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Why Do We Need New Treatments for Rheumatoid Arthritis?*

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Abstract

Rheumatoid arthritis is a chronic systemic autoimmune inflammatory disease characterized by progressive joint damage. The classical treatments of the disease such as myocrisin and sulphasalazine, are not always effective at controlling the disease. This has necessitated the development of novel agents for treating rheumatoid arthritis. Most of these drugs are biological in nature and are targeted at specific sites of the inflammatory cascade of reactions. A number of clinical trials have been conducted.

The clinical effects that have been observed are transient, necessitating repeated treatments and the risk of vaccination effects. Many of these agents have to be administered parenterally, production costs are very high. Consequently, chemical entities which can be taken orally need to be developed. Since the immune system is very complex with pleiotropic cytokines and redundancy in some of the regulatory networks, it may therefore be necessary to use multiple agents targeted at different specific sites of the inflammatory cascade or that different agents could be given at different stages of the disease, to induce disease remission and maintain the response to therapy. Cytokines such as tumour necrosis factor (TNF) and interleukin 1 (IL-1) play important physiological roles in the host's defence systems against infections and malignancy. The chronic inhibition of these cytokines by targeted therapies may therefore lead to the development of side effects. Thus, carefully controlled long-term studies will be required to assess the safety of selective targeting of processes involved in inflammation.

A more recent novel approach is to target hypoxic tissues with bioreductive agents. Thus, some of the established rheumatoid arthritis treatments could be linked to bioreductive agents and released in hypoxic tissues where inflammation is occurring. This review summarizes the important developments in the therapy of rheumatoid arthritis. There is no doubt that despite these developments we need to develop new and advanced treatment modalities for rheumatoid arthritis.

Rheumatoid arthritis is a chronic systemic inflammatory disorder characterized largely by predominant joint involvement. The incidence of the disease is two to three times greater in women than in men. Rheumatoid arthritis affects about 0·3 to 1·5% of the population worldwide. The joint inflammation often exhibits a remitting course in 90% of patients seen by rheumatologists but joint destruction and deformity, with variable degrees of incapacitation, ensue. In clinical practice, three forms of rheumatoid arthritis can be identified. In the first, patients have mild, self-limiting disease that usually resolves within one year. In the second, patients have mildly progressive disease that responds to conventional treatments with near normal clinical examination. In the third, the disease is more aggressive, difficult to control with drug treatment, and radiological deterioration develops with functional decline. Thus, rheumatoid arthritis has a significant long-term morbidity and is associated with early mortality. Extra-articular manifestations including vasculitis, ocular disease and alveolitis are an integral part of the disease and underline the systemic nature of the condition. Despite many years of intensive research, the cause of rheumatoid arthritis remains unknown. How-

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ever, recent progress in determining the underlying pathophysiological processes involved in the disease has resulted in new therapeutic avenues being opened. The current concept of the pathophysiology of rheumatoid arthritis is that inflammation and tissue destruction result from an unregulated cascade of complex cell-to-cell interactions initiated by macrophage activation induced by some unknown inflammatory trigger(s) (Chikanza 1996). These physiological events of acute inflammation are the same as those seen during inflammation induced by a host of other known inflammatory triggers (Chikanza 1996). T cells become activated upon engagement with antigen presenting cells (for example, macrophages) in association with HLA-DR glycoproteins and accessory molecules (Chikanza 1996). The activated macrophages produce the pro-inflammatory and pleotropic cytokines IL-1β and TNFα together with other cytokines (Akira et al 1990). TNFα is synthesized as a membrane-associated 26,000 molecular weight precursor (pro-TNFα) which is then proteolytically cleaved to release a mature soluble 17,000 molecular weight protein by a TNFα convertase enzyme (Kim et al 1993). TNFα acts in an autocrine and paracrine manner. IL-1β is also produced as an inactive 35 kDa cytosolic pro-IL-1β, which is post-translationally modified by an IL-1 converting enzyme (ICE) to the biologically active 17 kDa molecule that is secreted (Herzyk et al 1992; Wilson et al 1994). Second or co-stimulatory signals are required for maximal T cell activation and these include IL-1β, or other adhesion ligand pairs such as B7·1/B7·2, on macrophages and CD28 or CTLA-4 on T cells (Linsley et al 1992a). CTLA-4 is a homologue of CD28, which binds avidly to B7·1 and B7·2 (Figure 1). IL-1β and TNFα act via an autocrine and paracrine manner by binding to IL-1 and TNF receptors, respectively, which each exist as type 1 and 2, and are shed by inflammatory cells as soluble receptors (sIL-1R1, sIL-1R2, TNF-sR1 {p55},and TNF-sR2 {p75}, respectively). The shed soluble receptors act as negative regulators by inhibiting the effects of their respective cytokines (Seckinger et al 1990; Colotta et al 1993). A second wave of cytokine release mechanisms is also initiated by IL-1β and TNFα leading to the production of a variety of metalloproteinases such as collagenase, gelatinase and stromelysin, which in rheumatoid arthritis degrade cartilage and tissue matrix, contributing to the development of erosions (Mackay & Imhof 1993). The endothelium is activated and begins to express a complex array of adhesion molecules that facilitate the targeting of inflammatory cells to the inflammatory foci (Hanamaijer et al 1993). Endothelial cells proliferate and chemoattractants such as interleukin-8, platelet activating factor (PAF), C5a and LTB4 attract neutrophils and monocytes into the synovium. T cells and monocytes become fixed in the tissues, while neutrophils pass into the synovial fluid where they become activated. Pro-inflammatory factors such as leukotrienes, PAF, metalloproteinases and reactive oxygen species (ROS) that are involved in nuclear factor kappa B (NF-κB) activation and cytokine gene activation, are produced. The generation of ROS in rheumatoid arthritis joints is facilitated by the raised intra-articular pressure and associated hypoxia and also by the activation of inducible nitric oxide synthase (iNOS), which leads to the production of nitric oxide (Stevens et al 1991; Anggard 1994). In the rheumatoid synovium, fibroblast-like synoviocytes (FLS) respond to the hypoxic and pro-inflammatory synovial environment and evolve to cells that demonstrate anchorage-independent growth, oncogene expression and loss of contact inhibition (Firestein 1996). These cells, which predominate in the rheumatoid arthritis pannus, also secrete inflammatory cytokines and metalloproteinases and exhibit autonomous invasive behaviour (Firestein 1996) (Figure 1).

IL-1β and TNFα stimulate the hepatic acute phase response as well as the production of a host of pro-inflammatory neuroendocrine factors such as corticotrophin release hormone, prolactin and substance P; these augment the local acute inflammatory response, while cortisol produced by the adrenal glands dampens inflammation (Chikanza & Grossman 1996). The acute inflammatory reaction is negatively regulated by the release of interleukin-1 receptor antagonist (IL-1Ra) (which inhibits the binding of IL-1 to its receptors), soluble IL-1 receptors type 1 and 2 (sIL-1R1 and sIL-1R2), TNF soluble receptors type 1 and 2 (TNF-sR1 and TNF-sR2) (released by inflammatory cells), interleukin-10 and interleukin-4. In rheumatoid arthritis patients, the production of IL-1Ra is deficient (Firestein et al 1994; Chikanza et al 1995) while the levels of circulating cortisol are inappropriately down-regulated for the degree of ongoing inflammation (Chikanza 1996). Prolactin production is up-regulated while the cytokines TNFα and IL-1β are present in excessive and unbalanced amounts thus creating a mainly pro-inflammatory cytokine and hormonal milieu (Chikanza 1996). As a consequence, acute inflammation is not properly restrained and regulated, and inflammatory processes continue within a background of local and systemic physiological perturbations that lead to the development of chronic inflammatory disease. Recent studies have shown that TNFα and IL-1β appear to play central roles in perpetuating chronic

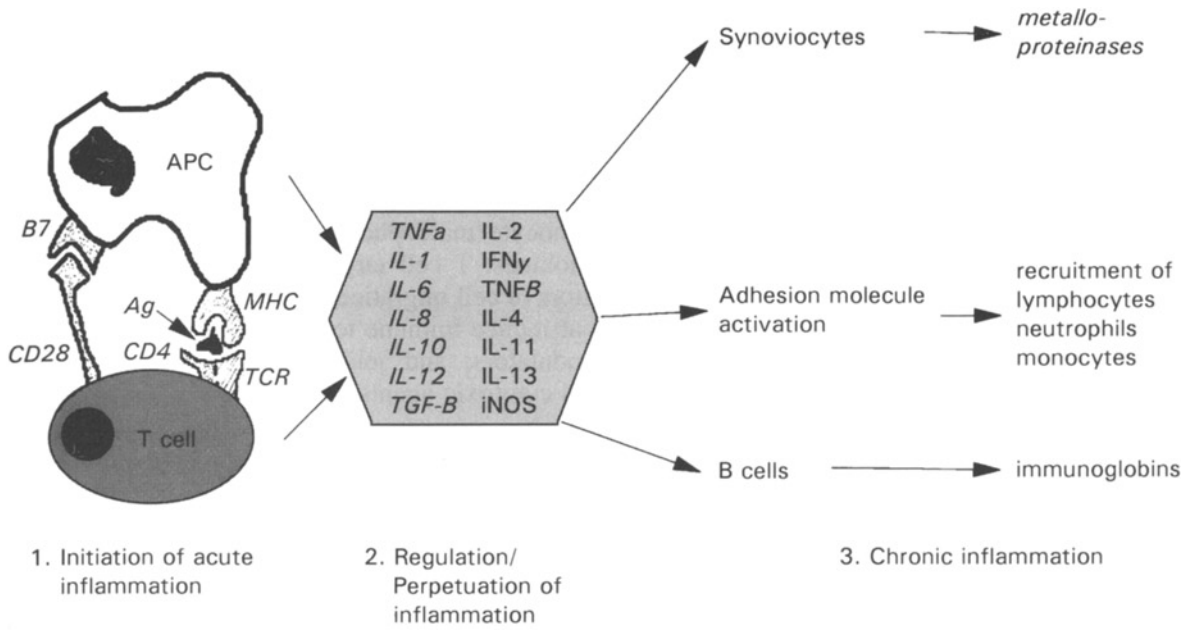

Figure 1. The inflammatory cells that are implicated in rheumatoid inflammation. APC, antigen presenting cells; Ag, antigen; MHC, major histocompatibility complex; TCR, T-cell receptor; iNOS, inducible nitric oxide synthase; TNF, tumour necrosis factor.

inflammation in rheumatoid arthritis and initiate signal transduction pathways that involve NF-κB and cFos/cJun complex (AP1) activation (Arend & Dayer 1995). On the other hand, the demonstration of altered microvascular dynamics within the joint synovium, synovial hypoxia and raised circulating NADH oxidase activity in rheumatoid arthritis patients (Stevens et al 1991; Edmonds et al 1993) also promotes the generation of ROS and repeated cycles of ischaemic reperfusion injury in the joints. Whether the raised circulating levels of arginine vasopressin seen in rheumatoid arthritis (Chikanza 1996) contribute to the altered microvascular dynamics associated with the disease remains to be determined.

The delineation of these processes of inflammation and the discovery of abnormal cytokine responses, raised intra-articular joint pressure, synovial hypoxia and neuroendocrine defects is now providing the scientific rationale for therapeutic intervention with biological, chemical and hormonal entities targeted at specific sites of the inflammatory cascade aimed at modulating inflammation. A number of the agents that have been developed for the treatment of rheumatoid arthritis are biological in nature, while others are chemical entities.

Existing Treatments for Rheumatoid Arthritis

None of the existing treatments can be considered to be curative or definitive therapies for the disease despite the demonstration of superior efficacy compared to placebo. The drugs that are currently available for rheumatoid arthritis (disease modifying antirheumatic drugs {DMARDs}) were developed empirically without much consideration for the basic physiological processes involved in inflammation. Their modes of action appear to be generalized in nature and doubt has been cast on their ability to influence the long-term course of the disease. Furthermore, long-term studies have shown significant morbidity and mortality in up to 90% of treated rheumatoid arthritis patients (Pincus 1992). Thus, a revised terminology for DMARDs has been suggested: SMARD (symptom modifying antirheumatic drug) and DCART (disease control antirheumatic drug therapy) (Boers et al 1994). As yet, no drug fulfils the criteria for the latter category. The term SMARD will be used instead of DMARD in this review. However, this classification does not take account of other treatments (such as NSAIDs), the mode of action, or the rapidity of the onset of action of antirheumatic drugs.

A classification based on a clear understanding of what we know about the physiology of inflammation and immune responses might be more appropriate. Thus it is suggested that antirheumatic drugs might be classified as follows.

Firstly antirheumatic agents could be classified according to speed of onset of action, for example, short-acting drugs (such as corticosteroids, NSAIDs or selective Cox 2 inhibitors) or delayed-action drugs (such as DMARDs).

The second group of drugs would be those that target the immune or inflammatory response: including those that target the innate response (such as NSAIDs, selective Cox 2 inhibitors or LTB4 inhibitors); and those that target the adaptive response (such as DMARDs, corticosteroids or biological agents).

The third category comprises drugs that are targeted to the joint and includes drugs that inhibit adhesion molecules and gene therapy; this category also includes targeted bioreductive agents. Since the adaptive and innate mechanisms are physiological responses to inflammatory or immune perturbations, therapies that are directed against these pathways are most likely to induce toxicity as they have a non-specific approach.

The safety and efficacy of SMARDs can be assessed in part by the length of time that patients stay on the given drug using life tables (Wolfe et al 1991). The median drug survival times for studies lasting more than two years were: azathioprine 2·27; sodium aurothiomalate 1·4; auranofin 1·16; sulphasalazine 1·1; D-penicillamine 1·42; hydroxychloroquine 1·59; methotrexate generally had the longest drug survival time of about 5·5 years. These studies have highlighted doubt about the usefulness of continuing treatment with current SMARDs beyond three years (Wolfe et al 1991). The 'specific' T cell drug cyclosporin A has been shown to be as effective in rheumatoid arthritis as the existing SMARDs, however it is associated with significant renal toxicity. Its mechanism of action might be attributed in part to its anti-pro-inflammatory hormonal effects mediated via the inhibition of the binding of prolactin to its receptors and enhancement of dehydroepiandrosterone and testosterone production. Combination therapy using two or more SMARDs together, has been tested in at least 30 trials with variable results. Recent meta-analysis of the controlled trials conducted to date has failed to demonstrate a clear advantage of the two-drug combination therapy (Felston et al 1994). It is therefore essential that more effective agents are developed that are more than just SMARDs but are of the DCART variety. Research into the events involved in inflammation suggests that for these agents to be effective, they would need to be targeted at specific sites of the inflammatory cascade. Inhibition of TNFα and IL-1β have become important therapeutic strategies for rheumatoid arthritis, while the use of bioreductive agents and hormonal manipulation also appear to be innovative and exciting therapeutic strategies.

Classification of Novel Therapies for Rheumatoid Arthritis

The potentially novel SMARDs and DCARTs that are targeted at specific sites of the inflammatory cascade in rheumatoid arthritis can be broadly divided into the following categories: inhibitors of monocyte/macrophage derived pro-inflammatory cytokines; T cell targeted biological agents; inhibitors of cell migration and ROS generation; agents that induce immune tolerance; hormonal immunomodulators; and joint-directed anti-inflammatory and cytotoxic agents.

T Cell Targeted Strategies

T cells have previously been thought to play a pivotal role in the pathogenesis of rheumatoid arthritis. Hence a number of biological agents that inhibit T cell function have been developed and assessed in patients with rheumatoid arthritis (Table 1). Although efficacy was demonstrated by the majority of these agents in open-label studies, double-blind studies have yielded less than encouraging results.

Inhibitors of Monocyte/Macrophage Derived Pro-inflammatory Cytokines

The precise functions of all the cytokines involved in the inflammatory cascade of reactions still have to be fully delineated. However, studies suggest that TNFα and IL-1β have a pivotal role in rheumatoid inflammation thus indicating that the monocyte/macrophage cells are pivotal in this disease process rather than the T cells (Brennan et al 1989; Elliot et al 1993, 1994a). To date, the area of drug development that has proved most promising has been the targeting of monocyte/macrophage associated pro-inflammatory mechanisms. The biological approaches focusing on inhibiting pro-inflammatory cytokines include: neutralizing anti-cytokine monoclonal antibodies; soluble cytokine receptors; cytokine receptor antagonists, cytokine fusion or cytokine receptor fusion constructs; inhibition of the post-translation and release of IL-1β and TNFα; and the use of counter-regulatory cytokines such as IL-10 and IL-4.

A number of these approaches have been developed and are being tested in clinical trials on patients with rheumatoid arthritis.

Monoclonal antibodies to TNFα

Neutralizing chimeric monoclonal antibodies to TNFα cA2 have provided the first evidence of the

Table 1. The list of novel T cell targeted biological agents studied to date in clinical trials in patients with rheumatoid arthritis.

Biological agent	Cell target epitope	Double-blind study result
Murine anti-CD7 mAb	CD7	poor open study results
Chimaeric anti-CD7 mAb	CD7	poor clinical benefit
Murine anti-CD4 mAb	CD4	too immunogenic
Chimeric (depleting) anti-CD4 mAb	CD4	poor
Humanized (nondepleting) anti-CD4 mAb	CD4	no significant changes
Primatized anti-CD4 mAb	CD4	some clinical response
CAMPATH-1H mAb	CD52	poor results even in open study
Murine anti-CD5 ricin toxin	CD5	no clinical benefit
Anti-IL-2 mAb	CD25	modest in 3 patients
DAB_{486}IL-2, DAB_{389}IL-2 fusion proteins	CD25	modest in open study
Autologous T cell vaccination	arthritogenic T cells	poor result
MHC antagonists	DR4/DR1 or HLA-DR	encouraging open label study

mAb = monoclonal antibody.

beneficial effect in rheumatoid arthritis of targeted therapy in an open study (Elliot et al 1993). The successful results seen in this open study were subsequently confirmed by a multicentre, double-blind study (Elliot et al 1994a). The inhibition of TNFα had a significant but short-lived anti-inflammatory effect. Repeated treatments with cA2 induced suppression of disease activity in eight patients. Four patients withdrew due to side effects, which included: urticaria, vasovagal attack, development of SLE-associated antibodies following cycle 2, and chronic sinusitis (Elliot et al 1994b). The magnitude of the response was maintained by each cycle of treatment with reductions in the number of active joints and c-reactive protein (CRP) of 70% and 85%, respectively. Serum levels of soluble E-selectin fell significantly in a dose-response manner, while the synovial expression of E-selectin also decreased (Paleolog et al 1995). To date, about 150 patients have been studied. Two patients have developed non-Hodgkin's lymphoma and the occurrence of IgM anti-dsDNA antibodies and pleuritis has been noted.

Humanized anti-TNFα monoclonal antibody CDP-571 also induces significant clinical improvements lasting between 4 to 8 weeks when given as a single infusion (Rankin et al 1994). The levels of IL-6 and stromelysin in plasma fell in parallel with the acute phase reactants (Rankin et al 1995). Five patients developed anti-cardiolipin antibodies, one anti-dsDNA antibodies and two positive anti-nuclear factor (ANA) tests with repeated dosing (total of four doses). The usefulness of this approach may be limited by the development of human antibodies to cA2 and CDP-571 with repeated treatments as well as the development of a lupus-type autoimmune phenomenon.

Soluble tumour necrosis factor receptor treatment

Inflammatory cells shed TNFα receptors type 1 p60 (p55 {TNF-sR1}) and type 2 p80 (p75 {TNF-sR2}) as soluble receptors. These act as natural inhibitors of TNFα. There is an imbalance in the synovial fluid levels of TNF-sR with respect to TNFα in patients with rheumatoid arthritis suggesting that therapy with TNF-sR1 and TNF-sR2 could have beneficial effects. Moreland et al (1996) conducted a phase I dose escalation study to evaluate the safety and efficacy of p80 TNF-sR2 in the treatment of rheumatoid arthritis. Sixteen patients were treated for four weeks with recombinant human TNF-sR2 (Moreland et al 1996). Modest clinical effects were observed, probably due to the short half life of TNF-sR2. The use of TNF-sR2:Fc protein construct (rhTNFR:Fc) has demonstrated significant improvements in both clinical and laboratory parameters (Hasler et al 1996; Sander et al 1996). No serious side-effects were observed. TNF-sR1:Fc is also undergoing clinical trials for rheumatoid arthritis. The outcome of long term studies is awaited. The efficacy of TNF-sR can also be enhanced by fusing it to polyethylene glycol (PEG) to form a dimer. Both TNF-sR1:PEG and TNF-sR2:PEG have been developed and shown to be effective in streptococcal cell wall arthritis in Lewis rats. They are now being tested as potential treatments for rheumatoid arthritis.

Chemical inhibition of TNFα production

The production costs for biological agents are high and most patients require repeated parenteral treatments. Most patients develop human antibodies to the agents leading to tachyphylaxis with diminished clinical efficacy following repeated treatments. Thus, there is a need to develop chemical entities that can be taken orally and that are

economical to produce. A number of chemical TNFα-targeted strategies are under development. The first relates to TNFα convertase inhibitors that inhibit the post-translation of TNFα and its release from the cells (Gearing et al 1994). These agents have been shown to be effective in animal models of arthritis. The second are phosphodiesterase type 4 inhibitors, following the observation that pentoxifylline, a non-specific phosphodiesterase (PDE) inhibitor, inhibits the release of TNFα in-vitro by increasing intracellular levels of cAMP (Thiel 1991). An open study of pentoxifylline in 14 patients with refractory severe rheumatoid arthritis showed a significant improvement (according to modified Paulus criteria) in 50% of patients (Maksymowych et al 1995) and this drug appeared to have a significant steroid-sparing effect in two patients. Phosphodiesterase isoenzymes (at least seven) have been identified and their substrate specificity and regulation are now characterized (Beavo & Reisfeld 1990). Phosphodiesterases are tissue specific and, PDE isoform specific drugs have been developed. PDE type 4 (PDE4) is present in human lymphoid and myeloid cells and also in the central nervous system tissue in low concentrations (Beavo & Reisfeld 1990). Inhibition of PDE4 exhibits several anti-inflammatory properties, which include inhibition of TNFα and IL-6 release (Semmler et al 1993), suppression of neutrophil adhesion and peroxide-induced injury of the endothelium (Suttorp et al 1993). By contrast IL-10 production is up-regulated. The development of experimental allergic encephalomyelitis in rats is also inhibited by PDE4 inhibitors (Sommer et al 1995). These observations suggest that these highly specific PDE4 inhibitors could be a potential therapy for rheumatoid arthritis. Although most of these drugs have been developed with asthma as the main indication, studies in asthma have been disappointing. We have recently evaluated the PDE4 inhibitor RP73401 developed by Rhone-Poulenc Rorer, in rheumatoid arthritis patients in a double-blind placebo-controlled dose-escalation study (Chikanza et al 1996). Although the changes in clinical or laboratory parameters of disease activity did not reach statistical significance, there was a trend towards improvement. RP73401 was able to inhibit the increase in CRP and IL-6 in a dose-dependent manner.

Soluble interleukin-1 receptor type 1 (sIL-1R1) treatment

The extra-cellular portions of IL-1R1 and IL-1R2 are shed by inflammatory cells as soluble receptors (sIL-1R1 and sIL-1R2) and these bind circulating IL-1β preventing it from exerting its effects on cells. They therefore function like antibodies to IL-1. This suggests that sIL-1R infusions may be an effective therapy for rheumatoid arthritis patients. A double-blind safety and efficacy study of recombinant human sIL-1R1 has been carried out in 23 patients (Drevlow et al 1996). No clinical benefits were observed and patients receiving the highest dose developed skin rashes, thus limiting the use of higher doses.

These observations may be related to the following considerations: firstly, rhIL-1R1 may diminish the IL-1-mediated enhancement of cortisol production that occurs as part of the negative feedback physiological response to inflammation (Besedovsky et al 1986); secondly sIL-1R1 binds more avidly and irreversibly to IL-1Ra than to IL-1β and IL-1α (Arend et al 1994; Svenson et al 1993) and thus its administration may worsen the deficient IL-1Ra levels shifting the balance towards more excess IL-1β. IL-1Ra and cortisol production are deficient in rheumatoid arthritis patients. Thus, shIL-1R1 treatment of rheumatoid arthritis may potentially worsen the disease activity if given in much higher doses than used in this study. This conjecture is supported by the observations that, in rabbits, immunoneutralization of IL-1Ra exacerbates colitis and, in patients, one out 12 patients who received intra-articular rhIL-1R1 developed worsening synovitis whereas none in the placebo group did (Pope et al 1997). IL-1R2 does not participate in signal transduction and binds more avidly to IL-1β and IL-1α than to IL-1Ra (Sadouk et al 1994). It therefore functions as a decoy molecule for IL-1 and may be a more appropriate biological trial agent in rheumatoid arthritis.

Interleukin-1 receptor antagonist treatment

IL-1Ra is a naturally occurring inhibitor of IL-1β and IL-1α that binds more avidly to IL-1R1 than to IL-1R2: but it is a pure antagonist (Arend & Dayer 1990). During active inflammation there is often a 50 to 100 fold excess of IL-1Ra over IL-1β (Chikanza et al 1995). The circulating levels of IL-1Ra in rheumatoid arthritis patients are deficient in relation to those of IL-1β (Chikanza et al 1995), thus increasing therapeutic options.

Phase 1 studies in which recombinant IL-1Ra was given subcutaneously daily for 28 days have shown that this approach is effective (Lebsack et al 1991). Significant improvements in disease activity were also seen in a dose-escalation, phase 2, double-blind, multicentre study of 175 rheumatoid arthritis patients (Lebsack et al 1993). Side-effects such as mild skin reactions at the injection sites and

soft tissue infections at distant sites were seen in a few patients. A much larger multicentre trial of 475 patients treated for 6 months has also confirmed these observations (Bresnihan et al 1996).

Another way of correcting the deficient production of IL-1Ra relative to IL-1β would be to enhance the endogenous production of the protein in sites of inflammation using gene therapy. This concept has been tried in experimental animal models of arthritis (Roessler et al 1993; Hartman et al 1993). The direct inoculation of cDNA for IL-Ra placed in an adenoviral vector into the joint, induced an increased production of IL-1Ra by macrophages and synovial fibroblasts. In the indirect approach, rabbit isolated synovial cells were infected with retroviral vector carrying IL-1Ra cDNA ex vivo. The infected cells were re-injected into the joints and they successfully colonized the joints and produced biologically active IL-1Ra for a period of up to five weeks. This locally produced IL-1Ra inhibited the inflammatory responses induced by IL-1β injected into the joints (Bandara et al 1993). Gene therapy still has its problems, including infection with vector. Nevertheless, this approach is a novel and exciting development for the therapy of rheumatoid arthritis.

Inhibition of Interleukin-1 converting enzyme

Interleukin-1 converting enzyme (ICE) is required for the post-translational processing of the immature 35 kDa IL-1β to generate the biologically active 17 kDa form of IL-1β that is secreted (Herzyk et al 1992; Wilson et al 1994). ICE has therefore become a potential therapeutic target. A number of companies are developing ICE inhibitors. More recently, two ICE inhibitors designated VE-13,045 and VE-16,084 have been shown to inhibit in vitro the production of IL-1β by lipopolysaccharide (LPS) stimulated human and murine splenic monocytes while in vivo, they inhibited the LPS induced increase in serum IL-1β and IL-1α in mice by 25–80%. VE-13,045 reduced the onset of type 2 collagen-induced arthritis inflammation and the overall disease activity severity by 60% (Harding et al 1995). This approach is therefore also promising and clinical trials using suitable compounds are awaited.

Interleukin-10 as a therapy for rheumatoid arthritis

IL-10 can inhibit the secretion of both IL-1β and TNFα in-vitro and in-vivo as well as increasing the production of natural cytokine inhibitors (Isomaki et al 1996). IL-10-deficient mice develop chronic enterocolitis, while immunoneutralization of IL-10 results in severe collagen-induced arthritis. IL-10 has been used to suppress the induction of experimental allergic encephalitis in Lewis rats and administration of IL-10 had a beneficial effect on murine streptococcal cell wall arthritis (Joosten et al 1995). Of particular interest was the observation that the expression of IL-1Ra mRNA was up-regulated in the joints of treated animals. In-vitro, IL-10 also inhibits metalloproteinase secretion while stimulating tissue metalloproteinase inhibitor production by human monocytes (Joosten et al 1995). In-vitro, IL-10 up-regulates IL-1Ra and TNFsR1 and TNFsR2 production (Kuhlmann et al 1994). A recent study has shown that IL-10 can be safely administered in normal human volunteers without side-effects. Although high levels of IL-10 are found in the synovial fluid from rheumatoid arthritis patients, these do not appear to be sufficient to exert clinically relevant anti-inflammatory effects. Thus, IL-10 treatment could be of potential benefit for the treatment of rheumatoid arthritis. Clinical studies in rheumatoid arthritis are in progress. Other anti-inflammatory cytokines being considered for rheumatoid arthritis therapy include IL-4 and IL-13. These cytokines exert similar effects to those of IL-10 in-vitro. Although interferon-γ has immunomodulatory effects, studies in rheumatoid arthritis have not shown it to be effective clinically.

Joint-directed Therapeutic Strategies

Inhibition of adhesion molecules

Anti-ICAM-1 monoclonal antibody treatment. Leucocyte adherence is a necessary prerequisite for inflammatory cell homing and migration into inflammatory joints and other foci. Of the diverse repertoire of adhesion molecules, the ligand pair LFA-1 (CD11a/CD18) expressed on leucocytes and ICAM-1 (CD54) expressed on the endothelial cells, plays a central role in adhesive interactions that mediate transendothelial migration of T cells. Hence, inhibiting adhesion molecules is another form of targeted therapy for rheumatoid arthritis. Murine anti-ICAM-1 (CD54) monoclonal antibodies have been shown to be effective in 32 patients with refractory rheumatoid arthritis in an open-label, dose-escalation study (Kavanaugh et al 1994a). This treatment induced a peripheral CD4 lymphocytosis while some patients developed transient cutaneous anergy. Another open study of ten patients with early rheumatoid arthritis demonstrated similar findings (Kavanaugh et al 1994b). Patients who received a second infusion developed serum sickness including urticaria, petechial rashes, myalgia and angiodema (Kavanaugh et al 1994c). This observation might be

related to the nature of the monoclonal antibodies but nevertheless this approach should be re-tested again using a humanised anti-ICAM-1 monoclonal antibody.

Gene therapy. A gene therapeutic approach is being explored by ISIS Pharmaceuticals, the company has developed an anti-sense inhibitor of ICAM-1 called ISIS-2302 as a treatment for inflammatory disorders including rheumatoid arthritis. Phase 1 trials in normal volunteers have been completed and phase 2 studies to establish proof of concept have been initiated and results are pending. IL-1Ra gene therapy is discussed above.

Antioxidants, free-radical scavengers and nitric oxide production inhibitors

A number of antioxidants, free-radical scavengers and iNOS inhibitors aimed at inhibiting the generation of ROS are under development. Interest in these compounds for the therapy of rheumatoid arthritis comes from the observations that ischaemic reperfusion injury plays a role, in part, in perpetuating chronic joint inflammation (Edmonds et al 1993) and also the observation that nitric oxide plays a role in the immunopathology of adjuvant arthritis. The inhibition of nitric oxide production by iNOS inhibitors ameliorates adjuvant arthritis in Sprague-Dawley rats and antigen induced arthritis in rabbits (Santos et al 1995). These compounds could thus prove to be beneficial in rheumatoid arthritis. The antioxidant drug CV-3611 (Takeda Chemical Industries Ltd) is now in phase 3 clinical trials for the treatment of inflammation and the results of these studies are awaited.

The development of targeted bioreductive drugs that would home in on the hypoxic inflammatory synovial pannus is an interesting concept. The inflamed rheumatoid synovium is hypoxic (Edmonds et al 1993). Tissue hypoxia is associated with enhanced reductive metabolism. Thus, regions of hypoxia can be targeted by compounds that are selectively activated by reductive metabolism. Two approaches may be adopted. Bioreductive cytotoxic drugs can be activated to effect chemical synovectomy, or non-toxic delivery systems may be used to target known anti-rheumatic drugs to the inflamed synovium.

Bioreductive cytotoxic agents. Bioreductive cytotoxic agents require metabolic reduction to generate cytotoxic metabolites. The initial one electron reduction step is reversible by oxygen, thus further reduction to cytotoxic species occurs preferentially under hypoxic conditions. Consequently, hypoxia enhances bioreductive targeting of drugs. In this respect, metronidazole and the anti-cancer agent mitomycin C represent the first generation of targeted bioreductives. The efficacy of metronidazole in rheumatoid arthritis was first demonstrated in 1964 and since then there have been a number of studies (Pybus 1964). Its use is limited by associated nausea, cytokine release syndrome and neuropathy. In the past two decades, research into bioreductive therapy has increased our understanding of the intricacies associated with this approach. Metronidazole has a low one electron reduction potential, which makes it particularly difficult for human enzyme complement to activate it. Its effectiveness as an antibiotic is reflected by its comparative reduction by anaerobic bacteria. Based upon the reductase enzyme in the rheumatoid arthritis synovium, a more moderate one electron reduction potential is far more preferable. Thus, a thorough investigation of the therapeutic profile of alternative nitroimidazoles that have this range of reduction potential is warranted. Suitable candidate drugs are available from both the established pool and those that have been developed recently within the laboratory.

Bioreductives for targeting other drugs. A number of the currently available drugs are toxic. Toxicity could be reduced by delivering the drugs to the sites of inflammation. An optimal approach to such drug targeting could be achieved by taking advantage of the hypoxic milieu of the rheumatoid arthritis joint synovium. By coupling an established anti-rheumatic drug to a bioreductive agent, it may be possible to selectively target the inflamed hypoxic rheumatoid arthritis synovium. For this to be an effective inert delivery system, the bioreductive moiety should not be activated to a cytotoxic. In practice, steroid-bioreductive conjugates that safely release the steroid in the hypoxic tissues are being developed (Blake et al 1997). The leading compound in this class of delivery agents is a steroid conjugate of an oxidised form of vitamin E. The conjugate has been designed so that reduction causes steroid release and intramolecular cyclisation to generate the parent vitamin E. Thus, both the steroid and an antioxidant are delivered selectively to the rheumatoid joint.

Induction of Immune Tolerance

Immune tolerance can be induced by the ingestion of a given antigen or by the induction of T cell anergy by interfering with the macrophage/monocyte T cell interactions during antigen presentation. The rationale for oral tolerance therapy comes from the observations in animal models of

human disease that the ingestion of a specific antigen can induce peripheral tolerance to it via the induction of Th2 cells with increased IL-4, IL-10 and TGFβ cytokine production. This is exemplified by the observation that administration of collagen type 2, which is restricted to articular cartilage and the eye, reduces the severity of adjuvant arthritis.

CD28-B7 co-stimulatory blockade by CTLA-4Ig
The maximal activation of T cells requires two independent signalling events, one mediated via the T cell receptor engagement by the antigen presenting cell and the second through the cognate interactions with co-stimulatory molecules expressed on T cells and macrophages (antigen presenting cells). The CD28/B7 pathway is crucial for induction of T cell proliferation (Linsley & Ledbetter 1993). Inhibition of this pathway induces T cell anergy and immune tolerance. This pathway can be blocked by CTLA-4Ig, a soluble form of CTLA-4, which binds with high affinity to the CD28 ligands B7·1 and B7·2 (Linsley et al 1992b). CTLA-4Ig treatment in the BB rat prevented clinical and histological manifestations of collagen-induced arthritis (Knoerzer et al 1995). In a mouse model of experimental allergic encephalitis, CTLA-4Ig administration suppressed the disease by a mechanism that involved the inhibition of Th1 (IL-2 and INFγ production) while sparing Th2 (IL-4, IL-10 and IL-13) cytokines (Khoury et al 1995). Th2 cytokines inhibit macrophage production of pro-inflammatory cytokines such as IL-1β and TNFα. These observations suggest that CTLA-4Ig may be a potential therapy for rheumatoid arthritis.

Oral collagen therapy in rheumatoid arthritis
Trentham et al (1993) were the first to demonstrate that ingesting chicken collagen type 2 induces a trend towards improvement in patients with active rheumatoid arthritis. However, a second large double-blind study of 90 patients with early disease using bovine collagen type 2, which has a higher homology with the human type, has demonstrated no significant efficacy of this approach (Sieper et al 1996). Thus, the usefulness of collagen type 2 oral therapy in rheumatoid arthritis remains questionable.

Subreum
The protein extract from the bacterium *E. coli* (subreum) has immunomodulatory properties. In animal models of adjuvant arthritis it ameliorates the severity of the disease and has various effects on cytokine production. Subreum was effective in rheumatoid arthritis in an open trial (Rosenthal & Plattner 1981). Subsequent six- and 12-month placebo-controlled studies have confirmed this observation and shown subreum to be as effective as D-penicillamine (Brackertz & Vischer 1989; Hauzeur & Applebom 1989; Vischer 1988; Verstraenten et al 1990). The optimum dose is approximately 24 mg day^{-1}. Animal models suggest that the mode of action is similar to that of oral collagen (Staines et al 1992). However, its precise role in the drug therapy of rheumatoid arthritis has yet to be established.

Other Cellular Targets

Inhibition of metalloproteinases (MMPs)
MMPs degrade tissue matrix and this contributes to cartilage damage and the development of bone erosions. Thus, the inhibition of MMPs may be of therapeutic potential for rheumatoid arthritis. MMP-targeted strategies under consideration include: exogenous administration of tissue inhibitor of MMP (TIMP) (Carmichael et al 1989), enhancing endogenous production of TIMP by retinoids (Clark et al 1987) and the inhibition of MMPs by synthetic peptides, antibiotics such as tetracyclines and related compounds (Greenwald et al 1987; Tilley et al 1995; Kloppenburg et al 1994). It is interesting to note that minocycline has recently been shown to be effective in rheumatoid arthritis (O'Dell et al 1997). Metalloproteinases are important for the physiological remodelling of tissue matrix. Thus, their inhibition could potentially interfere with blood vessel formation and contribute to tissue hypoxia and the development of ischaemic reperfusion injury. It is worth noting that therapy with the metalloproteinase inhibitor, marimastat has been associated with the development of tenosynovitis.

Induction of apoptosis
Since the synovium in rheumatoid arthritis is hyperplastic and expresses Fas, another approach could be the induction of apoptosis in the synovial cells and inflammatory cellular infiltrate. In animal models of arthritis, this approach significantly reduces joint inflammation (Williams et al 1994). Apoptosis is induced by intra-articular instillation of anti-Fas monoclonal antibody. Cross-linking of Fas molecules by anti-Fas monoclonal antibody or ligands induces apoptosis.

Hormonal Immunomodulation

The neuroendocrine defects described to date in rheumatoid arthritis include: inappropriately down-regulated levels of cortisol for the degree of joint

inflammation; high levels of circulating prolactin; deficient hormonal levels of androgen; increased local production of corticotrophic releasing hormone (CRH) in joints where it is pro-inflammatory within the joint micro-environment. Since androgens and cortisol are anti-inflammatory while prolactin is pro-inflammatory, the pro-inflammatory hormonal balance in rheumatoid arthritis promotes and contributes to rheumatoid inflammation. Thus, altering this hormonal balance could have major therapeutic effects. This concept has been tested extensively in animal models of autoimmune disease. The inhibition of prolactin and treatment with androgens ameliorates adjuvant arthritis. Treatment with corticosteroids at the onset of arthritis in Lewis rats, abolishes the development of chronic arthritis. Corticosteroids are effective in the treatment of rheumatoid arthritis but cause side-effects with time. However, a recent study has shown the usefulness of low dose corticosteroids. It might be that corticosteroids need to be given in high doses at the onset of disease to prevent the development of the chronic phase of rheumatoid arthritis. Prolactin inhibition (pituitary) is as effective as treatment with D-penicillamine but, interestingly, does not suppress acute phase markers. This observation might be related to the fact that T cells produce prolactin themselves and for this approach to be successful, this source of hormone needs to be inhibited. Variable results have been obtained in studies of rheumatoid arthritis using androgens. These observations highlight the need to look at the hormone profile as a whole and show that targeting just a single neuroendocrine defect may not be the way forward.

Conclusions

There is a need to continue the development of novel drugs for rheumatoid arthritis that are curative in nature i.e. DCARTs. The systematic dissection of events involved in the inflammatory cascade is providing the scientific rationale for specific targeting of individual disease-promoting cytokines and sites of the inflammatory cascade as a means of modulating rheumatoid arthritis. However, one must not loose the foresight that cytokines have important physiological roles in normal homoeostasis and disease states. The selective blockade of disease-causing or potentiating cytokines could therefore induce potentially serious adverse effects. Selecting targeting could be avoided by, for instance, the co-administration of anti-TNF monoclonal antibody with a T cell-directed biological agent such as anti-CD4 monoclonal antibody. This approach has been shown to be beneficial in collagen-induced arthritis and reduced the development of anti-TNF globulin responses (Williams et al 1994) but is as yet to be proven in rheumatoid arthritis. Long-term blockade of TNFα could lead to the development of infections and lupus-type autoimmune phenomenon and possibly malignancy. Already, the long-term use of neutralizing anti-TNFα antibodies has been associated with the development of non-Hodgkin's lymphoma, and anti-dsDNA antibodies with pleuritis. Despite these potential drawbacks, there is no doubt that TNFα blockade has been a major recent development in the treatment of rheumatoid arthritis. It is most likely that PDE4 inhibitors, which inhibit TNFα release while enhancing IL-10 and IL-1Ra production, could replace the use of anti-TNF monoclonal antibody and/or IL-1Ra as a treatment of rheumatoid arthritis if they prove to be effective, as they can be taken orally. The use of TNF-sR constructs as treatment for rheumatoid arthritis appears quite promising too. However, the demonstration of the development of antibodies to rhTNFR:Fc could potentially lead to a diminution of efficacy as a consequence of tachyphylaxis with repeated treatments. This could limit the usefulness of this approach. Whether autoimmune phenomenon could develop as has been associated in long-term trials of anti-TNFα antibodies, remains to be determined.

A cogent case could be made for the use of combination targeted biological and chemical agents in rheumatoid arthritis or the addition of a targeted therapeutic agent(s) to traditional SMARD therapy. Different stages of the disease might respond more sensitively to different specific targeted agents. This conjecture is supported by the recent observation that different stages of type 2 collagen-induced arthritis in DBA/1 mice have different TNFα and IL-1 dependencies and that blocking TNFα was effective in the early stages of the disease (Joosten et al 1996). In the latter stages of the disease, blockade of IL-1β and IL-1α with monoclonal antibodies was most effective. Continuous IL-1Ra treatment was necessary to achieve an optimal response. Thus, in conclusion, despite the significant strides that have been made in the therapy of rheumatoid arthritis, there is still a need to develop other exciting novel therapeutic strategies.

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