

# Spreading spondyloarthritis: are ILCs cytokine shuttles from base camp gut?

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A series of discoveries has transformed concepts of spondyloarthritis and proinflammatory cytokine interleukin-23 (IL-23) has taken centre stage. The IL-23 frenzy kicked off with the identification of single nucleotide polymorphisms in the IL-23 receptor (IL-23R) gene that are associated with ankylosing spondylitis (AS)<sup>1</sup> and also with related disorders such as psoriasis, psoriatic arthritis and Crohn's disease. The most striking and direct evidence comes from an in vivo IL-23 overexpression study in mice that phenocopies the human disease and its Janus-faced characteristics: joint inflammation and structural damage presenting as new bone formation.<sup>2</sup> Patient studies reported increased serum levels of IL-23 in AS<sup>3-5</sup> and the presence of IL-23-positive cells was shown in facet joints of patients with AS.<sup>6</sup> Direct clinical evidence comes from a prospective, open-label clinical trial with ustekinumab, an antibody binding to the shared p40 subunit of IL-23 and IL-12<sup>7</sup> and successful clinical trials targeting IL-17, one of the downstream cytokines associated with IL-23 signalling.<sup>8</sup> Key to the hypothesis and evidence proposed in the mouse model is the presence of an IL-23 receptor-positive T-cell population in the enthesitis of mice. As enthesitis is one of the main characteristics of AS and was proposed as the primary lesion,<sup>9</sup> it is hard to ignore the potential key role of such cells. However, until now, these IL-23 receptor-positive cells have not yet been demonstrated in human samples.

Ciccia *et al*<sup>10</sup> report on the presence of a population of IL-23R-positive innate lymphoid cells (ILCs) in the gut, peripheral blood, synovial fluid and bone marrow of patients with AS. Numbers of such cells are increased as compared with different controls and their surface characteristics show similarities with the

mouse cells identified earlier. However, these ILCs are a rare cell type whose significance in human disease is as yet uncertain. In two previous studies Ciccia *et al*<sup>11,12</sup> showed increased IL-23 expression in the gut of patients with AS as compared with healthy controls. In AS synovium and peripheral blood cells, however, no differences in expression of IL-23 were found.<sup>13,14</sup>

They characterised these cells as  $\text{Lyn}^{-}\text{IL-23R}^{+}\text{Nkp44}^{+}\text{Tbet}^{+}\text{RORc}^{-}$  cells and the induced production of cytokines IL-17 and IL-22 led them to conclude that these are ILC3 cells. Unlike conventional ILC3 and IL-23R<sup>+</sup> enthesial T cells, these cells seemingly do not express RORc but do express the transcription factor Tbet which can, according to the authors, possibly be explained by a specific stage of differentiation of these cells. This finding makes them somewhat atypical, even for ILC3s. The reasons for these differences between 'conventional' characteristics of ILCs and the spondyloarthritis-associated cells are unclear and whether these changes precede onset of inflammation or are rather a secondary to already established inflammation remains to be determined. Further clarification of the exact nature of these cells clearly needs to be carried out. Hence, fine details in small subpopulations of cells may still represent a static, rather than dynamic, view on cell populations that are actively part of host defence or disease.

The expansion of these ILC3 cells in patients with AS with acute and chronic gut inflammation was significantly correlated with the disease activity as assessed by the Bath Ankylosing Spondylitis Disease Activity Index. In patients with AS without gut inflammation the upregulation of ILC3s was not detected. The authors reported an expansion of  $\text{Nkp44}^{+}\text{ILC3s}$  in gut samples of patients with AS and in the peripheral blood, synovial fluid and the bone marrow of patients with AS. ILC3s are defined by their capacity to produce the interleukins IL-17 and IL-22,<sup>15</sup> portrayed as critical cytokines in the pathogenesis of AS. In the study, gut ILC3 cells were demonstrated to produce IL-17 and IL-22 and a small percentage of cells expressed both cytokines. In the peripheral blood, the majority

of ILC3s produced IL-22 and only a small subset of ILC3s produced IL-17 or the combination of IL-22 and IL-17. Among synovial fluid and bone marrow, mononuclear ILC3s produced exclusively IL-22. The reasons for these differential patterns according to localisation are unclear and could reflect tissue-imprinted cytokine patterns. These data suggest that ILCs could be an important supplier of IL-17 and IL-22 in AS, which is also one of the implicit conclusions of this study by Ciccia *et al*.

Insights into the role of IL-17 in spondyloarthritis are dynamically evolving with a shift from a focus on adaptive immune cells towards more innate immune populations. Indeed, IL-17-producing CD4<sup>+</sup> cells, also known as Th17 cells, were shown to be elevated in HLA-B27/human  $\beta 2\text{m}$  transgenic rats<sup>16</sup> and in the lymph nodes of an AS mouse model.<sup>17</sup> Moreover, there are studies demonstrating an increased amount of IL-17-producing CD4<sup>+</sup> cells in the blood of patients with AS as compared with controls<sup>18-21</sup> although this observation is not consistent.<sup>22-24</sup> IL-17-producing cells were also found in the subchondral bone marrow cells of affected facet joints of patients with AS.<sup>6</sup> Interestingly, the majority of these IL-17-positive cells were rather innate immune cells (CD15<sup>+</sup> neutrophils and MPO<sup>+</sup> cells of the myeloid lineage) than CD4<sup>+</sup> T cells.<sup>6</sup> Obviously, the most convincing proof of principle that IL-17 plays a key role in spondyloarthritis is found in the clinical trials evaluating the effect of secukinumab, a monoclonal anti-IL-17A antibody that rapidly reduced clinical and biological signs of active AS.<sup>8</sup>

IL-22 has also earned its place in the spotlight as it has been associated with enthesitis/arthritis development in mice and with upregulation of genes potentially involved in the new bone formation process (such as Wnts and bone morphogenetic proteins).<sup>2</sup> However, the role for IL-22 in AS and related disorders seems to be tissue dependent—as demonstrated in a study by Benham *et al*<sup>25</sup> using the SKG mouse model. In this mouse model curdlan injection in SKG mice leads to IL-23-dependent axial and peripheral arthritis and ileitis. When IL-22 was neutralised by an IL-22 antibody, the mice developed a reduced severity of enthesitis but an exacerbation of ileitis.<sup>25</sup> This finding highlights that the effects of interleukins in the pathogenesis of AS are not always straightforward but can vary from tissue to tissue and be influenced by tissue-dependent environmental factors. It is important to note that the current

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attention towards IL-23, IL-17 and IL-22 to some extent neglects the critical role of tumour necrosis factor (TNF) in this type of diseases. A TNF overexpression model mimics many features of the disease<sup>26</sup> and TNF inhibition is currently the mainstay approach with high and sustained efficacy in SpA. Bringing together the different proinflammatory cytokines in one paradigm is therefore an interesting challenge.

Detailed histological analysis of the gut biopsies drives the authors to propose the hypothesis that IL-23 receptor-positive ILC3s differentiate in the gut and then migrate to extraintestinal sites where they produce IL-17 and IL-22. This hypothesis was formulated in part on the abundant expression of  $\alpha 4\beta 7$  integrin on ILC3s, a  $\beta 7$  integrin with marked gut tropism, even though earlier work demonstrated its presence on synovial T cells in SpA synovium.<sup>27</sup> Although this hypothesis touches the limits of careful observation in human samples without additional mechanistic experiments, the concept is certainly worth considering. Moreover, earlier this year Mackley *et al*<sup>28</sup> provided the first in vivo evidence of ILC3 traffic starting from the gut in mice. The concept is also in line with old and new insights into the role of gut inflammation in spondyloarthritis. Five per cent to 10% of patients with AS suffer from clinically apparent inflammatory bowel disease (IBD) and even a much larger proportion of AS patients suffer from subclinical gut inflammation as already suggested in 1985.<sup>29</sup> In a recent study by Van Praet *et al*<sup>30</sup> 46.2% of patients with AS showed microscopic gut inflammation of which 16.9% was acute and 29.2% was chronic inflammation. Patients with AS with chronic gut inflammation also have a higher degree of bone marrow oedema, as seen on MRI, further supporting a link between mucosal inflammation and progressive disease in AS.<sup>31</sup> The gut is a barrier tissue continuously faced with the challenge of maintaining the symbiotic gut microbiome while avoiding local and systemic disease development by pathogenic microorganisms. New technologies can assess the gut microbiome in a systematic way. Different efforts are underway to link the microbiome with gut inflammation and joint disease in SpA and the results are eagerly awaited.

ILC3s are required for human immunity against extracellular bacteria and also play a role in chronic inflammation due to its proinflammatory characteristics.<sup>15</sup> Keeping in mind that intestinal bacteria could play a role in the pathogenesis of AS, as described above, and that AS is a

chronic inflammatory disease, the hypothesis that ILC3s are directly involved in the pathogenesis of AS sounds plausible. Of course, further research is necessary to, first, confirm the data and, second, further explore this hypothesis. If the hypothesis about the migration of IL-23 sensitised gut-resident ILC3s is correct, one of the key residual questions, for example, is how HLA-B27, the strongest risk factor for AS, fits in this story. Since HLA-B27 is a major histocompatibility complex class I molecule it will present endogenous antigens/peptides originating from the cytoplasm on the cell surface. These antigens/peptides include self-peptides and can derive from viruses and bacteria. This peptide presentation could then possibly be the link to the gut microbiome. As the HLA-B27 gene is not linked to IBD, another possibility is that HLA-B27 does not play a role in disease induction in the gut, but only plays a role in inflammation at the joint level. Another question is how to explain joint inflammation in patients with AS without currently detectable gut inflammation. Notwithstanding the many additional questions, the paradigm proposed by Ciccía *et al* focusing on ILC3 cells as cytokine shuttle travelling from gut to blood, synovium and bone marrow in AS is an interesting new approach to the unravelling of spondyloarthritis' pathogenesis.

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