Macrophage polarization in spondyloarthritis
Ambru, C.A.

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Scope of the thesis
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SPONDYLOARTHRITIS
Spondyloarthritis (SpA) is the second most frequent form of chronic inflammatory arthritis after rheumatoid arthritis (RA) with an overall prevalence of approximately 1% [1,2]. The disease starts usually between 20 and 40 years of age and leads to life-long symptoms and functional impairment. The seminal features of SpA are inflammation of the spine (spondylitis), asymmetric peripheral arthritis and/or enthesitis, extra-articular involvement (eye, skin, gut), osteoproliferation as evidenced by radiologic sacro-iliitis, and familial aggregation based on genetic susceptibility factors such as HLA-B27. The diagnose of SpA is mainly based on a combination of several of these features since, in contrast to RA, SpA is not characterized by elevated acute phase proteins or disease specific autoantibodies. Moreover, radiographic changes of the spine and sacroiliac joints are pathognomonic but occur late in the disease course and not in all patients. MRI and HLA-B27 typing can be useful in patients with a moderately high clinical suspicion of SpA [3].

The phenotypic presentation of SpA is diverse and, accordingly, SpA has been traditionally sub-classified in clinically distinct subtypes. Ankylosing spondylitis (AS), which is the prototype of SpA, was first described in 1892 by the Russian neurologist Bechterew as “a chronic ankylosing inflammation of the large joints and the spine”. The other SpA subtypes are: reactive arthritis (where articular symptoms are triggered by an urogenital or gastrointestinal bacterial infection), inflammatory bowel disease (IBD)-associated SpA (where articular symptoms accompany Crohn’s disease or colitis ulcerosa), psoriatic arthritis (PsA), undifferentiated SpA, HLA-B27 associated uveitis, and juvenile onset SpA. More recently, ASAS has proposed new classification criteria for axial and peripheral SpA which allow to better capture the whole disease spectrum, including early and non-prototypical forms of the disease [3]. This novel classification is in agreement with the increasing genetic, experimental and clinical evidence that the different phenotypic SpA subtypes do not represent distinct pathophysiological entities, although pathogenetic differences may exist between axial and peripheral disease [4].

PATHOGENESIS OF SPA
A combination between genetic risk factors (HLA-B27 and a number of genes involved in innate immune recognition and cytokine signaling) and environmental factors such as microtrauma [5] and microorganisms [6,7] is thought to be responsible for the development of this immune-mediated inflammatory disease [8]. In chapter 2 we extensively discuss the existing evidence that supports the classification of SpA among the autoinflammatory (innate immune system-driven) rather than the autoimmune (acquired immune system-driven) diseases, such as rheumatoid arthritis (RA) of systemic lupus erythematosus (SLE) [9].
Peripheral synovitis displays a similar macroscopic appearance in SpA and RA (Figure 1). It was previously shown that the level of inflammation and the number of inflammatory cells infiltrating the synovial membrane is also similar in both diseases [10-13]. Interestingly, CD163-expressing macrophages were the predominant macrophage population in the inflamed peripheral joints of SpA patients [10-13]. Macrophages are known to play a major role in chronic synovitis, since their number in the inflamed synovium correlates with the disease activity [14,15] and decreases after clinically efficient treatment in both RA [16-19] and SpA [20-22]. Furthermore, macrophage depletion in animal models of arthritis was reported to have an anti-inflammatory effect [23-25]. Finally, the efficiency of TNF blockade in many immune mediated diseases also pleads for a major pathogenic role of macrophages, as main producers of TNF.

![Figure 1: Arthroscopy of peripheral joints.](image)

The inflamed synovium in both SpA and RA is characterized by synovial surface projections (villi), hypervascularization and tortuous small vessels.

Since CD163 is in vitro up-regulated by IL-10 [26], corticosteroids and M-CSF [27] and was reported to have anti-inflammatory properties [28-30], but the role of CD163+ macrophages in SpA synovitis is not understood, in this thesis we investigated the correlation between in vitro characterized macrophage phenotype and function and macrophage phenotype and function in vivo (Figure 2).

An important concept that we use in several studies is the distinction between macrophage polarization and macrophage activation. Macrophage polarization consists of changes in the morphology, phenotype and the ability to respond to activating stimuli, while macrophage activation implies reaching full functional capacity as reaction to various stimuli, such as TLR ligands.

According to a simplified in vitro polarization model, macrophages can undergo classical (M1) or alternative (M2) activation. Originally, M1, which is mainly induced by IFN-γ (MΦ_{IFN-γ}), was described to have a pro-inflammatory function, while M2 had mostly a
Scope of the thesis

regulatory role. Later, M2 was further subdivided in IL-4- (MΦ_{IL-4}) and IL-10-polarized macrophages (MΦ_{IL-10}). A third M2 subset was described to be induced by immune complexes (ICs) and TLR ligands [31-33].

Figure 2. Conceptual design of this thesis. In this thesis we analyzed the correlation between the phenotype and function of in vitro polarized human macrophages and the phenotype and function of synovial macrophages in SpA

OUTLINE OF THE THESIS

As different polarized macrophage subsets have been phenotypically characterized in mouse but not in humans, in chapter 3 we characterize macrophage phenotype in vitro, by validating phenotypic markers for three prototypical macrophage subsets: MΦ_{IFN-γ}, MΦ_{IL-4}, and MΦ_{IL-10}.

In chapter 4 we analysed the expression of the in vitro validated phenotypic markers on peripheral blood-derived macrophages from SpA and RA patients in order to investigate whether differences in synovial macrophage phenotype are due to an intrinsic myeloid cell defect. To evaluate the predominance and distribution of specific macrophage subsets in the inflamed synovium from SpA compared to RA patients, we next measured the expression of these markers on synovial tissue macrophages from both diseases.

In chapter 5 we studied the correlation between in vitro polarized macrophage phenotype and in vitro cytokine production. Since ICs in combination with TLR ligands were previously reported to induce alternative activation in mouse macrophages [34,35], we also evaluated
the effect of ICs on the phenotype and cytokine production of polarized human macrophages. In chapter 6 we studied the expression of angiopoietin-2 (Ang-2) receptors on polarized macrophages and the in vitro effect of angiopoietin-2 (Ang-2) on macrophage cytokine production and function. We also investigated in vivo the role of Ang-2 in a mouse arthritis model.

In chapter 7 we studied the induction of an unfolded protein response (UPR) in human macrophages. HLA-B27, the major genetic risk factor for SpA, has the tendency to misfold inside the endoplasmic reticulum (ER), thereby rendering the ER susceptible to generating an UPR as a response to cell activation by TLR ligands [36]. In human dendritic cells stimulated with chemical agents and in rat macrophages overexpressing HLA-B27, the UPR was characterized by an increased production of pro-inflammatory cytokines, and especially IL-23 [37-39]. First we measured the response of healthy donor MΦ$_{\text{IFN-\gamma}}$ and MΦ$_{\text{IL-10}}$ to chemical ER stress induced by thapsigargin. Second we compared the expression of ER stress markers and pro- and anti-inflammatory cytokines by MΦ$_{\text{IFN-\gamma}}$ and MΦ$_{\text{IL-10}}$ derived from peripheral blood between HLA-B27+ and HLA-B27- SpA patients, RA patients and healthy donors.

In chapter 8 we addressed macrophage function in vivo, as reflected by the local inflammatory milieu in SpA and RA. In order to establish the existence of a specific macrophage signature, we measured the levels of pro- and anti-inflammatory cytokines in synovial fluid from SpA and RA patients. Furthermore, we investigate whether SpA and RA synovial fluid modulates the phenotype of peripheral blood-derived macrophages from healthy donors.

Chapter 9 provides a discussion of the findings described in this thesis and of the future perspectives.

REFERENCES


