

From ubiquitous antigens to joint-specific inflammation: could local vascular permeability be the missing link?

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The mechanisms underlying organ-specific autoimmune diseases are incompletely understood. In particular, it is still unclear how autoantibodies, often against ubiquitous antigens, can cause pathology preferentially in certain tissues. Recently, Binstadt and colleagues, using observations in the K/BxN murine arthritis model, suggested novel pathways underlying the site-specific localization of inflammation driven by immune complexes. In this commentary, we discuss these findings, the problems related to their general applicability and the implications for future research.

Introduction

The reasons behind the preferential tissue pathology observed in 'organ-specific' autoimmune conditions are not fully understood. Nonetheless, two important explanations are the tissue specificity of various infective agents (i.e. hepatic, neuro- or lympho-tropic viruses) and the breakage of immune tolerance relating to local exogenous or endogenous antigens. These possibilities can overlap, and it has been suggested that organ-specific parenchymal cells 'aberrantly' expressing HLA class II antigen-presenting molecules (in response to the same or different infective agents) would result in chronic stimulation of the immune system, the unmasking of tissue-specific and or cryptic antigens ('epitope spreading') and autoimmune pathology. Additionally, in recent years there has been an increasing realization of the important contribution of the vascular system to tissue-specific pathology.

For example, it has become clear that different subsets of effector memory T and B cells are recruited and retained in relevant tissues through the use of 'tissue-specific' cell-adhesion molecules (homing receptors) and vascular 'addressins' in addition to chemokine-receptor interactions [1]. Classical examples include $\alpha_4\beta_7$ -mucosal addressin cell-adhesion molecule (MAdCAM)-1 and CCL17-CCR9 interactions in gut homing, and cutaneous lymphocyte-associated antigen (CLA)-E-selectin and CCL25-CCR4 in skin homing [1]. There is also evidence that the acquisition of 'topographical memory' is imprinted by dendritic cells in secondary lymphoid organs, where a combinatorial access code would be

generated to target pathogenic immune cells to the relevant tissues through specific interactions with preferentially expressed and presented molecules by the local microvascular endothelium [2]. In addition, Binstadt and colleagues recently presented evidence supporting another important function of the vascular endothelium in determining tissue-specific pathology, namely that the transfer of arthritogenic antibodies from K/BxN mice causes macromolecular vasopermeability localized to sites destined to develop arthritis in normal mice [3]. In this commentary, we discuss these findings, the problems related to their general applicability and the implications for future research (key points summarized in Box 1).

Box 1. Key Points

- **Selective endothelial permeability in tissue-specific pathology.** Observations in the K/BxN mouse model of arthritis, in which the passive transfer of serum produces transient disease, have shown that IC formation can drive joint-specific changes in vascular endothelial permeability to macromolecules. The underlying mechanisms have yet to be fully defined but seem to involve vasoactive amines as the downstream effector molecules. This suggests a model for the generation of joint-specific disease by antibodies to ubiquitous antigens, and might also explain why transient arthritis is a feature of many, notably viral, systemic conditions.
- **Future directions.** The mechanisms involved in differential changes in endothelial permeability are still poorly understood. Further work is required to distinguish the cellular and molecular components of the pathway(s) which release systemic vasoactive mediators in response to IC formation, in addition to what underlies the differential sensitivity of tissue vascular endothelial cells to these molecules. Also, it is still unknown whether IC-mediated vascular permeability drives tissue specificity in compartments other than the joint. For example, can antibodies against Ro/La ubiquitous ribonucleoprotein particles, observed in Sjögren's syndrome, cause salivary- and lacrimal-gland pathology? Another important question is whether the IC-mediated effect can facilitate the development of joint pathology triggered by antibodies specific to antigens other than GPI (e.g. anti-citrullinated proteins).
- **Applicability to human diseases.** Compared with the K/BxN mouse model, human inflammatory arthritis is usually characterized by more-complex patterns of joint inflammation, such as the absence of involvement of the distal interphalangeal joints of the hands in rheumatoid arthritis, or the asymmetric patterns and spinal involvement often seen in sero-negative arthritis. These conditions are also associated with heterogeneous extra-articular manifestations. Whether the same or different mechanisms are factors for articular and extra-articular pathology, and whether the findings in the K/BxN mouse can be extrapolated to the human disease have yet to be elucidated.

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Microvascular permeability: a regulatory mechanism for joint-specific pathology?

The K/BxN mouse, first described a decade ago by the same group [4], develops a spontaneous and aggressive arthritis that has several histological similarities to human rheumatoid arthritis (RA). These mice have T- and B-cell reactivity to the ubiquitously-expressed cytoplasmic enzyme glucose-6-phosphate isomerase (GPI), the expression of which is also seen at joint surfaces; the T cells carry a transgene-encoded T-cell receptor derived from a T-cell hybridoma recognizing a bovine ribonuclease peptide sequence. The transfer of serum or polyclonal antibodies from these mice to unaffected recipients produces a transient arthritis, histologically similar to that seen in the donors, with a preference for the distal joints [5]. The observations that Fc receptors (FcRs) and the alternative complement pathway were necessary for arthritis development highlighted the potential role of immune complexes (ICs) [6]. The injection of non-specific ICs also resulted in the increased localization of antibodies to distal joints, a process that depended on neutrophils, mast cells and FcRs (although not complement) [7]. This led to the proposal of a model whereby IC activation of neutrophil FcRs resulted in the release of inflammatory mediators with consequent increases in local vascular permeability and subsequent amplification through mast-cell FcRs, antigen binding and complement activation [7]. In the work discussed here, they further refine these observations using several experiments [3].

First, using an intravital microscopy model, they showed that the intravenous administration of specific (anti-GPI) or non-specific ICs resulted in a rapid increase in endothelial permeability to macromolecules. However, only specific ICs determined arthritis, indicating the need for antigen presence at the pathology site. The effect on vascular permeability was rapid, peaking at 2–3 min, and was confined to the distal extremities. In addition, complement was not required for the increase in permeability (although, as already mentioned, it is important for tissue pathology) but neutrophils (through Fc γ RIII-independent mechanisms), mast cells and, possibly, other unidentified cells resistant to ablation by radiation (through Fc γ RIII-dependent mechanisms) were necessary. Furthermore, surgical ligation of the portal circulation resulted in the loss of the permeability response. Because this response was retained in splenectomised animals, it is likely that Fc γ RIII stimulation in cells in the liver (or the gut, a possibility not eliminated by these experiments) is necessary. Finally, to investigate the potential role of vasoactive amines in this process, they found that specific antagonism of the histamine H1 or serotonin 5-HT_{2A} receptor significantly downregulated this increased vascular leakage. Also, the systemic administration of histamine or serotonin resulted in increased vascular permeability in a tissue distribution similar to that induced by immune complexes, although this did not result in arthritis. Although the vasopermeability effect was not essential to arthritis development, it seems to be an important amplifying step because Fc γ RIII-deficient animals did develop less-severe disease.

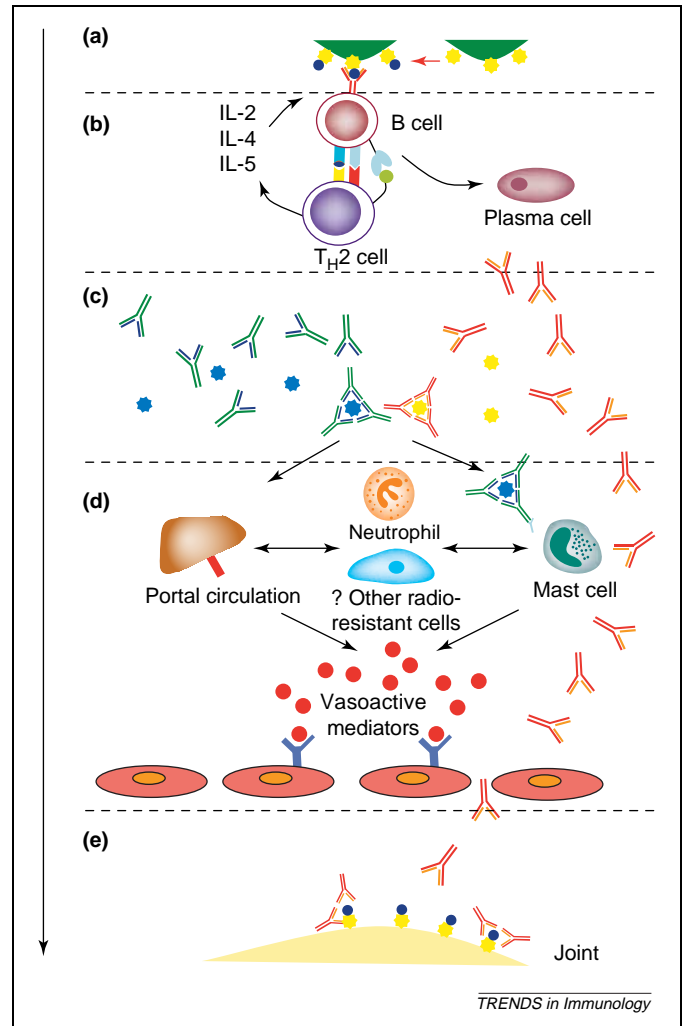


Figure 1. Putative model for site-specific inflammation triggered by sensitization to non-specific antigen. (a) An environmental insult, in association with other (e.g. genetic) risk factors, results in the modification of amino acid residues or novel antigenic exposure, for example, citrullination (blue) of arginine residues (yellow). (b) B-cell sensitization and autoantibody production is followed by a period of latency. (c) A trigger, such as infection, results in the formation of immune complexes that can be non-joint-specific (green) or formed from arthritogenic antibodies (red). (d) The activation of FcR receptors on unidentified cells in the portal circulation and, possibly, mast cells results in the secretion of systemic vasoactive amines by a mechanism that also depends on neutrophils. These have a selective effect on endothelial permeability to macromolecules in the joints with the resultant extravasation of arthritogenic autoantibodies. (e) At the inflammation site, binding of antigen by antibody results in the activation of the alternative complement pathway, with the release of the anaphylatoxin C5a and consequent pro-inflammatory and chemotactic effects on multiple cell types resulting in local pathology.

Thus, this paper [3] proposes an additional mechanism of localizing the ‘autoimmune attack’ to a specific tissue through the preferential local vasopermeability mediated by circulating ICs (Figure 1). However, several questions are still unanswered.

Unanswered questions and future research

It is unclear what underlies the selective endothelial response to the distal extremities. It is possible that the endothelial cells at this site are more sensitive to vasoactive mediators (e.g. due to the increased expression of specific receptors) although a role of, for instance, local mast cells cannot be ruled out. In fact, the precise role of mast cells and neutrophils (in a Fc γ RIII-independent

manner) is still undefined, as are the radio-resistant cells and the systemic mediators released from the portal circulation. The increased systemic levels of histamine are also seen in animals with portal circulation ligation, which suggests the presence of two or more synergistic mechanisms. Whether inhibiting these pathways can prevent or alleviate arthritis in these animals also requires further investigation.

Of most relevance, it is not yet established whether: (i) these observations can be generalized to the capacity of immune complexes to precipitate disease in organs other than the joint; and (ii) specificity to antigens other than GPI can cause arthritis.

Considering the first question, can antibodies, for example, those against the Ro/La ubiquitous ribonucleoprotein particles, observed in Sjögren's syndrome (SS), cause salivary and lacrimal gland pathology? Previous work has shown that the passive transfer of purified immunoglobulin (Ig)G from patients with Sjögren's syndrome can alter salivary gland function in some mouse strains, although the underlying mechanisms are unclear [8]. Furthermore, immunization with short peptides from the 60-kDa Ro antigen recapitulates SS pathology in BALB/c mice [9]. Though the underlying pathogenetic pathways in this model are still unknown, it would be interesting to examine whether IC-mediated local vascular permeability has a role. This is a crucial question because it still unknown whether the IC-mediated effects described by Binstadt and colleagues are restricted to the joint environment owing to specific physiopathological conditions (i.e. low oxygen tension, differential expression of vasoactive amine receptors, etc.) or are applicable to other vascular beds; although Binstadt *et al.* note the absence of a permeability response in the skin and caecum, this does not exclude an effect elsewhere, for example, in the salivary glands.

Further questions are raised by other examples from human autoimmune disease. A recent paper described the association between the presence of antibodies to double-stranded DNA (dsDNA), a subgroup of which also have activity against a glutamate receptor, and neurocognitive impairment in a mouse model [10]. This was seen only following permeabilization of the blood–brain barrier by the systemic administration of lipopolysaccharide. Equivalently, progressive neurocognitive decline that might not correlate with disease activity or treatment can be seen in a subgroup of patients with systemic lupus erythematosus (SLE). However, whether dsDNA is responsible in these patients and what factors could regulate their exposure to CNS sites protected by the blood–brain barrier are unknown. In another example, enhanced vascular macromolecular permeability has also been observed as an early change in a murine model of autoimmune type I diabetes [11]. Again, the underlying mechanisms are unknown.

Regarding the second question of IC specificity to antigens other than GPI, this is important because elevated serum anti-GPI antibodies have not been consistently detectable in human RA [12,13]. By contrast, antibodies to citrullinated proteins (anti-CCP), which are

present in 80% of patients, can predict the development of RA in early arthritis [14] and can be detected in the serum of patients up to 10 years before disease onset [15]. Citrulline is produced by the post-translational modification of arginine residues and, like GPI, can be detected in the synovium of RA patients [16]. Furthermore, the citrullination of proteins enhances the arthritogenicity of autoantigens such as collagen and fibrinogen [16,17]. The observation that smoking can upregulate citrullinated proteins in the lungs, and the increased risk of RA in smokers (which is significantly higher in smokers carrying the HLA-DR shared-epitope alleles) with elevated anti-CCP antibodies, has led to the proposal of a pathogenetic link [18]: sensitization at a different site (namely, the citrullination of proteins in the lung secondary to smoking) would trigger an immune response that, in genetically predisposed individuals, would precipitate arthritis through the recognition of citrullinated antigens in the joint. Whether anti-CCP antibodies form ICs with citrullinated proteins in the circulation, and whether these cause joint-specific vasopermeability, have yet to be established. However, the K/BxN model suggests this might not be necessary; for example, during infection, the formation of non-specific ICs could be sufficient to stimulate a vascular endothelial permeability response in the joint with the subsequent localization of pathogenic antibodies (e.g. anti-GPI or anti-CCP) and the triggering of arthritis.

Verifying these concepts would take us a step forward to the possibility of therapeutic intervention in patients with identifiable risk factors for RA and, possibly, to the ultimate goal of RA research: disease prevention.

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