I am aware of the great personal honour of being invited to give the Heberden Oration, and wish to acknowledge my gratitude to numerous colleagues over the years for their work, support and collaboration. This event, long established in the traditions of our rheumatological society, symbolizes the importance that is attached for the pursuit of scientific knowledge for the purpose of understanding the causes of rheumatic diseases. It provides an opportunity for the Orator to take stock of his field of study and view his contributions in some perspective.

In my field of study, namely, rheumatoid arthritis (RA) and connective tissue diseases, the driving force for the past five or six decades has been the hunch that disease is triggered in susceptible individuals by an environmental agent. This, together with the observation that autoimmune phenomena are commonly observed in patients, has focused research on the cells and products of the immune system. By describing some aspects of such work, and for the sake of brevity, concentrating on rheumatoid arthritis, I want to convince you that as a result of the inspiration provided by intellectual ideas on the mechanisms leading to disease and the increasing accessibility to powerful clinical, molecular and cellular tools for study, we are on the threshold of major advances in unravelling the hitherto mysterious causes of these disorders.

**HLA class II antigens: susceptibility factors, initiators, and regulators of autoimmune responses**

Epidemiological studies predict that genetic inheritability plays a part in the development of RA but that several genes are involved in conferring susceptibility [1]. The observation that the HLA class II antigens HLA-DW4 and DR4 were over-represented in Caucasian patients with rheumatoid arthritis has been amply confirmed by many studies including a large study on 440 patients in Britain to which our unit contributed [2, 3]. The HLA association suggests that one of the genes involved is either a polymorphic gene expressed as the $\beta$-chain of the HLA-DR4 molecule itself, or a polymorphic gene in proximity to it on a stretch of DNA on the short arm of the sixth chromosome.

In recent studies of seminal importance by Bjorkman and her colleagues [4], it proved possible to utilize X-ray diffraction analysis of crystals of an HLA molecule to show that the protein domain distal to the cell membrane forms a cleft or groove-like structure, 25Å long by 10Å wide by 11Å deep, the floor of which is a platform consisting of $\beta$-strands of peptides and the walls of which are formed by spring-like $\alpha$-helical peptides. This groove is not only the site for antigen binding but is involved in interacting with the T-cell receptor [5]. These studies form the basis of the general concept that antigen presentation to T-cells involves the formation of a trimolecular complex consisting of the HLA molecules, antigen, and T-cell receptor in which the specificity and diversity of the reaction is dictated by the amino acid structure of the three interacting molecules (Fig. 1).

In the context of these observations it has emerged that polymorphisms of class II genes coding the variable $\beta$-chain which forms one of the $\alpha$-helical walls of the groove are important in defining disease susceptibility. In the case of insulin-dependent diabetes mellitus, critical susceptibility or resistance is conferred by amino acid residue 57 on the DQ$\beta$ chain such that presence of aspartic acid correlates with resistance [6]. In the case of RA, susceptibility correlates with the amino acid sequence of DR$\beta$1 third hypervariable region from position 70 to 75, which also falls within the $\alpha$-helical structure of the
Fig. 1.—Antigen processed by an antigen-presenting cell (APC) is bound to the groove-like structure formed by α- and β-chains of HLA molecule. Antigen (Ag) bound to this groove forms a trimolecular complex with the α, β-chain of T-cell receptor. CD3 molecules transduce an activation signal. CD4 provides further adhesion between the cells by binding to HLA, and the adhesion molecules LFA-3 and CD2 also interact. In providing better cellular contact, APCs synthesize IL-1 (and TNF) which are involved in signalling T-cell activation; IFN-γ produced by T-cells augments gene regulation of HLA molecules.

groove, and includes the presence of glutamine at position 70 and arginine or lysine at position 71, and is present in the HLA-DR4 subtypes DW4, DW14 and DW15, as well as HLA-DR1, all of which have been found to be increased in RA patients (reviewed in [7]).

The fact that the antigen and T-cell binding site of the HLA molecule is implicated in susceptibility to RA may help to explain several observations that we and others have made and which point to functional pathways of aetio-pathogenetic importance. These include the following:

1. In a study on RA patients we found that the frequency of HLA-DR4 rose to 92% in RA patients with vasculitis and other forms of extra-articular disease whose serum contained rheumatoid factor and immune complexes [8]. We have analysed immune complexes from such patients and shown them to be enriched in all classes of RF including IgM, IgG, and IgE [9–13]. This suggests that B-cells making RF are at least partly dependent on T-cell help, and implies that both B- and T-cell receptors bind to the antigens on Fc-gamma (the antigen of RF). In the case of antigen presentation to T-cells, the antigen would be first bound to an HLA class II antigen and in RA one would expect that this would be the β-chain of HLA-DR4 or DR-1. The reported finding of a correlation between in vitro responses to collagen type II and DR4 may similarly indicate that in addition to antigens on Fc-gamma, collagen type II antigens presented to T-cells in RA are preferentially bound by HLA-DR4 [14]. In the light of this, it is of interest that we have successfully cloned T-cells reactive with collagen II from the synovium obtained at surgery on a rheumatoid patient on three occasions over 2 years [15].

In other studies we find support for the concept that the HLA phenotypes may determine specific autoimmune responses by the best fit of antigens to HLA and presentation to T-cells. Thus our findings that IgG anti-SS-B (La) antibodies occur almost exclusively in HLA-DR3 Caucasians [16], and that normal individuals whose peripheral blood lymphocytes secrete the highest levels of anti-La antibodies under the influence of a polyclonal mitogen are HLA-DR3 [17], strongly hint that La antigens are presented to T-cells by HLA-DR3 prior to triggering of B-cells by the La antigen. Direct evidence for this hypothesis could be obtained by presenting appropriate autoantigogenic peptides with defined HLA molecules to T-cell clones. One might predict that, if successful, this would lead to a fruitful search for synthetic peptide analogues of natural autoantigens, so as to blockade artificially the HLA groove and prevent the harmful consequences of autoantigen binding.

2. Overexpression of HLA molecules at disease site would lead to localization and perpetuation of immune responses in joints of RA patients. Immunohistological examination of synovia has shown that HLA molecules of all isotypes (DP, DQ and DR) are expressed at high levels in the rheumatoid joint [18, 19]. This finding has been quantitated and confirmed by measuring mRNA levels of HLA class II molecules expressed by synovial cells. In these experiments we showed elevated levels of HLA-DR, DP and DR persisting for up to 5 days in culture [20], and since the half-life of mRNA was only 30 minutes, the experiments suggested that gene regulation was dependent upon cellular interactions. My colleague, Marc Feldmann, has suggested that persistent up-regulation of HLA class II genes could be viewed as a step of central importance in establishing a self-perpetuating immune reaction dependent upon cellular interactions involving antigen-presenting cells and T-lymphocytes, and mediated by cytokines [21]. This reaction may be a feature of many autoimmune diseases and is not specific for RA. However, recognition molecules and ligands would differ in the various forms of autoimmune diseases and
include antigens present in target tissues. Whether in RA the ligands are autoantigens, foreign antigens, or a combination of both is open to speculation at this stage. However, once established, HLA expression could be itself responsible for the enhanced ability of cells derived from joints to mount a vigorous autologous mixed lymphocyte reaction of the type described by Zvaifler's group [22].

In an attempt to ascertain whether particular HLA isotypes may play a special role in perpetuating chronic synovitis, we were surprised to find that, in RA biopsies of synovium taken at an early stage of disease, HLA-DR, DP, and DR were already all equally expressed. In contrast, biopsies from patients with reactive arthritis at an early stage, whose arthritis ran a self-limiting course lasting up to 20 weeks, showed extensive expression of HLA-DR, but relatively little HLA-DQ expression [23]. We have no satisfactory explanation for this finding at this stage but were interested in a recent report that gold-induced remission of RA was accompanied by a differential suppression of HLA-DQ [24]. One is tempted to suggest that if HLA-DR molecules initiate autoimmunity, understanding DQ regulation may shed light on factors which terminate it.

3. Molecular mimicry between HLA-DR and the Epstein-Barr virus (EBV) may be of aetiological importance. For some years we have been impressed by the remarkably elevated levels of IgG antibodies to rheumatoid arthritis nuclear antigen (RANA) in patients with RA and have speculated on the significance of this finding [25, 26]. This was originally interpreted as possibly indicating a link between EBV infection and RA [27], but since we documented a handful of patients with classical RA who had no serological evidence of previous EBV infection, this agent could not be the sole cause of RA. We have subsequently shown that anti-RANA antibody is a result of reactions with epitopes on Epstein-Barr nuclear antigen (EBNA-1) and the synthetic glycine and alanine-rich antigen p62 represented in EBNA-1 is the main target epitope recognized by anti-RANA antibodies [28].

The paradox is that IgG anti-RANA antibodies contrast with similar or marginally elevated levels of antibodies to EBNA-1 or to EBV-viral capsid antigens in RA [25, 29, 30]. This pattern is not consistent with a persistent EBV infection of the type proposed on the basis of substantially increased levels to all EBV-viral antigens in nasopharyngeal carcinoma [31]. Clearly the dissociation of antibody responses to EBV antigens in RA must have some definable basis, and, if understood, could shed light on the link between RA and this DNA virus.

One explanation of the observations is the possibility that RANA may show immunological identity to a (non-EBV) nuclear antigen, and we have found some support for this idea in our studies [25]. Thus in RA an autoimmune response to the nuclear antigen (indistinguishable serologically) may summate with an anti-RANA response. An equally plausible theory is that since T-cell regulation of EBV-infection is defective in RA [32] the infection load with the virus may be marginally, but sufficiently, elevated to induce RANA antigens and polyclonal proliferation of B-cells without increasing productive and replicative EBV infection. The increased RANA antigen turnover may in turn provide the immunogen in RA, although this scheme does not explain why anti-EBNA antibodies are not raised in parallel with anti-RANA antibodies.

Recently, Carson's group from the Scripps Clinic published an hypothesis on the link between RA and EBV, based upon an observed homology between the HLA-DR β1 chain sequence from positions 69 to 74 in the hypervariable region, which confers RA susceptibility, discussed above, and a 6-amino-acid stretch on gp 110 viral capsid antigen of EBV [33]. This sequence present in the HLA-antigen groove might be expected to interact with the T-cell receptor. The immunological consequence of such an homology is difficult to predict, but it is likely that during immune repertoire development HLA-DR4 individuals would delete T-cells which bind such a peptide and thereby acquire immunological tolerance to this shared auto- and EBV epitope. If gp 110 were an important target of T-cell regulation of EBV, such a tolerance mechanism could form the basis of the described permissive state for EBV persistence and alter the dynamics of immune responses to the virus and its antigens. Alternatively, as suggested by the authors of the hypothesis, overrepresentation of shared HLA-DR β1 epitopes could be responsible for triggering autologous mixed lymphocyte reactive T-cells in joints [33]. Whether EBV is pathogenic in RA remains unproven, but it is possible that by pursuing the investigations further, important insights into disease mechanisms will emerge.

The significance of B-cell activation with special reference to CD3+ B-cells

Since the discovery 50 years ago by Eric
Waaler that rheumatoid factor was characteristically present in the blood of RA patients [34], a great deal of interest has centred on the role of B-lymphocytes in this disease [35]. Immunoglobulin-producing cells were demonstrated in rheumatoid synovia by Mellors et al. [36] 30 years ago and IgG rheumatoid factor was described as forming a major part of the B-cell response in RA synovium by Natvig and Munthe [37] 13 years ago.

In 1982, we found an increase in a subset of B-lymphocytes in RA which form rosettes with mouse erythrocytes [38]. The finding assumed special significance when we learned that this B-cell subset could be selectively stimulated by EBV to produce RF in vitro bearing a cross-reactive idiotype [39] and was the predominant B-cell during fetal development [40]. The B-lymphocytes bearing the mouse erythrocyte binding receptor turned out also to express the CD5 antigen in fetal B-cells and in chronic lymphatic leukaemia [40, 41]. We were soon able to show that the frequency of CD5+ B-cells was increased in RA [42]. Meanwhile, the observations of the Herzenberg group on murine Ly-1 B-cells, the equivalent of CD5+ B-cells in man, had not escaped our attention. In a series of published papers which were highly relevant to the findings in RA, they had shown that CD5+ B-cells in the murine system secreted IgM autoantibodies, were genetically regulated, overexpressed in mouse strains susceptible to autoimmunity and in a lineage of B-cells originating from the peritoneum distinct from ‘bone marrow derived adult’ B-cells (reviewed in [43, 44]) (Fig. 2).

In our original experiments showing increased CD5 B-cells in the blood of RA patients [42] we had used the technique of double-colour immunofluorescence microscopy. Subsequently we have used laser flow cytometry for documenting CD5+ B-cells, since it offers a more objective, sensitive, and quantitative technique for phenotyping cells, and because a fluorescence-activated cell sorter allows us to obtain pure populations of CD5+ B and CD5 negative (CD5-) B-cells for functional analysis. Using these techniques, we have been evaluating CD5+ B-cells in health and disease and have come to interim conclusions which are summarized below.

1. CD5+ B-cells predominate in fetal life and account for a significant proportion (~50%) of circulating B-cells in neonatal cord blood. We do not have any information on the stage in childhood or young life when this population is overtaken by the conventional CD5− B-cells, but in the majority of adults, CD5+ B-cells only account for 0–21% of the circulating B-cell pool [45] (Fig. 3).

2. In some apparently healthy adults, CD5+ B-cells are found in increased numbers and such
individuals notably include relatives of patients with RA and connective tissue diseases. This raises the possibility that, as exemplified in the murine system, the numbers of CD5+ B-cells are genetically regulated and could represent a susceptibility marker of disease. We have begun to address this issue, and in a preliminary and limited study on families of RA patients, have some evidence in support of the concept that a high frequency of CD5+ B-cells in adult life is under genetic influence. This is in accord with published evidence of concordance of CD5+ B-cell numbers in pairs of identical twins of whom one had RA [46].

3. In about 50% of our adult RA patients, CD5+ B-cells are increased over the range found in most normal adults [42, 45], and in these patients the frequency of the CD5+ B-cells is remarkably stable over time and bears no relationship to clinical assessment of disease activity, ESR, or levels of C-reactive protein. Some drugs such as gold or D-penicillamine do not appear to alter their numbers, but azathioprine does. This finding, together with the family study and twin data, is consistent with the high frequency of CD5+ B-cells in adult life being a genetic marker of RA.

4. Despite the above finding we are uncertain of the extent to which CD5 antigen on B-cells is only expressed on a distinct subpopulation of B-cells, because there is no doubt that CD5 can be induced on many B-cells by stimulation with phorbol ester in vitro. Thus CD5 antigen may be constitutively expressed as a lineage of B-cells and is potentially inducible on all B-cells. If the former represented a genetically determined trait and the latter a consequence of B-cell activation in vivo, measuring CD5+ B-cells becomes a confounding variable in a disease such as RA in which both attributes may be in operation. The lack of suitability of the CD5 marker is further highlighted by our observation that differentiation of B-cells into plasma cells is associated with loss of CD5 [45], and thus CD5 cannot be used to evaluate the lineage of plasma cells in RA joints; moreover, we do not yet know whether CD5+ B-cells class-switch, and if they do, whether CD5 is retained.

5. The function of CD5+ B-cells from neonatal cord and RA blood has been studied following separation in a cell sorter. Our findings support the findings of other groups [47] that IgM rheumatoid factor synthesis is enriched in this fraction of B-cells. It has also been claimed that IgM secreted by CD5+ B-cell lines from cord blood is polyreactive with autoantigen and could represent 'natural' antibodies that occur in normal blood [48]. In contrast, it has been found that monospecific IgM rheumatoid factor was secreted by CD5+ B-cells derived from RA blood [49], suggesting that selective pressures (or mutations) may play a part in autoantibodies secreted in disease.

6. In an attempt to address the questions (a) are CD5 cells represented in joints of RA patients and (b) are such B-cells making RF or natural antibodies, we have successfully fused B-cells from RA joints with a heteromyeloma fusion partner to form stable immunoglobulin-secreting hybridomas [50]. Our data show that a proportion of B-cells activated in the joint are CD5+, a gratifying and surprising finding, since the majority of B-cells in RA joints have usually differentiated into plasma cells with a consequent loss of CD5 antigen. One such hybridoma secretes a polyreactive antibody which includes RF activity and utilizes V\textsubscript{\lambda}III cross-reactive idiotypes detected by the C-7 and 17-109 monoclonal antibodies. The other two CD5+ B-cell hybridomas make antibodies of unknown antigen specificity.

7. We would also like to answer a broader question; namely, are all B-cells activated in RA joints derived from CD5+ B-cell (or autoimmune B-cell) precursors? To put it another way, we could hypothesize that autoantibodies producing B-cells at sites of disease are derived from a subset of the B-cell repertoire that may bear a unique and specific marker, and that CD5 antigen is one such marker. For reasons already discussed, we do not believe it likely that CD5 is a suitable marker for investigating this question and other novel markers for such B-cells are being sought. However, it seems quite plausible that resting fetal-type B-cells which bear the CD5 marker may indeed include the potentially autoreactive cells which normally make non-pathogenic (natural) and high connectivity autoantibodies which are part of the idiotypic network. It also seems possible that upon exposure to viruses and bacteria bearing homologous sequences to autoantigens (i.e. exhibiting molecular mimicry) such B-cells could be activated, undergo mutations in their variable region genes, class-switch from IgM to IgG and IgE, and give rise to pathogenic autoantibodies. Activated B-cells bearing receptors for local autoantigens could also present them to T-cells, and drive the immune response (Fig. 3). Because B-cells possess immunoglobulin receptors which...
specifically bind local autoantigens (such as collagen type II) they assume a greater importance as antigen presenting cells than the macrophage or dendritic cells, which take up antigen by non-specific mechanisms. The ability of B-cells to take up, process, and present antigen to T-cells has been experimentally validated, as has their greater efficiency than macrophages for eliciting an equivalent T-cell response [51].

The ability to form stable B-cell hybridomas secreting both IgM and IgG antibodies from synovium, without any extrinsic stimulation, has given us an opportunity to begin to seek evidence of their natural ligands in RA and to study inter-relationships between B-cells at a clonal level. For example, in a fusion from one RA patient, over 27 stable hybridomas secreting milligram quantities of monoclonal immunoglobulin have been cloned and already permit some general comments to be made [52].

First, it appears that 16 of the 27 hybridomas secrete autoantibodies; these include immunoglobulins with weak binding to autoantigens such as proteoglycans, suggesting that some of the reactions may be due to non-specific binding or cross-reactive antibodies. Of these antibodies, 11 are polyreactive, and 5 are monospecific (Table I). However, the rest do not react with any of a panel of autoantigens. This raises the possibility that either the major autoantigen remains to be defined, or that extrinsic antigens are involved. We are approaching this by making a list of candidate autoantigens and extrinsic agents (such as EBV, retroviruses, etc.) and testing them systematically.

Second, it appears that rheumatoid factors do not account for more than 30% of the clones, and that cross-reactive idiotypes representing V\textsubscript{H}III or V\textsubscript{\mu}I are slightly more common. Third, by immunochemical characterization of immunoglobulins and by defining antigen specificity, we can show that only a small number of B-cell hybridomas are clearly derived from sister B-cells (i.e. there is some evidence of clonal expansion) but the rest appear clonally unrelated. This suggests that the B-cell response is at best oligoclonal, or possibly even polyclonal. Using molecular biological techniques (e.g. Northern blotting for V-gene usage and mRNA sequencing) we shall be able to evaluate the clonal inter-relationships and the extent of mutations present in the variable gene segments of activated B-cells in joints. This approach should also permit us to comment on the possibility of preferential usage of certain variable segment genes in

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>RF IgG</td>
<td>5 (all weak)*</td>
</tr>
<tr>
<td>IgM</td>
<td>1 (strong)</td>
</tr>
<tr>
<td>Coll. type II</td>
<td>10 (1 strong, 9 weak)</td>
</tr>
<tr>
<td>Proteoglycans</td>
<td>11 (1 strong, 10 weak)</td>
</tr>
<tr>
<td>Cytoskeletal proteins</td>
<td>4 (1 weak, 3 strong)</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>3 (2 strong, 1 weak)</td>
</tr>
<tr>
<td>Monospecific</td>
<td>5</td>
</tr>
<tr>
<td>Multispecific</td>
<td>11</td>
</tr>
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</table>

*Degree of binding

The autoantibody specificities of monoclonal antibodies derived from fusing synovial B-cells with a heteromyeloma cell line are shown. The donor suffers from classical seropositive RA. 'Monospecific' means that reaction with a single antigen was observed; 'multispecific' means reaction with more than one (unrelated) antigen was observed. Binding reactions were graded: weak = low level in which non-specific binding cannot be excluded; strong = a definite positive result.
In an attempt to answer these questions, Professor Feldmann's group, in collaboration with mine, has been approaching this by cloning T-cells from RA synovia and by studying the T-cell receptor of infiltrating cells. In preliminary studies on a single RA patient undergoing surgery on joints, it proved possible to study synovial T-cells from more than one synovial specimen, with concordant results suggesting that we were dealing with a significant result. In this study, T-cell clones were derived by selecting cells already activated in vivo (i.e. IL-2 receptor bearing cells) and analysing their proliferative response to a large panel of autoantigens [54]. Amongst a large panel of T-cell clones, two specificities were found: (1) four clones reacted to autologous leucocytes, i.e. gave a positive reaction in an autologous mixed lymphocyte reaction (AMLR); and (2) three clones recognized collagen type II. AMLR has been studied in RA by other groups [55] and considered to be of importance in maintaining a chronic state of autoimmunity. However, we were more interested in the collagen type II specific clones, since this collagen is only found in cartilage and the finding of reactive T-cells would explain the localization of the immune response to joints. Also, collagen type II can induce an RA-like disease in experimental animals which is T-cell dependent [56]. However, the extent to which collagen type II reactivity is of primary importance has not been established.

Molecular analysis of the T-cell receptor has been undertaken by other groups and it has been suggested that the T-cell response in RA synovium is oligoclonal [57, 58]. We have used two monoclonal antibodies which react with the \( \beta \)-chain of T-cell receptors to enumerate the number of \( \text{V} \beta 5 \) and \( \text{V} \beta 8 \) cells enriched in the RA joint [59]. In our study there was evidence of presence of T-cells bearing both these markers, and in some instances, there was enrichment of numbers over that seen in blood. However, there was no consistent \( \text{V} \beta \)-gene usage and the findings were consistent with the conclusion that although some T-cell clones may be expanded, the T-cell response was at best oligoclonal or even polyclonal—a conclusion not dissimilar to that reached with our B-cell work.

Recent work has shown two distinct T-cell receptors: the 'conventional' type, consisting of a heterodimer of \( \alpha \)- and \( \beta \)-chains, and the fetal-type heterodimer of \( \gamma \)- and \( \delta \)-chains (Fig. 4). During the course of examining the phenotype of T-cells infiltrating the synovium, we noted that 10–20% were negative for CD4 and CD8. The 'double negative' T-cell population includes T-cells which use \( \gamma \)- and \( \delta \)-chains to form their T-cell receptor. In a study using flow cytometry and monoclonal antibodies, we were able to show that the 'double negative' T-cell population included T-cells on which \( \gamma \delta \)-chains were expressed. Approximately half the RA patients had more \( \gamma \delta + \) T-cells in the joints than in blood taken simultaneously [60], suggesting that there may be a ligand in the RA joint to which they bind.

The significance of \( \gamma \delta + \) T-cells in RA is unclear at present, but because the phenotype represents a 'fetal type' of cell in the thymus, and because CD5+ B-cells are also predominant during fetal life, we were interested to examine simultaneously for the two types of cell. Blood from three groups was studied: normal individuals, patients with RA, and patients with primary Sjögren's syndrome. The latter groups included selected patients previously known to possess high or normal numbers of CD5+ B-cells. The results obtained clearly showed a strong concordance in the numbers of \( \gamma \delta \)-T-cells and CD5+ B-cells, such that almost all the patients with raised CD5+ B-cells also showed raised numbers of \( \gamma \delta \)-T-cells [61]. This concordance was not observed in neonatal cord blood in which a high number of CD5+ B-cells were found without an increase in \( \gamma \delta \)-T-cells. Thus the proposed genetic regulation of numbers of CD5+ B-cells may not apply to \( \gamma \delta \)-T-cells. We have formed the impression that, unlike CD5+ B-cell numbers, which remain constant over time, \( \gamma \delta \)-T-cell numbers vary during the course of disease and reflect disease activity.

The increase of CD5+ B- and \( \gamma \delta + \) T-cells in the blood and their presence in RA joints raise many questions which we hope to investigate in

![Fig. 4.—B-cells, especially when activated, are able to capture antigen by specifically combining with the antibody receptor, thereby mopping up small amounts of antigen. Following intracellular processing, antigen is presented in the HLA groove to a T-cell receptor.](https://academic.oup.com/rheumatology/article-abstract/28/6/466/1776079)
the future. For example, are the two cell types interdependent because one produces an essential growth factor for the other, or are they both recognizing epitopes on the same ligand (an idiotype or autoantigen) in joints (Fig. 5)? Is their involvement reflecting a breakdown in patients with RA of an immune response whose main function is to maintain tolerance to autoantigens via the immune network? Or are we merely witnessing the recapitulation of T- and B-cells which form part of an alternative, phyogenetically more primitive and less well adapted, immune system working hand-in-hand with the conventional T- and B-cells? Whatever the answers, pursuit of these questions is now possible with precision and should lead to important clues to the initiation of disease.

**Cytokines: mediators of immune and inflammatory reactions**

In 1969, whilst I was a research fellow at the Kennedy Institute and working with Dumonde’s group, the importance of soluble mediators produced by lymphocytes, and termed ‘lymphotokines’, was only just being discovered [62, 63]. The importance of these factors and of soluble cellular factors in general, termed ‘cytokines’, was quickly appreciated, but the field moved apace once the technology of cloning DNA which encodes the peptide cytokines and sequencing purified cytokines became established in the past 5 years. The field has more recently advanced into cloning the receptors of cytokines and, as a result, a good number of molecules have been defined, which include cytokines and receptors with diverse biological functions [64].

Cytokines which are proving to be of interest in rheumatology include the interleukins (IL-), tumour necrosis factor (TNF), interferons (IFN), platelet-derived growth factor (PDGF), transforming growth factors (TGF) and colony-stimulating factors (CSF). These are glycosylated proteins, mostly in the molecular weight range 15-35 kDa. At one time it was thought that certain cytokines were derived only from one type of cell; for example, γ-interferon, lymphotoxin, IL-2, IL-3, IL-4 and IL-5 were all considered to be products of T-cells, whilst IL-1 and TNF were thought to be macrophage-derived. This is no longer thought to be the case and in general many different cells under certain conditions may synthesize and secrete many of the cytokines. Similarly, it used to be thought that each cytokine possessed one specific activity, but it is now recognized that these molecules are multifunctional by virtue of their cognate receptors being present on many cell types—albeit in different numbers and with varying affinities, so that specificity of activity is apparent under certain conditions.

The activities of cytokines may be essentially viewed as leading to regulation of cellular function and cellular interactions. The type of processes involved include cell activation (e.g. proliferation, differentiation) as well as inhibition of cell function (e.g. inhibition of synthesis of molecules). Of these, effects of cytokines on immune and inflammatory reactions are of obvious importance. Less obvious, but equally important, are effects on connective tissue and on the liver in inducing acute phase proteins characteristically associated with rheumatic
inflammation. It has also emerged that whilst some cytokines regulate function of cells producing them, e.g. IL-2 controls proliferation of T-cells synthesizing the molecule (an 'autocrine' effect), others lead to effects on other cells in their microenvironment, e.g. IL-1 and TNF, produced by macrophages, stimulate T- and B-lymphocytes in the synovium and chondrocytes in adjacent cartilage (a 'paracrine' effect). Cytokines are detectable in the blood (e.g. TNF in septicemia); lead to more widespread effects, and in this respect behave like hormones (an 'endocrine' effect).

Considerable attention is being paid to the detection of cytokines that are detectable in diseased tissues and body fluids, the range of cytokines being limited by the assays available for detecting them. Two main approaches are currently in use, namely: detection of cytokines as peptides by tests of biological function (cell proliferation, immunoglobulin synthesis, etc.), or by immunoassays; or detection of cytokines at mRNA level by using specific complementary DNA probes. Ideally, both methods should be used since detection at mRNA level does not always reflect the quality of peptide synthesized, and because once synthesized, peptides are taken up by receptors, degraded, eliminated in the circulation or bound to inhibitors and their measurement is not an accurate reflection of their production.

In conjunction with Professor Feldmann's group, our first step has been to catalogue as many cytokines as possible at both mRNA and protein level in RA joints (Table II). Currently the best documented are IL-1, IL-6 and TNF, and since all these have important implications for RA, I shall confine my comments to these.

IL-1 is a cytokine whose biological activities as a soluble factor had been investigated by different research workers and known nominally in the context of their own field of interest, as endogenous pyrogen, catabolin, and T-cell activation factor [65]. A unification of these multifunctional activities into one molecule occurred following its molecular characterization, and we have now come to appreciate that two forms of it exist, termed IL-1α and IL-1β. There is some sequence homology between the two forms, although their activities appear to be the same, reflecting the fact that the same receptor binds to both IL-1α and IL-1β [66]. As is known to this audience, IL-1 has been found in RA joint fluids [67] and in the synovium [68] and since its effects include mediation of the cellular phase of inflammation, fibrosis, destruction of cartilage in vitro, induction of arthritis in experimental animals following injection into joints and important effects on stimulation of T- and B-cells [69-71], its relevance to RA is obvious. We have been interested in three principal questions: (1) is there evidence of local production of the two forms in the synovium and what cells make it; (2) is there evidence that regulation of its production is abnormal, and (3) what are likely inducers of IL-1 in the joint?

In order to answer the first question, we have investigated mononuclear cell suspensions in culture, either separated on density gradients from the synovial fluid or extracted from synovium following collagenase and DNase digestion of tissue. The RNA from these cells is examined for specific IL-1α and β in RNA by hybridization with radiolabelled probes [72]. We find that both forms of IL-1 are detectable in high quantities and in some samples, greater amounts of IL-1α are present than can be induced in normal mononuclear cells by powerful stimulants in vitro. Further, if we culture the mononuclear cells from joints, without exogenous stimulation, high levels of IL-1 mRNA continue to be synthesized for up to 5 days. Since the usual kinetics of mRNA production in maximally stimulated cells is much shorter, and we believe that the degradation of mRNA is not responsible for persistence, the possibility arises that continual production is dependent on an inducer of IL-1 being present in the culture system. Our mononuclear cell suspensions are in fact a heterogeneous cell mixture consisting

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<th><strong>TABLE II</strong></th>
<th><strong>Cytokine Expression in Rheumatoid Joints</strong></th>
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<tbody>
<tr>
<td><strong>mRNA</strong></td>
<td><strong>Protein</strong></td>
</tr>
<tr>
<td>IL-1α</td>
<td>++</td>
</tr>
<tr>
<td>IL-1β</td>
<td>++</td>
</tr>
<tr>
<td>TNFα</td>
<td>++</td>
</tr>
<tr>
<td>PDGF-A</td>
<td>++</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>+</td>
</tr>
<tr>
<td>TGF-α</td>
<td>?</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>+</td>
</tr>
<tr>
<td>IL-6</td>
<td>++</td>
</tr>
<tr>
<td>IFN-α</td>
<td>?</td>
</tr>
</tbody>
</table>

++, high; +, moderate; ±, low; ?, not ascertained.

In this table data are summarized from our and other laboratories. For each cytokine included, the mRNA expressed in mononuclear cells has been quantitated by slot-blot analysis, the protein by bio- or immunoassay. Abbreviations: IL, interleukin; TNF, tumour necrosis factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; IFN, interferon; CSF, colony stimulating factor; GM, granulocyte, macrophage.
mainly of cells which have the phenotype of T-lymphocytes (CD3) and monocytes (CD14); this raises the possibility that the inducer is either an autocrine or paracrine cytokine.

In order to examine the cell types which synthesize IL-1 we have used the technique of immunohistology employing specific polyclonal F(ab)_2 anti-IL-1 antibodies or monoclonal anti-IL-1α and β antibodies. In the synovium, cells staining positively appear to be predominantly macrophages, although the technique of tissue section staining with this antibody is insufficiently developed to give a definitive report at this stage. Much more reliable results have been obtained on mononuclear cells separated from peripheral blood, where we have found large cells with a dendritic appearance which contain IL-1α and constitute up to 10% of the total (mononuclear cell population [73]).

In defining possible inducers of IL-1 our work has highlighted the importance of two other molecules also known to be present in RA joints, namely, immune complexes and TNFα. IgG–IgM and IgA–IgM rheumatoid factor cryoprecipitable immune complexes were shown to induce IL-1, and this activity was enhanced by γ-interferon and TNFα [74]. Hitherto, immune complexes have been thought to be pathogenic by virtue of their complement-fixing properties in RA [11, 12]; we can now add another important pathway of action via IL-1 production. Since TNF is also plentiful in the RA joint and has a profile of activities similar to IL-1, and is an inducer of IL-1, it is a cytokine of considerable importance in RA.

In recent on-going experiments, we have been investigating whether neutralizing TNFα activity with specific antibody leads to any change in IL-1 production. When mononuclear cells from RA synovium are cultured without any exogenous stimulation, the high IL-1 production is completely suppressed by the addition of anti-TNFα [75]. This experiment not only proves that TNFα is an important inducer of IL-1, but suggests a possible approach to therapy.

IL-6 is another molecule that we have studied. By using assays for the protein, IL-6 has been demonstrated in elevated amounts in RA fluids [76]. Using cDNA probes IL-6 mRNA is also elevated in mononuclear cells in RA joints [76]. Specific F(ab)_2, anti-IL-6 antibodies have been made by immunizing rabbits with an immunogenic peptide of IL-6 and used in an immunohistological analysis of synovium [77]. In our studies, IL-6-containing cells appear to reside in

The importance of IL-6 lies in its action on B-lymphocytes. It has been demonstrated that IL-6 leads to the secretion of immunoglobulins by activated and proliferating cells [78]. Its abundance in RA synovium may explain why the great majority of B-cells appears terminally differentiated into plasma cells, and why large amounts of immunoglobulin are synthesized locally [79]. The importance of B-cells in the RA process has been previously discussed, and in relation to antigen presentation to T-cells, it should be noted that activated B-cells are much more effective than are resting cells [80].

While many of the experiments I have described fit in with our current concepts of the dynamics of cellular pathology in RA, there are some observations that remain enigmatic. Thus despite morphological evidence of T-cell activation and the presence in mRNA of IL-2 [81], practically no IL-2 is demonstrable as a peptide [82]. Similarly, despite high levels of HLA class II expression suggesting the local action of γ-IFN, only low amounts of γ-IFN are demonstrable at both mRNA and protein level [82]. In preliminary work we have had inconsistent results of the presence of lymphotoxin, and can only demonstrate low levels of IL-3 (Table II). These data do appear to suggest that production of T-cell-derived lymphokines is impaired in RA, although the complexity of effects of inhibitors and binding to cell-associated and soluble receptors means much more work will be required before a coherent picture can emerge.

Implications for the future

I want to suggest that an important aspect of current immunological research is that after decades of unfulfilled promise, it is possible to begin to define new therapeutic targets and novel therapies for RA. The problem in moving forward is going to be not a dearth of ideas but an embarrassment of riches. I will merely pick out a few possibilities without elaborating on them.

Starting with the possibility of interfering with antigen presentation to T-cells, we can consider a number of strategies for interfering with the trimolecular interaction of HLA molecules, peptide antigens or T-cell receptor. For example, one could use monoclonal antibodies to HLA molecules, T-cell receptor, or to molecules expressed on activated T-cells (e.g. IL-2
receptor), or to accessory adhesion molecules (e.g. CD2). Alternatively, one might design synthetic (harmless) peptides which are processed and bind to the HLA-antigen groove and prevent the presentation of peptides involved in stimulating the T-cells in disease. If B-cells are important in antigen presentation to T-cells, one might consider deleting those known to make autoantibodies and expressed in joints; CD5+ B-cells are a candidate.

A different approach would be to attempt to regulate the cytokines produced in excess and believed to be harmful, e.g. IL-1, TNFα, and IL-6. This could be achieved by using monoclonal antibodies and in light of the effect of anti-TNF in reducing IL-1, this would be a possible candidate. However, experience with murine monoclonals suggests that because they are immunogenic in man, antibodies would probably be formed and neutralize their effect. Humanized antibodies may be a way of bypassing this problem but tissue penetration to sites of production of cytokines may not be good and repeated injections could be required for a lifetime.

The definition of suitable targets may be best defined by using a biological approach and will no doubt preoccupy many of us in the years to come. However, ideally, simple chemical drugs will need to be developed which interfere with the production, release, or receptor-uptake of cytokines.

In concluding, I would like to reiterate my basic belief and tenet today. Research in cellular and molecular biology has reached a stage where rich rewards may be reaped for the benefit of our patients. Progress in the future will depend not only on ingenuity and application of knowledge, but on a suitable collaboration between scientists and clinicians, working in an economically viable, intellectually disciplined, and scientifically capable environment.

‘Then slowly climb the many-winding way, And frequent turn to linger as you go, From loftier rocks new loveliness survey . . . ’

Childe Harold’s Pilgrimage, Byron, Canto I–xx.

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References

11. Erhardt CC, Mumford P, Maini RN. Differences in immunochemical characteristics of cryoglobulins in rheumatoid arthritis and


36. Mellors RC, Heimer R, Corcos J, *et al.* Cel...
37. Natvig JB, Munthe E. Self-associating IgG rheumatoid factor represents a major re-
response of plasma cells in rheumatoid inflammatory tissue. Ann NY Acad Sci 1975; 256:
88-95.
38. Room GRW, Plater-Zyberk C, Clarke MF, Maini RN. B-lymphocyte subpopulation
which forms rosettes with mouse erythro-
39. Fong S, Vaughan JH, Carson DA. Two different rheumatoid factor producing cell
populations distinguished by the mouse erythrocyte receptor and responsiveness to
40. Caligaris-Cappio F, Gobbi M, Boffill M, Janossy G. Infrequent normal B lympho-
cytes express features of B chronic lympho-
623-8.
in the human fetus. J Immunol 1985; 134:
1531-8.
42. Plater-Zyberk C, Maini RN, Lam K, Kennedy TD, Janossy G. A rheumatoid arthritis B cell
subset expresses a phenotype similar to that
in chronic lymphocytic leukaemia. Arthritis
81-102.
44. Hayakawa K, Hardy RR. Normal, auto-
immune and malignant CD5+B cells: the
Ly-1 lineage? Ann Rev Immunol 1988; 6:
197-218.
45. Plater-Zyberk C, Maini RN. Phenotypic and
functional features of CD5+B lymphocytes
in rheumatoid arthritis. Scand J Rheumatol
1988; 75: 76-83.
46. Kipps TJ, Vaughan JH. Genetic influence on
the levels of circulating CD5-B lymphocytes.
47. Casali P, Burastero SE, Nakamura M, Inghi-
rami G, Notkins AL. Human lymphocytes
making rheumatoid factor and antibody to
ssDNA belong to Leu-1+ B cell subset. Science 1987; 236: 77-80.
48. Nakamura M, Burastero SE, Notkins AL,
Casali P. Human monoclonal lymphocytes
making rheumatoid factor-like antibodies
from CD56 Leu-D+ B cells are polyreactive.
49. Burastero SE, Casali P, Wilder RL, Notkins
AL. Monoreactive high affinity and polyre-
active low affinity rheumatoid factors are produced by CD5+ B cells from patients
50. Brown CMS, Plater-Zyberk C, Maini RN.
Stable hybridomas from rheumatoid syn-
ovium secrete IgM rheumatoid factor, multi-
specific autoantibody and monoclonal cationic IGG1. Br J Rheumatol 1988; 27
(Abs Suppl 2): 120.
51. Chesnut RW, Grey HM. Antigen presentation
by B cells and its significance in T-B interac-
52. Brown CMS, Plater-Zyberk C, Maini RN. In-
vestigation of locally synthesized antibodies
in the rheumatoid synovial membrane. Clin
53. Bona CA. V genes encoding autoantibodies:
molecular and phenotypic characteristics.
54. Feldmann M, Londeli M, Leech Z, Brennan F,
Savill C, Maini RN. Analysis of T cell clones
in rheumatoid arthritis. Springer Semin Im-
matoid arthritis: a disease of T lymphocyte-
macrophage immunoregulation. Lancet 1981;
ii: 839-42.
56. Stuart JM, Townes AS, Kang AH. Collagen
autoimmune arthritis. Ann Rev Immunol
57. Savill C, Damle PJ, Kioussis D, et al. A minor-
ity of patients with rheumatoid arthritis show
a dominant rearrangement of T cell receptor
B chain genes in synovial lymphocytes.
58. Stamenkovic I, Stegagno M, Wright KA, et al.
Clonal dominance among T lymphocyte in-
filtrates in arthritis. Proc Nail Acad Sci USA
genicity of T cell receptor idiotypes in rheu-
73: 417-23.
cells expressing y6 chain receptors in rheu-
matoid arthritis. J Autoimmunity 1988; 1:
319-26.
61. Plater-Zyberk C, Brennan FM, Feldmann M,
Maini RN. 'Fetal-type' B and T lymphocytes
in rheumatoid arthritis and primary Sjögren's
syndrome. J Autoimmunity 1989; Suppl 2:
233-41.
62. Maini RN, Bryceson AD, Woltencroft RA,
63. Hamblin AS, Maini RN. An evaluation of lymph-
okine measurement in man. In: Thomp-
son RA, ed. Recent advances in clinical
immunology. 2. Edinburgh: Churchill
64. Buchan G, Barrett K, Turner M, Maini RN,
Allard S, Feldmann M. The role of cytokines
and interleukins in rheumatoid arthritis. In:
Decker J, Scott JT, eds. Perspectives in rheu-
matology 1989. Chichester: Current Medical
Literature Ltd, J Wiley & Son (in press).


