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Macrophage in Enthesis: A Likely Contributing Factor to Enthesitis through IL-23 in Ankylosing Spondylitis

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Abstract

Ankylosing Spondylitis (AS) is a chronic inflammatory disease that characterized by enthesitis and subsequent syndesmophytes in spinal joints and peripheral joints. However, the exact pathogenesis of AS is still unknown. Recent studies indicate that serum concentrations of interleukin-23 (IL-23) are elevated in AS and the expression of IL-23 in vivo is sufficient to phenocopy the human disease such as spondyloarthropathy (SpA), with the specific and characteristic development of enthesitis and entheseal new bone formation in the initial complete absence of synovitis. But it remains unclear what might be the cause that is related to the elevation of IL-23 in enthesis. Macrophage, which is a primary source of IL-23 in response to inflammatory stimulation, has been detected around the abnormal vascular structure at enthesis in SpA. Besides, the plasticity and polarization of macrophage plays a vital role in local inflammation and immune response. Here, we proposed a hypothesis that macrophages residing in and migrating through the abnormal vascular structure to enthesis in the inflammatory state are an essential source of IL-2, which alone can induce enthesitis and promote abnormal new bone formation in AS.

Keywords: Macrophage; Enthesitis; Inflammation; Ankylosing spondylitis

Introduction

Ankylosing spondylitis is a common inflammatory disease which often affects the function of axial joints and peripheral joints. Patients frequently suffer early symptoms of back pain and arthritis for many years [1]. While exercise can improve the joint functions, rest may have reverse effect. Previous studies suggest that spondyloarthropathies are characterized by earliest enthesitis and synovitis, in which blood vessels participate [2]. Previous study found that 0.5% of the western population is affect by SpA [3]. A recent study shows about 54% and 38% of the Latin America and Europe AS patients are affected by enthesitis [4]. The aetiology of AS is undefined but is thought to be immune-related and has a strong association with the HLA-B27 gene. About 90% of AS patients express the HLA-B27 positive genotype [5].

The site where a ligament, tendon, muscle or joint capsule is attached to the skeleton is known as an enthesis [6]. A study of 2007 by Benjamin et al. introduces two types of entheses: fibrous entheses, which comprise a direct insertion of dense fibrous connective in bone; and fibrocartilaginous entheses, where an intermediate tissue between dense connective tissue and cartilage is found [6]. Most entheses are fibrocartilaginous, which encompasses four zones-dense fibrous connective tissue, uncalcified fibrocartilage, calcified fibrocartilage, and bone. Fibrous entheses and fibrocartilaginous entheses are separated by a calcification front known as the tidemark [7]. Historically, normal enthesis are avascular in their fibrocartilaginous regions, but tissue microdamage to enthesis is common and appears to be associated with tissue repair responses and blood vessel ingrowth [8]. Anatomically, the primary role of entheses are to maintain the stability of insert site between tendons, ligaments and the skeleton in order to transmit mechanical force and dissipate mechanical stress. Due to their special locations, entheses are prone to overuse injuries [9]. Tennis, golfer's elbow and allied disorders are well described in sports medicine. Similarly, these symptoms are differently present in SpA. Mechanical stress injury may explain the clinical differences of inflammatory changes of entheses but why it is prone to occur especially in the SpA? Immune dysfunction is considered and several types of immune cells are included. Dendritic cell, $CD4^+$ or $CD8^+$ T cell, macrophage, haematopoietic cell and Th17 are reported in different inflammatory sites [3]. However, previous studies indicate that macrophage that carries scavenger receptor CD163 at the cell surface are predominant cells in the overlaying synovium and enthesis of SpA [10,11]. This may present a relative curial role macrophage in enthesis inflammation.

Macrophages, an important phagocytic and antigen presenting cells, play an essential role in innate immunity and host defense. Macrophages play key roles in wound repair by regulating extracellular matrix turnover. Moreover, macrophages can recruit and alter the functions of other cell types [12]. Monocyte-macrophage lineage mainly originates in bone marrow and matures in blood vessels. The matured macrophages can migrate to multiple body tissues and are characterized by considerable diversity and plasticity. In response to various signals, macrophage may undergo classical M1 activation or alternative M2 activation [13] M1 macrophages tend to promote tissue injury while M2 macrophages are often linked with the mechanisms of wound repair and fibrosis. In tumor development, altered macrophage differentiation and immune dysfunction have close association with the disease progression. Tumor–associated macrophages (TAMs) express high levels of M2 macrophage markers such as IL-10, Transforming growth factor beta (TGFβ), arginase 1 (ARG1) and relative low levels of M1 macrophage-induced

inflammation(Interleucin-2 (IL-2), tumor necrosis factor-α (TNF-α), Interleucin-6 (IL-6)) [14,15]. In Rheumatoid Arthritis (RA), pro-inflammatory macrophages are found both in Synovial Tissue (ST) and Synovial Fluid (SF), which can produce amount of inflammatory cytokine in response to Lipopolysaccharide (LPS), TNF-α or (Interleucin-1-β) IL-1-β. These results suggest that pro-inflammatory M1 macrophage is the likely a main contributor to local tissue injury to immune disease such as spondyloarthropathy.

Macrophages, which arise in spondyloarthropathies peripheral blood and early inflammatory lesions [16], have been proved to be associated with the HLA-B27 gene. HLA-B27$^+$ bone marrow macrophages from transgenic rats stimulated *in vitro* with Unfolded Protein Response (UPR)-inducer can produce increased IFN-β and IL-23 in response to lipopolysaccharide (LPS), and HLA-B27 misfolding and UPR activation in macrophages can result in increased secretion of the pro-Th17 cytokine IL-2, an pivotal cytokine in the pathogenesis of SpA [17]. Moreover, a recent study found that within fibrous tissue from AS and OA facet joints, IL-23 was predominantly produced by CD163$^+$ and CD68$^+$ macrophages. This reveals a potential role of macrophages in the HLA-B27-related immune diseases by producing excessive IL-23 [18].

Several studies have indicated that T lymphocytes and macrophages predominate in the inflammatory lesions of sacroiliac joints in SpA [3,16,19]. And both of the two cells types can produce certain amounts of IL-23. However, studies on HLA-B27/human β2-microglobulin transgenic rats showed a minor role of CD8$^+$ T cells in entheses inflammation, moreover, depletion of CD8$^+$ T cells in HLA-B27 transgenic rats had no impact on the start and severity of experimental arthritis [20,21]. The result indicated macrophages may be more important in the enthesis. And between the two types of cells, macrophages are detected around the abnormal vascular structure in inflammatory sites [6]. According to the fact that macrophage migrate via angiogenesis in many inflammatory disease [22], we may conclude that macrophage migrate to enthesis through the abnormal vascular structure in SpA in a similar way. Besides, there are a certain amount of macrophages resident in normal tissue including enthesis [23], and the two sources of macrophages may function together in response to stimulation. However, further studies are needed to prove it.

The relationship between IL-23 and SpA has already been proved, as polymorphisms in the IL-23 receptor are associated with ankylosing spondylitis and higher levels of IL-23 are found in in vitro-derived macrophages in this disease [24,25]. Moreover, the gene HLA-B27, which is strongly associated with spondyloarthropathy, has also been shown to upregulate IL-23 production [26,27]. Recently, Sherlock et al. reported that in vivo IL-23 alone was sufficient for inducing enthesitis and recapitulated the features of human SpA in mice by acting on ROR-γt+CD3+C4-CD8-entheseal resident T cell [23]. This shows a key role of IL-23 in the pathogenesis of SpA and indicates a potential therapy for this disease by inhibition of IL-23. But the specific mechanism of increased IL-23 production in SpA enthesis is still unclear. An attractive hypothesis is that IL-23 is released from distant immune cell populations in the gut and, perhaps, the lung [28]. As an important IL-23 producing innate immune cell, we believe that macrophage raised in enthesis is a likely contributing factor for the expression of IL-23 and drives enthesitis. Other researchers consider factors such gut microbiome, HLA-B27 UPR, biomechanical stress as the origin of IL-23 in the disease state [6,29,30], and these factors may also function through macrophage. However, all of these hypotheses require further studies.

The Hypotheses

Macrophage recruitment is vital during inflammatory repair and immune response, and macrophage plasticity and polarization lay the foundation for its function. During inflammation progression, monocyte attractants such as chemokine superfamily (in particular CCL2/MICP-1), Colony-Stimulating Factor-1 (CSF-1) and vascular endothelial growth factor (VEGF) can be found at higher expression levels in lesions [31-33], which greatly promote the migration of immune cell. Similarly, in SpA, such inflammatory factors are also found in abnormal expression, which may provide migration momentum and direction for T cell, B cell, macrophage, neutrophils or dendritic cells to lesions. Most prior studies have focused on the genetic mechanisms of AS to find new associated genes. And important factors, such as HLA-B27, ERAP1 (endoplasmic reticulum associated aminopeptidase-1), IL-23R, IL-10, TNF-associated receptor family genes have been shown associated with the pathogenesis of AS. However, the abnormal expression of these gene occurred in T cells, B cells or macrophages may produce different effects. Based on the evidence, we propose that macrophage residing in enthesis and migrating through the abnormal vascular structure to enthesis from distance would be an important source of IL-2, which would drive enthesitis *in vivo*. Thus, the hypothesis raises a new possible pathogenesis of enthesitis in SpA and provides a potential therapeutic target.

Evaluation of the Hypothesis

Enthesitis is a distinct characterization of spondyloarthropathy and the cells that are involved in inflammatory enthesis are the key to reveal SpA. Although different inflammatory cells are detected, macrophages seem to play a special role. The migration function of macrophage to inflammatory site is the basis of the hypothesis. Macrophages have been found increased in SpA enthesis and would produce higher levels of IL-23 in response to other stimulator factors. And IL-2, a central cytokine in the pathogenesis of SpA, is reported to act on a RORγt$^+$ CD3$^+$ CD4$^-$CD8$^-$ entheseal resident T cell recently. However, the source of IL-23 is a vital matter. Many hypotheses are proposed and here, we suppose that macrophage is likely a contributing factor for IL-23 produce in enthesis. We may test the hypothesis by inhibiting the function of inflammatory macrophage locally in enthesis. The IL-23 level may decrease and the progression of inflammation may slow down. However, inhibition of the inflammatory macrophages functions may cause other problems. Further studies are needed and the hypothesis can also be challenged.

Implications of the Hypothesis

Although different mechanisms may be involved in enthesitis of SpA, all these hypotheses need further evidence. We propose a new hypothesis that macrophage also participates in the development of enthesitis by producing higher levels of IL-23. This hypothesis may have implications in at least three aspects. Firstly, elucidates the role of macrophage in enthesis inflammation of SpA. Secondly, helps to find new possible sources of IL-23 in enthesis. Thirdly, provides a possible up-stream signal of IL23/IL-17 axis in SpA and presents potential therapeutic targets. Inhibition of macrophage function locally may weaken enthesitis by secreting lower levels of IL-23 and thus mitigating the local inflammation. However, inhibition of macrophage function may interfere with normal immune function and further experiments are needed.

Discussion

It is well established that Tumor-Associated Macrophages (TAMs) can affect diverse aspects of cancer progression including tumor cell growth and survival, invasion, metastasis, angiogenesis, inflammation and immunoregulation [34]. A large number of TAMs have been found in breast cancer. And TAMs intact with the surrounding tumor cells mainly through three pathways [35]. Firstly, secrete proangiogenic factors such as VEGF to promote new blood vessels that provide nutrition for tumor cells. Secondly, function as channels of transport into the extracellular matrix. Thirdly, release factors to decrease the local pro-inflammatory antitumor response, thus fascinating the escape of tumor cells. Besides, hypoxia stimulates macrophages to produce higher VEGF and inhibits T-cell mediated-immune responses, thus enhancing the evasion of tumor cells and metastasis. The powerful function of macrophages lays the foundation for its role in canner and other immune disease.

Studies have demonstrated higher levels of IL-23 and IL-17 in the serum of SpA such as active ankylosing spondylitis [36]. But what may account for the increase of IL-23 especially in enthesis? The unfolded protein response (UPR) in HLA–B27+ macrophages might be a factor, and ankylosing spondylitis macrophages have been showed to produce greater interleukin-23 in response to lipopolysaccharide (LPS) [37]. However, the result is insufficient to explain the exact mechanisms of increased IL-23 in enthesis of SpA, and further evidence is needed. Other factors such as gut microbiome stimulation, Biomechanical stress are also considered. Similarly, macrophage in enthesis is a likely contributing factor for IL-23 producing and thus promotes enthesitis.

Macrophage is an important innate immune cell and has close association with SpA. In this article, we propose a new hypothesis that macrophage migrating to or residing in enthesis in responding to stress may produce an amount of IL-23. Thus, a novel source of IL-23 can be determined. And a possible therapeutic target by inhibiting inflammatory macrophage may be sufficient and effective on enthesis-related disease.

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