In the 25 yr since the demonstration that specific recognition of antigens by T lymphocytes requires interaction with a self major histocompatibility complex (MHC) molecule [1], significant advances have been made in our understanding of this process. Initial observations of MHC associations with autoimmune disease involved the class I allele HLA-B27 with spondyloarthropathies in both adults and children [2, 3]. Subsequently, part of the inherited susceptibility to other arthritides was mapped to the MHC, in particular the association of severe rheumatoid arthritis (RA) with class II genes coding for HLA-DRβ molecules [4, 5], which share a common sequence in positions 67–74 [6]. Strong associations with MHC DRB1 alleles have also been recognized in the chronic arthritides of children, now collectively known as juvenile idiopathic arthritis (JIA). These differ from the RA-associated alleles and map to specific subgroups of disease, such as the young-onset oligoarticular disease which is associated with HLA-DRB1*0801, 1101, and 1301 [7, 8]. However, it is clear from genome-wide screening that the genetic component of these arthritides is polygenic [9, 10]. What is remarkable is that so many different MHC proteins confer an increased risk of some form of autoimmune disease, suggesting that common mechanisms for the breaking of tolerance exist, and directly implicating T cells in the disease processes.

The demonstration that the inflamed synovium in many arthritides contains a dense infiltrate of highly activated T cells which differ phenotypically from peripheral blood T cells [11–13] led to the T cell hypothesis of arthritis [14]: the concept of ‘arthritogenic peptides’ (whether self, or foreign but cross-reactive with self), whose recognition by T cells contributes to synovitis. However, the large number of studies looking for evidence of antigen-specific expansion of T cells within the joint have yielded conflicting results [15], casting doubt upon this hypothesis [16].

The cloning [17] and subsequent crystallization of the T cell receptor (TCR) [18, 19] have allowed visualization of the interaction between the TCR and its compound ligand, the peptide/MHC complex (p/MHC) [20]. Both
chains of the TCR heterodimer are variable molecules, and like Ig molecules are coded for by tandem arrays of rearranging genes. As expected from mutagenesis and sequence comparison studies, the most variable loops (CDR3) of the TCR\textalpha{} and \textbeta{} chains interact directly with antigenic peptide [21]. If the specific recognition of p/MHC drives the pathogenesis of arthritis, it has been widely held that the inflamed joint should contain expanded clones of T cells bearing a particular TCR, and also that related TCR sequences would be demonstrable. Furthermore, ‘public’ TCR sequences or BV usage should be common between different clones, or even between patients. Although restricted TCR usage has been seen in the response to given peptides [22, 23], it is clear that a single TCR may recognize a set of related peptides [24] and that some peptide-specific responses are remarkably diverse [25, 26]. In addition much evidence suggests that many of the T cells which enter an inflamed tissue are ‘bystanders’, recruited through their surface phenotype and ability to cross the endothelial barrier (under gradients such as chemokines, selectins and other leuco-attractant molecules), rather than by their antigen specificity [27, 28]. Even within a pathogenic lesion where T cells are known to contribute to autoimmune pathology, the percentage of antigen-specific T cells may be very low [29]. These facts do not preclude there being specific antigen recognition events which are critical to the pathogenesis of arthritis, but rather provide reasons for caution in over-interpretation of the data. Our recent understanding of functional divisions between different T cell populations and how they regulate each other, as well as new techniques to dissect these populations, should allow us to refine the T cell hypothesis in the future. Meanwhile, what can we learn from the myriad of studies thus far?

Early studies using Southern blotting to detect rearranged DNA [30, 31], or flow cytometric analysis with monoclonal antibodies to different TCRBV families of TCR [32–34] frequently suggested T cell expansions within the joint. Subsequently more sensitive methods using anchored polymerase chain reaction (PCR) or TCRBV family-specific PCR primers and probes were used [35–39] with conflicting results. In addition, a large number of studies have used PCR and DNA sequencing looking for both clonal expansions and shared or common motifs in the CDR3 sequences of expressed TCR [40–43]. These methods may still have been too insensitive for the detection of significant clones. The theoretical naive T cell repertoire, estimated at \(10^{13}\) different TCR, is larger than the number of T cells in an individual [17]. The study of T cell clone size has been greatly facilitated by the recent development of p/MHC oligomers (in general as tetramers) for staining reagents [44, 45]. These studies have shown that even large T cell clones which expand in response to viral infection are typically less than 1\% of the CD8\(^+\) population [46]. Memory CD4\(^+\) clones are often at far lower frequency than this [47, 48].

It is clear then that in order to investigate the role of antigen-specific T cells in the pathogenesis of arthritis, methods are required which are highly sensitive, which cover the whole expressed TCR repertoire, and which can ‘track’ specific clones in different sites or over time. The recognition that the CDR3 region of the TCR is critical to antigen recognition, and is also unique to a clone of T cells, has led to the development of methods for tracking T cells by their CDR3 sequences. In general, these use TCR family-specific PCR and most groups have analysed only the TCRBV chain. The interpretation of TCRBV PCR presents considerable difficulty since the TCRBV genes do not have tight allelic exclusion [49, 50]. CDR3-based methods are of two types: those which follow TCR by CDR3 length, and those which harness specific CDR3 sequence differences allowing a heteroduplex (HD) or single-stranded conformation polymorphism (SSCP) approach to be used.

When TCR amplification products from a polyclonal T cell population are analysed on a high-resolution gel they produce a normal distribution of PCR products differing in their CDR3 lengths. This method has become known as spectratyping [51] or immunoscope [52] and sensitivity is increased by using a TCRJB run-off PCR. Where clonal expansions occur, the Gaussian curve is distorted, and this has been used to demonstrate multiple expanded clones in synovial fluid T cells from patients with RA and reactive arthritis [53, 54]. The method provides CDR3 length but not sequence information, and favours detection of clones of long or short CDR3 lengths. In contrast, methods based upon CDR3 sequence differences, such as SSCP or HD, do not give length information but identify individual clones and are more sensitive for the detection of low-frequency clones [48]. Using a modified HD method [55] the synovial fluid (SF) T cells from patients with oligoarticular JIA have been shown to be highly oligoclonal in all TCRBV analysed, with identical clones present in the effusions of different joints [56]. These clones persist over many years and recur with a flare even after therapeutic joint injection (Wedderburn, unpublished observations). Surprisingly this oligoclonality is non-overlapping with peripheral blood: thus a large peripheral blood CD8\(^+\) clone in a child with JIA was not detectable in the inflamed joints, indicating that synovial T cell clonality is not simply due to amplification of ‘common’ clones from peripheral blood mononuclear cells [56]. Similar conclusions have been reached for RA using SSCP [57, 58]. Both these techniques allow the tracking of T cell clones, both over time and in different samples, by their specific and unique CDR3 regions.

The recognition that inflammatory T cells are a phenotypically selected subpopulation compared with PBMC, expressing high levels of CD45RO, HLA-DR and other activation markers [12, 13], has led some groups to compare the expressed TCR repertoire of synovial fluid T cells to a particular subset of PBMC. Although CD45RO\(^+\) T cells show a more restricted TCR repertoire than PBMC as a whole [59, 60], CDR3 analysis showed that the expressed TCR found in CD45RO\(^+\) synovial fluid T cells differed significantly from those of
CD45RO + PBMC [61]. A similar approach has been used to analyse Fas + [62] or IL-2R (CD25) + synovial T cells [31].

A ‘meta-analysis’ of this large collection of studies is made difficult by significant methodological differences such as whether T cells were from synovial fluid or tissue, or were initially expanded in vitro in the presence of IL-2 or mitogens, and the method used for TCR analysis. Despite this caveat and the conflicting nature of the data, some common themes have emerged. The inflammatory T cells found in both synovial fluid and synovial tissue in general express TCR from all TCRBV families, but within each family use a restricted set of TCR compared with PBMC. The clones which persist often occur in several joints, and are often not easily detectable in the PBMC from whence they arose. In many studies these expansions make up very large proportions of a particular TCRBV. The majority of these data arise from few patients; however, if taken together it would appear that the evidence for ‘public’ TCR or CDR3 motifs (common to many patients), which contribute to this oligoclonality, is lacking.

The prevailing interpretation of these data is that expanded clones within the synovium are evidence of an antigen-driven process, with specific recognition of p/MHC on antigen-presenting cells (such as dendritic cells or inflamed synoviocytes) within the joint. An alternative explanation remains a theoretical possibility. The ‘demands’ made upon a T cell to enter the joint across the inflamed endothelium, and then to survive there, are stringent. If such requirements can be met by only a small fraction of T cells, it is likely that these cells will have a survival advantage within the confined environment, outlive their fellow T cells and by default become expanded relative to other T cells. Such a scenario would not, a priori, require continued antigen-specific recognition.

The interpretation of TCR data is made difficult by the lack of clear evidence for common autoantigens, although many candidates, including collagen, Hcgp39 and heat shock proteins, have been implicated (reviewed in [63]). Here, animal models may usefully inform our thinking. In collagen-induced arthritis, administration of a single self peptide which is recognized by highly restricted T cells, can lead to a breakdown of tolerance and the cascade of responses leading to arthritis, yet the joint still contains many T cell clones [64]. More remarkable is the recent demonstration that a single transgenic TCR may lead to arthritis, even though the T cell recognition does not involve joint-specific antigens [65]: again TCR recognition of p/MHC is pathogenic but synovial T cells are heterogeneous. If these models are at least in part homologous to human arthritis, it is clear that the pathogenic TCR may be a small fraction of infiltrating T cells, or that the critical recognition of p/MHC may occur outside the joint. More recently, the approach of using mice made transgenic for human MHC class II genes (with or without human CD4) has yielded novel insights, including how different MHC alleles handle particular peptides and lead to different cytokine or disease profiles (reviewed in [66]).

The fact that within the inflamed joint T cells frequently show a marked skew in both their cytokine production, whether Th1-like (in RA and JIA) [67, 68] or Th2-like (in reactive arthritis) [69] and also in chemo-kine receptor expression [68, 70], suggests that recruitment mechanisms may be non-antigen specific. The realization that activated or memory T cells are in fact a complex set of committed populations which may be separable by markers such as CD7 [71], CD27 [72] or CCR7 [73] expression, should allow the careful dissection of the functional phenotype of cells which survive within a chronically inflamed site. The combination of our knowledge of the biochemistry of the TCR, new techniques to track specific T cells, and an understanding of their functional diversity should allow us to address the question of antigen-specific recognition by T cells in arthritis and their contribution to pathogenesis directly. Paradoxically it may be that the oligoclonality which has been frequently observed in T cells infiltrating these chronically inflamed sites will provide more information about the mechanisms of persistence of arthritis than the specificity of the antigens involved in its initiation.

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References

4. Wordsworth BP, Lanchbury JS, Sakkas LI, Welsh KI, Panayi GS, Bell JI. HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DRB1 is the major susceptibility locus within the HLA class II region. Proc Natl Acad Sci USA 1989;86:10049–53.

Editorials


55. Wack A, Montagna D, Dellabona P, Casorati G. An
et al.
56. Wedderburn LR, Maini MK, Patel A, Beverley PCL, mononuclear cells expressing the chemokine receptor
57. Yamamoto K, Sakoda H, Nakajima T et al.
58. Ikeda Y, Masuko K, Nakai Y et al.