-17A protein. We developed an *in vitro* model to test the mechanisms of IL-17A uptake and release by mast cells.

In **chapter 7** we questioned if mast cells, as an important source of IL-17, are a potential therapeutic target in SpA. Based on the preclinical data obtained in chapter 4, we designed a proof-of-concept, randomized placebo-controlled clinical trial with the tyrosine kinase inhibitor nilotinib. We evaluated both the clinical and the immunopathological effects of mast cell targeting in SpA.

In **chapter 8** we summarized the findings presented in this thesis. We integrated the knowledge obtained in this study into the current view on pathophysiology of SpA and discussed the current strategies and the future perspectives.
REFERENCES


