

An Enthesitis Based Model for the Pathogenesis of Spondyloarthropathy. Additive Effects of Microbial Adjuvant and Biomechanical Factors at Disease Sites

The spondyloarthropathies (SpA) are a heterogeneous group of diseases of poorly defined pathogenesis that include ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), enteropathic arthritis, and undifferentiated arthritis. These diseases share common features including spinal and peripheral joint inflammation and also multisystem disease involving the eye, heart, skin, intestinal tract, and lung, and associations with microbes and HLA-B27. Collectively, SpA are immensely important as they serve as a model that involves interaction between triggering microorganisms and the host immune response¹, but in the case of SpA a unifying disease model explaining the known anatomical and microbial factors has not been proposed.

Models for other diseases have been developed by investigating the relationship between the immune system and factors at the anatomical sites of disease, an example of this being celiac disease, where gut lamina propria enzymes interact with dietary protein antigens, leading to autoimmunity². Defining a unified anatomical basis for SpA has not been possible because the spectrum of skeletal abnormalities is varied and includes spinal inflammation (sacroiliitis and spondylitis), synovitis, dactylitis, lytic bone lesions, periostitis, osteitis, and enthesitis (inflammation at points where tendon, ligament, and joint capsules are inserted to bone)¹. Entheses are ubiquitous throughout the skeletal system, and while it has long been appreciated that enthesitis was the primary lesion in AS, many of the skeletal features remained difficult to explain in relationship to enthesitis. However, the enthesitis lesion, which is clinically focal in nature, is pathologically associated with a diffuse inflammation in the adjacent bone marrow and soft tissue³⁻⁵, and these changes may represent the forerunner of radiographic osteitis, periostitis, bone lysis, and new bone formation that are typical of SpA but not rheumatoid arthritis (RA). Based on the frequency, extent, and distribution of these enthesial changes in synovial joints and elsewhere we have proposed that enthesitis associated pathology links the diverse skeletal pathology in SpA⁶. This article proposes a disease model for SpA based on the enthesitis lesion.

THE PHYSIOLOGY OF THE ENTESIS

The anatomy, physiology, and biochemistry of the entesis have not been investigated in great detail, which may relate to the relative inaccessibility of enthesial tissue and because the importance of enthesitis as a skeletal phenomenon has been

overlooked. Nevertheless, several important observations have been made in relationship to the normal anatomy and physiology of the entesis, but disease pathogenesis has not been addressed in relationship to these. In comparison to other skeletal locations, the entesis is a site of repetitive biomechanical stressing forces that are applied during the course of normal muscle, ligament, and tendon action⁶. The entesis and adjacent regions of the capsule, tendons, and ligaments can undergo significant functional adaptation with increased bulk in response to stressing⁷. Similarly, the adjacent bone reacts to stressing with the formation of surface spurs that are observed on radiographic or histologic assessment⁸. Anatomically, certain entheses flare out toward their insertions and attach to a larger area of bone⁹, which may contribute to the diffuse pathology noted adjacent to entheses.

The consequence of sustained biomechanical stressing about these various joint structures is tissue microtrauma. Indeed, histologic changes suggestive of microtrauma with evidence of an associated healing response have been reported in normal enthesial tissue⁸.

FUNCTIONAL SIMILARITIES OF ENTESIS AND OTHER DISEASE SITES

Aortic root involvement, anterior uveal tract disease in the eye, psoriasis, and occasional lung apex involvement are the extraskeletal disease manifestations of SpA. It has been suggested that these changes are due to autoreactive lymphocytes attacking commonly expressed antigens, an observation that is supported in part by experimental data^{10,11}. However, these sites also share remarkable biomechanical properties with the entesis; that is, the aortic root, the ciliary body in the eye, skin extensor surfaces, and the lung apex are subject to intermittent biomechanical stressing throughout life (Figure 1). The cutaneous regions most characteristically involved in psoriasis, namely over the elbows and the knees, are subject to comparatively high biomechanical stressing compared to skin at other sites¹². Therefore, just like the entesis, these are regions where higher repetitive forces are acting than in neighboring tissue. Recent computerized tomography studies have confirmed that lung apex inflammation in AS does not reflect rare episodes of incidental tuberculosis infection, but was in fact seen in 8% of cases¹³. The localization of lung inflammation in AS shows a predilection for the lung apex only — the site where most distention of the pulmonary tissue

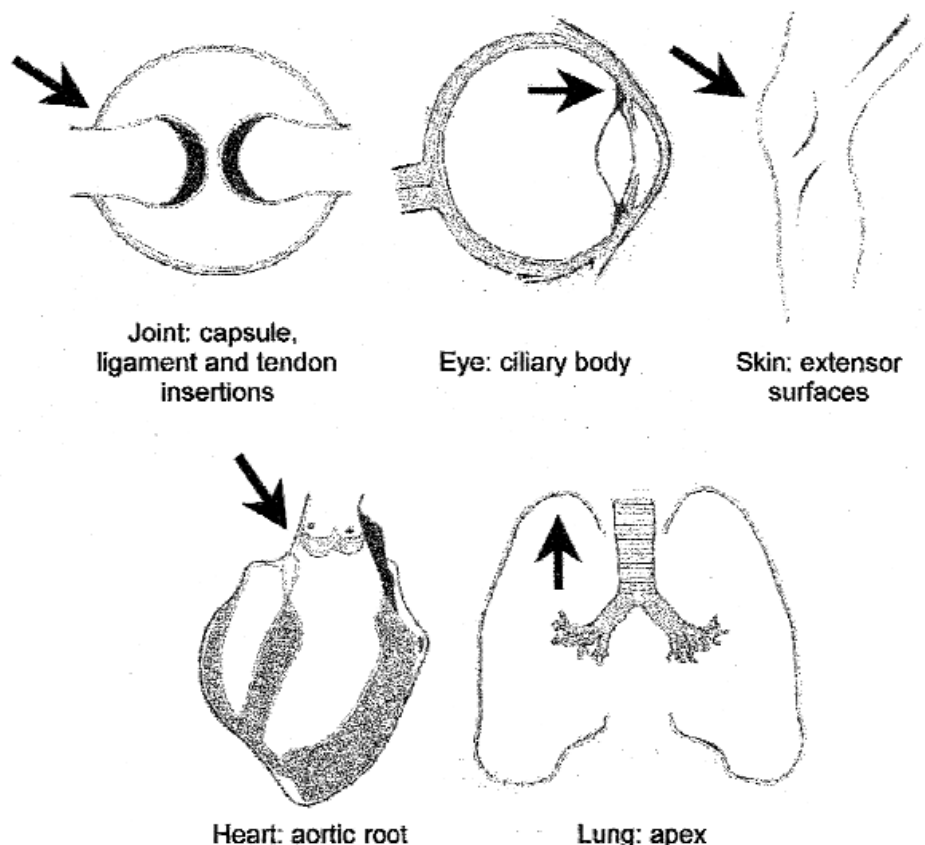


Figure 1. Sites of disease in human SpA. All diseased sites are subject to repeated cyclical biomechanical stressing and are also sites of mechanical failure when there are defects in extracellular matrix proteins. Disease localization in SpA may be related more to biomechanical factors than to shared autoantigens at these diverse sites.

bined effects of anatomical and microbial factors at diseased sites could upregulate proinflammatory cytokines and transcription factors to a degree that could shift the balance of normal tissue homeostasis from repair to an inflammatory response (Figure 2).

Because of the wealth of data available on NF- κ B in the setting of biomechanical stressing and healing responses and in microbial immunity we have focused our arguments on this pivotal transcription factor. Indeed, NF- κ B possesses the dual capability of regulating the inflammatory responses and also being a consistent theme linking mechanical stimuli to gene expression¹⁹. One of the principal proinflammatory cytokines produced following NF- κ B activation is TNF- α , and it is tempting to speculate that these factors could ultimately be the basis for upregulation of TNF- α in SpA and therefore underscore the response of SpA to anti-TNF- α therapy. However, the additive or synergistic effects between adjuvant and biomechanical factors leading to proinflammatory gene expression may be more generalized and involve other proinflammatory molecules.

These biomechanical and microbial factors at disease sites provide an explanation for how scant bacteria or their constituent macromolecules could incite an inflammatory reaction. They also provide an explanation for the pattern of

disease localization to skeletal and extraskeletal sites in SpA.

Recently, it has been suggested that immune activation occurs in response to "danger signals" that, experimentally, include infection, trauma, or proinflammatory cytokines, but these signals remain poorly defined for most disease³². The above observation in human SpA suggests that the immune system may be inappropriately activated by the additive effect of different danger signals including cytokines derived from the healing response and biomechanical stressing (host factors) and adjuvant (environmental factors).

This concept could also have implications, not just for SpA, but also for mechanisms of RA, where there is also evidence for adjuvant in the synovium³³. This model may also be of general relevance to chronic diseases such as atherosclerosis, where biomechanical and microbial factors contribute to disease, although their interrelationship is presently ill defined³⁴. For example, *Chlamydia pneumoniae* may be important in the pathogenesis of atherosclerosis, and recent observations suggest that *Chlamydia* derived adjuvant could contribute to inflammation in the atherosclerotic response³⁵. Therefore, bacterial products derived from distant sites of infection or colonization could represent a general mechanism that could interact with local biomechanical factors to induce inflammatory disease.

occurs during breathing. It is noteworthy that disease localization in SpA is similar to Marfan's syndrome, where weakening of the connective tissue at the same sites due to a defect in the fibrillin protein results in joint dislocation and aortic root, eye, and lung apex disease¹⁴, demonstrating the importance of repetitive biomechanical stressing at different sites.

Finally, it should be noted that, in some cases, microscopic vascular and inflammatory changes have been reported in the synovium in normal large weight bearing joints¹⁵, which are sites of increased shear stressing and also the most commonly involved joints in SpA. This suggests that mechanical factors independent of enthesitis could play a direct role in joint disease.

Some sites of inflammation in SpA including that in the genitourinary and gastrointestinal tract are less readily explained in relationship to biomechanical stressing. However, these mucosal locations usually represent the site of triggering infections or their resident microbes may lead to disease independently of biomechanical stress response.

ANATOMICAL FACTORS IN DISEASE LOCALIZATION

There are a number of functional consequences of the enthesitis that could contribute to localization of the inflammatory response in SpA.

- (1) The effect of biomechanical stressing on proinflammatory gene expression.
- (2) The effects of tissue microtrauma and associated healing response on proinflammatory gene expression.
- (3) The altered vascularity at the sites of disease.
- (4) The deposition of bacteria or their macromolecules at these sites of stressing and microscopic inflammation.

Biomechanical stressing. Repeated cyclical biomechanical stressing at the enthesitis is an anticipated consequence of joint use. This has not been investigated in detail, but the consequences of biomechanical stressing in culture systems have been evaluated, and marked changes in gene expression following stress have been reported with an estimated 600 stress-responsive genes¹⁶. Shear stressing activates transcription of a number of genes including platelet derived growth factor, tissue plasminogen activator, and a number of adhesion molecules¹⁷. Changes in gene expression in response to biomechanical stress are due in part to upregulating the activity of several nuclear transcription factors including activator protein-1 (AP-1) and nuclear factor κ B (NF- κ B)¹⁸ and several others¹⁹. This indicates that biomechanical stressing can directly upregulate a number of molecules and transcription factors that also play prominent roles in the inflammatory cascade.

Tissue microtrauma. Tissue microtrauma with evidence for a healing response and microscopic inflammation may occur at a normal enthesitis and is probably a response to biomechanical stressing⁹. The healing and inflammatory responses are closely linked and are mediated by common inflammatory cells and cytokines including interleukin (IL-1), tumor necrosis factor- α

(TNF- α), transforming growth factor- β (TGF- β), chemokines such as interleukin 8 and others playing pivotal roles in both processes²⁰. Therefore, in addition to playing a direct role in cytokine responses at the enthesitis, the indirect effects of biomechanical stressing with tissue damage will regulate transcription factor and proinflammatory cytokine expression.

Altered vascularity. A further important consequence of the healing response associated with inflammation is the associated increase in vascularity. Schulz and colleagues found in animal studies that sites of joint stressing with altered vascularity seem to be sites that favor preferential deposition of particulate matter and bacteria²¹. In joints this could lead to the preferential deposition of bacterial molecules at these sites.

Deposition of bacteria. Bacterial colonization or preceding bacterial infection at sites remote from involved joints are striking features of both human SpA and animal models of disease²². However, replicating bacteria have not been recovered from the synovial cavity, and with the possible exception of *Chlamydia trachomatis*, evidence is lacking for viable bacteria within the joint²³. However, studies have shown that certain bacterial constituents including lipopolysaccharide, immunostimulatory bacterial DNA²⁴, and heat shock proteins²⁵ in the absence of viable microbes are capable of triggering joint inflammation. It has long been appreciated that these adjuvant properties of bacteria can lead to immune activation²⁶, but it is difficult to reconcile this with such marked inflammatory changes at characteristic sites in SpA. We have recently reported that, even with prolonged culture of enthesal derived tissue, bacteria cannot be grown, suggesting that viable organisms are not present in the primary skeletal lesion either²⁷.

Recently the molecular basis for immune system activation by adjuvant has been shown to occur via the NF- κ B signaling cascade. In humans the cell membrane receptor for lipopolysaccharide has been identified as Toll-like receptor 2, which leads to NF- κ B transcription²⁸. It has been proposed that bacterial CpG DNA motifs result in a reactive oxygen species burst leading to NF- κ B activation²⁹. Other bacterial molecules including bacterial heat shock proteins also upregulate NF- κ B transcription³⁰, and other hitherto poorly characterized adjuvants may similarly contribute to immune activation³¹. The utilization of common signaling cascades in response to different adjuvants may enhance the chances of immune reactivity against microbes present in low concentrations, as is the case in SpA.

A MULTIHIT MODEL FOR IMMUNE ACTIVATION IN SPONDYLOARTHROPATHY

Thus far we have drawn attention to the similar effects that biomechanical stressing, microtrauma, and microbial factors at the enthesitis have on proinflammatory transcription factor and gene expression. On its own, small amounts of adjuvant may be insufficient to activate the immune response, but the ability of adjuvants to activate the immune response can be greatly enhanced by proinflammatory cytokines²⁶. The com-

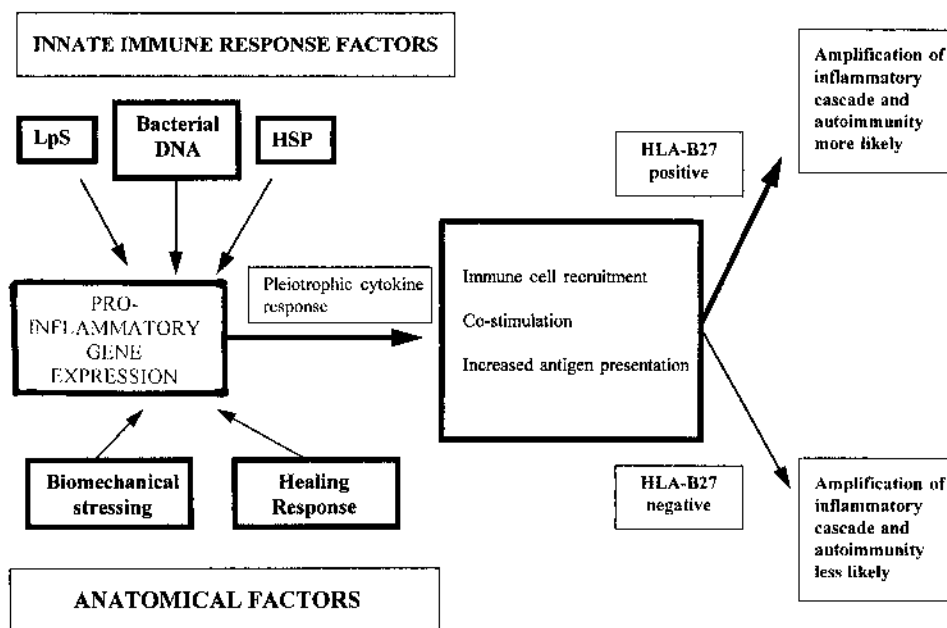


Figure 2. Mechanisms of immune activation without viable microbes in SpA. Immune activation depends on the net input from stimulatory and inhibitory signals. This illustrates how the combination of bacterial adjuvants and mechanical factors could act in a synergistic manner, through common proinflammatory transcription factors and cytokines. These factors could directly activate the innate immune response and could provide the necessary co-stimulatory signal for an acquired immune response, especially in HLA-B27 positive subjects. As the major genetic contribution to SpA resides outside the MHC, this model also shows how factors determining gut permeability and the magnitude of individual cytokine responses and functional polymorphisms could contribute to disease. LpS: lipopolysaccharide, HSP: heat shock proteins.

What are the factors that lead to disease in some subjects but not others? The SpA are heterogeneous, but 2 such factors are fairly well defined. First, in those subjects who possess the HLA-B27 gene, the presentation of an undefined peptide could activate the acquired immune response. This could convert a low level subclinical response at the enthesis into a florid clinically detectable inflammatory reaction. A second factor is abnormal mucosal permeability such as is evident in AS or inflammatory bowel disease associated arthritis. These factors could, by increasing the amounts of bacterial adjuvant that reach the mechanically stressed sites, trigger inflammation. Other genetic factors leading to disease remain ill defined, but genetic control of the stress and healing responses may lead to new factors being identified.

Clearly, some musculoskeletal sites are not particularly associated with SpA, despite being sites of significant biomechanical stressing. For example, the metatarsophalangeal joint in the foot is not that prone to SpA or psoriasis, although the adjacent skin is clearly subject to considerable biomechanical stressing. Further, artery bifurcations are sites of altered stress and are prone to atherosclerotic disease, but there are no data suggesting accelerated atherosclerosis at these sites in SpA. Possible factors that may be important in disease localization to the enthesis include (1) the type of stress — enthesal insertions are subject to compressive and shear forces that may differ from stress at other sites; (2) common antigens expressed adjacent to enthesis and other disease prone sites that lead to autoimmunity.

IMPLICATIONS

A corollary of the concept that biomechanical stress and microtrauma is important in SpA is that bed rest may be an important part of the therapy of resistant cases. However, rest is beneficial for virtually all types of inflammatory arthritis and it is therefore difficult to make specific extrapolations about SpA. Athletes are especially prone to higher levels of mechanical stress and microtrauma, and it is therefore possible that some with episodic “mechanical” enthesopathies do in fact have significant inflammatory disease. Further, subtle degrees of joint malalignment, which could lead to increased stress, may be contributory factors for disease localization to specific joints.

To date the pathogenesis of SpA has in the main been addressed from specimens of inflamed synovial tissue, by investigating the role of microbes and exploring the role of HLA-B27. The factors within the joints and other disease sites that lead to disease localization there in the first place have not been investigated. Future studies need to look at the pattern of gene expression from cells in subjects with SpA compared to controls, and to determine if there is a difference in the magnitude of inflammatory gene expression. Studies determining the degree of microtrauma in normal entheses and adjacent bone in healthy and SpA subjects could also shed light on the role of microtrauma at these sites.

The spondyloarthropathies serve as a disease model for understanding the interaction between microorganisms and

genetically susceptible hosts. In the proposed enthesitis based model, the additive interactions between biomechanical factors and the innate immune response to bacterial products result in disease localization. This model for SpA may have more general implications for chronic inflammatory disorders.

DENNIS MCGONAGLE, MRCPI,
Lecturer in Rheumatology, MRC Clinical Scientist,
University of Leeds, Honorary Consultant,
Halifax General Hospital;

LUKE STOCKWIN, BSc,
Research Fellow, School of Biochemistry and Molecular Biology,
University of Leeds;

JOHN ISAACS, PhD, FRCP,
Senior Lecturer in Rheumatology;

PAUL EMERY, MD, FRCP,
ARC Professor in Rheumatology, Department of Rheumatology,
University of Leeds.

Address reprint requests to Dr. D. McGonagle, Department of Rheumatology,
36 Clarendon Road, University of Leeds, Leeds LS2 9NZ, UK. E-mail:
d.g.mcgonagle@leeds.ac.uk

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