Molecular and cellular mechanisms of joint destruction in rheumatoid arthritis: two cellular mechanisms explain joint destruction?

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Rheumatoid arthritis (RA) is a chronic systemic disorder of unknown aetiology. Most of its serious and debilitating sequelae derive from progressive destruction of joints. Although early and late stages of this disease may involve distinct processes, the affected joints of symptomatic patients characteristically exhibit overlapping manifestations of three pathobiological phenomena (fig 1):

(1) *Inflammation* appears to be the basis of joint pain and several systemic manifestations. A large number of inflammatory mediators, including arachidonic acid metabolites, vasoactive amines, and neuropeptides, have been identified in the affected joints of patients with RA, and cytokines such as tumour necrosis factor α and interleukins 1 and 6 (IL-1 and -6) have been implicated in the perpetuation of rheumatoid synovitis.^{1 2}

(2) *Abnormal cellular and humoral immune responses* represent another hallmark of RA. Autoantibodies, particularly rheumatoid factors (RFs) and antibodies directed against collagen type II, are often present.³ Moreover, accumulation of T lymphocytes, particularly those expressing the CD4+, CDw29 (UCHL 1+/4B4+) memory phenotype of the helper-inducer subset, occurs in the synovium. Some of these T cells express activation markers on their surfaces. Evidence pointing to a critical role for activated T cells in the pathogenesis of RA has fostered the development of several new therapeutic strategies.⁴

(3) *Synovial hyperplasia* is characterised by proliferating, synovial cells with a transformed appearance⁵ and large numbers of infiltrating macrophages.⁶

The conundrum of RA has puzzled researchers for decades. What is the initial step in the pathogenesis of the disease? The association of certain major histocompatibility complex (MHC) class II molecules with RA has been repeatedly demonstrated (see below). As MHC class II molecules are known to function in the restricted presentation of antigenic peptides to CD4+ T cells,⁷ in the shaping of the CD4+ T cell receptor repertoire,⁸ as well as the presentation of superantigens to CD4+ cells,⁹ the T cell is presumed to have a central role in the pathogenesis of RA. Harris, for example, has suggested that T cells are associated with 'stage 1' of the disease.¹⁰ On the other hand, Firestein and Zvaifler have challenged the importance of T cells in RA based on observations that only

Figure 1 Overlapping manifestations of three pathobiological phenomena present in the affected joints of patients with symptomatic rheumatoid arthritis.

negligible levels of T cell specific lymphokines (for example, IL-2, IL-3, IL-4, interferon γ , and tumour necrosis factor β) can be detected in rheumatoid joints.¹¹ The latter data can be accommodated by an alternative hypothesis—namely, that induction of RA is favoured by a 'hole' present in the T cell repertoire of susceptible subjects, which results in impaired handling of an aetiological agent(s), such as a virus. It is of interest in this regard that patients with AIDS expressing HLA-B35 respond to HIV-I with a more accelerated clinical course than patients with AIDS lacking this phenotype.¹²

With regard to the role of inflammation in the pathogenesis of RA (fig 1), our laboratory has been investigating approaches suitable for detecting early evidence of joint destruction, such as the presence of collagen type II fragments in synovial fluid and serum. Although these studies were aimed at the development of an indicator system for monitoring disease activity, they also provided insights into the cellular basis of joint destruction in RA. Surprisingly, we found that the presence of collagen type II fragments in synovial phagocytes did not correlate with the number of white blood cells in the synovial fluid,¹³ suggesting that cartilage destruction in RA may proceed through pathways independent of classic inflammation. Indeed, increasing evidence indicates that synovial cells with a transformed appearance participate in the cartilage/bone destruction characteristic of

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RA. In this review we discuss recent findings supporting roles for both T cell dependent immune responses and hyperplastic synovial cells in the pathogenesis of RA.

T cell responses in rheumatoid arthritis

Evidence implicating T cells in the pathogenesis of RA includes the aforementioned strong association between RA and DR alleles sharing a common amino acid sequence motif in the third hypervariability region of their DR β 1 chains¹⁴; the prominence of T cells in synovial mononuclear cell infiltrates,¹⁵ 10–15% of which characteristically express activation markers such as the interleukin 2 receptor (IL-2R)¹⁶; apparent efficacy of T cell directed treatments in RA, including cyclosporin¹⁷ and experimental monoclonal antibodies to CD4^{18–20}; and resolution of pre-existing RA in patients developing AIDS.^{21–22}

What are T cells responding to in RA? Although a definitive answer remains elusive, some insights have emerged from molecular studies of T cell receptor diversity in affected joint tissue. Using a quantitative polymerase chain reaction technique, Paliard and colleagues noted expansion of V β 14+ T cells in the synovial fluid of each of nine patients with active RA, despite low expression of V β 14 among peripheral blood T cells in these patients.²³ Sequence analyses disclosed oligoclonality of V β 14 transcripts in RA synovial fluid. In contrast, enhanced expression of V β 14 was not found among synovial fluid T cells in three arthritic controls (two with Reiter's disease, one with psoriatic arthritis). The results were interpreted as being consistent with an initial activation of V β 14+ T cells by a superantigen followed by local expansion of certain V β 14+ T cell clones by antigens present in the joints of these rheumatoid patients. More recently, Uematsu and coworkers analysed the synovial fluid T cell receptor repertoire of a patient with RA using an inverted polymerase chain reaction approach.²⁴ This powerful technique obviates the need for V region specific or V region consensus primers, a source of potential bias. Although considerable heterogeneity of synovial fluid T cell receptor V α and V β gene usage was noted, a modest two- to threefold enrichment (relative to peripheral blood) of V β 2.1 and V β 3.1 transcripts was detected in this patient.

Reasoning that analysis of activated T cells might be more informative, Howell *et al* isolated IL-2R+ synovial T cells from five patients with RA and found V β 3, V β 14, and V β 17 were expressed in most of the synovial samples.²⁵ Dominant rearrangements were seen within these V β families (unlike peripheral blood), consistent with oligoclonal expansion of activated T cells. Evidence indicating oligoclonal expansion of IL-2 responsive synovial T cells in RA has also been obtained using restriction fragment analysis, but patterns of V α and V β gene expression were not examined in that report.²⁶

Preferential expression of particular V β families among synovial T cells in RA is consistent with exposure to a superantigen(s). Apparently conflicting results regarding patterns of T cell receptor V β expression in RA might reflect the dominant influence of different superantigens. Indeed, distinct patterns of selective V β family activation have been shown for different streptococcal²⁷ and staphylococcal²⁸ toxin superantigens. The parallel increase in V β 3, V β 14, and V β 17 seen among activated synovial T cells in some patients with RA is of particular interest as these V β families exhibit considerable sequence homology, especially in the fourth complementarity determining region, implicated as the V β binding site for several superantigens.^{29–31} That this pattern of V β family expansion might reflect the influence of a superantigen is supported by the finding that *Mycoplasma arthritidis* mitogen activates human T cells bearing V β 3 and V β 17.³²

While a role for superantigens in local T cell activation in RA is an attractive hypothesis, it is difficult to reconcile the available T cell receptor repertoire data with that explanation alone. Oligoclonal expansion seen in the aforementioned studies is more consistent with local antigen induced responses. Recent evidence from a study of autoimmune thyroid disease supports this view. Restricted T cell receptor V gene expression has been convincingly shown among intrathyroidal T cells of patients with autoimmune thyroid disease,³³ and, clearly, a substantial fraction of these T cells is reactive with thyroidal antigens.³⁴

Little information, however, exists about the specificity of synovial T cells in RA. This issue is complicated by evidence that only a fraction of synovial T cells in RA seems to be activated (for example, those cells bearing IL-2R). Nonetheless, it is intriguing that Londei and colleagues found that about 12% of T cell clones, isolated from two synovial samples obtained at an interval of 21 months from the same joint of a patient with RA, were reactive with collagen type II.³⁵ The epitope specificity of T cells reactive with collagen type II in RA is unknown. A DR7 restricted CD4+ T cell clone directed against type II collagen, isolated from a healthy subject, recognised an epitope spanning amino acids 271–285 of the α 1(II) chain located in cyanogen bromide fragment 11 of the molecule.³⁶ Interestingly, α 1(II)-cyanogen bromide fragment 11 is thus far the only collagen II fragment clearly shown to be arthritogenic in animal studies. Heat shock proteins are also an attractive target antigen for T cells in RA. These evolutionarily conserved proteins are often the dominant target antigens for immune responses directed against a broad array of microorganisms. In the case of adjuvant arthritis, T cells reactive with an epitope contained in the 65 kilodalton heat shock protein of *Mycobacterium tuberculosis* can transfer the disease and presumably cross react with an as yet unidentified autoantigen in joint tissue of recipients.^{37–38} Local expression of human 65 kilodalton heat shock protein has been shown in inflamed rheumatoid

synovium.³⁹ Cytotoxic T cell clones cross reactive with *Mycobacteria bovis* and human 65 kilodalton heat shock protein have been isolated from the joints of patients with RA⁴⁰; however, the frequency of T cells reactive with the mycobacterial 65 kilodalton heat shock protein seems to be similar in both peripheral blood and synovial fluid of patients with RA.⁴¹ Thus a role for T cells reactive with heat shock proteins in the pathogenesis of RA remains unproved.

T cell migration into rheumatoid synovium

Expression of cell adhesion molecules on endothelial cells and complementary ligands on the surface of circulating leucocytes seems to have a critical role in determining the site of leucocyte emigration and the nature of the leucocytes migrating into an inflammatory lesion. Butcher has proposed that leucocyte-endothelial cell recognition requires at least three steps: primary adhesion (transient), leucocyte activation, and activation dependent binding with subsequent leucocyte transmigration.⁴² This model implies that the specificity of endothelial cell/leucocyte interactions is determined by unique combinations of adhesion molecule/ligand interactions operating at each step. Although the adhesion molecule/ligand pairs taking part in the leucocyte/endothelial cell primary adhesion event, also referred to as 'rolling', have not been fully elucidated, it seems that selectin/carbohydrate ligands mediate this event in the case of neutrophils.^{43 44} Interestingly, synovial endothelial cells isolated from patients with RA constitutively express endothelial leucocyte adhesion molecule type 1 (ELAM-1) and upregulate expression of this selectin after exposure to IL-1.⁴⁵

Kavanaugh and colleagues obtained evidence that the interaction of leucocyte function associated antigen 1 (LFA-1) with its ligand, intercellular adhesion molecule 1 (ICAM-1), probably has a key role in trans-endothelial migration of T cells.⁴⁶ ICAM-1 is also expressed on isolated rheumatoid synovial endothelial cells and is subject to regulation by IL-1.⁴⁵ Binding of T cells to endothelial cells in the absence of expressed LFA-1 occurred through the interaction of very late antigen 4 (VLA-4) and its ligand, vascular cell adhesion molecule 1 (VCAM-1).⁴⁶ More recently, Oppenheimer-Marks *et al* extended these observations by demonstrating that VCAM-1/VLA-4 interactions predominately mediate binding of T cells to endothelial cells exposed to IL-1, whereas LFA-1/ICAM-1 interactions are crucial to subsequent transendothelial migration of the T cell.⁴⁷

The predominance of memory T cells in rheumatoid synovium had been thought to reflect in situ activation of T cells. Enhanced surface expression of integrins (including VLA-4) on memory (CD45R0+) T cells as compared with virgin (CD45R0-) T cells, however, would be expected to lead to selective migration of memory cells into the inflamed

synovium of patients with RA.^{48 49} Indeed, CD45R0+ T cells adhere better to endothelial cells than do CD45R0- cells.⁵⁰ Retention of these cells in the synovium is probably enhanced by subsequent interactions between adhesion molecules and matrix molecule ligands such as VLA-4 and fibronectin. Local release of cytokines such as IL-1 and IL-8 with consequent upregulation of endothelial cell adhesion molecules probably promotes differential binding and transmigration of memory T cells into rheumatoid synovium. It is therefore likely that a substantial fraction of infiltrating cells in RA may be 'innocent bystander' cells, a possibility which may explain the aforementioned difficulty in detecting T cell derived cytokines in rheumatoid synovium. That only a fraction of synovial T cells in RA is activated may at least partially explain difficulties in identifying consistent patterns of T cell receptor V region gene usage among unselected synovial T cells.

Emerging evidence indicates that adhesion molecules can also function as costimulatory receptors for T cells (and other cells such as monocytes and neutrophils). Engagement of several integrins potentiates T cell proliferation induced by cross linking of the T cell receptor/CD3 complex on T cells.⁵¹⁻⁵³ Binding of the $\alpha 5 \beta 1$ integrin to fibronectin induces expression of AP-1, a transcription factor necessary for IL-2 gene expression.⁵⁴ Another integrin, $\alpha v \beta 3$, functions as a costimulatory molecule for IL-4 production in $\gamma \delta$ T cells.⁵⁵ Thus interactions between T cell integrins and their respective matrix molecule ligands can exert important influences on the activation and function of these cells.

T cell proteases

Transendothelial migration of activated T cells involves as yet less defined proteolytic processes. Specialised granules within the cytoplasm of cytolytic T lymphocytes contain a unique set of proteins that are responsible for the cytolytic activity of these cells (for review, see ref 56). The major constituents are the pore-forming protein perforin (cytolysin) and several highly homologous serine proteases, termed granzymes. With regard to the molecular mechanisms involved in the migration of T cells through vascular endothelial basement membranes, we were able to show that T cell specific serine proteinase 1 (granzyme A) partially degrades basement membrane collagen type IV by cleavage of the $\alpha 2(IV)$ chain within the native molecule,⁵⁷ raising the possibility that this protease may facilitate the movement of T cells through vascular basement membranes into inflammatory foci. Interestingly, both CD4+ and CD8+ lymphocytes from the synovial fluid of patients with RA express perforin and granzyme A.⁵⁸

Humoral responses in RA

As RF is characteristically present in the serum and synovial fluid of about 80% of patients

with RA, efforts have been directed towards delineating the molecular basis of expression of this prototypic autoantibody in RA. Whether polyclonal or antigen driven mechanisms contribute to RF expression in RA remains uncertain; however, RF present in autoimmune MRL/lpr mice exhibits patterns of variable region somatic mutations most consistent with an antigen driven response.⁵⁹ Similar conclusions have been reached for antibodies to DNA also present in these mice.⁶⁰ A useful approach for studying the molecular basis of RF expression in man has been the development of structurally defined monoclonal antibody anti-idiotypic reagents. Two such monoclonal antibodies, designated 17·109⁶¹ and 6B6·6,⁶² recognise distinct V κ III light chain idiotypes expressed by about two thirds of IgM κ RF paraproteins.⁶³ The 17·109 and 6B6·6 cross reactive idiotypes (CRIs) are probably encoded by distinct germline V κ genes designated Humkv 325⁶⁴ and Humkv 328⁶⁵ respectively. 6B6·6 CRI (+) light chains are members of the V κ IIIa sub-subgroup, whereas 17·109 (CRI)(+) light chains are members of the V κ IIIb sub-subgroup.⁶¹⁻⁶³

Efforts have also been directed toward defining CRI shared by heavy chain V regions of RF paraproteins. For example, monoclonal antibody G6 recognises a V_H1 CRI encoded by the germline V_H gene closely related or identical to hv3005.⁶⁶⁻⁶⁹ Another subgroup of RF contains heavy chains recognised by monoclonal antibodies LC1, 5-14, and 6-10; these CRIs are encoded by a V_H4 gene(s) yet to be precisely identified.⁷⁰ The demonstration that a limited number of germline immunoglobulin V region genes (for example, Humkv 325 and Humkv 328) encode a substantial fraction of IgM RF paraproteins suggested that these V genes might also contribute to the expression of RF in RA. Serological analyses of serum RF using monoclonal antibodies identifying germline encoded RF associated CRIs (for example, 17·109 and 6B6·6) have indicated that a substantial proportion of patients with RA synthesise some RF bearing either the 17·109 or 6B6·6 V κ III CRI.^{62 71 72} Only a negligible fraction of RF in individual serum samples, however, appeared to express either CRI. These observations are in striking contrast with the dominance of these idiotypes among RF paraproteins and suggest either that germline Humkv 325 and Humkv 328 (or closely related genes) encode only a small proportion of RF in RA or that extensive mutation of genes encoding RF may occur in the disease. Circumstantial evidence supporting a role for somatic mutation in accounting for the low proportion of 6B6·6 and 17·109 CRI(+) RF in RA has come from studies of a cDNA library generated from synovial cell mRNA of a patient with RA expressing low levels of 17·109 and 6B6·6 CRI(+) RF in her serum and synovial fluid.⁷³ Unexpectedly, 27% of κ light chain clones sampled from this patient's synovial library were drawn from the two germline V region elements, Humkv 325 and Humkv 328. These clones showed con-

siderable somatic mutations with a pattern most consistent with antigen driven selection.⁷³ Unfortunately, the design of this study did not permit analysis of the specificity of the intact antibodies using these light chain sequences.

An alternative approach which is being used to investigate the V region diversity of RF in RA entails analysis of V region sequences of RF elaborated by cloned B cell lines (or hybridomas) obtained from healthy controls and patients with RA. These studies, still in their infancy, indicate that multiple V_H and V_L genes can encode RF in RA. Both essentially unmodified germline sequences and mutated V region sequences, consistent with antigen selection, have been found.^{69 74-76} It therefore seems that the highly restricted pattern of V gene utilisation, seen among RF paraproteins, is not shared by RF secreting B cell lines rescued from healthy subjects and patients with RA. Caution must be exercised in interpreting these results, however, as the precise relation between cloned B cell lines and autoantibody expression in vivo is far from established.

Although serological studies have the advantage of directly analysing RF expression in vivo, they are constrained by the limited number of monoclonal antibodies recognising structurally defined CRIs that are currently available. Moreover, this approach is unable to distinguish between the influence of somatic mutation of a particular germline gene (with consequent loss of germline encoded CRIs) and the use of alternative germline genes. Application of both serological and cell line analyses to investigation of RF expressed in the same subject offers the most promise of ultimately elucidating the genetic origin of RF in RA. Moreover, such studies should reveal the relative importance of antigen selection versus polyclonal activation in expression of these autoantibodies.

Animal models

One major difficulty in elucidating the early features and sequence of events in the pathogenesis of RA has been the lack of an appropriate animal model. Following the original discovery in Dr Kang's laboratory that immunisation of rodents with collagen type II induces an inflammatory polyarthritis,⁷⁷ subsequent investigations have shown that the disease can be transferred by T cell clones, that T cell deficient animals do not develop arthritis, and that the disease can be abrogated with antibodies to CD4 (for review see refs 78 and 79). The direct relevance of the type II collagen arthritis model to the pathogenesis of RA remains uncertain, though the presence of T cells reactive with collagen type II in the synovium of patients with RA is of interest in this regard.

Arthritis can also be induced by the parenteral administration of a group A streptococcal cell wall peptidoglycan group-specific polysaccharide.⁸⁰ This latter model has provided important insights into the role of cytokines and growth factors in the regulation

of synovial cell growth associated with joint destruction.⁸¹ Moreover, this model is of further interest because an abnormally functioning hypothalamic-pituitary-adrenal axis, reflected by low endogenous corticosteroid production, contributes to disease susceptibility.⁸² A provocative preliminary report suggests that patients with RA also have a defective hypothalamic-pituitary-adrenal axis response to immune stimuli which is not attributable to chronic inflammation itself as it was not seen in patients with osteomyelitis.⁸³

With Dr John Mountz, our group recently developed a superantigen arthritis model using a T cell receptor V β 8.2 transgenic mouse in which all T cells are reactive with staphylococcal enterotoxin β by virtue of expression of the V β 8.2 T cell receptor transgene.⁸⁴ The arthritis develops within 20 days of an intra-articular injection of a single dose of staphylococcal enterotoxin β ; the recipient lpr transgenic mice exhibit a tolerance defect in T cells recognising the staphylococcal enterotoxin β -MHC class II molecule complex, which biases the T cell response away from anergy induction and towards activation. Presumably, sustained activation of T cells in this model triggers a chronic inflammatory response. Interestingly, staphylococcal enterotoxin A induces expression of IL-6 and IL-8 in rheumatoid synovial cells.⁸⁵

Recently, infectious models have been developed with the hope of providing insight into the pathogenesis of chronic inflammatory arthritis. Infectious agents, both bacterial and viral, have long been known to have the potential for triggering inflammatory polyarthritides. In this regard, caprine arthritis-encephalitis virus, a lentivirus in the subfamily of retroviruses, infects cells of the macrophage lineage in goats and triggers a progressive arthropathy.⁸⁶ The Lyme arthritis spirochaete, *Borrelia burgdorferi*, has been shown to induce both a progressive and destructive polyarthritis,⁸⁷ as well as severe systemic lesions in severe combined immunodeficiency mice.⁸⁸ These studies may facilitate re-evaluation of the role of infectious agents in the pathogenesis of chronic inflammatory arthritis.

Synovial hyperplasia

One major difficulty in elucidating the early events in spontaneous destructive arthritis has been overcome by using the MRL-lpr/lpr mouse strain, because these mice develop a spontaneous RA-like destruction of articular cartilage and bone in addition to expressing RFs and antibodies against collagen type II.⁸⁹ The initial disease in joints of MRL-lpr/lpr mice resembles the synovial cells with a transformed appearance described by Fassbender in human RA.⁵ Most strikingly, the initial proliferation, attachment, and invasion of these cells into cartilage or bone, or both, appears in the absence of inflammatory cells at the sites of destruction, including polymorphonuclear leucocytes, lymphocytes, and mast cells. After initial cartilage and bone destruction, inflammatory cells then appear in

the subsynovium and are largely associated with the development of vascular lesions resembling the features of rheumatoid pannus in the human joint. The pathological similarities between the joint lesions in MRL-lpr/lpr mice and human RA prompted us to undertake a more detailed characterisation of the synovial cells involved in joint destruction in RA. Immunohistological studies indicated that most of the cells with a transformed appearance (50–70%) in RA seem to be derived from the monocyte/macrophage (M ϕ) lineage. A much smaller proportion of cells (5–10%) proliferates in vitro, particularly large stellate shaped cells with a transformed appearance.⁶ These data suggest that only a small proportion of the resident synovial cells in RA proliferates and that most cells in rheumatoid synovial hyperplasia originate from bone marrow.^{6, 90} Based on these observations, a major goal of our laboratory has been to study the role of transcriptional factors in regulating synovial cell proliferation and function.

In view of evidence that several cellular proto-oncogenes have a control role in regulating cell proliferation and that certain oncogenes encoding nuclear proteins, such as myc and myb, cooperate with an activated ras gene in the transformation of cells,⁹¹ we examined the expression of these proto-oncogenes in synovial tissue from patients with destructive RA.⁶ Ras and myc oncoproteins could be detected in about 70% of the patients with RA and were largely restricted to the synovial cells attached to sites of cartilage and bone destruction.⁹² Despite the fact that cooperation between oncogenes has been described in other systems,⁹³ it is not established in rheumatoid synovial cells. Interestingly, however, the cysteine proteinase cathepsin L, previously shown to be the major ras induced protein in ras transformed cells,⁹⁴ colocalised with ras and myc expression in about 50% of the cases of RA studied.⁹² Negligible expression of these oncogenes was detected in inflammatory osteoarthritis. Subsequent studies also showed that cathepsin B occurred at sites of synovial attachment to cartilage and bone in rheumatoid joints.⁹⁵ The secretion of active forms of cathepsins B and L by ras oncogene-expressing Kirsten virus transformed fibroblasts⁹⁶ and the colocalisation of ras and myc with these cathepsins in RA suggest that these oncoproteins are involved in the upregulation of cysteine proteinases in rheumatoid synovium. Evidence that cathepsins B and L degrade cartilage collagen types II, IX, and XI⁹⁷ and cartilage proteoglycan aggregates⁹⁸ suggests that these proteinases contribute to joint destruction in RA. Earlier studies have convincingly implicated collagenase in rheumatoid joint destruction.⁹⁹ In this regard, fos and the early growth response gene 1 (egr-1), an inducible gene encoding a zinc finger protein containing two AP-1 binding sites and a sequence homologous to the response element of the c-fos promoter,¹⁰⁰ have been identified in collagenase-producing rheumatoid synovial lining cells.¹⁰¹ Although the role of fos, jun, and

the AP-1 complex in cell proliferation and transformation is just beginning to emerge,¹⁰² it is of interest that an AP-1 site and *fos* are not only involved in the transcriptional control of collagenase but have also been implicated in the basal expression of stromelysin, the other major metalloproteinase that degrades matrix proteins.¹⁰³

In view of the observations that both in human RA^{5 104} and in the spontaneous MRL-lpr/lpr arthropathy¹⁰⁵ cartilage and bone erosions occur exclusively at sites of synovial attachment, it seems likely that a critical cell-matrix interaction(s) occurs. It is well established that various cell types express characteristic patterns of transmembrane adhesion receptors (see above) which recognise and interact with specific types of collagen and collagen associated glycoproteins (for review see ref 106) and, therefore, it is of current interest to delineate the molecular and structural basis of attachment of rheumatoid synovial cells to cartilage and bone matrix components. It should be stressed that fragments of degraded matrix molecules may also regulate expression of connective tissue degrading enzymes. This concept was fostered by the discovery that partially degraded but not native fibronectin induces collagenase and stromelysin expression in synovial fibroblasts following interaction with fibronectin receptors.¹⁰⁷ These data suggest that the synthesis of proteolytic enzymes in the rheumatoid joint is not only under the control of certain cytokines^{79 81} but is probably also regulated by products generated by the destructive process itself. Both mechanisms may be crucial to the perpetuation of synovial inflammation.

Based on the observation that synovial hyperplasia in RA is associated with a transformed appearing phenotype of the proliferating synovial cells expressing *ras* and

myc oncogenes,¹⁰⁸ we searched for evidence of transforming factors, especially retroviral antigens, in the rheumatoid synovium. Immunohistology showed reactivity with antibodies against HTLV-I p19 and p24 in the synovium, but as the same patients were seronegative for HTLV-I we concluded that antigens cross reactive with or related to these HTLV-I constituents were present in the synovium.¹⁰⁹ Studies in our laboratory designed to detect collagen fibrils in synovial fluid,¹³ however, led to the unanticipated detection of virus-like particles within the dense meshwork of proteinaceous aggregates present in rheumatoid synovial fluids.¹¹⁰ Subsequent studies, including immunoelectron microscopy, have shown the presence in five of eight cases of RA of spherical particles 200 nm in diameter and a distinct eccentrically located internal core with an average size of 70 nm, lacking epitopes for HTLV-I p19 and HIV-I p24 (Stransky G, Aicher W K, Moreland L W, *et al*, unpublished data). These particles bear a striking resemblance to type C retroviral particles but do not resemble any of the known human retroviruses, including particles derived from known endogenous retroviral structures.¹¹¹ A retroviral origin for these particles is not only suggested by their morphological appearance but also by the concomitant spontaneous and constitutive expression of the cellular zinc finger transcription factor Z-225 in short and long term cultures of rheumatoid synovial cells.¹¹² The constitutive expression of Z-225, resembling *egr-1*, has only been reported for cells transformed by HTLV-I or HTLV-II.¹¹³ In addition, recent studies have shown that synoviocytes derived from rheumatoid tissue exhibit reverse transcriptase activity.¹¹⁴ Thus we are attracted to the possibility that a hitherto unknown retrovirus may play a part in the pathogenesis of RA. Figure 2 depicts a

Figure 2 Hypothesis for the aetiopathogenesis of rheumatoid arthritis. PDGF=platelet derived growth factor; IL-1/6=interleukin 1/6; TNFα=tumour necrosis factor α; GM-CSF=granulocyte-macrophage colony stimulating factor.

working *hypothesis* for the aetiopathogenesis of RA. Such a hypothesis could account for the two dominant cellular processes operating in rheumatoid joint destruction (synovial hyperplasia and T cell dependent immune responses) and would be consistent with a genetic basis for susceptibility to the disease.⁸ Moreover, it could explain the upregulation of proto-oncogenes in the transformed appearing proliferating synovial lining as well as the abnormal humoral and cellular immune responses. In this regard, it is of interest that an inflammatory arthropathy resembling certain features of RA occurs in HTLV-I transgenic mice.¹¹⁵ It is also intriguing that HTLV transactivator proteins tat and tax are able to induce the expression of several cellular genes which have been implicated in various aspects of the pathogenesis of RA, including c-fos,¹¹⁶ granulocyte-macrophage colony stimulating factor,¹¹⁷ the c-sis proto-oncogene encoding for the platelet derived growth factor B chain¹¹⁸ and MHC class II genes.¹¹⁹ Last, but not least, this hypothesis would also explain why current antirheumatic treatment is of limited effectiveness in halting progression of joint destruction in RA.

- 1 Lipsky P E, Davis L S, Cush J J, Oppenheimer-Marks N. The role of cytokines in the pathogenesis of rheumatoid arthritis. *Springer Semin Immunopathol* 1989; 11: 123–62.
- 2 Arend W, Koopman W J, eds. Molecular biology and the immunopathogenesis of rheumatoid arthritis. Keystone Symposia on Molecular Cellular Biology. *J Cell Biochem* 1991; suppl 15E: 141–87.
- 3 Gay S, Koopman W J. Immunopathology of rheumatoid arthritis. *Current Rheumatology* 1989; 1: 8–14.
- 4 Kingsley G, Pitzalis C, Panayi G S. Immunogenetic and cellular immune mechanisms in rheumatoid arthritis: relevance to new therapeutic strategies. *Br J Rheumatol* 1990; 29: 58–64.
- 5 Fassbender H G. Histomorphologic basis of articular cartilage destruction in rheumatoid arthritis. *Coll Relat Res* 1983; 3: 141–55.
- 6 Gay S, Gay R E. Cellular basis and oncogene expression of rheumatoid joint destruction. *Rheumatol Int* 1989; 9: 105–13.
- 7 Buus S, Sette A, Colon S M, Miles C, Grey H M. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science* 1987; 235: 1353–8.
- 8 Kappler J W, Roehen M, Marrack P. T cell tolerance by clonal elimination in the thymus. *Cell* 1987; 49: 273–80.
- 9 Herman A, Kappler J W, Marrack P, Pullen A M. Superantigens: mechanism of T-cell stimulation and role in immune responses. *Annu Rev Immunol* 1991; 9: 745–72.
- 10 Harris E D Jr. Rheumatoid arthritis: pathophysiology and implications for therapy. *N Engl J Med* 1990; 322: 1277–89.
- 11 Firestein G S, Zvaifler N J. How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum* 1990; 33: 768–73.
- 12 Itescu S, Mathur-Wagh U, Skovron M L, Brancato L J. HLA-B35 is associated with accelerated progression to AIDS. *J Acquir Immune Defic Syndr* 1992; 5: 37–45.
- 13 Moreland L W, Stewart T, Gay R E, Huang G-Q, McGee N, Gay S. Immunohistologic demonstration of type II collagen in synovial fluid phagocytes of osteoarthritis and rheumatoid arthritis patients. *Arthritis Rheum* 1989; 32: 1458–64.
- 14 Gregersen P K, Silver J, Winchester R J. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987; 30: 1205–13.
- 15 Van Boxel J A, Paget S A. Predominantly T-cell infiltrate in rheumatoid synovial membrane. *N Engl J Med* 1975; 293: 517–20.
- 16 Burmester G R, Jahn B, Gramatzki M, Zacher J, Kalden J R. Activated T cells in vivo and in vitro; divergence in expression of Tac and Ia antigens in the nonblastoid small T cells of inflammation and normal T cells activated in vitro. *J Immunol* 1984; 133: 1230–4.
- 17 Shiroky J B, Yocum D E, Wilder R L, Klippel J H. Experimental basis of innovative therapies of rheumatoid arthritis. *Concepts Immunopathol* 1989; 7: 106–44.
- 18 Herzog C, Walker C, Müller W, et al. Anti-CD4 antibody treatment of patients with rheumatoid arthritis: I. Effect on clinical course and circulating T cells. *J Autoimmun* 1989; 2: 627–42.
- 19 Horneff G, Burmester G R, Emmrich F, Kalden J R. Treatment of rheumatoid arthritis with an anti-CD4 monoclonal antibody. *Arthritis Rheum* 1991; 34: 129–40.
- 20 Reiter C, Kakavand B, Rieber E P, Schattenkirchner M, Riethmüller G, Krüger K. Treatment of rheumatoid arthritis with monoclonal CD4 antibody M-T151. *Arthritis Rheum* 1991; 34: 525–36.
- 21 Calabrese L H, Wilke W S, Perkins A D, Tubbs R R. Rheumatoid arthritis complicated by infection with human immunodeficiency virus and the development of Sjögren's syndrome. *Arthritis Rheum* 1989; 32: 1453–7.
- 22 Bijlsma J W J, Derksen R W H M, Huber-Bruning O, Borleffs J C C. Does AIDS 'cure' rheumatoid arthritis? *Ann Rheum Dis* 1988; 47: 350–1.
- 23 Paliard X, West S G, Lafferty J A, et al. Evidence for the effects of a superantigen in rheumatoid arthritis. *Science* 1991; 253: 325–9.
- 24 Uematsu Y, Wege H, Straus A, et al. The T-cell-receptor repertoire in the synovial fluid of a patient with rheumatoid arthritis is polyclonal. *Proc Natl Acad Sci USA* 1991; 88: 8534–8.
- 25 Howell M D, Diveley J P, Lundeen K A, et al. Limited T-cell receptor β -chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis. *Proc Natl Acad Sci USA* 1991; 88: 10921–5.
- 26 Cooper S M, Dier D L, Roessner K D, Budd R C, Nicklas J A. Diversity of rheumatoid synovial tissue T cells by T cell receptor analysis. Oligoclonal expansion in interleukin-2-responsive cells. *Arthritis Rheum* 1991; 34: 537–46.
- 27 Abe J, Forrester J, Nakahara T, Lafferty J A, Kotzin B L, Leung D Y M. Selective stimulation of human T cells with streptococcal erythrogenic toxins A and B. *J Immunol* 1991; 146: 3747–50.
- 28 Choi Y, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J. Interaction of *Staphylococcus aureus* toxin 'superantigens' with human T cells. *Proc Natl Acad Sci USA* 1989; 86: 8941–5.
- 29 Jores R, Alzari P M, Meo T. Resolution of hypervariable regions in T-cell receptor beta chains by a modified Wu-Kabat index of amino acid diversity. *Proc Natl Acad Sci USA* 1990; 87: 9138–42.
- 30 Choi Y, Herman A, DiGiusto D, Wade T, Marrack P, Kappler J. Residues of the variable region of the T-cell-receptor beta-chain that interact with *S. aureus* toxin superantigens. *Nature* 1990; 346: 471–3.
- 31 Cazenave P A, March PN, Jouvin-Marche E, et al. V beta 17 gene polymorphisms in wild-derived mouse strains: two amino acid substitutions in the V beta 17 region greatly alter T cell receptor specificity. *Cell* 1990; 63: 717–28.
- 32 Baccala R, Smith L R, Vestberg M, Peterson P A, Cole B C, Theofilopoulos A N. Mycoplasma arthritis mitogen: VB engaged in mice, rats, and humans, and requirements of HLA-DR α for presentation. *Arthritis Rheum* 1992; 35: 434–42.
- 33 Davies T F, Martin A, Concepcion E S, Graves P, Cohen L, Ben-Nun A. Evidence of limited variability of antigen receptors on intrathyroidal T cells in autoimmune thyroid disease. *N Engl J Med* 1991; 325: 238–44.
- 34 Dayan C M, Londei M, Corcoran A E, et al. Autoantigen recognition by thyroid-infiltrating T cells in Graves disease. *Proc Natl Acad Sci USA* 1991; 88: 7415–9.
- 35 Londei M, Savill C M, Verhoef A, et al. Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proc Natl Acad Sci USA* 1989; 86: 636–40.
- 36 Yan T, Burkhardt H, Ritter H, et al. Specificity and T cell receptor β chain usage of a human collagen type II-reactive T cell clone derived from a healthy individual. *Eur J Immunol* 1992; 22: 51–6.
- 37 Van Eden W, Thole J E R, van der Zee R, et al. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* 1988; 331: 171–3.
- 38 Van Eden W, Holoshitz J, Weve Z, Frenkel A, Klajman A, Cohen I R. Arthritis induced by a T-lymphocyte clone that responds to mycobacterium tuberculosis and to cartilage proteoglycans. *Proc Natl Acad Sci USA* 1985; 82: 5117–20.
- 39 Karlsson-Parra A, Söderström K, Ferm M, Ivanyi J, Kiessling R, Klareskog L. Presence of human 65 kD heat shock protein (hsp) in inflamed joints and subcutaneous nodules of RA patients. *Scand J Immunol* 1990; 31: 283–8.
- 40 Li S G, Quayle A J, Shen Y, et al. Mycobacteria and human heat shock protein-specific cytotoxic lymphocytes in rheumatoid synovial inflammation. *Arthritis Rheum* 1992; 35: 270–81.
- 41 Fischer H P, Sharrock C E M, Colston M J, Panayi G S. Limiting dilution analysis of proliferative T cell responses to mycobacterial 65-kDa heat-shock protein fails to show significant frequency differences between synovial fluid and peripheral blood of patients with rheumatoid arthritis. *Eur J Immunol* 1991; 21: 2937–41.
- 42 Butcher E C. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1991; 67: 1033–6.
- 43 von Andrian U H, Chambers J D, McEvoy L M, Bargatze R F, Arfors K-E, Butcher E C. Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and leukocyte β 2 integrins in vivo. *Proc Natl Acad Sci USA* 1991; 88: 7538–42.

- 44 Smith C W, Kishimoto T K, Abbass O, *et al.* Chemotactic factors regulate lectin adhesion molecule 1 (LECAM-1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. *J Clin Invest* 1991; 87: 609-18.
- 45 Abbot S E, Kaul A, Stevens C R, Blake D R. Isolation and culture of synovial microvascular endothelial cells. Characterization and assessment of adhesion molecule expression. *Arthritis Rheum* 1992; 35: 401-5.
- 46 Kavanaugh A F, Lightfoot E, Lipsky P E, Oppenheimer-Marks N. Role of CD11/CD18 in adhesion and transendothelial migration of T cells. Analysis utilizing CD18-deficient T cell clones. *J Immunol* 1991; 146: 4149-56.
- 47 Oppenheimer-Marks N, Davis L S, Bogue D T, Ramberg J, Lipsky P E. Differential utilization of ICAM-1 and VCAM-1 during the adhesion and transendothelial migration of human T lymphocytes. *J Immunol* 1991; 147: 2913-21.
- 48 Sanders M E, Makgoba M W, Sharrow S O, *et al.* Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, Pgp-1) and have enhanced IFN- γ production. *J Immunol* 1988; 140: 1401-7.
- 49 Shimizu Y, Van Seventer G A, Horgan K J, Shaw S. Regulated expression and binding of three VLA (B1) integrin receptors on T cells. *Nature* 1990; 345: 250-3.
- 50 Pitzalis C, Kingsley G, Haskard D, Panayi G. The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: evidence for regulation by selective endothelial and homotypic adhesion. *Eur J Immunol* 1988; 18: 1397-404.
- 51 Matsuyama Y, Yamada A, Kay J, *et al.* Activation of CD4 cells by fibronectin and anti-CD3 antibody. *J Exp Med* 1989; 170: 1133-48.
- 52 Shimizu Y, van Seventer G A, Horgan K J, Shaw S. Costimulation of proliferative responses of resting CD4⁺ T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. *J Immunol* 1990; 145: 59-67.
- 53 Burky L C, Jakubowski A, Newman B M, Rosa M D, Chirasso G, Lobb R. Signaling by vascular adhesion molecule-1 (VCAM-1) through VLA-4 promotes CD-3 dependent T cell proliferation. *Eur J Immunol* 1991; 21: 2871-5.
- 54 Yamada A, Nikaida T, Nojima Y, Schlossman S F, Morimoto C. Activation of CD4 T lymphocytes. Interaction of fibronectin with VLA-5 receptor on CD4 cells induces the AP-1 transcription factor. *J Immunol* 1991; 146: 53-6.
- 55 Roberts K, Yokoyama W M, Kehn P J, Shevach E M. The vitronectin receptor serves as an accessory molecule for the activation of a subset of gamma/delta T cells. *J Exp Med* 1991; 173: 231-40.
- 56 Griffiths G M, Mueller C. Expression of perforin and granzymes in vivo: potential diagnostic markers for activated cytotoxic cells. *Immunol Today* 1991; 12: 415-9.
- 57 Simon M M, Kramer M D, Prester M, Gay S. Mouse T-cell associated serine proteinase 1 degrades collagen type IV: a structural basis for the migration of lymphocytes through vascular basement membranes. *Immunology* 1991; 73: 117-9.
- 58 Griffiths G M, Alpert S, Lambert E, McGuire J, Weissman I L. Perforin and granzyme A expression identifying cytolytic lymphocytes in rheumatoid arthritis. *Proc Natl Acad Sci USA* 1992; 89: 549-53.
- 59 Schlomchik M J, Marshak-Rothstein A, Wolfowicz C B, Rothstein T L, Weigert M G. The role of clonal selection and somatic mutation in autoimmunity. *Nature* 1987; 328: 805-11.
- 60 Schlomchik M J, Mascelli M, Shan H, *et al.* Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc Natl Acad Sci USA* 1987; 84: 9150-4.
- 61 Carson D A, Fong S. A common idiotype on human rheumatoid factors identified by a hybridoma antibody. *Mol Immunol* 1983; 20: 1081-7.
- 62 Schrohenloher R E, Accavitti M A, Bhowan A S, Koopman W J. Monoclonal antibody 6B6-6 defines a cross-reactive kappa light chain idiotype on human monoclonal and polyclonal rheumatoid factors. *Arthritis Rheum* 1990; 33: 187-98.
- 63 Crowley J J, Goldfien R D, Schrohenloher R E, *et al.* Incidence of three cross-reactive idiotypes on human rheumatoid factor proteins. *J Immunol* 1988; 140: 3411-8.
- 64 Radoux V, Chen P P, Sorge J A, Carson D A. A conserved human germline V κ gene directly encodes rheumatoid factor light chains. *J Exp Med* 1986; 164: 2119-24.
- 65 Chen P P, Robbins D L, Jirik F R, Kipps T J, Carson D A. Isolation and characterization of a light chain variable region for human rheumatoid factors. *J Exp Med* 1987; 166: 1900-5.
- 66 Mageed R A, Dearlove M, Goodall D M, Jefferis R. Immunogenic and antigenic epitopes of immunoglobulins. XVII. Monoclonal anti-idiotypes to the heavy chain of human rheumatoid factors. *Rheumatol Int* 1986; 6: 179-83.
- 67 Chen P P, Liu M-F, Glass C A, Sinha S, Kipps T J, Carson D A. Characterization of two Ig V κ genes which are homologous to human rheumatoid factors. *Arthritis Rheum* 1989; 32: 72-6.
- 68 Crowley J J, Mageed R A, Silverman G J, *et al.* The incidence of a new human cross-reactive idiotype linked to subgroup V κ III heavy chains. *Mol Immunol* 1990; 27: 87-94.
- 69 Pascual V, Randen I, Thompson K, *et al.* The complete nucleotide sequences of the heavy chain variable regions of six monospecific rheumatoid factors derived from Epstein-Barr virus-transformed B cells isolated from the synovial tissue of patients with rheumatoid arthritis. *J Clin Invest* 1990; 86: 1320-8.
- 70 Silver G J, Schrohenloher R E, Accavitti M A, Koopman W J, Carson D A. Structural characterization of the second major cross-reactive idiotype group of human rheumatoid factors: association with the V κ 4 gene family. *Arthritis Rheum* 1990; 33: 1347-60.
- 71 Koopman W J, Schrohenloher R E, Carson D A. Dissociation of expression of two rheumatoid factor cross-reactive κ L chain idiotypes in rheumatoid arthritis. *J Immunol* 1990; 144: 3468-72.
- 72 Kouri T, Crowley J, Aho K, *et al.* Occurrence of two germline-related rheumatoid factor idiotypes in rheumatoid arthritis and in non-rheumatoid seropositive individuals. *Clin Exp Immunol* 1990; 82: 250-6.
- 73 Lee S K, Bridges S L Jr, Koopman W J, Schroeder H W Jr. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. *Arthritis Rheum* 1992; 35: 905-13.
- 74 Ezaki I, Kanda H, Sakai K, *et al.* Restricted diversity of the variable region nucleotide sequences of the heavy and light chains of a human rheumatoid factor. *Arthritis Rheum* 1991; 34: 343-50.
- 75 Victor K D, Randen I, Thompson K, *et al.* Rheumatoid factors isolated from patients with autoimmune disorders are derived from germline genes distinct from those encoding the Wa, Po, and Bla cross-reactive idiotypes. *J Clin Invest* 1991; 87: 1603-13.
- 76 Olee T, Lu E W, Huang D-F, *et al.* Genetic analysis of self-associating immunoglobulin G rheumatoid factors from two rheumatoid synovia implicates an antigen-driven response. *J Exp Med* 1992; 175: 831-42.
- 77 Trentham D E, Townes A S, Kang A H. Autoimmunity to type II collagen: an experimental model of arthritis. *J Exp Med* 1977; 146: 857-69.
- 78 Stuart J M, Watson W C, Kang A H. Collagen autoimmunity and arthritis. *FASEB J* 1988; 2: 2950-6.
- 79 Firestein G S. Cytokines in autoimmune diseases. *Concepts Immunopathol* 1992; 8: 129-60.
- 80 Wilder R L. Streptococcal wall-induced polyarthritis in rats. In: Greenwald R A, Diamond H S, eds. *CRC handbook of animal models for the rheumatic disease*. Vol I. Boca Raton, FL: CRC Press, 1988: 33-40.
- 81 Remmers E F, Lafyatis R, Kumkumian G K, *et al.* Cytokines and growth regulation of synoviocytes from patients with rheumatoid arthritis and rats with streptococcal cell wall arthritis. *Growth Factors* 1990; 2: 179-88.
- 82 Sternberg E M, Young W S III, Bernardini R, *et al.* A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell-wall-induced arthritis in Lewis rats. *Proc Natl Acad Sci USA* 1989; 86: 4771-5.
- 83 Panayi G S, Chikanza I C, Petrou P, Kingsley G, Chrousos G. Defective hypothalamic-pituitary-adrenal axis function in rheumatoid arthritis is not a consequence of chronic inflammation per se. *FASEB J* 1991; 6: A1448.
- 84 Mountz J D, Zhou T, Gay R, *et al.* Genetic and environment requirements for development of inflammatory arthritis. *Arthritis Rheum* 1991; 34(suppl): 26.
- 85 Mourad W, Mehindate K, Schall T J, McColl S R. Engagement of major histocompatibility complex class II molecules by superantigen induces inflammatory cytokine gene expression in human rheumatoid fibroblast-like synoviocytes. *J Exp Med* 1992; 175: 613-6.
- 86 Banks K L, Jutila M A, Jacobs C A, Michaels F H. Augmentation of lymphocyte and macrophage proliferation by caprine arthritis-encephalitis virus contributes to the development of progressive arthritis. *Rheumatol Int* 1989; 9: 123-8.
- 87 Schaible U E, Kramer M D, Museteanu C, Zimmer G, Mossmann H, Simon M M. The severe combined immunodeficiency (SCID) mouse: a laboratory model for the analysis of Lyme arthritis and carditis. *J Exp Med* 1989; 170: 1427-32.
- 88 Schaible U E, Gay S, Museteanu C, *et al.* Lyme borreliosis in the severe combined immunodeficiency (scid) mice manifests predominantly in the joints, heart and liver. *Am J Pathol* 1990; 137: 811-20.
- 89 Koopman W J, Gay S. The MRL-lpr/lpr mouse: A model for the study of rheumatoid arthritis. *Scand J Rheumatol Suppl* 1988; 75: 284-9.
- 90 Henderson B, Revell P A, Edwards J C W. Synovial lining hyperplasia in rheumatoid arthritis: dogma and fact. *Ann Rheum Dis* 1988; 47: 348-9.
- 91 Land H, Parada L F, Weinberg R A. Tumorigenic conversion of primary fibroblasts requires at least two cooperating oncogenes. *Nature* 1983; 304: 596-602.
- 92 Trabandt A, Aicher W K, Gay R E, *et al.* Expression of the collagenolytic and ras-induced cysteine proteinase cathepsin L and proliferation-associated oncogenes in synovial cells of MRL/l mice and patients with rheumatoid arthritis. *Matrix* 1990; 10: 349-61.
- 93 Hunter T. Cooperation between oncogenes. *Cell* 1991; 64: 249-70.
- 94 Joseph L J, Chang L C, Stamenkovich D, Sukhatme V P. Complete nucleotide and deduced amino acid sequences of human and murine preprocathepsin L. An abundant

- transcript induced by transformation of fibroblasts. *J Clin Invest* 1988; 81: 1621-9.
- 95 Trabandt A, Gay RE, Fassbender H G, Gay S. Cathepsin B in synovial cells at the site of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 1991; 34: 1441-51.
 - 96 Mason R W, Wilcox D, Wikstrom P, Shaw E N. The identification of active forms of cysteine proteinases in Kirsten-virus-transformed mouse fibroblasts by use of a specific radiolabelled inhibitor. *Biochem J* 1989; 257: 125-9.
 - 97 Maciewicz RA, Wotton S F, Etherington D J, Duance V C. Susceptibility of the cartilage collagens type II, IX and XI to degradation by the cysteine proteinases, cathepsin B and L. *FEBS Lett* 1990; 269: 189-93.
 - 98 Nguyen Q, Mort J S, Roughley P J. Cartilage proteoglycan aggregate is degraded more extensively by cathepsin L than by cathepsin B. *Biochem J* 1990; 266: 569-73.
 - 99 Krane S M, Conca W, Stephenson M L, Amento E P, Goldring M B. Mechanisms of matrix degradation in rheumatoid arthritis. *Ann NY Acad Sci* 1990; 580: 340-54.
 - 100 Sukhatme V P. The Egr transcription factor family: from signal transduction to kidney differentiation. *Kidney Int* 1992; 41: 550-3.
 - 101 Trabandt A, Aicher W K, Gay R E, Sukhatme V P, Fassbender H G, Gay S. Spontaneous expression of immediately early response genes c-fos and egr-1 in collagenase-producing rheumatoid synovial fibroblasts. *Rheumatol Int* 1992; 12: 53-9.
 - 102 Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1991; 1072: 129-57.
 - 103 Buttice G, Quinones S, Kurkinen M. The AP-1 site is required for basal expression but is not necessary for TPA-response of the stromelysin gene. *Nucleic Acids Res* 1991; 19: 3723-31.
 - 104 Allard S A, Muirden K D, Maini R N. Correlation of histopathological features of pannus with patterns of damage in different joints in rheumatoid arthritis. *Ann Rheum Dis* 1991; 50: 278-83.
 - 105 Tanaka A, O'Sullivan F X, Koopman W J, Gay S. Etiopathogenesis of the rheumatoid arthritis-like disease in MRL/l mice: II. Ultrastructural basis of joint destruction. *J Rheumatol* 1988; 15: 10-16.
 - 106 Gay S, Gay R E. Connective tissue structure and function. In: Wyngaarden J B, Smith L H Jr, Bennett J C, eds. *Cecil textbook of medicine*. 19th ed. Philadelphia: Saunders, 1992: 1491-6.
 - 107 Werb Z, Tremble P M, Behrendtsen O, Crowley E, Damsky C H. Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J Cell Biol* 1989; 109: 877-89.
 - 108 Gay S, Tanaka A, Tarkowski A, Gay R E, Fassbender H G. Expression of oncogenes ras and myc in proliferating synovial lining cells in rheumatoid arthritis. *Arthritis Rheum* 1988; 31: R40.
 - 109 Ziegler B, Huang G-Q, Gay R E, Fassbender H G, Gay S. Immunohistological localization of HTLV-I p19- and p24-related antigens in synovial joints of patients with rheumatoid arthritis. *Am J Pathol* 1989; 135: 1-5.
 - 110 Stransky G, Moreland L W, Gay R E, Gay S. Virus-like particles (VLP) in synovial fluids from patients with rheumatoid arthritis (RA). *Arthritis Rheum* 1990; 33: S143.
 - 111 Larsson E, Kato N, Cohen M. Human endogenous proviruses. *Curr Top Microbiol Immunol* 1989; 148: 115-32.
 - 112 Aicher W K, Gay R E, Stransky G, et al. Detection of Z-225 message in a cDNA library and synovial lining cells of patients with rheumatoid arthritis. *Arthritis Rheum* 1991; 34: D68.
 - 113 Wright J J, Gunter K C, Mitsuya H, Irving S G, Kelly K, Siebenlist U. Expression of a zinc finger gene in HTLV-I- and HTLV-II-transformed cells. *Science* 1990; 248: 588-91.
 - 114 Aicher W K, Stransky G, Gay R E, Gay S. Synovial lining cells derived from patients with rheumatoid arthritis (RA) produce reverse transcriptase. *Arthritis Rheum* 1991; 34(suppl): 177.
 - 115 Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 1991; 253: 1026-8.
 - 116 Fujii M, Sassone-Corsi P, Verma I M. c-fos promoter trans-activation by the tax₁ protein of human T-cell leukemia virus type I. *Proc Natl Acad Sci USA* 1988; 85: 3526-30.
 - 117 Nimer S D, Gasson J C, Hu K, et al. Activation of the GM-CSF promoter by HTLV-I and -II tax proteins. *Oncogene* 1989; 4: 671-7.
 - 118 Ratner L. Regulation of expression of the c-sis proto-oncogene. *Nucleic Acids Res* 1989; 17: 4101-5.
 - 119 Hirayama M, Miyadai T, Yokochi T, et al. Infection of human T-lymphotropic virus type I to astrocytes in vitro with induction of the class II major histocompatibility complex. *Neurosci Lett* 1988; 92: 34-9.