
Influence of the gut and cytokine patterns in spondyloarthropathy

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ABSTRACT

Clinical and histological studies have previously shown that spondyloarthropathy (SpA) patients can have sub-clinical gut inflammation. This gut inflammation is related to enterocolitis in Crohn's disease (CD) and may evolve to overt inflammatory bowel disease in a subset of these patients. Moreover, there is an intriguing clinical link between gut inflammation and peripheral joint inflammation. In order to explore immunologically these concepts, recent studies have characterized phenotypically and functionally the inflammatory cells in both the gut and the synovium of SpA patients and have provided a number of new insights. Firstly, they confirm histological and pre-histological alterations of the gut immune system in SpA, which are redundant of CD and which are linked to alterations of the peripheral joints. Secondly, both the acquired and the innate immune system contribute to these alterations, with an important role for both T cells and macrophages and their cytokines. Thirdly, interpretation of these data support the hypothesis that gut and joint inflammation in SpA are induced by the combination of an impaired anti-bacterial host defence and an uncontrolled pro-inflammatory response of the innate immune system. The insights provided by the study of the gut immunology in SpA have contributed to develop new therapeutic strategies, with TNF blockade as prototype.

Introduction

Spondyloarthropathy (SpA) is a group of autoimmune disorders of the joint which includes ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease (IBD), and undifferentiated SpA. The different types of SpA share a number of common clinical, radiological, and genetic features that are clearly distinct

from RA. Clinically, SpA is characterized by both axial and peripheral joint involvement. The involvement of the axial skeleton with sacroiliitis and/or spondylitis is probably the most important hallmark. Peripheral joint involvement is mostly mono- or pauciarticular and asymmetric. It affects predominantly the lower limbs (knees and ankles) and is frequently associated with enthesitis (Achilles tendon, fascia plantaris). Radiologically, the evolution of both axial and peripheral joint disease is characterized rather by bone formation than bone erosions, leading eventually to ankylosis. New imaging techniques argue for bone oedema and osteitis at the site of synovitis and/or enthesitis. Finally, there is a strong genetic predisposition for SpA, as illustrated by the familial clustering and the overlap between different disease entities in the same patient. HLA-B27, the best known genetic factor, is found in 90% of the AS patients versus 8% in the overall population.

These well-known clinical, radiological and genetic features of SpA are not only important for diagnosis but also provide some pathogenetic clues. As to the pathogenesis, however, another group of clinical features is of major importance, namely the extra-articular manifestations. These include acute anterior uveitis, urogenital involvement, skin lesions (psoriasis, erythema nodosum), and inflammatory gut lesions which can evolve to inflammatory bowel disease. The aim of the present review is to give an overview of the role of the gut in the pathogenesis of SpA and to highlight the relationship with Crohn's disease (CD). Special attention will be paid to the cytokine patterns, both of T cells and macrophages, and the implications for innovative therapies in SpA.

Gut inflammation in SpA

Clinical evidence for the involvement of the gut in SpA is provided by gastro-

intestinal ReA, a subtype of SpA which is known to be triggered by infections of the gut with bacterial strains such as *Yersinia enterocolitica*, *Salmonella typhimurium* and *enteritidis*, *Shigella flexneri*, and *Campylobacter jejuni* (1). On the other hand, musculoskeletal manifestations compatible with SpA can be seen in IBD patients. Some recent studies confirm that an important number of IBD patients present clinical or radiological arthritis signs (30-40%), with inflammatory back pain in 30%, synovitis or tendinitis in 15%, and sacroiliitis in 20-25% (2-6). These observations suggest that not only overt infection of the gut but also subclinical and chronic gut inflammation, probably related to a more generalized loss of tolerance to bacterial antigens, can lead to arthritis in genetically susceptible individuals. This concept is illustrated by the spontaneous development of an SpA-like disease in HLA-B27 transgenic rats, which however remain free of gut and joint inflammation when kept in germ-free conditions (7).

In human SpA, subclinical gut inflammation revealed by ileoscopy was first reported by Mielants and Veys (8). Systemic ileocolonoscopy studies in SpA patients without clinical gut involvement confirmed this finding and demonstrated the presence of inflammatory gut lesions in colon and terminal ileum in 60% of patients with AS, in 90% of patients with ReA, and in 65% of patients with UspA (9-13). Gut inflammation was also documented in psoriatic arthritis (14) and in HLA-B27 positive acute anterior uveitis (15). These findings were confirmed in different forms of SpA by several authors (16-20).

The histological gut lesions can be divided into acute and chronic inflammation (21, 22). The acute type resembles acute bacterial enterocolitis: the mucosal architecture is well preserved, but the ileal villi and crypt epithelial cells are infiltrated by polymorphonuclear cells. There is also an increased number of infiltrating inflammatory cells in the lamina propria, mainly granulocytes, lymphocytes and plasma cells. This acute type of inflammation is mostly seen in patients with entero-

genic ReA. The chronic type of inflammation resembles the chronic ileocolitis as seen in Crohn's disease (CD): crypt distortion, atrophy of the villous surface of the colonic mucosa, blunting and fusion of villi, increased mixed lamina propria cellularity, and basal lymphoid aggregates in the lamina propria. In some cases aphthoid ulcers, branching and pyloric metaplasia of the crypts, and sarcoid-like granuloma's can be seen. This chronic type of inflammation is preferentially found in patients with AS.

In the follow-up of SpA patients who underwent an ileocolonoscopy, two most interesting phenomena were observed. Firstly, after 2 to 9 years 6.5% of the patients developed overt CD, although they had no clinical signs of IBD at the first visit. All these patients evolved into AS and most of them had persistent peripheral arthritis. Secondly, in patients in whom a second ileocolonoscopy was performed, remission of joint inflammation always was associated with a disappearance of the gut inflammation, whereas persistence of locomotor inflammation mostly was associated with the persistence of gut inflammation (11,23). These clinical and histological observations lead to a two-folded hypothesis: on one hand, there is an important overlap between the subclinical gut inflammation in SpA and the overt enterocolitis in CD, suggesting that SpA may serve as a unique human model for early IBD; on the other hand, there exists a link between gut and peripheral joint inflammation, which may provide new clues on the pathogenesis of SpA. Phenotypical and functional characterization of inflammatory cells in the gut and the joint of SpA patients is of major importance to further explore these hypotheses.

T cell cytokines in SpA

Several factors favour an important role for T cells in the pathogenesis of SpA. Firstly, the inflammatory infiltrate of the intestinal mucosa, the synovium, and the enthesis in SpA is rich in T cells, more particularly CD8+ T cells (24-26). Secondly, athymic HLA-B27 transgenic rats fail to develop gut

inflammation and arthritis/spondylitis resembling human SpA. Transfer of T cells from euthymic donors to nude rats induces disease, as does engraftment of the thymus. The most efficient transfer occurs with purified CD4 positive T cells, although CD8 positive T cells also can transfer disease (27). Thirdly, T cells reactive to certain bacterial antigens derived from *Yersinia* and *Salmonella* have been described in the synovial fluid of patients with enterogenic ReA and AS (28, 29).

This last observation also suggests a possible recirculation of pathogenic T cells between the gut and the peripheral joint, which could at least partially explain the linkage between both disease localizations. In a recent study, gut-derived lymphocytes from IBD patients were demonstrated to be able to bind to synovial vessels using multiple homing receptors and their corresponding endothelial ligands, including vascular adhesion protein-1 (30). Another set of adhesion molecules, the beta 7 integrins which are expressed on gut-derived lymphocytes, appear also to have an important role in homing of T cells in CD and SpA. This is illustrated by the abnormal expression of both alphaEbeta7 on gut-derived lymphocyte cell lines and of its ligand E-cadherin in SpA and CD gut mucosa (31-33). Moreover, there is also a differential expression of the integrins alphaEbeta7 and alpha4beta7 on synovium-derived T cell lines in SpA, while one of the ligands of alpha4beta7, vascular cell adhesion molecule 1, is highly expressed in SpA synovium (34,35). A formal proof of concept for the recirculation hypothesis has been put forward by the identification of identical T-cell expansions in the colon mucosa and the synovium of a patient with enterogenic spondyloarthritis (36).

Considering the potential role of T cells in the pathogenesis of SpA and the possible recirculation between gut and joint, a lot of effort has been put over the last years in the functional characterization of the T cell cytokine profiles in SpA and CD. Classically, T cells are functionally divided into T helper 1 (Th1) lymphocytes, which produce IFN and IL-2, and Th2 cells, which

secrete IL-4, IL-5, and IL-10.

There are numerous reports on the Th1/Th2 cytokine profiles in peripheral blood and gut mucosa of CD patients, but the data are often conflicting. This may be due to the technical approaches since techniques such as ELISA, ELISPOT and RT-PCR largely ignore the cellular source of the measured cytokine. In contrast, the detection of intracellular cytokines by flow cytometry assures the cellular origin of the cytokines, but one can not exclude that the stimulation *in vitro* does not reflect the physiological behaviour of the T cells *in situ*. Although CD is generally considered as a Th1 mediated disease, recent studies using flow cytometry indicated a relative decrease of the production of Th1 cytokines in both peripheral blood lymphocytes and colonic lamina propria lymphocytes in CD compared to healthy controls (37, 38).

Interestingly, similar observations were made in SpA. Using the same approach as for CD, it was demonstrated that mucosal lymphocytes from the gut of SpA patients depicted also an impaired Th1 profile, although more than half of them had no clinical or histological signs of gut inflammation (39). Low secretion of IFN γ , IL-2, and/or TNF α and an increase of the IL-10 production by T cells was also reported in peripheral blood of patients with ReA (40), AS (41), and other types of SpA (42) compared to both healthy controls and patients with rheumatoid arthritis (RA). Finally, analysis of synovial fluid T cells and synovial membrane specimens in SpA and RA confirmed that the decreased Th1 (IFN γ)/Th2 (IL-4) ratio also extended to the joint of SpA patients (43, 44).

Taken together, these studies indicate that there is an impaired Th1/Th2 balance in both the gut of patients with CD, the gut of patients with SpA, and the peripheral blood and joint of patients with SpA. Thus, functional characterization of T cells in SpA highlights again the similarity between gut inflammation in SpA and CD as well as the relationship between the gut and the joint in SpA. Moreover, these findings fit into the concept that a defective Th1 response, certainly at the mucosal

site, may impair the immune defence against intracellular bacteria and thereby contribute to a decreased immune tolerance against bacterial antigens. This is believed to be a crucial trigger for inflammation and/or autoimmunity in ReA, but also probably in other types of SpA and in CD. Illustrating this theory, it was demonstrated that low secretion of TNF α , but no other T cell cytokines, correlated with chronicity in reactive arthritis (45).

Macrophage-derived cytokines in SpA

Another important aspect of the normal mucosal immune defence is the innate immunity. In CD, it was demonstrated that not only lymphocyte-mediated antibacterial activity but also phagocytosis and killing exerted by polymorphonuclear cells and monocytes were significantly reduced (46). Consequently, it was suggested that several defects in the innate immune system may contribute to the pathogenesis of CD (47, 48).

In SpA, recent reports also support the concept of abnormality of the innate immune system, more precisely abnormal expression of macrophage scavenger receptors. The macrophage receptor with collagenous structure (MARCO), which plays a role in the defence against gram negative bacteria, is upregulated on PBMC of patients developing ReA but is low in the synovial compartment in SpA compared to RA, thus suggesting a defective host defense mechanism in the SpA joint (49). Another scavenger receptor, CD163, was investigated in the gut and the joint of patients with SpA. Macrophages expressing CD163 are increased in the gut of patients with CD but also in the gut of SpA patients, even when they have no clinical or histological gut inflammation (50). This illustrates again the relationship between both diseases, but also the fact that alterations of the innate immune system may be early phenomena in the inflammation cascade. Moreover, the same subset of macrophages was demonstrated to be selectively increased in SpA synovium compared to RA synovium and appeared thus to be another

candidate for a role in the gut-synovium axis (51).

Functionally, macrophages may contribute to the pathogenesis of gut and joint inflammation in SpA in different ways, such as by defective clearance of bacteria or by presentation of autoantigens to T cells. However, since they are the major source of pro- and anti-inflammatory cytokines such as TNF α and IL-10, functional phenotyping is of major interest. CD163 positive macrophages produce high amount of TNF α but low levels of IL-10 after LPS stimulation (51). This may lead to a disturbed balance between pro- and anti-inflammatory cytokines, resulting in uncontrolled intestinal and synovial inflammation. The role of low IL-10 is further supported by the influence of IL10.G microsatellites on the development of reactive arthritis and by the intestinal inflammation in IL10 gene-deficient mice (52,53). On the other hand, clear TNF α message has been demonstrated in the sacroiliacal joint of AS patients and in synovium of PsA patients (54,55). The formal prove of the crucial role of these cytokines has been given by the trials investigating TNF α blockade in SpA. Based on previous experience with anti-TNF α in CD (56, 57), a manifest improvement of both gut and joint inflammation was shown in CD patients with associated SpA (58). Consequently, two open label trials extended these findings to patients with AS and other types of SpA (59, 60). These were recently confirmed by double-blind, placebo-controlled trials (61-63). Of interest, there is also some evidence that these therapies have a structure modifying effect on inflamed synovium and enthesitis (64, 65). Moreover, TNF α blockade restored the impaired Th1 profile of SpA patients (42), suggesting that this alteration of the T cell function may be secondary to abnormalities in the macrophage-derived cytokine balance. There are also some preliminary therapeutic data confirming that a relative defect of the anti-inflammatory cytokine IL-10 may play a role in the pathogenesis of gut and joint inflammation. In mice models there was a clear benefit of IL-10 on gut inflammation (66-

68), but this was not unequivocally confirmed in all studies in human CD (69-71). In SpA, there is one double-blind, placebo controlled study with recombinant human IL-10 in psoriatic arthritis indicating improvement of a number of biological parameters such as monokine production and inflammatory changes of the synovial membrane (72). Taken together, these data point to the major contribution of the balance of pro- and anti-inflammatory monokines in the pathogenesis of SpA.

Conclusions

Over the last years, several studies have confirmed biologically and immunologically the relationship between CD and SpA on one hand, and between gut and joint inflammation in these diseases on the other hand. Whereas T cells and their cytokines are still believed to play a role in this concept, recent evidence points mainly to alterations of the innate immune system and, more precisely, macrophages and their products. These data could lead to the hypothesis that the combination of an impaired bacterial clearance and an uncontrolled inflammatory response in genetically susceptible hosts can lead to a breakdown of the normal immunological tolerance and to a pathogenic cellular immune response to specific bacterial species, thereby inducing inflammation in the gut and the joint. Tuning down the uncontrolled inflammatory response by TNF blockade has proven to be a very effective clinical strategy in SpA, but one may raise concerns as to further impairment of the host defense and increased risk for major infections in these patients. Therefore, further exploration of other strategies to restore the monokine balance and to improve the normal immune response against bacteria remains mandatory.

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