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Review

Arthritis in space and time – To boldly go!

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ABSTRACT

Despite the profound impact of biologics on the treatment of rheumatoid arthritis (RA), long lasting disease remission remains elusive. We propose that this is a consequence of failing to target the right molecular pathway in the most relevant patient group at the appropriate time and place in disease progression. A limitation to testing this approach is the availability of disease models representing the discrete steps in autoimmune pathogenesis. A particular example is the paucity of models to dissect the conditions permissive for the breach of self-tolerance, which would subsequently allow identification and testing of therapeutics for re-establishment of self-tolerance. We conclude that a detailed understanding of the location and timing of events leading to the systemic breach of self-tolerance and subsequent progression to tissue specific pathology are required if rational application of existing drugs and identification of novel targets is to be achieved. This will take the personalised medicine revolution into the realms of contextualised medicine, whereby the right drug is targeted to the right tissue, in the right patient, at the right time.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder whereby the immune system promotes articular damage. It is a disabling and painful condition, which can lead to severe loss of mobility due to pain and joint destruction. RA is a systemic disease, often affecting extra-articular tissues throughout the body including the skin, heart, lungs, and muscles [1–5]. With approximately 50% of RA patients are unable to work 10 years after the onset of their disease [6] and recent studies showing 0.44% of men and 1.16% of women in the UK population suffering from RA [7] and increased mortality in these patients [8], accurate diagnosis and monitoring of the condition is critical. The processes that initiate and perpetuate RA are currently unclear and consequently the pursuit of novel therapies for RA has been mostly empirical. However, over the last 15 years improvements in molecular biology and biochemistry, together with improved understanding of cytokine biology, have identified molecules such as TNF as playing a central role in RA pathogenesis. These studies were greatly facilitated by the availability of ex vivo synovial membranes from the clinic as well as the availability of an animal model, collagen induced arthritis (CIA), for in vivo studies. As a direct result of improving our understanding of the basic biological/pathological mechanisms involved in RA we have continued to identify and validate new therapeutic strategies. Subsequent to TNF blockade, targeting IL-6 is in advanced clinical development and additional cytokine targets such as IL-17 and IL-15 [9–12] appear promising. While these treatments have revolutionised the treatment of RA, some patients remain refractory to biologic intervention [13]. Thus, while the control of inflammation has been mainly successful, the critical objective in rheumatoid therapeutics is now to re-establish peripheral tolerance and thereby maintain therapeutic remission. If achieved, this could bring about therapeutic induction of drug free remission. The advent of anti-CD20 (rituximab; potentially modifying B cell APC function) and of CTLA4-Ig in RA suggests that such effects may be achievable, although these approaches are not optimal [14]. In particular, these strategies result in non-specific effects on immune function, potentially leading to suppression of beneficial immune function such as those pertaining to defence against infectious agents and neoplasms. Our incomplete understanding of the mechanisms of breach of self-tolerance has critically impaired the development of such novel therapeutics. Specifically, there is a need for models and approaches that allow detailed analysis of the processes that regulate tolerance in arthritis in vivo. Such models should facilitate identification of the major cellular and molecular interactions involved in RA pathogenesis and analysis of their integration prior, and subsequent, to breach of self-tolerance to gain insights into the steps required to re-educate the immune system. In this instance, early clinical intervention is likely to be essential if a patient's immune system is to be re-programmed. While approaches such as the case control

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consortium [15] have established a number of predictive genetic variations associated with the development of RA, they will not inform when and where to intervene for a given pathway. Understanding the events surrounding the breach of self-tolerance associated with RA could therefore reveal markers associated with the onset of preclinical disease and signal a window of early intervention that would prevent the initiation of the cascade of events leading to symptomatic disease.

2. What is tolerance and breach of tolerance?

Tolerance is defined as a state of antigen specific unresponsiveness to subsequent exposure to that antigen in an immunogenic form in an otherwise immunocompetent organism. This usually comprises components of central and peripheral tolerance. Specifically, central tolerance, mediated by mechanisms such as clonal deletion and/or anergy, is established during the education of developing T and B cells. Peripheral tolerance exists to regulate responses that were not centrally tolerised (due to lack of exposure to these in primary lymphoid organs) or are peripherally breached (for general reviews of central and peripheral tolerance see Refs. [16–20]). Proposed mechanisms include deletion, anergy, ignorance and active regulation. Dysregulation of either central or peripheral tolerance has been associated with the development of autoimmunity in both animal models and patient studies [21–27].

Disease models have contributed significantly to a detailed mechanistic understanding of human autoimmune disease in general. Unlike many other conditions, animal models of RA have tended to focus on the later stages of disease pathogenesis. However, recent clinical studies demonstrating the predictive utility of anti-citrullinated protein/peptide antibodies (ACPA) for subsequent development of RA reveal that the most critical checkpoint, breach of self-tolerance, occurs months or years prior to clinical presentation [28–30]. This is the least well studied or understood aspect of the disease, due to the limitations of existing rodent models to dissect events surrounding the induction of autoimmunity. For example, the majority of animal studies employ powerful, non-physiological adjuvants or a large number of self-reactive TCR transgenic cells to drive breach of tolerance to exogenously administered self-antigens [31–35]. Therefore, little is known about the immunological processes that lead to the spontaneous loss of tolerance that is characteristic of human disease. This represents a bottleneck to the clinical development of prevention and early intervention strategies.

3. Factors involved in breach of self-tolerance

A critical role for adaptive immunity in the pathogenesis of RA is supported by the presence of activated T cells in the synovial lesion, by long established association with *HLADR04* and by recent genome wide scanning studies implicating *ptpn22*, *cd40*, *ctla4* and *cd28* [36–38]. Definitive proof of concept for adaptive immune involvement resides in the proven efficacy of therapeutics such as rituximab and abatacept, targeting B and T lymphocytes, respectively [39,40]. The conglomeration of genetic and environmental factors promoting breach of self-tolerance, autoimmunity and progression to pathology are legion, yet our understanding of their integration is limited (see summary in Table 1).

3.1. Genetics

The description of the Human Leukocyte Antigen (HLA) association with RA is the strongest evidence for the genetic basis of disease [41]. Most patients with RA express particular HLA-DR alleles such as HLA-DRB1*0401, *0404, *0405, *0408, *0101, *0102, *1001 and *1402 [42]. RA associated HLA-DR alleles share a highly conserved amino acid motif (^{70}QRRAA^{74}, ^{70}RRRAA^{74} or ^{70}QKRAA^{74}) expressed in the third hypervariable region of the DRB1 chain, termed the shared epitope (SE) [42]. Apart from MHC, the best established locus of susceptibility for RA is protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) which encodes Lyp, a tyrosine phosphatase known to be a powerful inhibitor of T cell activation [22]. The minor allele of a single nucleotide polymorphism (SNP) in PTPN22 has been linked to autoimmunity [24]. This SNP has been suggested to result in a gain of function, increasing the T cell activation threshold, which leads to a failure of thymic negative selection. Genome wide association studies (GWAS) have confirmed the association of HLA-DR1 and PTPN22 with RA [25]. Other candidate genes are CTLA-4, the α and β chain of the IL-2 receptor (IL-2RA and IL-2RB), genes of the TNF pathway (TNFAIP2 (tumour necrosis factor, alpha-induced protein)) and in the regulation of T-cell function by granzyme B (GZMB) [25].

3.2. Environment

The amount of data relating to environmental factors that contribute to the development of the disease is surprisingly scarce. Smoking is the environmental factor most strongly linked to an increased risk of developing RA [43–47]. A link has been demonstrated between the HLA-DRB1 shared epitope (SE), citrullination and smoking [48,49]. Antibodies to antigens modified by citrullination, through deamination of arginine to citrulline (anti-cyclin citrullinated peptide (anti-CCP) antibodies), are present in approximately two-thirds of RA patients but are rare in other inflammatory conditions [12]. It has been proposed that smoking triggers citrullination by activation of peptidylarginine diaminase (PAD) [49] in alveolar antigen presenting cells (APCs), enabling efficient antigen presentation of the post-translationally modified peptides for which the immune system has not developed tolerance [44,50]. In addition, it has been reported that the conversion of arginine to citrulline at the peptide side-chain position interacting with the SE significantly increases peptide-MHC affinity, which could lead to an immune response in individuals carrying the susceptible HLA-DRB1 alleles [51]. Recently great attention has been given to the immunomodulatory role of mucosal microflora [52,53]. Interest-

Table 1
Environmental factors and RA.

Environmental factor	Effect	References
Smoking	Increased risk, dependent on magnitude and length of habit, association with anti-CCP antibodies	[43,45,46,48,50,156]
Alcohol	May decrease risk, lower risk for anti-CCP positive RA	[156,157]
High birth weight	Increased risk	[158]
Oral contraceptives	Lowers the risk of RF positivity	[159]
Breast feeding	Reduced risk	[160]
Socioeconomic status	Inverse association between socioeconomic status, measured by occupational class and education and RA	[161]
Geography	Location of birth and current residence is associated with differential risk of RA	[162]
Microbiota	Intestinal flora could be protective, *P. gingivalis* promotes disease	[52–55]

ingly, patients with early RA have different intestinal microflora than non-RA patients [54]. It has been proposed that normal intestinal microflora may protect against the development of inflammatory diseases [53,55,56]. Mice deficient in a G-protein coupled receptor that recognises products of fibre metabolism by gut microbes developed exacerbated arthritis in the KxB/N serum-induced arthritis model, which suggests that commensal bacteria might be required for regulation of the immune response. Other reports suggest that components of the microflora drive arthritis development [57,58]. A prime example of a possible link between the mucosal microflora and RA pathogenesis is *Porphyromonas gingivalis*. *P. gingivalis* has been linked to the development of immunity against citrullinated proteins due to its ability to produce citrullinated epitopes [59–62] and its presence in an environment highly analogous to RA, characterised by bone erosion and chronic inflammation.

4. Establishment of breach of self-tolerance and autoimmunity

Though adaptive immunity appears to be a central component of RA pathogenesis, clearly a variety of cell types contribute to disease. For example, it has recently been proposed that the progression to pathology in RA is a four-step process, which does not require antigen-specific recognition events. These four steps have been proposed as (i) T cell activation regardless of antigen specificity, (ii) local events inducing tissue-specific accumulation of activated T cells, (iii) enhanced sensitivity to T cell-derived cytokines in populations of cells in affected tissue, and (iv) activation of a cytokine-dependent IL-6 amplification loop, "IL-6 amplifier", triggered by $CD4^+$ T cell-derived cytokines such as IL-17A [63]. However, to some extent this is a question of semantics, as there is considerable evidence to support not only roles for non-immune components, but also innate and adaptive immunity and importantly, their integration as summarised in Fig. 1 and detailed below.

5. Non-immune cells

It is becoming increasingly clear that cells not conventionally considered immune cells have significant impact on the organisation, localisation and function of the immune system.

5.1. Osteoclasts

Osteoclasts are multinucleated cells of haemopoeitic origin, the primary bone reabsorbing cells, and are essential for the remodelling of bone throughout life [64]. These giant cells contain up to 20 nuclei [65] and only a few clinical conditions induce their local formation, one of these being RA. Synovial inflammation can drive osteoclastogenesis [65,66]; the synovial membrane contains many monocytes/macrophages that can undergo osteoclast differentiation under the appropriate conditions. Cell types considered very important in providing differentiation signals for monocytes to become osteoclasts are fibroblasts and T cells. Fibroblasts are the cells of the pannus, a major component of the hyperplastic synovial membrane and express receptor activator of nuclear factor κB (NFκB) ligand (RANKL), which is a major driver of osteoclast formation [67]. T lymphocytes, apart from RANKL, express cytokines such as IL-17 that support osteoclast formation [68]. Other cytokines present in the synovial environment such as TNF, IL-1 and IL-6 also are important in RANKL upregulation and possibly on osteoclast formation [65]. From models of arthritis it is evident that osteoclast formation is an early and rapid pathological event [69], which eventually leads to the destruction of the joint.

5.2. Synovial fibroblasts

Synovial fibroblasts, together with synovial macrophages, are the main cellular components of the synovial membrane. RA synovial fibroblasts are now considered active drivers of RA pathology [70]. The physiological function of these cells is to provide the joint

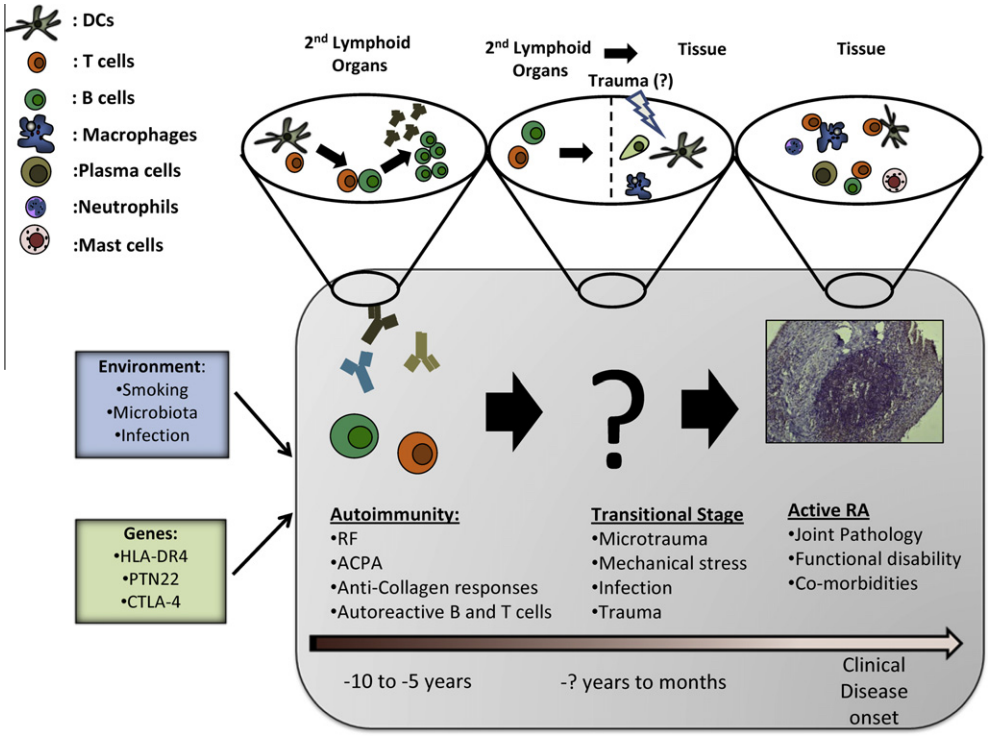

Fig. 1. Pathogenesis of rheumatoid arthritis? Summary of potential factors involved in inflammatory arthritis, how they may integrate at different stages of disease pathogenesis, highlighting uncertainty regarding the mechanisms underlying transition from the prearticular to acute pathology state.

cavity and cartilage with plasma proteins and lubricating molecules such as hyaluronic acid. Human synovial fibroblasts contribute to disease pathology through the production of inflammatory mediators and chemokines, such as VEGF, IL-15, interferon-β (IFNβ), IL-8, CXCL2, CCL8, CCL5, CXCL10 [71–74] and degradative enzymes, notably cathepsins and matrix metalloproteases [75–77]. It should be noted that RA synovial fibroblasts differ considerably from healthy joint fibroblasts. RA fibroblasts have an activated phenotype, which is characterised by morphological differences, long-term growth, reduced apoptosis and an altered response to various stimuli. A recent study demonstrated that RA fibroblasts were able to spread pathology by invading unaffected joints, an ability lacking in non-RA fibroblasts [78]. Various mechanisms could be involved in the development of this phenotype, amongst them cytokines and growth factors (FGF, IL-17, IL-18, TNF and IL-1) [70,79–82], articular hypoxia of the rheumatoid joint activating the production of pro-angiogenic and pro-inflammatory factors [83], and expression of proto-oncogenes and tumour suppressors [84].

6. Innate immune cells

6.1. Monocytes/macrophages

The predominance of macrophage-derived cytokines in the synovial compartment signifies the importance of this cell type in the pathology of RA. In the normal synovial membrane, macrophages predominate in the lining where they scavenge debris from articular structures and eliminate all microorganisms entering via the blood or following trauma [85]. In the inflamed synovial membrane activated macrophages are one of the most abundant cell types [85] and the degree of macrophage infiltration directly correlates with clinical status and progression of joint damage [86,87]. At the tissue level, pre-activated monocytes infiltrate the synovial membrane, mature into macrophages, which are activated and interact with other synovial cells [88]. Activated macrophages contribute to the progression of pathology through production of a panoply of mediators such as pro-inflammatory cytokines (e.g., TNF-α, IL-1, IL-6 and GM-CSF), chemoattractants and chemokines (e.g., IL-8, MCP-1 and MIP-1) and matrix metalloproteinases (e.g., MMP9 and MMP12) which may all contribute to tissue destruction [48]. Clearly, macrophages may also play a role in antigen presentation, though it appears that the major player in this function to link innate and adaptive immunity is the dendritic cell (DC).

6.2. Dendritic cells

DCs comprise a complicated population of heterogeneous APCs that are critical for the initiation of the adaptive immune response and the maintenance of both central and peripheral tolerance [89]. DCs in both human and mice can be divided into subsets according to tissue distribution, function and phenotype [90]. DCs have been identified in RA synovial fluid and synovial tissue by several groups but their origin, function and potential role in the pathogenesis are not fully understood [91,51]. A major subdivision can be made between conventional and plasmacytoid DCs (cDCs and pDCs, respectively) [90]. Contrary to other APCs, DCs can prime naïve T cells for helper and cytotoxic functions, are essential for the generation of primary antibody responses, and are powerful enhancers of natural killer cells [92]. DCs can potentially play a number of roles in the pathogenesis of RA. Firstly, DCs could prime autoimmune responses by presenting self-antigens. Our group has demonstrated that the presentation of collagen derived peptides by mature bone marrow derived DCs is sufficient to induce arthritis in DBA/1 mice [93]. More importantly, in a model of pre-clinical arthritis, we have demonstrated that conventional DCs are the cells that orchestrate the initial breach of self-tolerance [94]. Secondly, DCs could infiltrate the synovial tissue and fluid where they could take up and present antigen locally, perpetuating the disease, however there are no direct evidence to support this [95]. Furthermore, DCs, alongside with other immune cells and synoviocytes produce inflammatory mediators that drive RA pathology [96].

DCs are critical for peripheral and central tolerance. Both cDCs and pDCs have been suggested to have tolerogenic abilities in different environmental settings. We have previously demonstrated, using a breach of self-tolerance model in the context of arthritis, that pDCs can function to limit self-reactivity and the consequent pathology [96]. To further support a regulatory role for pDCs a tolerogenic $CCR9^+$ pDC population, which can suppress acute host versus graft disease, has been also demonstrated [97]. Even though there is an incomplete understanding of how DCs are involved in RA pathology, DCs therapies are currently under development with some success in murine models [98–100], whereas clinical trials have been initiated in UK (http://www.news.bbc.co.uk/1/hi/health/7560535.stm) and Australia (http://www.uq.edu.au/news/?article=13128). It should also be noted that the success/or not of DC therapeutics will no doubt rely on important spatiotemporal information, i.e., do the DCs need to be targeted to the secondary lymphoid tissues or somatic tissue, and how effective this approach is at different stages of disease progression?

7. Adaptive immune cells

7.1. B cells

A critical role for B cells in RA pathogenesis is now supported by the clear association of particular autoantibody specificities, elevation of B cell survival factors (e.g., APRIL, BAFF and IL-21) and altered circulating B cell subpopulations [101,102], with progression to disease and the therapeutic effectiveness of B cell depletion. More circumstantial evidence includes the localisation of B cells and their activation and formation of ectopic germinal centres in RA synovial membranes. The first reported contribution of B cells, helped by the accessibility of peripheral blood, was the identification of rheumatoid factor (RF), an autoantibody against the Fc portion of human IgG. RF is found in about 80% of RA patients but it is not specific to RA and can be found in other autoimmune conditions and even in 10% of healthy individuals [1]. There are differences between RF in health and disease. In healthy individuals, the RF isotype is IgM and is produced by B1 cells as "natural" antibody with low affinity and polyreactivity. In contrast, in RA patients, RF has undergone affinity maturation and class switching [103]. The association of cognate T cell/B cell interactions with disease is further highlighted by the diagnostic value of class switched anti-CCP antibodies [104]. B cell infiltration is less prominent in samples of synovial tissue that lack a defined level of organisation of immune cells, whereas they are more significant in samples characterised by large and well organised mononuclear aggregates [105]. In synovial membranes, B cells are the major source of lymphotoxin-B (LT-β), an important cytokine in normal lymphoid organogenesis [106] suggesting a significant role of B cells in ectopic lymphoid tissue organisation. A defined lymphoid architecture in synovial tissue, with B cells in close proximity to T cells, provides the appropriate microenvironment for B cell activation. In addition, increased B cell activation markers, such as Blys and APRIL, have been reported in RA synovial membrane [107]. B cells can act as efficient APCs in an antigen specific manner to stimulate T cells and to allow optimal $CD4^+$ T cell memory. RF^+ B cells can take up antigen-IgG immune complexes via their membrane Ig receptors, which are IgG specific. B cells then process and present peptides from the antigen and provide T cell activation and help,

which could lead to responses against self-antigens [108]. Finally, B cells are also a source of various cytokines, most importantly IL-6, TNF and LT, following BCR ligation and CD40 activation [109]. These cytokines have a profound autocrine impact on B cell activity and differentiation, and serve to amplify the ongoing immune response. As noted above, the importance of B cells in RA pathology is defined by the effectiveness of B cell depletion therapies. Rituximab is a potent CD20-specific monoclonal antibody, which can kill B cells from the pre-B-cell stage to the pre-plasma-cell stage [110]. Several B cell depletion agents are now under investigation with the re-establishment of immunological tolerance as a major goal.

7.2. T cells

The evidence for T cell involvement in RA include the association of HLA-DR4 and HLA-DR1 alleles, the effects of T cell derived cytokines, T-cell dependent experimental models of arthritis, and the anti-arthritic effects of T-cell directed therapies. Numerous animal models confirm the genetic evidence for T-cell involvement in RA. An intrinsic defect in TcR signalling, where a spontaneous point mutation alters the encoding of an SH-2 domain of ZAP70, can lead to T-cell dependent arthritis in mice [21]. On the other hand, immunization with glucose-6-phosphate isomerase induces T-cell dependent polyarthritis in DBA/1 mice [111]. We have developed a model of breach of self-tolerance, where a Th1 response to irrelevant antigen (OVA) results in arthropathy associated with spontaneous induction of autoreactive T and B cell responses [112]. Recent studies suggest that a new subset of effector T-cells distinct from T helper (Th) 1 and Th2 subsets, producing IL-17 and termed Th17, characterises the pathology of rheumatoid arthritis in animal models at least. The contribution of an additional T cell subset (Treg) must also be considered, where the balance of active regulation by $FoxP3^+$ regulatory T cells and pathogenic Th subsets impact on disease progression [113]. T cells have also been detected in RA synovial membrane and are mainly activated memory $CD4^+$ $CD45RO^+$ cells [114]. In patients with RA and ankylosing spondylitis, IL-17^+ and IL-22^+ $CD4^+$ T cells could be detected in the circulation and were increased compared to healthy controls [115]. Development of this Th17 responses is likely to occur early in disease pathogenesis, with dysregulated Th17 related cytokine production evident in VERA (very early RA) patients [116]. Various T-cell directed therapies have been developed with the greatest success being abatacept, a fully human recombinant fusion protein consisting of the extracellular domain of endogenous inhibitory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4) and the Fc domain of human IgG_1 [114].

8. Cellular interactions in RA

The interactions between the cell types noted above need to be choreographed to elicit an adaptive immune response. The induction of an adaptive immune response begins when an antigen is ingested by immature DCs in the presence of pathogen associated molecular patterns (PAMPS) or danger associated molecular patterns (DAMPS) [117–119]. The activated DC are carried away from the involved tissue in lymph, along with their antigen cargo, to enter peripheral lymphoid tissue in which they can interact with naïve T cells and initiate the adaptive immune response [119,120]. Activated DCs will interact with antigen specific T cells, which in response will proliferate and differentiate into effector Th cells [119,121–123]. We hypothesise that these events are identical or similar in autoimmunity, but why they are dysregulated to allow responses to self-antigens remains unclear. In both of these situations our current knowledge has depended on static analysis of cells in/from fixed tissues. The important subtleties of the dynamic and localised nature of cellular interactions of the immune system deep within tissues have only become tractable following recent technological developments, and this allows us to answer critically important questions. Obviously movement and interaction of the components of the immune system occur in specialised compartments in vivo, however it is clear that these interactions are also highly dynamic. For example, studies using intravital-multiphoton microscopy have demonstrated that the movement behaviour of T cells in intact lymph nodes, following primary and secondary exposure to immunogenic or tolerogenic forms of antigen, are quantitatively different [124,125]. More recent studies have also begun to confirm the long suspected importance of immune surveillance and reveal the underlying molecular mechanisms involved in this process. These studies also highlight the importance of scale within the immune system, as migration and localisation are important at levels from cell through organ to organism. At one end of the scale, the requirement for T cell migration into B cell follicles for the evolution of a productive immune response has long been appreciated (reviewed in [126,127]) and the role of this process in the pathogenesis of a range of autoimmune diseases is seen as an increasingly important therapeutic target [128]. Indeed, we have recently revealed that abatacept (CTLA-4-Ig) may act by suppression of T cell follicular migration [129]. At the other end of the scale, the migration of various cell types around the body and in and out of joints is perceived as crucial to the pathogenesis of RA [130] but remains relatively understudied. In both cases, determining the molecules (e.g., chemokines and integrins) responsible for regulation of migration and localisation will be the key steps to therapeutic intervention [131].

9. Localisation of pathological consequences

How, why and whether the systemic immune responses initiated and expanded in secondary lymphoid organs are subsequently focused on the joints in RA are crucial questions. The answers may lie in the biomechanics of the joint itself, the relationship between address codes of eliciting and target organ and whether we consider RA to be a tissue specific or systemic disease. Importantly, we need to consider the location and timing of the key events of the initiation, establishment, maintenance and regulation of autoimmune responses in RA.

10. Why joints?

The major clinical sign of RA is joint pathology, which manifests as a symmetric polyarthritis with associated swelling and pain in multiple joints, often initially in the joints of the hand, wrist and feet [132]. This is recapitulated in many animal models, however why the systemic autoimmunity that characterises the preclinical phase of the disease eventually targets the joints is still unknown. Interestingly, our own studies using an adoptive transfer model of arthropathy reveal early involvement of the articular environment was prerequisite for development of autoreactive responses as immunisation in other systemic sites did not lead to autoimmune arthritis (Benson, R.A., unpublished data). Reasons related to the environment and function of the joint, namely biomechanical stress, hypoxia, and trauma could potentially explain its preferential involvement in RA. Joint overuse and misuse in conjunction with trauma have been linked to the development of osteoarthritis [133], however their role in RA development is not clear. Interestingly, a case control study links physical trauma with RA onset [134], whereas in experimental arthritis development of the disease was associated with joint microbleeding [135]. We could speculate that local microtrauma or infection leads to inflammation, damage, antigen release and activation of resident DCs, which

in genetically susceptible and environmentally conditioned individuals, target the autoimmune response to the joint. In experimental arthritis hypoxia-induced cell death was linked to the release of DAMPS, such as HMGB1, that perpetuated the inflammatory response [136]. Whether local trauma, in the relatively hypoxic joint environment, can initiate events like these is unknown, however it would be intriguing to hypothesise that cell death and sterile damage-related signals dictate tissue localisation.

11. Limitations to current models

Major contributions to the understanding of RA pathogenesis have been made using mouse models of disease. These have allowed some of the intricacies of cellular interactions and cytokine networks to be deciphered that promote inflammatory arthropathies. However, some of the most widely used animal models have important limitations. The contribution of $CD4^+$ T cells to immune mediated arthritis has clearly been demonstrated in the collagen induced arthritis model (CIA) [137]. While serum transfer from CIA mice results in arthritic inflammation in susceptible mice without stimulation of collagen specific T cells, disease is transient and mostly does not result in cartilage erosion [138,139]. A collagen-derived peptide, CII 250–270, has already been used in an antigen specific approach for prevention/treatment of arthritis in CIA [140]. Oral administration of this peptide suppresses antigen specific T cell and antibody responses. Crucially, further characterisation has revealed that the immunogenic CII peptide 256–270 binds to I-Aq via the same key anchor residues (positions 263 and 264) as when binding DR1 and DR4 [141–143]. A recent study has demonstrated the effectiveness of antigen specific intervention in CIA utilising this peptide. A monomeric murine recombinant T cell receptor ligand containing single chain two domain I-Aq molecules covalently linked to the immunogenic CII peptide reduced clinical and histological signs of CIA and induced long term tolerance when given as a prophylactic [144]. The effectiveness of this ingenious antigen specific therapy has also been proven in EAE [145,146]. Unfortunately, this does not portray the complexity of human disease, where spontaneously arising auto-reactive T cells are likely to be of multiple antigen specificities. These studies rely on breaching existing tolerance to a single self-antigen before re-establishing tolerance to the same antigen. Identification of antigen specificity in spontaneously arising autoimmune arthritis would be considerably more beneficial in understanding how breach of tolerance is likely to occur in human RA. Spontaneously arising arthropathy is seen in the K/BxN mouse [35]. In this model, disease occurs in the F1 progeny of NOD mice crossed with the KRN TCR transgenic mouse. In this system the transgenic TCR shows reactivity with glucose-6-phosphate isomerase (GPI) in the context of $I\text{-}A^{g7}$. Pathogenesis relies on T cell activation of B cells and their production of complement fixing GPI specific antibody. A newly described spontaneous arthritis model where HA is expressed as a self-antigen under an MHC class II promoter is driven by HA specific TCR Tg CD4 T cells [147]. While both of these models develop arthritis spontaneously and share many characteristics with human disease, they both utilise a single specificity TCR transgenic to initiate/maintain disease. Ideally, multiple T cell antigen specificities should be involved in a disease model. Such spontaneously arising auto-reactive T cell responses are evident in SKG mice [21,148]. Here, a point mutation in the gene encoding the TCR signalling molecule ZAP-70 results in altered thymic selection. These mice have high titres of rheumatoid factor, anti-type II collagen, anti-CCP and heat shock protein reactive antibodies, demonstrating multiple antigen specific response. However, the antigens recognised by T cells remain unknown.

Understanding the timing, location and mechanisms of the 'breach of tolerance' that we hypothesise leads to RA, will require the ability to identify and track spontaneously arising arthritogenic cells in mice and humans. We have developed a novel model of immune mediated arthritis in which stimulation of a trackable, T cell population with irrelevant antigen specificity, induces local articular inflammation and leads to spontaneous breach of B and T cell tolerance [112,149]. This spontaneous breach of self-tolerance is dependent on conventional dendritic cells [94], can be exacerbated by depletion of plasmacytoid dendritic cells [96] and does not require dual, therefore potentially autoreactive, TcR expression by the inciting transferred T cell population (confirmed by adoptive transfer of DO11.10 SCID TcR tg T cells which can only express a single TcR specific for OVA, Benson, R.A., unpublished data). This initial phase of inflammation is self-resolving over a period of 14 days and represents induction of a preclinical/arthropathy susceptible phase. Despite minimal evidence for footpad inflammation after this point, autoantibody titres continue to rise, making mice more susceptible to disease following a second footpad rechallenge, resembling flares in human disease. This aspect of the model directly reflects human disease in which rheumatoid factor is detectable long before presentation with clinical symptoms. Deciphering the development of pathophysiological T cell responses in relation to a previous inflammatory event also makes this model particularly relevant to human disease where clinical presentation and later disease flares are often associated with infectious events. No peptide sequence homology between ovalbumin and type II collagen has been detected but the production of auto-reactive T cell and antibody responses is both site and antigen specific, relying on introduction of the priming antigen (OVA) via the footpad. The spontaneous nature by which autoreactive T cells are primed in this model represents a major advantage over "antigen immunisation" models. Additionally, this autoimmune response can be studied in the context of a trackable initiating event, the OVA specific inflammation. Definition of target antigens and subsequent construction of recombinant T cell receptor ligands will facilitate identification/tracking of auto-reactive $CD4^+$ T cells allowing delineation of events leading to their priming and subsequent disease. Tracking of auto-reactive T cells will enable definition of the immune context in which they are primed. Once auto-reactive T cells can be identified, the APCs these clones interact with can be characterised (which cell type; their activation state; tissues they have come from and how they acquire antigen), thus facilitating delineation of the conditions promoting autoreactivity.

12. Importance of imaging

Imaging techniques (e.g., PET, SPECT and MRI) have impacted on arthritis from a diagnostic and assessment of pathology point of view [150,151]. In contrast, we have emphasised in the preceding sections the need to perform imaging with cellular resolution. The most effective approach to acquire this type of data is through in vivo optical imaging. Optical imaging will allow us to undertake the important, detailed, kinetic studies of cellular behaviour required in both lymphoid and disease relevant tissues during initiation, maintenance and resolution/regulation of autoimmunity and pathology. For clinical assessment and treatment of RA, it is clear that we must develop imaging modalities that maximise our spatial resolution within the joint. To this end, the need for high sensitivity, non-invasive imaging is paramount. With conventional multiphoton microscopy analysis being limited to a few hundred nanometres beneath the surface, multiphoton endoscopy (e.g., GRIN imaging) allows unparalleled imaging within the joint. In conjunction with this, second harmonic generation (SHG) signals

produced intrinsically in the body by collagen and elastin fibres in the joint may be visualised without the need for extrinsic fluorophores. This approach has recently been applied to study morphological and structural alterations occurring in the joint during the onset of arthritis in the SKG murine model [152]. Alternatively, non-invasive approaches with intrinsically deeper imaging potential include surface enhanced resonance Raman scattering (SERRS) imaging which can detect signal at depths of around 1–2 cm in mice [153]. Using these microendoscopic approaches have enabled imaging of human muscle dynamics [154] as well as deep brain imaging of the effect of tumour growth on gliomas in mice [155]. The relatively small sizes (300–1000 μm) of these lenses, suggests their potential for future studies of cell or structural fluorescence in joints.

Future developments are required in technologies such as light activated probes and drugs, far red fluorophores allowing deeper tissue imaging, enhancers/modulators of reporter fluorescent proteins, the ability to image and analyse multiple signals and finally, but maybe most importantly the ability to analyse the complex data sets that will be generated.

13. Future perspectives

Reductionist approaches have provided a list of cells and molecules involved in autoimmunity. The challenge now is to integrate this information into a working model of RA pathogenesis (e.g., Fig. 1), accounting for temporal and tissue specific aspects of disease. Developments in personalised medicine, in terms of earlier diagnosis, mean that it is now possible to intervene at the earliest stages of disease to reduce progression to pathology. Continued advances should mean that we could advance to a stage where the initial breach of tolerance is prevented. Although this is the ultimate aim, advancing our understanding of breach of tolerance in spatiotemporal in vivo systems has the additional advantage of rationalising use of existing therapeutics, i.e., *right drug, right time, right place, right person*.

References

[1] Firestein, G.S. (2003) Evolving concepts of rheumatoid arthritis. Nature 423, 356–361.
[2] Jorizzo, J.L. and Daniels, J.C. (1983) Dermatologic conditions reported in patients with rheumatoid arthritis. J. Am. Acad. Dermatol. 8, 439–457.
[3] Wolfe, F. and Michaud, K. (2004) Heart failure in rheumatoid arthritis: rates, predictors, and the effect of anti-tumor necrosis factor therapy. Am. J. Med. 116, 305–311.
[4] Geddes, D.M., Webley, M. and Emerson, P.A. (1979) Airways obstruction in rheumatoid arthritis. Ann. Rheum. Dis. 38, 222–225.
[5] Herbison, G.J., Ditunno, J.F. and Jaweed, M.M. (1987) Muscle atrophy in rheumatoid arthritis. J. Rheumatol. Suppl. 14 (Suppl. 15), 78–81.
[6] Seymour, H.E., Worsley, A., Smith, J.M. and Thomas, S.H. (2001) Anti-TNF agents for rheumatoid arthritis. Br. J. Clin. Pharmacol. 51, 201–208.
[7] Symmons, D. et al. (2002) The prevalence of rheumatoid arthritis in the United Kingdom: new estimates for a new century. Rheumatology (Oxford) 41, 793–800.
[8] Michaud, K. and Wolfe, F. (2007) Comorbidities in rheumatoid arthritis. Best Pract. Res. Clin. Rheumatol. 21, 885–906.
[9] Hueber, A.J. et al. (2010) Mast cells express IL-17A in rheumatoid arthritis synovium. J. Immunol. 184, 3336–3340.
[10] Lemos, H.P. et al. (2009) Prostaglandin mediates IL-23/IL-17-induced neutrophil migration in inflammation by inhibiting IL-12 and IFNgamma production. Proc. Natl. Acad. Sci. USA 106, 5954–5959.
[11] McInnes, I.B. et al. (1996) The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. Nat. Med. 2, 175–182.
[12] McInnes, I.B., Leung, B.P., Sturrock, R.D., Field, M. and Liew, F.Y. (1997) Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. Nat. Med. 3, 189–195.
[13] Feldmann, M. (2002) Development of anti-TNF therapy for rheumatoid arthritis. Nat. Rev. Immunol. 2, 364–371.
[14] Breedveld, F.C. and Kalden, J.R. (2004) Appropriate and effective management of rheumatoid arthritis. Ann. Rheum. Dis. 63, 627–633.
[15] Barton, A., Thomson, W. and Ke, X. (2008) Wellcome Trust Case Control Consortium; YEAR Consortium; BIRAC Consortium. Rheumatoid arthritis susceptibility loci at chromosomes 10p15, 12q13 and 22q13. Nat. Genet. 40, 1156–1159.
[16] Bluestone, J.A. (2011) Mechanisms of tolerance. Immunol. Rev. 241, 5–19, doi:10.1111/j.1600-065X.2011.01019.x.
[17] Gallegos, A.M. and Bevan, M.J. (2006) Central tolerance: good but imperfect. Immunol. Rev. 209, 290–296.
[18] Hogquist, K.A., Baldwin, T.A. and Jameson, S.C. (2005) Central tolerance: learning self-control in the thymus. Nat. Rev. Immunol. 5, 772–782.
[19] Basten, A. and Silveira, P.A. (2010) B-cell tolerance: mechanisms and implications. Curr. Opin. Immunol. 22, 566–574.
[20] Mueller, D.L. (2010) Mechanisms maintaining peripheral tolerance. Nat. Immunol. 11, 21–27.
[21] Sakaguchi, N. et al. (2003) Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature 426, 454–460.
[22] Michou, L. et al. (2007) Linkage proof for PTPN22, a rheumatoid arthritis susceptibility gene and a human autoimmunity gene. Proc. Natl. Acad. Sci. 104, 1649–1654.
[23] Chung, S.A. and Criswell, L.A. (2007) PTPN22: its role in SLE and autoimmunity. Autoimmunity 40, 582–590.
[24] Bowes, J. and Barton, A. (2008) Recent advances in the genetics of RA susceptibility. Rheumatology 47, 399–402.
[25] The Wellcome Trust Case Control Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. Nature 447, 661–678.
[26] Brunkow, M.E. et al. (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat. Genet. 27, 68–73.
[27] Bennett, C.L. et al. (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat. Genet. 27, 20–21.
[28] Nielen, M.M. et al. (2005) Antibodies to citrullinated human fibrinogen (ACF) have diagnostic and prognostic value in early arthritis. Ann. Rheum. Dis. 64, 1199–1204.
[29] Majka, D.S. et al. (2008) Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. Ann. Rheum. Dis. 67, 801–807.
[30] Rantapaa-Dahlqvist, S. et al. (2003) Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 48, 2741–2749.
[31] Brand, D.D., Latham, K.A. and Rosloniec, E.F. (2007) Collagen-induced arthritis. Nat. Protoc. 2, 1269–1275.
[32] Anthony, D.D. and Haqqi, T.M. (1999) Collagen-induced arthritis in mice: an animal model to study the pathogenesis of rheumatoid arthritis. Clin. Exp. Rheumatol. 17, 240–244.
[33] Swanborg, R.H. (1995) Animal models of human disease. Experimental autoimmune encephalomyelitis in rodents as a model for human demyelinating disease. Clin. Immunol. Immunopathol. 77, 4–13.
[34] Kouskoff, V. et al. (1996) Organ-specific disease provoked by systemic autoimmunity. Cell 87, 811–822.
[35] Monach, P. et al. (2007) The K/BxN mouse model of inflammatory arthritis: theory and practice. Methods Mol. Med. 136, 269–282.
[36] Raychaudhuri, S. et al. (2008) Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat. Genet. 40, 1216–1223.
[37] Seidl, C. et al. (1998) CTLA4 codon 17 dimorphism in patients with rheumatoid arthritis. Tissue Antigens 51, 62–66.
[38] Raychaudhuri, S. et al. (2009) Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. Nat. Genet. 41, 1313–1318.
[39] De Vita, S. et al. (2002) Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenetic role of B cells. Arthritis Rheum. 46, 2029–2033.
[40] Kremer, J.M. et al. (2003) Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N. Engl. J. Med. 349, 1907–1915.
[41] Gregersen, P.K. (1999) Genetics of rheumatoid arthritis: confronting complexity. Arthritis Res. 1, 37–44.
[42] Auger, I., Roudier, C., Guis, S., Balandraud, N. and Roudier, J. (2007) HLA-DRB1*0404 is strongly associated with anti-calpastatin antibodies in rheumatoid arthritis. Ann. Rheum. Dis. 66, 1588–1593.
[43] Alan, J.S., Jason, N. and Alexander, J.M. (1996) Cigarette smoking increases the risk of rheumatoid arthritis: results from a nationwide study of disease-discordant twins. Arthritis Rheum. 39, 732–735.
[44] Klareskog, L., Padyukov, L., Rnnelid, J. and Alfredsson, L. (2006) Genes, environment and immunity in the development of rheumatoid arthritis. Curr. Opin. Immunol. 18, 650–655.
[45] Saag, K.G. et al. (1997) Cigarette smoking and rheumatoid arthritis severity. Ann. Rheum. Dis. 56, 463–469.
[46] Costenbader, K.H., Feskanich, D., Mandl, L.A. and Karlson, E.W. (2006) Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. Am. J. Med. 119, 503.
[47] Stolt, P. et al. (2003) Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann. Rheum. Dis. 62, 835–841.

[48] Padyukov, L. et al. (2004) A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum. 50, 3085–3092.
[49] Klareskog, L. et al. (2006) A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 54, 38–46.
[50] Woodruff, P.G. et al. (2005) A distinctive alveolar macrophage activation state induced by cigarette smoking. Am. J. Respir. Crit. Care Med. 172, 1383–1392.
[51] Hill, J.A. et al. (2003) Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC Class II molecule. J. Immunol. 171, 538–541.
[52] Maslowski, K.M. and Mackay, C.R. (2011) Diet, gut microbiota and immune responses. Nat. Immunol. 12, 5–9.
[53] Wen, L. et al. (2008) Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 455, 1109–1113.
[54] Eerola, E. et al. (1994) Intestinal flora in early rheumatoid arthritis. Rheumatology 33, 1030–1038.
[55] Maslowski, K.M. et al. (2009) Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461, 1282–1286.
[56] Mazmanian, S.K., Round, J.L. and Kasper, D.L. (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 453, 620–625.
[57] Wu, H.J. et al. (2010) Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 32, 815–827.
[58] Yoshitomi, H. et al. (2005) A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. J. Exp. Med. 201, 949–960.
[59] Lundberg, K. et al. (2008) Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. Arthritis Rheum. 58, 3009–3019.
[60] Klareskog, L., Ronnelid, J., Lundberg, K., Padyukov, L. and Alfredsson, L. (2008) Immunity to citrullinated proteins in rheumatoid arthritis. Annu. Rev. Immunol. 26, 651–675.
[61] Wegner, N. et al. (2010) Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum. 62, 2662–2672.
[62] Mikuls, T.R. et al. (2009) Antibody responses to *Porphyromonas gingivalis* (*P. gingivalis*) in subjects with rheumatoid arthritis and periodontitis. Int. Immunopharmacol. 9, 38–42.
[63] Ogura, H. et al. (2008) Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. Immunity 29, 628–636.
[64] Teitelbaum, S.L. (2000) Bone resorption by osteoclasts. Science 289, 1504–1508.
[65] Schett, G. (2007) Cells of the synovium in rheumatoid arthritis. Osteoclasts. Arthritis Res. Ther. 9, 203.
[66] Gravallese, E.M. et al. (1998) Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am. J. Pathol. 152, 943–951.
[67] Ellen, M.G. et al. (2000) Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum. 43, 250–258.
[68] Sato, K. et al. (2006) Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J. Exp. Med. 203, 2673–2682.
[69] Georg, S. et al. (2005) Analysis of the kinetics of osteoclastogenesis in arthritic rats. Arthritis Rheum. 52, 3192–3201.
[70] Muller-Ladner, U., Ospelt, C., Gay, S., Distler, O. and Pap, T. (2009) Cells of the synovium in rheumatoid arthritis. Synovial fibroblasts. Arthritis Res. Ther. 9, 223–233.
[71] Cho, M.L. et al. (2007) Toll-like receptor 2 ligand mediates the upregulation of angiogenic factor, vascular endothelial growth factor and interleukin-8/CXCL8 in human rheumatoid synovial fibroblasts. Immunol. Lett. 108, 121–128.
[72] Jung, Y.O. et al. (2007) Toll-like receptor 2 and 4 combination engagement upregulate IL-15 synergistically in human rheumatoid synovial fibroblasts. Immunol. Lett. 109, 21–27.
[73] Pierer, M. et al. (2004) Chemokine secretion of rheumatoid arthritis synovial fibroblasts stimulated by toll-like receptor 2 ligands. J. Immunol. 172, 1256–1265.
[74] Fabia, B., Olivier, S., Renate, E.G., Steffen, G. and Diego, K. (2005) RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via toll-like receptor 3. Arthritis Rheum. 52, 2656–2665.
[75] Gernot, M.K. et al. (1995) Comparative analysis of cathepsin l, cathepsin d, and collagenase messenger rna expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis, by in situ hybridization. Arthritis Rheum. 38, 976–984.
[76] Honda, S. et al. (2001) Expression of membrane-type 1 matrix metalloproteinase in rheumatoid synovial cells. Clin. Exp. Immunol. 126, 131–136.
[77] Thomas, P. et al. (2000) Differential expression pattern of membrane-type matrix metalloproteinases in rheumatoid arthritis. Arthritis Rheum. 43, 1226–1232.
[78] Lefevre, S. et al. (2009) Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. Nat. Med. 15, 1414–1420.
[79] Hwang, S.Y. et al. (2004) IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways. Arthritis Res. Ther. 6, R120–R128.
[80] Mohammad, A.A. et al. (2007) Interleukin-18 induces angiogenic factors in rheumatoid arthritis synovial tissue fibroblasts via distinct signaling pathways. Arthritis Rheum. 56, 1787–1797.
[81] Dayer, J.M., de Rochemonteix, B., Burrus, B., Demczuk, S. and Dinarello, C.A. (1986) Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. J. Clin. Invest. 77, 645–648.
[82] Dayer, J.M., Beutler, B. and Cerami, A. (1985) Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J. Exp. Med. 162, 2163–2168.
[83] Carol, H. et al. (2002) Hypoxia-induced production of stromal cell-derived factor 1 (CXCL12) and vascular endothelial growth factor by synovial fibroblasts. Arthritis Rheum. 46, 2587–2597.
[84] Case, J.P., Lafyatis, R., Remmers, E.F., Kumkumian, G.K. and Wilder, R.L. (1989) Transin/stromelysin expression in rheumatoid synovium. A transformation-associated metalloproteinase secreted by phenotypically invasive synoviocytes. Am. J. Pathol. 135, 1055–1064.
[85] Firestein, G.S. (2005) Immunologic mechanisms in the pathogenesis of rheumatoid arthritis. JCR 11 (3), S39–S44.
[86] Paul, P.T. et al. (1997) Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum. 40, 217–225.
[87] Diarmuid, M., Oliver, F. and Barry, B. (1996) Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum. 39, 115–124.
[88] Kinne, R., Stuhlmuller, B. and Burmester, G.R. (2007) Cells of the synovium in rheumatoid arthritis. Macrophages. Arthritis Res. Ther. 9, 224.
[89] Banchereau, J. and Steinman, R.M. (1998) Dendritic cells and the control of immunity. Nature 392, 245–252.
[90] Shortman, K. and Naik, S.H. (2007) Steady-state and inflammatory dendritic-cell development. Nat. Rev. Immunol. 7, 19–30.
[91] Thomas, R. et al. (1999) Dendritic cells and the pathogenesis of rheumatoid arthritis. J. Leukoc. Biol. 66, 286–292.
[92] Lutzky, V., Hannawi, S. and Thomas, R. (2007) Cells of the synovium in rheumatoid arthritis. Dendritic cells. Arthritis Res. Ther. 9.
[93] Leung, B.P. et al. (2002) A novel dendritic cell-induced model of erosive inflammatory arthritis: distinct roles for dendritic cells in T cell activation and induction of local inflammation. J. Immunol. 169, 7071–7077.
[94] Benson, R.A. et al. (2010) Identifying the cells breaching self-tolerance in autoimmunity. J. Immunol. 184, 6378–6385.
[95] Thomas, R., Davis, L.S. and Lipsky, P.E. (1994) Rheumatoid synovium is enriched in mature antigen-presenting dendritic cells. J. Immunol. 152, 2613–2623.
[96] Jongbloed, S.L. et al. (2009) Plasmacytoid dendritic cells regulate breach of self-tolerance in autoimmune arthritis. J. Immunol. 182, 963–968.
[97] Hadeiba, H. et al. (2008) CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. Nat. Immunol. 9, 1253–1260.
[98] van Duivenvoorde, L.M. et al. (2007) Immunomodulatory dendritic cells inhibit Th1 responses and arthritis via different mechanisms. J. Immunol. 179, 1506–1515.
[99] Leonie, M.v.-D. et al. (2004) Antigen-specific immunomodulation of collagen-induced arthritis with tumor necrosis factor-stimulated dendritic cells. Arthritis Rheum. 50, 3354–3364.
[100] Olivier, J. et al. (2009) Dendritic cells modulated by innate immunity improve collagen-induced arthritis and induce regulatory T cells in vivo. Immunology 126, 35–44.
[101] Moura, R.A. et al. (2010) Alterations on peripheral blood B-cell subpopulations in very early arthritis patients. Rheumatology (Oxford) 49, 1082–1092.
[102] Moura, R.A. et al. (2011) Cytokine pattern in very early rheumatoid arthritis favours B-cell activation and survival. Rheumatology (Oxford) 50, 278–282.
[103] Bugatti, S., Codullo, V., Caporali, R. and Montecucco, C. (2007) B cells in rheumatoid arthritis. Autoimmun. Rev. 6, 482–487.
[104] Verpoort, K.N. et al. (2006) Isotype distribution of ANTI-CYCLIC citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. Arthritis Rheum. 54, 3799–3808.
[105] Manzo, A. et al. (2005) Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis. Eur. J. Immunol. 35, 1347–1359.
[106] Takemura, S. et al. (2001) Lymphoid neogenesis in rheumatoid synovitis. J. Immunol. 167, 1072–1080.
[107] Seyler, T.M. et al. (2005) BLyS and APRIL in rheumatoid arthritis. J. Clin. Invest. 115, 3083–3092.
[108] Leadbetter, E.A. et al. (2002) Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416, 603–607.
[109] Duddy, M.E., Alter, A. and Bar-Or, A. (2004) Distinct profiles of human B cell effector cytokines: a role in immune regulation? J. Immunol. 172, 3422–3427.
[110] Edwards, J.C.W. and Cambridge, G. (2006) B-cell targeting in rheumatoid arthritis and other autoimmune diseases. Nat. Rev. Immunol. 6, 394–403.
[111] Schubert, D., Maier, B., Morawietz, L., Krenn, V. and Kamradt, T. (2004) Immunization with glucose-6-phosphate isomerase induces T cell-

dependent peripheral polyarthritis in genetically unaltered mice. J. Immunol. 172, 4503–4509.
[112] Maffia, P. et al. (2004) Inducing experimental arthritis and breaking self-tolerance to joint-specific antigens with trackable, ovalbumin-specific T cells. J. Immunol. 173, 151–156.
[113] Duarte, J., Agua-Doce, A., Oliveira, V.G., Fonseca, J.E. and Graca, L. (2010) Modulation of IL-17 and Foxp3 expression in the prevention of autoimmune arthritis in mice. PLoS ONE 5, e10558.
[114] Toh, M.-L. and Miossec, P. (2007) The role of T cells in rheumatoid arthritis: new subsets and new targets. Curr. Opin. Rheumatol. 19, 284–288.
[115] Shen, H., Goodall, J.C. and Hill Gaston, J.S. (2009) Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. Arthritis Rheum. 60, 1647–1656.
[116] Cascao, R. et al. (2010) Identification of a cytokine network sustaining neutrophil and Th17 activation in untreated early rheumatoid arthritis. Arthritis Res. Ther. 12, R196.
[117] De Smedt, T. et al. (1996) Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. J. Exp. Med. 184, 1413–1424.
[118] Cella, M., Engering, A., Pinet, V., Pieters, J. and Lanzavecchia, A. (1997) Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature 388, 782–787.
[119] Jenkins, M.K. et al. (2001) In vivo activation of antigen-specific CD4 T cells. Annu. Rev. Immunol. 19, 23–45.
[120] Mempel, T.R., Henrickson, S.E. and von Andrian, U.H. (2004) T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature 427, 154–159.
[121] Kearney, E.R., Pape, K.A., Loh, D.Y. and Jenkins, M.K. (1994) Visualization of peptide-specific T cell immunity and peripheral tolerance induction in vivo. Immunity 1, 327–339.
[122] McHeyzer-Williams, M.G. and Davis, M.M. (1995) Antigen-specific development of primary and memory T cells in vivo. Science 268, 106–111.
[123] Garside, P. et al. (1998) Visualization of specific B and T lymphocyte interactions in the lymph node. Science 281, 96–99.
[124] Rush, C.M. et al. (2009) Characterization of CD4+ T-cell-dendritic cell interactions during secondary antigen exposure in tolerance and priming. Immunology 128, 463–471.
[125] Zinselmeyer, B.H. et al. (2005) In situ characterization of CD4+ T cell behavior in mucosal and systemic lymphoid tissues during the induction of oral priming and tolerance. J. Exp. Med. 201, 1815–1823.
[126] Smith, K.M., Brewer, J.M., Mowat, A.M., Ron, Y. and Garside, P. (2004) The influence of follicular migration on T-cell differentiation. Immunology 111, 248–251.
[127] King, C., Tangye, S.G. and Mackay, C.R. (2008) T follicular helper (TFH) cells in normal and dysregulated immune responses. Annu. Rev. Immunol. 26, 741–766.
[128] Vinuesa, C.G. et al. (2005) A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 435, 452–458.
[129] Platt, A.M. et al. (2010) Abatacept limits breach of self-tolerance in a murine model of arthritis via effects on the generation of T follicular helper cells. J. Immunol. 185, 1558–1567.
[130] Garrood, T. and Pitzalis, C. (2006) Targeting the inflamed synovium: the quest for specificity. Arthritis Rheum. 54, 1055–1060.
[131] George, A.J., Lee, L. and Pitzalis, C. (2003) Isolating ligands specific for human vasculature using in vivo phage selection. Trends Biotechnol. 21, 199–203.
[132] Sweeney, S.E. and Firestein, G.S. (2004) Rheumatoid arthritis: regulation of synovial inflammation. Int. J. Biochem. Cell Biol. 36, 372–378.
[133] Sun, H.B. (2010) Mechanical loading, cartilage degradation, and arthritis. Ann. N.Y. Acad. Sci. 1211, 37–50.
[134] Al-Allaf, A.W., Sanders, P.A., Ogston, S.A. and Marks, J.S. (2001) A case control study examining the role of physical trauma in the onset of rheumatoid arthritis. Rheumatology 40, 262–266.
[135] Murakami, M. et al. (2011) Local microbleeding facilitates IL-6- and IL-17-dependent arthritis in the absence of tissue antigen recognition by activated T cells. J. Exp. Med. 208, 103–114.
[136] Hamada, T. et al. (2008) Extracellular high mobility group box chromosomal protein 1 is a coupling factor for hypoxia and inflammation in arthritis. Arthritis Rheum. 58, 2675–2685.
[137] Cho, Y.G., Cho, M.L., Min, S.Y. and Kim, H.Y. (2007) Type II collagen autoimmunity in a mouse model of human rheumatoid arthritis. Autoimmun. Rev. 7, 65–70.
[138] Holmdahl, R., Jansson, L., Larsson, A. and Jonsson, R. (1990) Arthritis in DBA/1 mice induced with passively transferred type II collagen immune serum. Immunohistopathology and serum levels of anti-type II collagen autoantibodies. Scand. J. Immunol. 31, 147–157.
[139] Stuart, J.M. and Dixon, F.J. (1983) Serum transfer of collagen-induced arthritis in mice. J. Exp. Med. 158, 378–392.
[140] Zhu, P. et al. (2007) Oral administration of type-II collagen peptide 250–270 suppresses specific cellular and humoral immune response in collagen-induced arthritis. Clin. Immunol. 122, 75–84.
[141] Michaelsson, E., Andersson, M., Engstrom, A. and Holmdahl, R. (1992) Identification of an immunodominant type-II collagen peptide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection. Eur. J. Immunol. 22, 1819–1825.
[142] Brand, D.D. et al. (1994) Characterization of the T cell determinants in the induction of autoimmune arthritis by bovine alpha 1(II)-CB11 in H-2q mice. J. Immunol. 152, 3088–3097.
[143] Rosloniec, E.F., Whittington, K.B., Zaller, D.M. and Kang, A.H. (2002) HLA-DR1 (DRB1*0101) and DR4 (DRB1*0401) use the same anchor residues for binding an immunodominant peptide derived from human type II collagen. J. Immunol. 168, 253–259.
[144] Huan, J. et al. (2008) MHC Class II derived recombinant T cell receptor ligands protect DBA/1LacJ mice from collagen-induced arthritis. J. Immunol. 180, 1249–1257.
[145] Huan, J. et al. (2004) Monomeric recombinant TCR ligand reduces relapse rate and severity of experimental autoimmune encephalomyelitis in SJL/J mice through cytokine switch. J. Immunol. 172, 4556–4566.
[146] Vandenbark, A.A. et al. (2003) Recombinant TCR ligand induces tolerance to myelin oligodendrocyte glycoprotein 35–55 peptide and reverses clinical and histological signs of chronic experimental autoimmune encephalomyelitis in HLA-DR2 transgenic mice. J. Immunol. 171, 127–133.
[147] Rankin, A.L. et al. (2008) CD4+ T cells recognizing a single self-peptide expressed by APCs induce spontaneous autoimmune arthritis. J. Immunol. 180, 833–841.
[148] Hata, H. et al. (2004) Distinct contribution of IL-6, TNF-alpha, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice. J. Clin. Invest. 114, 582–588.
[149] Nickdel, M.B. et al. (2009) Dissecting the contribution of innate and antigen-specific pathways to the breach of self-tolerance observed in a murine model of arthritis. Ann. Rheum. Dis. 68, 1059–1066.
[150] McBride, H.J. (2010) Nuclear imaging of autoimmunity: focus on IBD and RA. Autoimmunity 43, 539–549, doi:10.3109/08916931003674766.
[151] Kijowski, R. (2010) Clinical cartilage imaging of the knee and hip joints. AJR Am. J. Roentgenol. 195, 618–628.
[152] Caetano-Lopes, J. et al. (2010) Chronic arthritis leads to disturbances in the bone collagen network. Arthritis Res. Ther. 12, R9.
[153] Qian, X. et al. (2008) In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags. Nat. Biotechnol. 26, 83–90.
[154] Llewellyn, M.E., Barretto, R.P., Delp, S.L. and Schnitzer, M.J. (2008) Minimally invasive high-speed imaging of sarcomere contractile dynamics in mice and humans. Nature 454, 784–788.
[155] Barretto, R.P. et al. (2011) Time-lapse imaging of disease progression in deep brain areas using fluorescence microendoscopy. Nat. Med. 17, 223–228.
[156] Pedersen, M. et al. (2006) Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides. Arthritis Res. Ther. 8, R133.
[157] Kallberg, H. et al. (2009) Alcohol consumption is associated with decreased risk of rheumatoid arthritis: results from two Scandinavian case-control studies. Ann. Rheum. Dis. 68, 222–227.
[158] Mandl, L.A., Costenbader, K.H., Simard, J.F. and Karlson, E.W. (2009) Is birthweight associated with risk of rheumatoid arthritis? Data from a large cohort study. Ann. Rheum. Dis. 68, 514–518.
[159] Bhatia, S.S. et al. (2007) Rheumatoid factor seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis. Ann. Rheum. Dis. 66, 267–269.
[160] Pikwer, M. et al. (2009) Breast feeding, but not use of oral contraceptives, is associated with a reduced risk of rheumatoid arthritis. Ann. Rheum. Dis. 68, 526–530.
[161] Bengtsson, C. et al. (2005) Socioeconomic status and the risk of developing rheumatoid arthritis: results from the Swedish EIRA study. Ann. Rheum. Dis. 64, 1588–1594.
[162] Chang, S.C., Laden, F., Puett, R. and Karlson, E.W. (2008) Geographic variation in rheumatoid arthritis incidence among women in the United States. Arch. Intern. Med. 15, 1664–1670.