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A FISTFUL OF T CELLS

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SUMMARY
Evidence incriminating T cells in rheumatoid arthritis (RA) is strong but circumstantial—like a smoking gun at the scene of a crime. To investigate this, T lymphocytes were studied in health and disease. The effect of mutations in the groove of HLA-A2 on peptide presentation to T cells was studied to investigate normal T cell function. This allowed a detailed description of the interactions between individual MHC residues and antigens. Subsequently, T cells in the autoimmune disease, multiple sclerosis, were studied, to investigate the mechanisms for breakdown in peripheral tolerance. T-cell clones that recognized both autoantigens and viral proteins were isolated, suggesting that infection may trigger disease. Autoantigens would need to be defined to use this strategy in RA. T-cell responses to type II collagen, a candidate auto-antigen, were therefore studied in RA and an epitope successfully defined. The search for microbial ‘mimics’ triggering RA, and novel forms of immunotherapy are now possible—with potential rehabilitation of T cells.

Key words: T cells, Rheumatoid arthritis, Antigen presentation, Peptide, Autoimmune disease, Collagen.

The stranger arrived and silently got off his horse. Outside the saloon lay the sheriff, wounded. In the middle of the gathering crowd stood a man holding a smoking gun. He claimed to be innocent. Nobody had witnessed the crime. Whodunit? How can this be solved?

There are some similarities here with autoimmune disease. As we get closer to understanding the pathology, the answer seems to slip farther away and new questions pop up to confuse the situation. For example, there is a general assumption that rheumatoid arthritis (RA) is an autoimmune condition. However, the basic pathological mechanisms underlying this remain unclear. Indeed, some authorities still venture to suggest that it is not an autoimmune disease at all [1]! In fairness, the evidence for cellular autoimmunity, whilst certainly provocative, is not conclusive. Many years ago, a profound lymphocyte infiltration was observed on simple histological examinations of inflamed synovial tissue, even resembling the follicles normally seen in lymph nodes, suggesting that T cells are activated. For many years, clinical studies, designed to reduce either lymphocyte numbers or function, have occurred in parallel. A whole range of interventions, including thoracic duct drainage [2], total lymphoid irradiation [3], leucopheresis [4], monoclonal anti-CD4 antibody [5] and cyclosporin A [6], have been tried, each with some, albeit variable, success. More recently, genetic techniques have revealed an association between disease severity and progression in RA and a small sequence of amino acids on class II major histocompatibility complex (MHC) molecules, the ‘shared epitope’ [7–9]. Since the only known function of these molecules is to present antigen to T lymphocytes, this suggests that these cells have a role in the pathogenesis of rheumatoid disease.

Let us say that the T lymphocyte is the prime suspect in the crime of causing RA, and is holding the smoking gun. I would like to prosecute, but any decent jury needs more than circumstantial evidence to convict. The case that I will prepare for my prosecution must address two main questions. (1) The target: what particular antigens in autoimmune disease did the T cell see that it did not like? (2) The motive: why did the T cell turn bad and bypass immunological tolerance mechanisms to become activated?

To prepare my case, I have utilized new technologies that are constantly evolving in immunology. In particular, I have concentrated on studying the relationship between structure and function in the interaction of T-cell antigen receptor (TCR), antigen and MHC molecule [10]. This lies at the heart of both normal and abnormal immune responses. Solving it may provide the key to understanding autoimmune diseases such as RA. It may also allow rehabilitation of the T cell, by the development of novel, effective therapy where the immune response is modulated specifically and safely—to prevent or reduce disease.

In this paper, I will summarize my work on the presentation of antigens to T cells. Firstly, the rules governing peptide interactions with class I MHC and TCR will be investigated, culminating in the detailed functional analysis of peptide presentation to T cells. Secondly, such principles are applied directly to the study of two MHC class II-related autoimmune diseases: multiple sclerosis (MS) and RA. Here potential triggers for MS are characterized, and target self-peptides for RA studied, allowing the development of specific, novel therapies. By the end of this paper, the case will be complete. It is then for the jury to decide upon the verdict on the T cell.

ANTIGEN PRESENTATION BY CLASS I MHC

Introduction
Whilst the existence of ‘tissue-type’ or ‘immune response’ genes had been known for many years, their...
physiological role—other than that of confounding transplant surgeons—was unclear. Detailed studies with inbred animal strains localized these genes to an area termed the MHC. The seminal work of Zinkernagel and Doherty in the 1970s [11] revealed that T cells recognized antigen only when the presenting cells expressed the same MHC genes as did the T cell. This was soon refined to a description of MHC molecules being cell surface molecules that were able to bind antigens, in the form of short peptides [12, 13]. Since then, the search was on to discover exactly how antigens fitted into the MHC molecule. A major breakthrough occurred in 1987 when Bjorkman et al. [14, 15] resolved the crystal structure of HLA-A2, one of the commonest MHC molecules. They described a highly polymorphic groove or cleft, running along the membrane-distal surface of the MHC. This had two main features. Firstly, it was of the appropriate size and shape to accommodate peptide antigen, and also it was accessible for interaction with the TCR (Fig. 1). It was hoped that a detailed characterization of the molecular interactions underlying antigen presentation would lead to more efficient means of stimulating the immune system (for vaccination), and novel ways to switch off the immune system (in autoimmune disease).

X-ray crystallography, however powerful a tool, could not provide information about the dynamics of peptide/MHC interaction and initially was not of sufficient resolution to determine the exact orientation of peptide within the groove of the MHC molecule. Such information could best be gained by a functional analysis of peptide/MHC/TCR interactions. I set out to study this with Professor A. J. McMichael at Oxford University. At that time, the structure of class II MHC [responsible for presenting peptide antigen to T helper (TH) lymphocytes] was not known; however, the high sequence similarity suggested that it would be similar to class I MHC.

The optimal class I MHC-restricted peptide contains nine amino acids

A full study of peptide presentation requires definition of the optimal peptide (with respect to both sequence and length) for a particular MHC molecule. Perhaps surprisingly, this key detail was not known. The system I selected to study was the presentation of influenza A virus by HLA-A2 to human cytotoxic T lymphocytes (CTL). This was chosen because HLA-A2 was a common MHC molecule, it was structurally defined, and T-cell responses to influenza A virus protein, restricted by HLA-A2, had already been observed. Although this particular MHC molecule was not directly associated with rheumatological disease, it had a significant sequence similarity with HLA-B27, for which no formal structural data were known. Indeed, class II molecules were also expected to behave in a similar manner. Synthetic peptides based on a fragment of the influenza A matrix protein (FMP), known to stimulate CTL responses, were therefore synthesized and tested for their ability to be recognized by influenza A-specific, HLA-A2-restricted, T-cell lines.

Recognition by CTL was assessed by their ability to lyse target cells that had been surface labelled with $^{51}$Cr. Cell lysis, occurring as a consequence of CTL recognition, resulted in a loss of membrane-bound $^{51}$Cr, which could be measured with a $\gamma$ radiation detector. The nine amino acid (9mer) peptide, FMP 58–66, was presented by far the best [16].

Mutations in a cluster of residues in the $\alpha_2$ domain $\alpha$ helix of HLA-A2 abolish presentation of peptide

The system of HLA-A2-restricted peptide presentation was now sufficiently refined to study the role of individual residues of the MHC and peptide in antigen presentation. I was also able to use it to describe interactions between the accessory molecule CD8 and class I MHC [17] (not discussed further here). Previous work on individuals with naturally occurring mutations in class I MHC molecules indicated that the $\alpha_2$ domain may be important for peptide binding and hence CTL recognition. In collaboration with Professor Jeff Frelinger and Dr Masanori Matsui (University of North Carolina, Chapel Hill, NC, USA), I determined the effect of introducing a series of mutations spanning the $\alpha_2$ domain $\alpha$ helix of HLA-A2 on presentation of peptide to T cells [18]. This constituted the whole of one wall and one-half of the floor of the peptide-binding groove (Fig. 1).

The strategy for this large undertaking was to use the novel technique of 'saturation mutagenesis' as a tool to introduce a series of random amino acid...
mutations along the whole of the $\alpha_2$ domain $\alpha$-helix. In this method, short oligonucleotides were synthesized, the reservoirs of each base deliberately contaminated with a small equimolar mix of the other three bases. This was designed to result statistically in one coding change per oligo, which were then cloned into Escherichia coli, expanded and sequenced. Those containing appropriate mutations were selected for study by transfecting into Hmy2.C1R cells that did not normally express class I MHC. The result was a large panel of clones that expressed HLA-A2 with point mutations spanning a major part of the peptide-binding groove. The advantage of studying the effect of random substitutions at each position was that it reduced selection bias in choosing mutations to study.

These transfected cells were then used to present FMP 58–66 peptide to influenza A matrix peptide-specific, HLA-A2-restricted T cells, as before. The results revealed that a striking cluster of residues from positions 152 to 156 in the $\alpha_2$ domain $\alpha$ helix of HLA-A2 were critical both for peptide presentation and functional importance, if any, of the FMP 58–66 peptide to influenza A matrix peptide—away from both peptide and TCR. This region corresponds closely to positions 152–156 of class I MHC. This would suggest that the third allelic hypervariable region on class II MHC molecules might also be intimately associated with peptide presentation. Indeed, it may be responsible for binding the 'arthritogenic' peptide involved in the pathogenesis of RA.

$\text{MHC heavy chain interaction with } \beta_2 \text{ microglobulin is necessary for } \text{MHC class I function}$

Analysis of the effect of further mutations in the $\alpha_2$ domain of HLA-A2 revealed that some residues lying in the floor of the groove also affected antigen presentation [21]. The importance of these particular positions was unexpected, because their side chains pointed not upwards, for interaction with peptide, but rather down—away from both peptide and TCR. This area of the floor of the groove lay above the small $\beta_2$ microglobulin chain that was non-covalently associated with the heavy chain (Fig. 1). At that time, the functional importance, if any, of $\beta_2$ microglobulin was not known. This observation indicated that an interaction between the heavy and light chains was indeed necessary for class I MHC function. This has subsequently been confirmed, using mutant cell lines with defects in antigen presentation [22].

$\text{Pockets lining the groove of class I MHC bind peptide antigen and are important for peptide presentation}$

A number of depressions or pockets in the peptide-binding groove were observed in the crystal structure of HLA-A2 and other class I MHC molecules [14, 15]. These appeared to be of sufficient size, shape and charge to accommodate amino acid side chains of the peptide antigen; however, whether they had any important role in binding peptides was not known. The effect of selected mutations introduced into many of the pockets of HLA-A2 by site-directed mutagenesis was therefore studied in collaboration with Professor Jack Strominger and Dr France Latron (Dana Farber Cancer Institute, Harvard University, USA). Most of these substitutions led to a loss of peptide presentation, suggesting that they were indeed responsible for binding peptide—even those pockets that looked too ‘shallow’ on X-ray crystallographic examination [21, 23, 24].

These residues were also found to have the paradoxical effect of selectively interfering with either the presentation of peptide fragment, or whole virus, but not both. This suggests that they are important in the dynamics of peptide binding to MHC during breakdown of larger fragments to bound peptide [25].

These data illustrate the limitations of purely structural studies, and confirm the requirement for parallel functional work to elucidate the molecular mechanisms underlying how T cells are presented with antigen.

$\text{The detailed orientation and position of peptide within the groove of HLA-A2 is deduced}$

The ultimate goal of this class I MHC work was to deduce the position of peptide antigen within the
groove. The potential benefits of such a description are great. These would include constructing criteria for the prediction of other class I MHC allele-associated peptides, improving vaccine design, and potentially generating ‘blocking’ or ‘inhibitory’ peptides that bind MHC, but could not be recognized by TCR—resulting perhaps in the selective switching off of autoimmune responses (Fig. 3, see below).

Within the extensive panel of transfected cells, some expressed HLA-A2 molecules with altered electrostatic charges at either end of the groove, and resultant loss of peptide presentation. We hypothesized that the introduction of a complementary opposite charge in the amino acid of the peptide that bound to that part of the MHC where the mutation occurred may restore presentation. This would indicate that these two residues must interact and lie in close proximity. A panel of synthetic peptides based on the index FMP 58–66 peptide was therefore synthesized, with individual substitutions of positive and negatively charged amino acids at each position. They were not presented to T cells expressing normal HLA-A2, but some were presented by some of the abnormal HLA-A2 molecules.

Charge changes at either end of the peptide were then correlated with charge change in the HLA-A2 molecule. In particular, a peptide with a change of charge from neutral to a positive residue at position 66 could now be presented by an HLA-A2 molecule with a negative charge change at position 116 (on the left-hand side of the floor of the groove). This would indicate that these two residues lie in close proximity. Further analyses of other such charge change complementations were able to pin down the orientation and position of peptide in the groove in detail [26] (Fig. 4). Molecular modelling studies from these data reveal that the FMP 58–66 peptide lies in the groove of HLA-A2 in an elongated form from the amino to carboxyl ends, with a short ‘hump’ in the middle [21]. The relative importance of most of the amino acids could be determined by this charge change complementarity technique. Later, high-resolution X-ray techniques using purified recombinant class I molecules have confirmed that peptides lie in the groove of class I MHC molecules in such a manner [27].

CLASS II MHC AND MOLECULAR MIMICRY IN AUTOIMMUNE DISEASE

Multiple sclerosis

Multiple sclerosis: a ‘model’ autoimmune disease for RA. Multiple sclerosis really is a T-cell-mediated, autoimmune disease. It is associated with class II MHC (HLA-DR2), dense T-cell infiltrates are found in inflammatory plaques and, in striking contrast to RA, autoantigens recognized by T cells have been discovered and characterized (reviewed by Moots and Wucherpfennig [28]). The search for candidate antigens started by focusing on the target organ: the central nervous system. Various components of nerve sheaths, notably myelin basic protein (MBP), have been observed to contain T-cell epitopes in animals (and act as a target for T cells in experimental allergic encephalomyelitis (EAE) [28]). MBP-reactive T cells have also been observed in the blood of patients with MS where they are often clonally expanded and persistent [29]. In these key respects, considerably more is known about the immunology of MS than of RA. Since both diseases are T cell mediated and are associated with class II MHC molecules, I set out to investigate the potential mechanism whereby autoreactive T cells become activated and break immunological tolerance in MS. If this approach were successful, it may be possible to apply it to RA.

Molecular mimicry in MS. Potentially autoreactive T cells may escape from deletion in the thymus during maturation and are often found in normal people. The resultant ‘resting’ autoreactive T cells interact with the TCR in a manner that will either block TCR recognition, or even ‘switch off’ the TCR so that it is unable to respond to other peptides.

Fig. 3. — Peptide immunotherapy. One potential mechanism whereby altered synthetic peptides may be used to modify (or prevent) disease is illustrated here. The interaction between peptide autoantigen, MHC and TCR must first be characterized in detail (above). An analogue peptide is then synthesized (below). This would have selective alterations, designed to retain binding to the MHC, but interact with the TCR in a manner that will either block TCR recognition, or even ‘switch off’ the TCR so that it is unable to respond to other peptides.
M antigens, myocardial and glomerular tissue [30]. A comparable event in T cells would be somewhat more complicated. Here there would have to be a dual specificity of TCR for both self and foreign peptide, each presented by disease-specific MHC molecules such as HLA-DR2 for MS and HLA-DR4 in RA (Fig. 5). It has proved to be a considerable task to investigate this [28]. Many have searched in vain, hampered by the far greater complexity of antigen recognition for T cells, compared to antibody. As for humoral mimicry, computers have been used to search for linear sequences of similarity between self and foreign proteins. This approach was not successful, because unless the self and foreign sequences were identical (up to a 1 in 20th chance), a significant recognition would not have been anticipated. Over the last few years, we have learnt that TCR recognition is actually more degenerate, and cross-reactivity at the T-cell level may indeed be common (reviewed by Moots and Wucherpfennig [28]). Indeed, the same MHC molecule (murine I-Au) has been observed to present two dissimilar peptides to the same specific T-cell clones, leading to the term ‘space mimicry’ [31].

This approach was refined further by Wucherpfennig and Strominger [32]. T-cell clones reactive to human MBP were generated from patients with MS and used to characterize the structural requirements for recognition of the immunodominant MBP(85–99) peptide in terms of MHC binding and TCR recognition. A series of amino acid substitutions were then selectively introduced at each critical position and the effects on T-cell recognition analysed. From this, the sets of amino acids permitted at each of the critical residues were defined. These structural data, together with the knowledge that amino acid side chains required for binding to MHC molecules are degenerate, were used to search a protein sequence database of human pathogens. Candidate peptides were synthesized and tested for recognition by MBP-reactive human T-cell clones derived from the peripheral blood of patients with MS. Seven viral and one bacterial peptide could also efficiently stimulate MBP-specific T-cell clones, mimicking the immunodominant MBP(85–99) peptide [33], even in the absence of any obvious sequence homology with the MBP peptide. This indicated that some TCRs recognize not just a single peptide, as had been previously thought, but rather a set of structurally related peptides derived from different antigens.

The diverse nature of the viral peptides able to stimulate MBP-specific T-cell clones from these data would suggest that more than one single pathogen is able to trigger autoimmunity. This may explain why it has been so difficult to link conclusively the pathogenesis of individual autoimmune diseases such as RA to particular pathogens, in the face of considerable evidence for association of disease with infection in general [28]. Rather, it is more likely that a group of common microbial pathogens are involved in the pathogenesis of autoimmune processes. In this case, an immunological approach has considerably more potential than epidemiological methods to determine the triggers.

**Fig. 4.**—The detailed position of peptide in the groove of HLA-A2 is deduced. By assimilating mutagenesis and charge change complementation data, some of the rules governing the interaction of individual amino acids from both peptide antigen and MHC molecule can be defined. The detailed position of peptide within the groove can then be deduced, by running these data on a Silicon Graphics computer using the program CHARMM (performed by Dr S. P. Young, Birmingham University). Above: looking along the groove, the two α helices on either side. The top of the peptide can be seen to stick out and up, for interaction with the TCR. Below: the peptide/MHC complex is now rotated so that the α helices are parallel to the paper and superimposed. The ‘hump’ in the middle of the peptide can be clearly seen, with the two anchors to the MHC lying deep inside the groove, at either end.

Molecular mimicry. Both implicate microbial pathogens and are supported by some (albeit limited) epidemiological evidence. There is currently very little evidence to support the role of superantigen as a trigger of MS or RA.

Molecular mimicry depends upon a structural homology between self and foreign antigens. This would result in a cross-reactive T-cell response if there were sufficient similarities between the two peptide epitopes for activation, yet enough differences to overcome tolerance. In rheumatic fever, there is a cross-reaction at the level of antibody between group A streptococcal
Fig. 5.—Molecular mimicry. This potential mechanism explains how tolerance to self-proteins may be broken in autoimmune disease. Here, T cells make an appropriate immune response to microbial antigens produced during infection. One T-cell clone, however, with an additional specificity for a self-antigen may become activated. If, during normal circulation through the body, this activated T cell encounters the appropriate self-antigen, it would proliferate further, mediating inflammation and hence disease.

Cross-reactivity for MBP, herpes simplex type 1 and human coronavirus

The exciting discovery of the potential for molecular mimicry in MS was an important advance. However, if peptides synthesized in the laboratory from published sequences of viruses cross-react with the self-protein MBP for presentation to TCR by the disease-associated molecule HLA-DR2, can this occur in the more physiological circumstances when cells are infected with whole virus? Cells have complicated machinery for presenting antigens to T cells. Proteins are first broken down to long peptide fragments in discrete cellular compartments by specific proteases. These are loaded onto class II MHC molecules by helper proteins. Finally, after trimming of the peptide, it is exported to the cell surface in complex with the MHC, where it is available for recognition by a specific TCR [34]. Would this complicated, specific cellular mechanism result in the exposure of the same sequences, that seem to work well, when computer-predicted peptides are made in the test tube?

I addressed this question in the laboratory of Strominger and Wucherpfennig, by assembling a panel of human viruses, and testing them for T-cell cross-reactivity with MBP. Viruses were chosen in preference to bacteria because it was technically considerably easier to work with them in tissue culture. They were selected on account of their availability, capacity to infect the APC used in proliferation assays, genome size (hence potential to code for protein) and, in some cases, possession of potential ‘mimicry epitopes’ [33]. These included human coronavirus and a variety of human herpes group viruses.

MBP-specific T-cell lines were first set up from lymphocytes isolated from the peripheral blood of patients with MS (courtesy of Charles Poser, Beth Israel Hospital, Harvard University, Boston, USA). A total of 19 patients were studied. MBP-specific T-cell lines were grown from 11 of them, corresponding with other reports. However, in four of the patients with MBP-reactive T-cell lines there was also some recognition of human coronavirus (CV) 229E, a common cause of upper respiratory tract infections (Fig. 6). This was investigated further, by studying the other human coronavirus serotype OC43 and the murine MHV A-59. Only 229E was recognized by MBP-specific T-cell lines. Lymphocytes from the patient with the best cross-reactive response were studied in more detail. MBP-specific T-cell lines were made by limiting dilution. Three independent clones with good reactivity against MBP were tested against CV and the other viruses. In each case, the clones were able not only to recognize the MBP peptide (MBP 85–102), but also CV229E and herpes simplex type 1. Other coronaviruses were not recognized, nor were the other viruses, including herpes simplex type 2. In this case, the antigen receptor of the MBP-specific T-cell clones appeared to have a triple specificity! Preliminary time scale experiments indicated that one of the early HSV 1 proteins (expressed within the first 4 h after infection)
I determined to investigate the prevalence of T-cell responses to CII in RA, applying the lessons learnt from analysis of the structure/function relationships of MHC. One problem from previous studies using collagen was that T cells were actually reactive to a contaminant, pepsin, rather than collagen. In addition, bovine collagen was usually used, because of its better availability. As for other T-cell work, the purity of the protein antigen was critical. I obtained highly purified CII from Dr Vic Duance of the Connective Tissue Research Unit, Cardi University. Gel electrophoresis analysis suggested that this protein would be of sufficient purity for use in generating specific T-cell responses.

![Graph](image)

**Fig. 6.** - A T-cell clone with specificity for self and foreign antigens. T-cell recognition of antigen is assessed by the capacity to take-up [3H]thymidine during proliferation. Cellular [3H]thymidine is measured by gamma counter and expressed as counts per minute (c.p.m.). The greater this value, the better the recognition of antigen. The T-cell clone here (JG10A3) was isolated from a patient with MS. It is specific for the MBP peptide 85-102. It is, however, also able to recognize autologous APC infected with the human pathogens HSV 1 or CV 229E. (In the latter case, the proliferative response is better than for whole MBP or MBP peptide.)

contains the mimicry epitope. Work is now in progress to define this in detail and determine whether this phenomenon holds true for independent clones from other patients. It is exciting to consider that the triggers for MS may be elucidated by this method.

**T-cell responses in RA**

Despite hard searching, no definitive autoantigen has been found in RA, suggesting to some that T cells are not important in this disease. The same had been said for MS a few years before the discovery of T-cell reactivity to MBP. I believed that the recent technical improvements in methods for generating T-cell lines were now sufficient to try to search for autoantigens in RA. Various candidate autoantigens have been proposed, including heat shock proteins, cartilage link protein and other proteoglycans (reviewed by Moots and Wucherpfennig [28]). Perhaps the most compelling of these is type II collagen (CII). This is expressed almost exclusively in synovial joints and is able to induce an inflammatory erosive arthritis in rodents, which shares many features with RA. This animal disease, collagen-induced arthritis (CIA), is mediated, at least in part, by CII-reactive T cells [35]. Indeed, a large amount of effort has been invested in studying the potential for inducing oral tolerance, with some preliminary success [36]. Previous studies had suggested that T cells reactive to CII may exist in patients with RA, and indeed may persist [37], but little was known about the detail, if true.

The occurrence of autoreactive T cells alone would not be enough to link the smoking gun with the shooting: autoreactive T- and B-cell responses to a wide range of self-antigens may be found in many ‘normal’ people. However, a concentration of autoreactive T cells in the diseased compartment, the joint, would be somewhat more convincing.

**CII-specific T-cell responses in synovial fluid of patients with RA**

T lymphocytes were isolated from paired synovial fluid and blood samples from patients with RA and other forms of inflammatory arthritis. Patients were recruited from the Brigham and Women’s, New England Deaconess and New England Baptist Hospitals, Boston, MA, USA. Cells were grown in culture and tested for CII reactivity using a simple [3H]thymidine incorporation proliferation assay. T cells recognizing CII were maintained in culture and restimulated with further CII until stable T-cell lines emerged.

A degree of CII specificity was observed from T cells derived from synovial fluid in 11 out of 16 patients with RA. No reactivity was observed in synovial or peripheral blood T cells taken from any of the three patients with diagnoses other than RA. Type II collagen reactivity was concentrated in the joint (Fig. 7) with responses in blood (taken in parallel) only observed in two of those patients. No T-cell responses to CII were observed in the blood in the absence of a synovial fluid response.

![Graph](image)

**Fig. 7.** - CII-specific T-cell reactivity in RA. In this [3H]thymidine incorporation proliferation assay, a T-cell line, derived from synovial fluid (SF) lymphocytes of a patient with RA, is reactive to both CII and the CII peptide 258-272. No comparable reactivity is observed from peripheral blood lymphocytes isolated from this patient.
A human CII epitope is defined

The observation of a T-cell response to CII in patients with RA can now be characterized in more detail. The key to this is defining the peptide epitope. The approach that I employed for this was to use information on the dynamics of peptide class II MHC interaction to predict peptides from within CII that would be capable of binding disease-specific MHC molecules such as HLA-DR4. It is known that slightly longer peptides bind to class II compared to class I MHC, of the order of 15 amino acids. The dynamics of this interaction also appear to differ from class I MHC and there would appear to be more degeneracy in binding to particular class II molecules [38, 39]. Crystal structure analyses of a variety of class II MHC molecules indicate that relatively longer peptides are bound, overhanging the ends of the groove.

Unlike class I MHC, the main interaction is with the peptide backbone rather than amino acid side chain, reflecting the lower specificity [39]. However, peptide-binding pockets in class II MHC are present, for binding peptide amino acid side chains. Moreover, in vitro binding studies, where peptides with varying sequences are studied for their ability to bind specific class II molecules such as HLA-DR4, have suggested that certain motifs or anchor residues may be found in peptides that bind to individual class II molecules [40–42]. A computer search for potential HLA-DR4-restricted CII epitopes was performed on a human protein database using criteria for HLA-DR4-binding motifs from published data. Seven epitopes were predicted to bind to HLA-DR4. These were then synthesized and used to sensitize autologous antigen-presenting cells for presentation to CII-specific T-cell lines.

One peptide, comprising residues 258–272, was recognized by T-cell lines from patients with RA, and not by those from control patients with diagnoses of reactive arthritis, psoriatic arthritis or osteoarthritis. Recently, other work in transgenic mice engineered to express human HLA-DR4 and DR-1 molecules has supported the idea that the immunodominant CII peptide lies in this region [43, 44]. In addition to this, the same peptide appears to mediate the bulk of the anti-CII response in the H-2q mouse [45]. The characterization of such an immunodominant CII peptide, restricted by disease-associated MHC molecules in humans with disease, is of fundamental importance to the study of potential triggering mechanisms of RA and lays the foundation for molecular mimicry work to occur, as for MS.

POTENTIAL FOR IMMUNOTHERAPY

Understanding the molecular immunology of antigen presentation is a major achievement. By elucidating the fine specificity of events between the tri-molecular complex of peptide, MHC and TCR, we are able to apply the lessons learnt to autoimmunity. The discovery of molecular mimicry in MS and a self-peptide in RA may have significant clinical benefits. Indeed, immunotherapy already is a treatment of today. The outlook for inbred strains of mice with the murine model of MS, EAE, is now exceedingly good! With peptide epitopes and TCR specificity known, it is possible both to prevent disease and induce remission in established disease by the use of analogue peptides that are able either to block or actively ‘switch off’ activated autoaggressive T cells [46], and do this specifically and safely (Fig. 3). There are similar reports in the CIA model for RA [47]. Whilst this is obviously harder in humans—thankfully we are not inbred—it now becomes a possibility as the underlying mechanisms behind autoimmune disease become clearer.

CONCLUSIONS

T lymphocytes are at the same time good (protect us from disease), bad (induce autoimmune disease) and ugly (give us many clues, but show increasing levels of complexity the nearer we get to understanding them). Were Clint Eastwood a rheumatologist or immunologist, he would have solved this long ago, and moved on to other things. As scientists, we require a higher level of proof before we are convinced. This is now starting to happen. The rapid developments in immunological techniques over recent years are there for us to grasp, and apply to clinical problems such as RA and MS. Understanding, as we do now, the molecular immunology of antigen presentation in autoimmune disease, may have as great a consequence for the future of rheumatology as did the invention of the Winchester rifle for the Wild West.

I conclude that in this awful crime of crippling destruction of the joint in RA, there is good reason to charge the T lymphocyte. Using molecular biological techniques, I and others now have strong evidence to implicate the lymphocyte’s unique antigen recognition in the crime. The target is possibly an immunodominant epitope within type II collagen. What was the motivation? I believe that it was not revenge (supertagonism), but rather mistaken identity (molecular mimicry, as for MS). So what are the lessons for the future? Can the lymphocyte be rehabilitated? Perhaps better education, in the form of specific immunotherapy, rather than our current methods of punishment with relatively crude immunosuppression, will allow the lymphocyte to retain some honour and lead a more profitable life.

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