Role of T-cells in the development of arthritis

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ABSTRACT

1. Arthritis can be induced in rodents by priming T-cells to respond to a foreign antigen and then challenging with antigen intra-articularly. This may be a model of the situation in human reactive arthritis in which T-cell responses are induced by antigens from organisms which trigger reactive arthritis (e.g. Chlamydia trachomatis) and antigen finds its way to the joint, most probably within macrophages. Priming by previous exposure to antigens similar to those of the triggering organism could also play a part in pathogenesis. Genetic factors determining the nature and control of the immune response affect the severity and duration of the arthritis.

2. T-cell-dependent arthritis can be induced in rodents by immunization with an antigen known to be expressed in the joint (e.g. Type II collagen). Whether this is an important mechanism in human arthritis is still unclear, even though diseases such as rheumatoid arthritis are conventionally thought of as 'autoimmune'. No convincing candidate autoantigen has yet been identified in rheumatoid arthritis, and recent experiments in transgenic mice indicate that arthritis can be induced by an autoimmune T-cell response which does not target an antigen confined to the joint.

3. Adjuvant arthritis is a classical T-cell-dependent animal model of human arthritis; recently arthritis has been described as a rare complication in patients receiving adjuvant (intra-vesical live BCG organisms) for bladder cancer. Increasing attention is being paid to the role of adjuvants as 'danger signals', which allow the immune system to determine whether an antigenic challenge poses a threat. Inappropriate attachment of danger signals to self antigens may result in T-cell-mediated immune responses, which could play a part in the pathogenesis of arthritis.

4. Animal studies indicate that autoimmune/inflammatory diseases can be produced by imbalances within T-cell populations, and that certain T-cells also have the capacity to regulate inflammatory responses. Among the latter are T-cells specific for conserved epitopes within heat shock protein 60. The extent to which T-cells of this kind operate in human disease has yet to be determined.

INTRODUCTION

For some time there has been a lively debate among rheumatologists about the role of T-cells in the pathogenesis of inflammatory arthritides, particularly rheumatoid arthritis (RA) [1–6]. Some have argued, mainly on theoretical grounds, that T-cells must be critical and should be the principal target of therapeutic efforts. Others have pointed to the substantial production of macrophage-derived cytokines within the synovium, compared with meagre quantities of T-cell-derived cytokines, and argued for a minimal role of T-cells in the pathogenesis of synovitis. This argument has been fortified by the striking immediate therapeutic effects of...
inhibiting macrophage-derived cytokines such as interleukin (IL)-1 and tumour necrosis factor-α (TNF-α) [7,8], compared with the less impressive results of initial attempts at manipulating T-cell responses ([9,10], and references therein). However, as time goes on, this debate is being seen as ultimately sterile; in complex diseases involving the immune system it is unlikely that there is a single ‘guilty’ effector cell. This would be akin to believing that the Mafia consists solely of hit-men, paying no attention to the role of the godfathers, or indeed to the complex social milieu required for such criminal activity to flourish. This lesson is made clear in studies of infectious diseases; patients or mice who lack healthy T-cell responses are at risk from mycobacterial infection [11,12], but so are those whose macrophages fail to make IL-12 or fail to respond to interferon-γ [13–15,15a,15b]—both components of the response are critical to a successful outcome and may also be involved in immunopathology.

If it is important not to think of the pathogenic role of T-cells in isolation from other elements of the immune response, it is also necessary to reassess the nature of their role. The conventional model postulates a T-cell response to an arthritogenic antigen/peptide presented by the alleles of the major histocompatibility complex (MHC) which are associated with different forms of arthropathy (e.g. HLA-B27, HLA-DR4). This implies a major role for T-cells in disease initiation. Although this is by no means excluded, one of the hallmarks of the major human inflammatory arthropathies such as RA or spondyloarthropathy is their chronicity or persistence, and it is possible that T-cells have a critical role in this aspect. It is clear that most immune responses, including those associated with pathology, are satisfactorily brought under control. Despite much confusion about the precise properties of ‘suppressor’ T-cells, T-cells have a role in this down-regulatory process, and defects in this might be an important factor in chronicity. Thus both pathological responses to antigen and a failure of regulatory function need to be considered as ways in which T-cells could perform critical roles in human arthritis.

This review will highlight some of the advances in our understanding of the role of T-cells in the pathogenesis of joint inflammation, particularly lessons learned from animal models and the extent to which these may apply to human diseases.

**ARTHRITIS CAUSED BY ANTIGEN-SPECIFIC T-CELLS (Figure 1)**

**Animal model**

The clearest example of arthritis that is dependent on antigen-specific T-cells is the animal model of antigen-induced arthritis. Animals are primed to respond to a foreign antigen such as methylated BSA, so that subsequent intra-articular challenge with the same antigen produces arthritis [16,17]. This is not a surprising result, and the only requirement seems to be an antigen whose charge properties allow it to be retained in the joint by interaction with the negatively charged cartilage matrix.

**Human counterpart?**

Does this model really have any human counterpart? Deliberate priming and articular challenge with antigen is obviously not a common occurrence, but articular challenge with antigen does occur when antigens derived from infectious agents find their way to the synovium. The extent to which this occurs is now being realized, particularly for the organisms which are associated with reactive arthritis and Lyme disease. The presence of both Chlamydia and Gram-negative organisms (Salmonella, Yersinia, Shigella) has been demonstrated by immunofluorescence in synovial fluid and tissue [18–21], and DNA from both Chlamydia trachomatis and Borrelia burgdorferi has been detected by polymerase chain reaction [22–24]. Despite this the organisms generally cannot be cultivated from the tissue. How and why these organisms traffic to the joint is of great interest; for Gram-negative organisms, lipopolysaccharide (LPS) and bacterial proteins have been demonstrated in synovial mononuclear phagocytes. These have presumably encountered the organisms at the site of infection and subsequently travelled to the joint, since bacterial products can sometimes be demonstrated in phagocytes in peripheral blood [19]. Likewise for Chlamydia, which is an obligate intracellular organism - it is likely that the organisms arrive in the joint within macrophages. Recent evidence supports this notion; in rodents infected with Chlamydia at either genital or ocular sites, organisms could be detected in synovial tissue 7–14 days later (but not in adjacent muscle) [25,26]. This highlights an important property of synovium, which is that approximately 50% of the cells which make up the lining layer are macrophages recruited from peripheral blood. Thus ‘passenger’ antigens and organisms within macrophages will inevitably find their way to joints, with the dose being influenced by recruitment rates. Large joints would be expected to recruit greater numbers than small joints, and joints with some degree of inflammation due to minor trauma (as may occur routinely in weight-bearing joints) would also recruit greater numbers. These factors might account for the preferential involvement of large lower limb joints in reactive arthritis.

However, the fact that antigen finds its way to the synovium does not inevitably lead to arthritis. Indeed, it seems possible that some Chlamydiae might find their way to the synovium in most people infected by the organism, and in line with this idea, polymerase chain reaction has detected Chlamydiae in rheumatoid synovia.
Role of T-cells in the development of arthritis

Immunize with foreign antigen

T-cell dependent

ARTHRITIS

Challenge by injecting antigen into joints

MOUSE MODEL

Immunize with infectious agent in gut or genito-urinary tract

? Reactive

ARTHRITIS

Antigen traffic to joint

HUMAN COUNTERPART

Figure 1 Experimental rodent model of arthritis caused by antigen-specific T-cells, and its possible human counterpart

[27] (albeit in a lower proportion than in reactive arthritis), and even in one normal synovium (of 25 tested) [28]. Therefore, there must be additional factors which influence the immune response to the antigens deposited in the synovium. These could include dose of antigen, duration of antigen persistence within synovium, and effectiveness in eliminating infection outside synovium, so that further antigen deposition ceases. With regard to T-cell responses, the occurrence of arthritis could reflect the immunodominant antigens targeted by the immune response to the pathogen, and whether these antigens are available in synovium. In addition, the occurrence and duration of arthritis could be influenced by the cytokines produced by the T-cells responding to antigen, and the mechanisms whereby the immune response is terminated. In reactive arthritis, the relative importance of any of these factors is not yet known. Work is beginning on identifying the principal antigens recognized by synovial T-cells in reactive arthritis associated with particular bacteria such as Yersinia [29-31] or Chlamydia [32-34], but it has not yet been determined whether patients infected by the same organisms who do not develop arthritis respond to the same or different antigens.

Another factor to be considered is T-cell priming (i.e. previous exposure to the same antigen); in experimental antigen-induced arthritis, disease is dependent on previous priming of T-cell responses to the antigen used in the intra-articular challenge. Likewise, patients infected by an organism whose antigens find their way to the synovium, may already be primed to respond to certain antigens by previous encounter with the organism, or an organism which expresses similar antigens. This point is illustrated by the example of synovial T-cell responses to heat shock protein 60 (hsp60) in reactive arthritis after C. trachomatis infection. Mapping the epitopes recognized showed it to be identical to that in hsp60 from C. pneumoniae, a common respiratory pathogen which a significant proportion of patients will probably have encountered in the past [35]. Likewise, target antigens of responses to enteric pathogens are often those which are conserved in other Gram-negative bacteria including commensals such as Escherichia coli (again hsp60 is a good example). However, whether reactive arthritis is more common in patients primed by prior exposure to related pathogens remains to be determined.

In recent years it has been possible to subdivide T-cells, particularly in the mouse, into subsets according to the set of lymphokines that they produce when activated. These are often termed Th1 and Th2; the former characteristically make interferon-γ and are very important in delayed-type hypersensitivity responses and immunity to intracellular pathogens. The latter make IL-4 and are important in IgE-mediated allergic responses and immunity to parasitic infections. There have been suggestions that cytokines associated with the Th2 subset of T-cells are prominent in the synovium of patients with reactive arthritis (compared with for instance rheumatoid synovium) [36,37], although there are contrary reports [38]. It has been argued that clearance of reactive arthritis-associated pathogens requires a Th1-type T-cell response (principally production of interferon-γ), and murine models support this idea in chlamydial infection [39]. Thus a Th2 response could be regarded as inappropriate, allowing persistence of bacteria in the synovium or even at the site of infection [40]. Alternatively, since the disease resolves in the majority of patients with reactive arthritis (although often taking 12 months to do so), the appearance of anti-inflammatory Th2 cytokines may reflect an ability to control joint inflammation. IL-4 has been shown to down-regulate joint inflammation and
destruction in animal models of arthritis [41]. As yet, there are no data which distinguish between these two conflicting explanations of the significance of synovial Th2 cytokines.

Reactive arthritis is clearly associated with HLA-B27, but how B27 influences susceptibility to arthritis, and whether it is mediated by an effect on the synovial T-cell response to bacterial antigens remains unclear [42-44]. The association with B27 is most evident in cases which are considered to require referral to rheumatology clinics because of severity or duration. When mild cases in the community are identified, the incidence of B27 falls from the 60-70% seen for hospital cases to 20-30% [45], or even occasionally to zero in particular patient cohorts [46]. Thus B27 also predisposes to chronicity rather than the occurrence of arthritis per se; this predisposition is evident in the most chronic form of spondyloarthropathy, ankylosing spondylitis (to which a minority of patients with reactive arthritis progress), in which more than 90% of patients are B27 positive.

β-zygous loss of β2-microglobulin knock-out mice which express human HLA-B27 heavy chains (i.e. not complexed with β2-microglobulin, as is usually the case with class I HLA antigens). This has been put forward on the basis that B27 transgenic mice do not get arthritis unless they also have a gene-targeted homzygous loss of β2-microglobulin (β2-microglobulin knock-out mice). Conversely, disease is also seen in β2-microglobulin knock-out mice which express human β2-microglobulin, the latter complexing less well with B27. Finally the disease is dependent on CD4+ T-cells [53,54]. It is further proposed that T-cells which recognize bacterial peptides plus B27 cross-react with a self peptide present in the joint, but as yet there is no experimental evidence to support the idea that an autoimmune response of this kind is important in this model. A continuing response to bacterial peptides supplied to the joint could explain chronic arthritis equally well.

Summary

T-cell responses have a critical role in experimental antigen-induced arthritis. Reactive arthritis may be a human counterpart of this disease, driven by bacterial antigens which gain access to synovium. The influence of HLA-B27 in susceptibility to reactive arthritis remains unexplained, but B27 transgenic rodent models cast doubt on the idea that disease is primarily due to arthritogenic B27-restricted CD8+ T-cells.

ARTHRITIS CAUSED BY AUTOANTIGEN-SPECIFIC T-CELLS (Figure 2)

Candidate autoantigens

There are a number of animal models which depend on immunization with an autoantigen known to be present in the joint, including Type II collagen (CII) [55], proteoglycan [56], and more recently the cartilage protein, gp39 [57]. Conceptually these models are rather similar to those already discussed, with the obvious exception that intra-articular challenge with antigen is not required since it is already present. There is the expected influence of MHC in these models, particularly CII-induced arthritis, where only certain mouse class II alleles support T-cell responses to CII [58,59]. Elegant transgenic experiments have mutated non-responder alleles to the responder phenotype by changes in just four amino acids (from I-Aβ to I-Aβ), I-Aβ antigens in mouse being equivalent to HLA-DR in humans) and produced susceptibility to disease [60]. Other genes are also required, as shown by studies in the rat where mapping of susceptibility genes outside MHC is currently underway [61,62].

In all of these models arthritis is completely T-cell dependent, but there is an additional need to elicit antibodies to native CII in CII-induced arthritis [63]. Many manipulations of the T-cell response have ameliorated arthritis in the CII model [64-69]. However, these have usually only been effective before the development of arthritis rather than as curative measures. Interestingly, despite the overwhelming evidence of the central role of T-cells in CII-induced arthritis, it has sometimes proved difficult to detect CII-specific T-cells, particularly in the rat. This point may be relevant to studies of human arthritis.

Is there compelling evidence for human arthritis mediated by autoantigen-specific T-cells? Rheumatoid arthritis is usually classified as an ‘autoimmune’ condition with the underlying assumption that there is a target autoantigen. Type II collagen has been the most favoured candidate, but it has proved difficult in most cases to demonstrate CII-specific T-cells in patients with RA, in either blood or joint, although there are some positive findings [70,71]. Antibodies to CII are present in a proportion of patients [72], implying the existence of CII-specific helper T-cells, and indirect evidence has come from a therapeutic trial claiming that disease could be relieved by inducing tolerance to CII by oral administration [73]. This was tested because inducing oral tolerance is effective in the rodent model [74,75], but more recent trials in humans have not shown efficacy [76].
Role of T-cells in the development of arthritis

T-cell dependent ARTHRITIS

MOUSE MODEL

Immunize with antigen known to be in the joints

T-cell dependent ARTHRITIS

HUMAN COUNTERPART

Immunize with (a) foreign antigen cross reacting with joint component.
(b) joint component released from damaged joint

Figure 2 Experimental rodent model of arthritis caused by autoantigen-specific T-cells, and its possible human counterpart

The cartilage glycoprotein, gp39, has been suggested as a candidate autoantigen in RA [57]. Peptides within this protein which were predicted to bind to the HLA-DR allele associated with RA, DR4, were used to stimulate T-cells from patients with RA, and a proportion showed responses to one or more peptides. Responses were less frequent, but not absent, in controls, and responses in RA were not confined to DR4+ patients. Nevertheless these results led the investigators to immunize mice with gp39, when it was found to be arthritogenic. Thus the evidence implicating gp39 in RA is largely circumstantial and awaits further study.

T-cell responses to the cartilage proteoglycan aggrecan can be detected in patients with arthritis [77], and again inflammatory arthritis can be induced in mice using aggrecan [56,78,79]. In these experiments it is essential to use foetal aggrecan which lacks keratin or chondroitin sulphate side-chains, or to remove them enzymically, implying that a T-cell epitope (i.e. the short linear 8–10-amino-acid sequence which the T-cell recognizes) in the aggrecan core is normally hidden from the immune system by side chains. Interestingly, T-cell responses to aggrecan have mainly been documented in patients with ankylosing spondylitis [80], but there are recent reports of responses in patients with RA [81].

All of these studies indicate that a T-cell repertoire capable of recognizing a number of the components in the joint remains after thymic selection, the process which is designed to delete autoreactive T-cells. Presumably, under normal circumstances T-cells with this recognition specificity are not activated. However, various artificial manoeuvres can activate the cells and produce experimental arthritis - these include immunization with xenogeneic protein (e.g. rat CII in mice), the use of adjuvants (see below), and modification of the protein to reveal hidden or 'cryptic' epitopes. Such exposure of cryptic epitopes would be expected to occur as a consequence of joint damage and cartilage destruction, so the finding of T-cells specific for joint components in patients with arthritis does not necessarily imply that T-cells caused the arthritis. Responses to multiple joint components would be consistent with this hypothesis of immunity secondary to damage, but it is also possible that there are multiple targets of an arthritogenic T-cell-mediated autoimmune response. The parallel here would be with the spontaneous diabetes which develops in the non-obese diabetic (NOD) mouse, in which a T-cell-mediated destruction of islet $\beta$-cells is certainly responsible for disease, but where several components of the $\beta$-cell are targeted - glutamic acid decarboxylase, insulin, hsp60 and others [82].

Autoimmune arthritis with no joint-specific target antigen

Thus far the discussion has centred on which joint-specific autoantigen might be a candidate target in human arthritis, but recent experiments in transgenic mice question the assumption that a tissue-specific target is necessary (Figure 3). These experiments made T-cell receptor transgenic mice in which all T-cells express a receptor specific for the antigen bovine pancreatic ribonuclease presented by I-A$k$. In mice which express I-A$k$, nothing happens since no foreign antigen (ribonuclease) is available. However, when the same transgenic T-cell receptor was expressed in the NOD mouse, which has a unique MHC class II allele, I-A$k$ [83], the mice developed arthritis. Further investigation revealed that the transgenic T-cell receptor had a fortuitous cross-reaction with I-A$k$, i.e. transgenic T-cells were stimulated by interaction with the allogeneic I-A$k$ even in the absence of the
antigenic peptide from ribonuclease. T-cell receptors specific for antigenic peptide plus self MHC (i.e. ribonuclease peptide and I-A<sup>α</sup> in this example) are often able to cross-react with an allogeneic MHC antigen (i.e. I-A<sup>ε7</sup>), and these findings apply to both murine and human T-cells. In an I-A<sup>ε7</sup>-expressing mouse, the cells which interact with I-A<sup>ε7</sup> alone, or I-A<sup>ε7</sup> in combination with other self peptides, are normally deleted on encountering I-A<sup>ε7</sup> in the thymus, since they are autoreactive. However, in the T-cell receptor transgenic mouse, where all the T-cells have this specificity, some escape deletion, most probably a population of T-cells with low levels of T-cell receptor expression. The appearance of these cells in the periphery coincided with the onset of arthritis.

The disease was dependent on the presence of both CD4<sup>+</sup> T-cells and B-cells, but interestingly, in view of experience in human RA, treatment with anti-CD4 was ineffective unless administered well before the development of arthritis.

Given that the mice had T-cells which could respond to any cell expressing I-A<sup>ε7</sup>, why did they develop arthritis rather than any other form of autoimmune disease? It is possible that the T-cells recognize I-A<sup>ε7</sup> along with a specific self-peptide which is abundant in the synovium, since this would explain the occurrence of arthritis. There is no evidence for this however, and this disease appears to be an example of potentially 'systemic' autoimmunity being manifest in an organ-specific way. In the light of the discussion in the previous section showing the ready traffic of bacteria or their products to the synovium, synovial macrophages may be stimulated by subclinical bacterial infection or LPS to up-regulate class II MHC expression and co-stimulatory ligands, thus making them more likely to interact successfully with autoreactive T-cells. Joints are susceptible to certain cytokines, as illustrated by the arthritis which occurs in the transgenic mouse which expresses human TNF-α in an unregulated fashion [84]. Thus, autoreactive T-cells might interact with I-A<sup>ε7</sup>-positive synovial macrophages and induce TNF-α production.

**Summary**

Arthritis mediated by autoreactive T-cells can clearly be shown in experimental models, but requires an intervention to break self-tolerance. Thus far there is no autoantigen which has been conclusively demonstrated to be critical to the pathogenesis of any human arthritis, and it is unclear whether the responses to autoantigens which have been documented are secondary to joint damage rather than primary factors in pathogenesis. However, certain animal models teach us that an autoantigen-specific T-cell population may not be readily demonstrable, even in experimental situations where there is overwhelming evidence for its involvement in arthritis. Conversely, others show that arthritis does not necessarily require an immune response directed against a joint-specific antigen.

**ARTHRITIS CAUSED BY THE EFFECTS OF ADJUVANTS (Figure 4)**

**Animal models**

Experimental arthritis induced by adjuvants has been studied for the last three decades. However, in many ways a separate section dealing with adjuvants is somewhat misleading, since they are required in each of the experimental models described in the previous sections. Although immunologists usually concern themselves mainly with specific aspects of immune responses - what
antigen is recognized, can the epitope be identified, what receptors are responsible? Experimental induction of immune responses requires the action of adjuvants. These are mainly bacterial products, although synthetic adjuvants have also been developed. The function of adjuvants is to activate the innate immune system so that subsequent specific immune responses can take place [85]. One attractive idea is that the adjuvant alerts the immune system to the ‘danger’ which any antigenic challenge poses to the host [86]. Since the immune system is designed to counter threats from pathogens, the co-delivery of both antigen and adjuvant which occurs during bacterial infection ensures a satisfactory immune response. Antigen delivered without accompanying adjuvant may be perceived as non-threatening and an immune response not elicited, hence the usual need for adjuvant, acting as a surrogate ‘danger signal’, in most experimental immunization protocols.

In early work on adjuvant arthritis complete Freund’s adjuvant, comprising killed mycobacteria in oil, was used, providing both antigenic components (mycobacterial proteins) and adjuvant (oil and mycobacterial cell wall components). When the arthritis induced was examined in terms of T-cell specificity, it appeared that a response to mycobacterial antigens was mounted, and it was postulated that this cross-reacted with a self antigen present in the joint [87]. More recently it has become clear that adjuvant arthritis can be induced in the absence of exogenous antigen, using mineral oils [88], synthetic adjuvants [89] and pristane [90]. These models are wholly T-cell-dependent and show influences of MHC and non-MHC genes, with susceptibility varying in different inbred rodent strains. However, the specificity of the arthritogenic T-cells has not yet been defined in these models.

**Human counterpart?**

Is there a role for adjuvants in human arthritis? Again this is not known. ‘Adjuvant therapy’ in the form of live Mycobacterium bovis BCG delivered into the bladder is a well-accepted treatment for bladder cancer, and has been associated with inflammatory arthritis in a small proportion of patients [91–93]. The synovial fluid has been shown to contain T-cells specific for mycobacterial antigens [94], including hsp60, the antigen implicated in rat adjuvant arthritis. Whether bacterial antigens drive the arthritis, or whether the adjuvant properties of the mycobacteria allow an autoreactive response to joint components to develop, is unclear. By analogy with other, more conventional forms of reactive arthritis, it is likely that macrophages bearing mycobacterial antigens find their way to joints where either the antigenic or adjuvant components of the organism may have their effect. Equally, although conventional reactive arthritis was discussed in a previous section in terms of the delivery of specific antigen to the joint, in all cases bacterial products with adjuvant properties will also be delivered. Whether arthritis occurs will then depend on host factors, as demonstrated in rodent models. These will include MHC (DR4, B27) but also other genes. It will be particularly interesting to see whether susceptibility loci governing adjuvant arthritis in rodents are syntenic with loci conferring susceptibility to RA and/or ankylosing spondylitis, as these are identified by genome-wide screening techniques.

**Summary**

Adjuvants are critical to the initiation of T-cell-mediated responses to antigen, and provide a ‘context’ in which an immune response is deemed appropriate. In view of the
ability of adjuvant to induce arthritis in animal models, even in the absence of exogenous antigen, it is possible that natural adjuvants (such as LPS) also play an important role in human arthritis. Adjuvants may find their way to joints accompanying bacterial antigens, but may also stimulate immune responses within the lymphoid system, including autoimmune responses, which eventually result in arthritis.

ARTHRITIS RELATED TO A FAILURE OF REGULATORY T-CELLS (Figure 5)

One of the most striking features of most animal models of arthritis, whether involving exogenous or self antigens, is that they are essentially acute arthropathies in which joint inflammation resolves without permanent joint destruction. After resolution, resistance to subsequent induction of arthritis by the same stimulus is also seen. Since both the target antigen and T-cells capable of recognizing it usually persist, the resolution and resistant state must involve regulatory mechanisms, and T-cells have been implicated in such regulation. In animal models it is also possible to render the animal resistant to a subsequent arthritogenic challenge without actually inducing arthritis, and again T-cells have been shown to mediate this disease-resistant state. These experiments raise the possibility that some forms of human arthritis are related to an inability to resolve joint inflammation, or the lack of a protective T-cell population. In early arthritis clinics it is notoriously difficult to identify those patients who will progress to established RA, and to distinguish them from patients who will be able to resolve their synovitis. HLA-DRA is one of the factors which predicts disease persistence [95], and interestingly there are reports that DR4 is also associated with persistent arthritis in Lyme disease [96]. As noted previously, HLA-B27+ patients with reactive arthritis are more likely to develop chronic arthropathy. Therefore the influence of these disease-associated MHC alleles might be on T-cells which normally allow disease resolution, rather than on those responsible for its initiation.

Evidence implicating hsp60 in immunoregulation

Much of the work demonstrating the protective abilities of T-cells has been in the adjuvant arthritis model in Lewis rats. It was first reported that immunization with mycobacterial hsp60, previously shown to be a target antigen recognized by an arthritogenic T-cell clone, did not induce arthritis, but instead rendered animals resistant to subsequent arthritis induction with adjuvant [97]. The resistant state was transferable by T-cells, and subsequent experiments mapped an epitope in mycobacterial hsp60 which was able to induce the protective T-cell population [98,99]. Interestingly, this epitope was highly conserved in both mycobacterial and mammalian hsp60, so that the protective cells were in effect autoreactive, although their generation was dependent on immunization with the bacterial hsp60. Clearly, MHC alleles would influence the epitopes in hsp60 selected by the T-cell response, and consequently determine whether a concomitant autoreactive regulatory response would also be generated. It is now important to determine whether the production of autoreactive hsp60-specific T-cells is a general mechanism of immunoregulation [100], and if so, which mechanisms are involved. These could include the production of anti-inflammatory cyto-

Figure 5  Experimental rodent models of arthritis caused by a failure of regulatory T-cells

The data are insufficient to suggest a human counterpart with confidence.
kines (e.g. those made by the Th2 subset, as discussed above) in response to the increased expression of self hsp60 at sites of inflammation [101].

Similar experiments implicating immune responses to hsp60 in protection have been performed in other animal models of arthritis [102], including pristane-induced arthritis in mice [103], suggesting that the phenomenon is general. Initials that this mechanism may also apply in humans come from observations in juvenile chronic arthritis, where peripheral blood T-cell responses to human hsp60 have been correlated with subsequent resolution of arthritis [104]. Under this hypothesis chronic arthritis would be associated with a failure to mount an adequate response to self hsp60. Recent experiments in mice and humans show that a T-cell repertoire for the recognition of hsp60 survives thymic selection ([105]; J. R. Ramage, J. L. Young, and J. H. V. Gaston, unpublished work), but it is not yet known under what circumstances this repertoire is used, and whether it is mainly cross-reactive with bacterial hsp60. It has been suggested that immunization with hsp60 or hsp60-derived peptides might be tried in humans, following the efficacy of this approach in both experimental arthritis and diabetes [106].

Other animal models involving immunoregulation of autoimmunity
In addition to the work implicating recognition of self hsp60 in immunoregulation, other studies in the same experimental systems have suggested that disease can be prevented or ameliorated through direct effects on arthritogenic T-cells. Thus regulatory T-cells which recognize peptides derived from the T-cell receptor of disease-inducing T-cells may interfere with their action [107,108]. Other experimental systems have highlighted the extent to which interactions between T-cell subsets are normally required to avoid autoimmune disease. Disturbances in the balance between subsets can lead to a variety of diseases, although usually organ-specific autoimmunity (e.g. diabetes, thyroiditis) rather than arthritis. This is dramatically demonstrated by transfer of certain T-cell subsets into athymic rats (which lack any T-cells) [109]. CD4+ T-cells can be subdivided according to their expression of different isoforms of cell-surface molecule CD45. The isoforms are generated by differential splicing of three exons (A, B and C). The low-molecular-mass form, CD45RO, does not include any of the exons; different combinations of A, B and C expression are found in high-molecular-mass forms, and their characterization in different species depends on available antibodies. When CD4+ cells which express high levels of the high-molecular-mass isoform (CD4+ CD45RC hi cells in rats) are transferred, the recipients develop a wasting disease with inflammation affecting many organs including pancreas and thyroid [110]. Co-transfer of CD4+ CD45RC lo cells prevents disease. Parallel studies in mice show that transfer of the equivalent subset (CD4+ CD45RB hi) cells induces inflammatory bowel disease, abrogated by CD4+ CD45RB lo cells [111]. Imbalances in T-cell subsets may also result from therapy with immunosuppressants such as cyclophosphamide or irradiation; the former hastens the autoimmune pancreatic islet destruction which occurs spontaneously in the NOD mouse, but the effect can be overcome by transfer of T-cells from normal non-diabetic mice [112]. Irradiating certain strains of rat which have been thymectomized as adults produces diabetes and/or thyroiditis which can be prevented by transfer of normal CD4+ T-cells, again using the CD45RC hi subset [113]. The overall conclusion from these observations is that potential autoimmunity is normally kept at bay by the homeostatic interaction of T-cell subsets.

Is there evidence that failure to control potentially autoimmune T-cells is an important factor in human arthritis? Currently such evidence is lacking (hence the large question mark in Figure 5), mainly because the mechanisms of control have not yet been defined in detail so as to allow detection of abnormalities. However, RA is associated with other autoimmune diseases; both Sjogren’s syndrome and autoimmune thyroid disease occur at a higher frequency in patients with RA, suggesting a general failure to control autoreactive immune responses. Further evidence in favour of immunoregulatory abnormalities may come from therapeutic trials, and attempts are underway to stimulate regulatory cells by immunization with T-cell receptor peptides from putative arthritogenic T-cells [114].

Summary
The extent to which the immune system requires active regulation to avoid inflammatory autoimmune disease is now becoming evident. The possibility that defects in T-cell regulatory mechanisms might be the basis of some forms of human arthritis needs to be considered, but awaits better definition of the T-cell-dependent homoeostatic controls which normally operate.

CONCLUDING REMARKS
This review has sought to outline the ways in which T-cells might play a critical role in the pathogenesis of human inflammatory arthritis. Animal models have been useful for defining possible mechanisms; the challenge is now to determine which are operative in human disease. It is likely that several of the mechanisms may be involved in different diseases. A heterogeneous condition such as RA might consist of disease subsets with different abnormalities. Alternatively, different mechanisms may...
operate at different phases of the disease - there is no reason to assume that exactly the same immunopathological mechanisms operate in the early RA patient presenting with symmetrical synovitis and in the same patient 20 years later with Felty's syndrome and vasculitic ulcers. Progress might be expected in the following areas:

(i) definition of agents which can trigger synovitis, particularly infectious agents, and whether their action depends primarily on antigenic or adjuvant components;

(ii) identification of genes outside the MHC which predispose to persistent synovitis, and whether these influence regulatory T-cells;

(iii) definition of the normal process for resolving T-cell-mediated inflammatory responses.

Further understanding of any of these processes would be expected to lead to novel therapies. In the context of this review, it may not be surprising that initial and necessary naive attempts to treat RA with agents which deplete or inactivate T-cells in general, or substantial T-cell subsets, have had limited success. It would be an error to deduce from these results either that T-cells do not play a significant role in the pathogenesis of inflammatory arthritides, or that T-cell directed therapies will have little to offer.

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