The cytokine network in rheumatoid arthritis: definition of TNFα as a therapeutic target

Rheumatoid arthritis (RA) is one of the commonest human autoimmune diseases, affecting about 1% of the population in the UK. The autoimmune diseases are a group of chronic diseases in which the immune system, instead of performing its usual function of protecting the host from infectious agents, reacts specifically against some of the host tissues, the 'autoantigens'. This autoimmune response may be either a cell-mediated or a humoral response, or both, and leads to disease by generation of local inflammation and tissue damage. It is not understood why the usual mechanisms maintaining self non-reactivity have broken down in autoimmune diseases. However, the mechanism or 'pathogenesis' of these diseases is becoming understood [1–6].

In this article I will review progress in elucidating important molecular events in the pathogenesis of RA. The work spans over a decade; during this time my group has investigated which of the local protein molecular mediator molecules, now generically termed 'cytokines' were highly expressed in the rheumatoid joints. We found a large collection of pro-inflammatory and anti-inflammatory cytokines in all samples (recently reviewed [7,8]). We then studied the dysregulation of cytokine expression in cultures of RA joint cells in order to define potential targets for therapeutic intervention. This revealed that tumour necrosis factor (TNF)α, a trimeric protein of 51 kd, was the main driving factor in the production of other pro-inflammatory cytokines in the joint cultures [9]. This suggested that TNFα might be a useful therapeutic target [10]. This hypothesis has been successfully tested in animal models of arthritis, and more recently also in patients with long-standing and active RA. It has led to marked changes in our approaches both to the therapy of RA and in our expectations of a successful anti-rheumatic therapy.

The pathogenesis of rheumatoid arthritis

The synovial joints are the major sites of RA. However, in severe cases (ca10%), there is also extra-articular disease, with rheumatoid nodules, vasculitis and pulmonary fibrosis; more rarely (ca 1%) there is hypersplenism leading to low granulocyte and other blood counts (Felty syndrome). While RA is a well recognised cause of chronic pain and disability, it is much less recognised that in severe cases it is also a cause of death [11,12].

In all human autoimmune diseases, there is a clear-cut genetic component, discernible in twin and family studies. Because RA is a late-onset disease which varies in severity, it is unclear what percentage of its aetiology is genetically determined, but 50% is a common estimate based on the rate of concordance in identical twins [13]. Multiple genes are involved, with the HLA region being the most important. The HLA-DR4 (Dw4, 14 and 15 subtypes) and DR1 genes encode what has been termed a 'common epitope' in the β chain, spanning amino acids 70–74 with aminoacids QRRAA (in single letter code) the usual sequence [14]. This sequence is situated on the α helix forming one boundary of the peptide binding groove of HLA-DR. The only known function of DR is to bind peptides and present them to CD4+ T cells, so the DR4/DR1 genetic predisposition implies that immune recognition is important at some critical step in the development of RA [15]. There is now evidence that susceptible DR4 subtypes bind different peptides from those bound by non-susceptible DR4 subtypes [16]. However, it is not known at which step this occurs: it could range from an effect in the thymus on T cell repertoire development to an effect in the periphery in the recognition either of a potential disease-inducing pathogen or of an autoantigen. The last one is the most popular hypothesis.

In RA, as in most other autoimmune diseases, women are affected more often than men [17]. This has led to experimental analysis of the effect of sex steroids in animal models of arthritis which were shown to mediate the sex linkage. However, there is still a large, non-genetic component of the aetiology, and it thus appears likely that in genetically predisposed individuals non-genetically encoded events such as exposure to triggering agents, presumably micro-organisms, are important. The search for the relevant micro-organisms including mycoplasma, mycobacteria, Gram-negative bacteria and retroviruses [18,19] has not been successful, with candidates espoused by some workers, but not confirmed by others.

The cellular composition of the inflamed joints in RA is different from that of normal joints or of joints affected by osteoarthritis (OA). They are full of leukocytes. In the synovial membrane, the most abundant cells are T lymphocytes and macrophages, with fibroblasts, plasma cells, dendritic cells and endothelium

This article is an updated version of the Watson Smith lecture given at the Royal College of Physicians in November 1994 by **Professor Marc Feldmann**, Head of the Cytokine and Immunology Division, Kennedy Institute of Rheumatology, and Professor of Cellular Immunology, Charing Cross and Westminster Medical School. (Manuscript received September 1996.)

also prominent. The localisation of these cells is not random. The synovial lining layer, normally 1–2 cells thick, swells to 6–8 or more cells. These cells are a mixture of activated macrophages and fibroblasts. The lining layer often develops into villi. Deeper in, there is increased vascularity, with follicles of lymphoid cells, chiefly CD4+ T cells and macrophages. Scattered between the follicles are T cells, mostly CD8+, activated fibroblasts and macrophages. Many cells in the synovium express markers of activation [20]. Detailed descriptions of synovium have been published (eg Ref 21).

A major site of damage in the rheumatoid joint is where the inflamed synovium contacts the cartilage, grows over and erodes it, and then erodes into the bone (this is often termed the 'pannus'). Because of the difficulties in studying this site, from which arthroscopic biopsy is not possible, it is less well characterised than the rest of the synovium. Recent studies have suggested that there are two types of pannus:

- an 'active' form, which contains a lot of macrophages and produces a plethora of pro-inflammatory cytokines, including TNFα, and has upregulated the TNF receptors, and
- an 'inactive' form which produces fewer pro-inflammatory mediators which are not detectable by immunostaining, and chiefly produces the anti-inflammatory cytokine, transforming growth factor (TGF)β [22,23].

One of the major hallmarks of activation in RA synovium is the augmented expression of HLA class II antigens, first noted in the early 1980s independently by Janossy's and Klareskog's groups [24,25]. These antigens are constitutively expressed on B lymphocytes and dendritic cells, but after activation they are also expressed on T lymphocytes, macrophages, endothelium, fibroblasts, etc. The most powerful signals that induce class II expression in the latter cells are cytokines such as interferon (IFN)γ [26], granulocyte-macrophage colony-stimulating factor (GM-CSF) [27] and TNFα [28]. The observation that most cells in the RA joint express HLA-DR strongly suggests that some of these cytokines are active at that site, and thus likely to have been produced locally.

The over-expression of HLA-DR in the tissues of local auto-immune sites was also noted in other auto-immune diseases such as Graves' disease [29], Hashimoto's thyroiditis, insulin-dependent diabetes mellitus (IDDM) [30], pernicious anaemia, etc. It led us to publish in 1983 a general concept for the pathogenesis of human autoimmune disease, based initially on our Graves' disease work [1], but applicable to all autoimmune diseases with marked local features. This concept, represented schematically in Figure 1, has been a useful working hypothesis in our work on autoimmunity.

One of the major premises of this scheme is the importance of the antigen presenting cell/T cell interaction in the maintenance of the disease process. It was implicit that autoantigen reactive T cells should be present and activated at sites of autoimmunity; this was duly demonstrated, first in the thyroid infiltrate of Graves' disease patients by my colleague, Dr Marco Londei [31]. Activated T cells express receptors for interleukin-2 (IL-2), the major growth factor for T cells. Hence, by culturing cells extracted from Graves' disease thyroid with IL-2, there was selection for *in vivo* activated T cells. These were cloned in the absence of antigen, using IL-2 and irradiated antigen presenting cells; these clones were then screened for antigen reactivity using autologous thyroid follicular cells. Some of the cloned T cells proliferated specifically to autologous thyroid follicular cells and not to autologous blood or heterologous thyroid. These experiments confirmed a major premise of our hypothesis, namely,

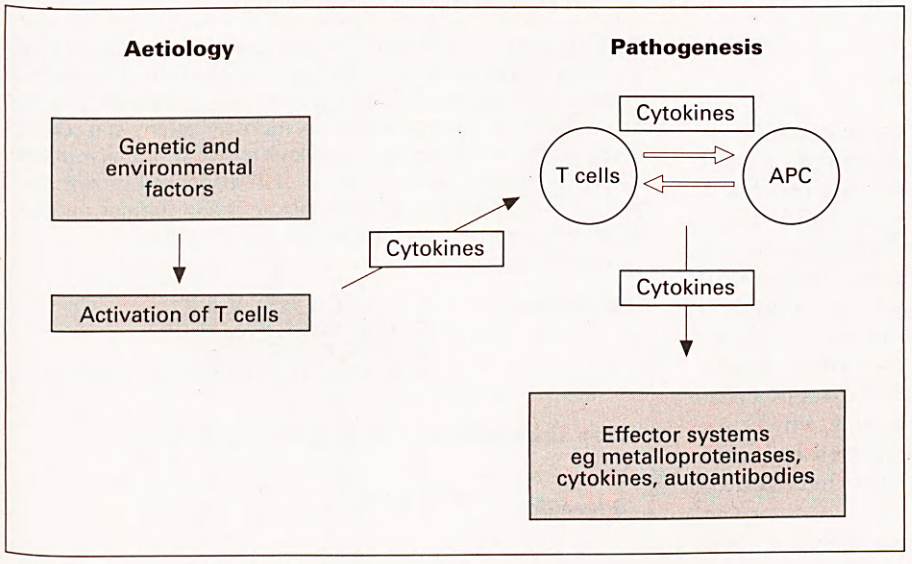


Fig 1. *Schematic representation of the mechanism of autoimmunity* (APC = antigen presenting cell) (scheme redrawn to reflect concepts described in Bottazzo *et al* [1])

that autoimmune sites contain activated autoantigen reactive T cells. Recent work has extended this to define the multiple proteins and epitopes recognised [32–34].

The role of cytokines in the pathogenesis of autoimmune disease is emphasised in the hypothesis illustrated in Fig 1. Cytokines are important at several stages. First, in the initial events, in activating the autoantigen presenting cells as well as the auto-antigen reactive T cells. Evidence to support this concept has accrued in patients where cytokine infusion with IFNα [35] and especially IL-2 [36] has precipitated the onset or relapse of several autoimmune diseases, most commonly thyroiditis. IFNγ precipitated relapses of multiple sclerosis [37]. The most clear-cut evidence came from using transgenic mice in which local IFNγ production has been targeted to the islets of Langerhans by using the insulin promoter [38]. Such mice develop an auto-immune diabetes, complete with autoantigen reactive T cells capable of lysing islet cells both *in vivo* and *in vitro*. Analogous results have recently been reported with IFNγ [39], but not with IL-2 [40] or TNFγ. With the latter two cytokines, other signals such as islet cell-expressed B7-1 (CD80), a potent costimulator of T cells which binds to CD28, were also needed to trigger IDDM [41] (review Ref 42).

Cytokines also have important roles in maintaining the activation of both the T cell and antigen-presenting cell systems. For example, IFNγ from T cells upregulates HLA class II expression and IL-1 and IL-6 derived from antigen-presenting cells help activate T cells. Furthermore, cytokines produced by either T cells or antigen-presenting cells were envisaged as important signals in activating the inflammatory and potentially destructive systems at the sites of the autoimmune process. For all these reasons, we became interested in the role of cytokines during our studies of the pathogenesis of RA. We sought to determine which one(s) were 'dominant' in the pathogenesis, and thus could be a target for therapy.

Cytokines

Over the years, a growing cluster of protein mediator molecules, important in immunity, inflammation, cell growth, differentiation and haemopoiesis has been uncovered. These mediators have been confusingly named, based on the first property reported, as 'interferons', 'interleukins', growth factors', 'cytotoxic factors', etc. There is now a tendency to put together all these proteins with related functions and call them 'cytokines'. They are chiefly local mediators, usually signalling interactions between closely adjacent cells. Cytokines are thus distinct from hormones, which are secreted into the blood stream by specialised cells and destined to work at long distances from their cell of origin.

Cytokines are chiefly produced after cell activation and act at low molarities on target cells expressing receptors of very high affinity. As the cytokine proteins became molecularly defined in the early 1980s by cloning of their complementary DNAs (cDNAs) (eg Refs 43,44), they could be used as new tools to examine the cytokine expression in the small pieces of human tissue available for study derived from autoimmune disease sites. RA was the obvious choice for the work because it was possible to obtain biopsies at the height of the disease process. The role of cytokines in other autoimmune diseases is described in a monograph my colleague, Dr Fionula Brennan, and I recently edited [45].

Cytokine expression in rheumatoid arthritis synovium

In a local disease it can be predicted that the most relevant cytokines would be those actively produced at that site. Local synthesis of cytokines can be measured in various ways. For sensitivity, and especially for specificity, we started to assay for messenger RNA (mRNA), using cDNA probes. Abundant mRNA was found in active RA joint samples for a range of cytokines [7,46,47]. Similar cytokine expression was noted in all active disease samples, regardless of duration of disease or mode of treatment—even in patients treated with corticosteroids which are potentially able to diminish cytokine synthesis (Fig 2).

Using normal lymphoid cell populations from mouse spleen or human blood mononuclear cells, cytokine expression after antigen or mitogen activation is usually transient, lasting for one or at most a few days after the activating stimulus. However, the regular presence of cytokines (eg IL-1, TNFα, IL-6) in all our RA synovial samples suggested that in the pathological site, in contrast to the normal situation, cytokine synthesis is relatively continuous. This concept was tested by culturing dissociated cells from

Fig 2. *Expression of cytokines in rheumatoid joints* (illustrating cytokines found abundantly and less frequently in rheumatoid synovial joint tissue) (G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL - interleukin; MCSF = macrophage colony-stimulating factor; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumour necrosis factor; VEGF = vascular endothelium growth factor)

Abundant:	IL-6, TNFα, IL-1, IL-8, IL-11 GM-CSF, PDGF, IFNγ, VEGF RANTES, G-CSF, M-CSF, IL-10, TGFβ, IL-13 and other chemokines
Less abundant:	IL-2, IL-3, LT, IFNγ, IL-12, IL-15
Sporadic:	IL-4

rheumatoid synovium *in vitro* without any extrinsic stimuli [46]. These studies showed that there was indeed high production of cytokine mRNA *in vitro* over a prolonged period of time, far longer than that detected using any combination of mitogenic stimuli.

At the time these studies were being conducted, assays for cytokine proteins were still chiefly bioassays. Because of the potency of cytokines, relevant assays usually need to be highly sensitive (in pg/ml range), although certain cytokines are considerably more abundant than that (eg IL-6, IL-8). Sufficiently sensitive binding assays, which employ antibodies and have high specificity, were not yet available. While bioassays have the virtue of detecting only active (and not degraded or neutralised cytokines), a major drawback is their lack of molecular specificity. Several cytokines can often have the same effect on a bioassay (eg IL-2, IL-4 and IL-7 on T cell proliferation). However, it was important to determine whether the bioactive cytokine proteins were also chronically expressed, because they are capable of signalling and activating other cells. A bioassay for IL-1, the mouse thymocyte costimulation assay, was therefore used to evaluate whether chronically elevated IL-1 mRNA also yielded augmented IL-1 protein. This was indeed the case, although it was essential to exclude the participation of other cytokines acting on the thymocyte assay (eg IL-6) by using neutralising antisera [9].

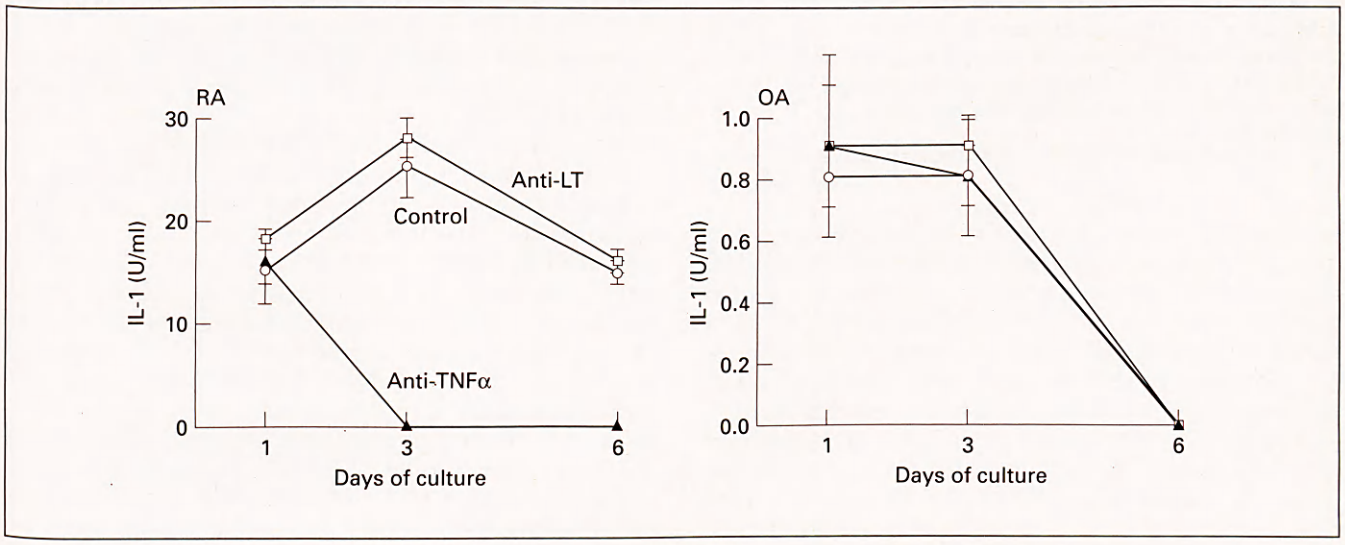
Cytokine regulation in the rheumatoid arthritis synovium

Certain cytokines, such as IL-1, are therefore chronically produced in dissociated synovial cultures throughout the culture period. These experiments have not been pursued beyond seven days, due to the change in composition of the cells in culture with a relative loss of T cells and macrophages, and overgrowth of fibroblasts in these simple media lacking optimal concentrations of lymphocyte growth factors. The chronic production of these pro-inflammatory cytokines supported the concept that their regulation at a pathological site is different from that in normal tissue. Furthermore, this chronic production took place in the *absence* of extrinsic stimulation, indicating that the signals regulating the chronic cytokine production were contained within the culture and were thus amenable to experimental analysis.

Considerable evidence was obtained by 1988 that IL-1 was a potent pro-inflammatory cytokine and likely to be of relevance in RA. It had been detected in RA synovial fluid [48], was produced in RA synovium and its cultures, and was capable of inducing arthritis if injected into rabbit knee joints [49]. Most importantly, it had been implicated in the destruction of cartilage and bone [50]. Thus, IL-1 became the focus of our attempts to study the mechanisms of cytokine upregulation in RA synovium.

Multiple potential IL-1 inducing signals were already known to be present in the RA synovium. These included immune complexes capable of inducing pro-inflammatory cytokines (eg IL-1, TNF) from cultures of human peripheral blood mononuclear cells [51], TNFα and lymphotoxin, reported by Dinarello and Cerami [52] to be potent IL inducers, and GM-CSF, all of them able to synergise with IFNγ. From the above data, we anticipated that IL-1 production in the RA synovial cultures would be under multifactorial control. To our surprise and excitement, we found that rabbit antibodies to TNFα, but not to the closely related protein lymphotoxin (also known as TNFβ), markedly inhibited bioactive IL-1 synthesis within

Fig 3. *Evidence that tumour necrosis factor (TNF)α drives production of interleukin (IL)-1 in rheumatoid synovium.* This figure illustrates that IL-1 production in synovial tissue in rheumatoid arthritis (RA) (left) but not in osteoarthritis (OA) (right) is dependent on TNFα (LT = lymphotoxin) (reproduced, with permission, from Ref 9)



three days [9]. This was consistent for multiple RA synovial samples, but not for OA synovial samples which produced much less IL-1 to start with (Fig 3). Both TNFα and IL-1 are strongly pro-inflammatory, and both have been implicated in the induction of cartilage and bone destruction [50,53]. The fact that blocking TNFα had the unexpected bonus of also blocking IL-1 made us realise that it was likely to have an important role in the pathogenesis of RA. It was also the first clue that TNFα might be a useful therapeutic target.

As other pro-inflammatory cytokines such as GM-CSF, IL-8 and IL-6 are abundant in the RA synovium, it was of interest to evaluate whether their production, like that of IL-1 also depended on the prior generation of TNFα. This was evaluated in the same manner as the regulation of IL-1 by TNFα, using dissociated synovial cultures and neutralising antibodies to TNFα. GM-CSF bioactivity [54] and IL-6 and IL-8 levels [55] were found to be greatly reduced in the presence of anti-TNFα, demonstrating that the production of a wide spectrum of pro-inflammatory cytokines was dependent on TNF. We now envisage the cytokine system in an active RA joint as a 'cascade' (Fig 4) with TNFα inducing IL-1, and both inducing IL-6, IL-8 and GM-CSF. This is based on the differential effects of antibodies to cytokines or of the IL-1 receptor antagonist [9,54,55]. Thus, in contrast to anti-TNFα which reduces IL-1 production, the IL-1 receptor antagonist neither reduces TNFα production nor, interestingly, IL-production. This indicates that the actions of TNFα and IL-1 are not reciprocal; while it is well documented that IL-1 in certain systems is an inducer of TNF (review Ref 56), it does not do so in RA cultures. Furthermore, autocrine stimulation of IL-1 by IL-1 is also not significant in RA joint cultures.

These results encouraged us to evaluate the expression of TNFα in more detail in the joints *in situ*. Immunostaining for TNFα protein showed that abundant TNFα was present in the lining layer and other parts of the synovial membrane, including the cartilage pannus junction where the erosions which lead to joint failure take place [57].

Inhibitory cytokines

Despite the fact that the first cytokines to be characterised (eg IL-1, TNFα) were pro-inflammatory, some of the subsequently described cytokines have inhibitory actions. They include TGFβ, IL-4, IL-10 and, more recently, IL-13 (Fig 5). The expression of these cytokines in RA synovium has been evaluated by a variety of techniques. IL-4 is rarely present [58]. In contrast, TGFβ [59] and IL-10 [60] were relatively abundant in the supernatants of short-term (24-hour) synovial cultures. The importance of these cytokines in regulating the pro-inflammatory cytokines was evaluated in RA synovial cultures. Additional TGFβ did not alter IL-1 or TNFα production [61], but additional IL-10 reduced it 2-3 fold within 24 hours [60]. In a reciprocal way, anti-IL-10 was found to augment IL-1 and TNF production, indicating that even late in the disease process IL-10 still acts as an endogenous regulator of disease activity [60].

Cytokine receptor expression

Cytokines act on high affinity receptors, so knowledge of cytokine expression alone is not informative, unless it is clear that the relevant receptors are always expressed. There is evidence that cytokine receptor expression is tightly regulated, for example those for IL-2 [62]. Resting T cells have very few high affinity IL-2 receptors. Thus, cytokine receptor expression was monitored as soon as cytokine receptor cDNAs and

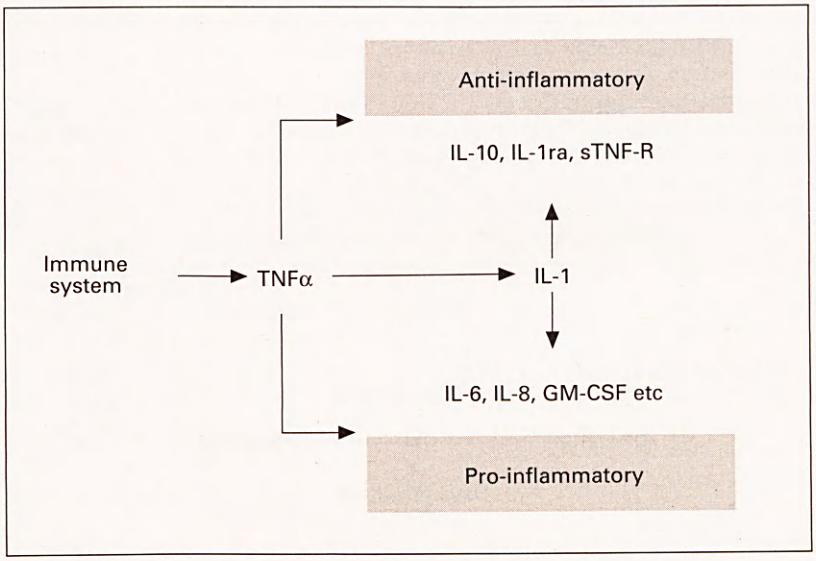


Fig 4. *Cytokine cascade in rheumatoid arthritis* (GM-CSF = granulocyte-macrophage colony-stimulating factor; IL-1ra = interleukin-1 receptor antagonist; sTNF-R = soluble tumour necrosis factor receptor) (reproduced, with permission, from Ref 8)

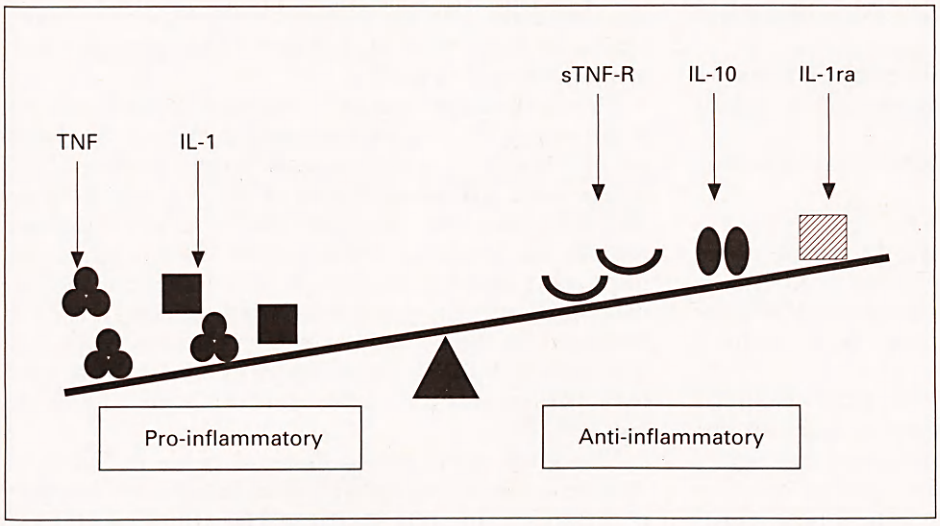


Fig 5. *Cytokine disequilibrium* (IL-1ra = interleukin-1 receptor antagonist; TNF = soluble tumour necrosis factor) (reproduced, with permission, from Ref 8)

relevant antibodies made it technically feasible. The IL-2 receptor α chain was the first cytokine receptor antibody and cDNA to be cloned, and it was found that IL-2R mRNA and protein was present in RA synovium, but at variable and not very high levels [46].

There are two distinct TNF receptors, termed p55 and p75 TNF-R. Expression of both receptors is augmented in RA synovium, as assessed either qualitatively *in situ* by immunohistology, or quantitatively in dissociated cells by flow cytometry [61]. In contrast, TNF-R levels were not raised either in RA blood or in OA synovium. The elevated expression of TNF-R was discernible by flow cytometry in both macrophages and lymphocytes. Elevated surface TNF-R facilitated signalling by TNF, and was thus 'pro-inflammatory' [63].

However, surface TNF-R is also the precursor of the soluble TNF-R (sTNF-R) found in body fluids, even in normal circumstances at levels (4–5 ng/ml) capable of neutralising TNF [64]. We have investigated the production of soluble TNF-R in RA patients in order to evaluate whether diminished production of these inhibitors may be involved in the prolonged TNF signalling uncovered by our studies of dissociated cultures of RA synovium. Elevated levels of sTNF-R, both p55 and p75, were found in a variety of arthritic conditions in serum and synovial fluid samples. Levels in synovial fluid were 2–3 times higher than in serum, suggesting that their production in synovium was contributing to serum levels. The highest levels occurred in RA, with lower levels in seronegative arthritides. These results suggest that production of sTNF-R in the synovium is upregulated, and that deficiencies in the production of TNF inhibitor are not a major part of the pathogenesis of RA [65].

In view of the elevated levels of sTNF-R in synovial fluid, it was of interest to know whether TNF-R could neutralise the TNF produced in the synovium. This analysis cannot be performed in the intact synovium, but can be mimicked in dissociated RA synovial cultures. There was bioactive TNFα in all RA cultures, with a median level of bioactivity of about 50%. This was not found in OA cultures, in which there was often no bioactive TNF (median bioactive TNF level, 35%). The actual levels of sTNF-R were higher in RA cultures than in OA cultures. To establish whether sTNF-R was partially neutralising TNFα, the lytic activity of rheumatoid synovial fluids was compared before and after incubation with monoclonal antibody to TNF-R. These antibodies to TNF-R augmented lytic activity, verifying the role of sTNF-R as an inhibitor of TNF. Overall, these results suggest that, while sTNF-R can inhibit some of the TNF, not enough is produced by synovial tissue in patients with active RA to do more than partially inhibit the effects of TNF on driving the disease process [66].

In summary, TNFα is of major importance in the cytokine cascade or 'network' which takes place in the RA synovium. We now envisage that the multiple pro- and anti-inflammatory forces are almost in equilibrium, just favouring the pro-inflammatory side, so giving rise to a chronic inflammatory disease (Fig 5). The results of these studies also suggest that TNFα would be a good therapeutic target.

Animal models of arthritis respond to anti-tumour necrosis factor

A restricted number of mouse strains develop an inflammatory arthritis if injected intradermally with collagen type II in Freund's complete adjuvant. We have used this model in DBA/1 mice; they develop erosions of cartilage and bone, which are difficult to distinguish from those at similar sites of human RA.

To further test the hypothesis that TNFα is of major importance in arthritis, we treated these mice *after* the onset of arthritis with a monoclonal anti-mouse TNF antibody. This led to a marked diminution of disease activity, as judged by:

- reduction in footpad activity (a marker of inflammation)
- lessened 'recruitment' of newly affected limbs (a marker of disease progression) and, most importantly,
- histological assessment of erosions of cartilage and bone.

This result *in vivo* supported the *in vitro* analysis described above, and was an important preliminary step leading towards the clinical trials of anti-TNF antibody therapy in RA [67]. Other groups have obtained similar results [68,69].

Other animal models of arthritis have also supported our concept of the importance of TNFα in the pathogenesis of arthritis. Keffer, Kollias and colleagues have generated interesting transgenic mice with dysregulated human TNFα production, which reproducibly develop arthritis [70]. Anti-TNF therapy has also been successful in rats with collagen-induced arthritis, streptococcal cell wall-induced arthritis and adjuvant arthritis, and in rabbits with antigen-induced arthritis (review Ref 7). It therefore seems likely that TNF generation is an important step in the development of virtually any chronic erosive arthritis.

Clinical trials of anti-tumour necrosis factor α antibody therapy

The work described above provided a rationale for testing, in patients with long-standing and active RA, whether blockade of TNFα would be beneficial. An 'open' (non-blinded) clinical trial was initiated in May 1992, using a chimaeric (human immunoglobulin G (IgG) murine Fv) high affinity anti-TNFα antibody, cA2, produced by Centocor Inc (Malvern, USA) (Fig 6) [71]. This was infused intravenously at a total dose of 20 mg/kg over a two-week period [72]. The results were uniform and spectacular, and confirmed the hypothesis (Fig 7). The details of the clinical trials were described by my colleague, Professor Ravinder Maini, in his Croonian Lecture [73], and in other recent reviews [7,74,75].

The median duration of benefit was 12 weeks (range 8–26)—long for a single course of treatment, but brief in the lifetime of a patient with chronic disease [72]. There were no cures. Some of these patients were treated again after relapse, and in every case the relapse was brought under control. This permits the important conclusion that the fundamental mechanism of the disease process does not change after TNF blockade (ie there is no 'tachyphylaxis'), and indicates that other pro-inflammatory cytokines do not take over the 'dominant' role of TNFα when TNFα is blocked [76].

The gold standard of clinical trials is the randomised, placebo-controlled double-blind trial, which is rightly considered the necessary proof of clinical efficacy of any therapy. cA2 was shown to be highly effective in such a multicentre trial [77]. The importance of TNFα in RA, and of the effectiveness of anti-TNF antibody therapy in this disease, has been further confirmed using a humanised antibody produced by Celltech in a dose-escalation, placebo-controlled clinical trial [78]. Trials with fusion proteins of human IgG and TNF receptor produced by Roche, (p55) and Immunex (p75), suggest that they are also effective.

What are the implications of successful anti-tumour necrosis factor therapy in rheumatoid arthritis?

The rapid expansion in the 1980s in the number of molecularly defined cytokines with several overlapping and similar properties made it difficult to understand how these molecules acted as efficient regulators of the immune and inflammatory responses. It was widely believed that if there are multiple pro-inflammatory cytokines with similar *in vitro* properties (eg IL-1, and TNFα) in a diseased tissue (eg a rheumatoid joint), blocking a single one of these cytokines is unlikely to

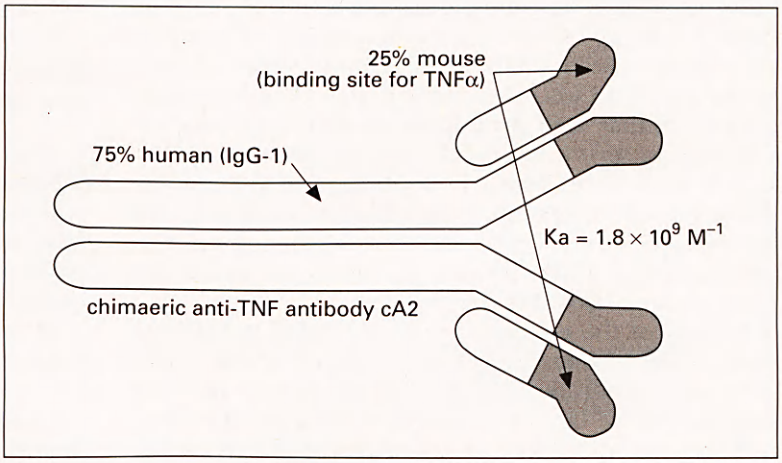


Fig 6. *Structure of a chimaeric antibody, cA2 (Centocor Inc)* (IgG = immunoglobulin G; TNF = tumour necrosis factor) (previously described by Knight *et al* [71])

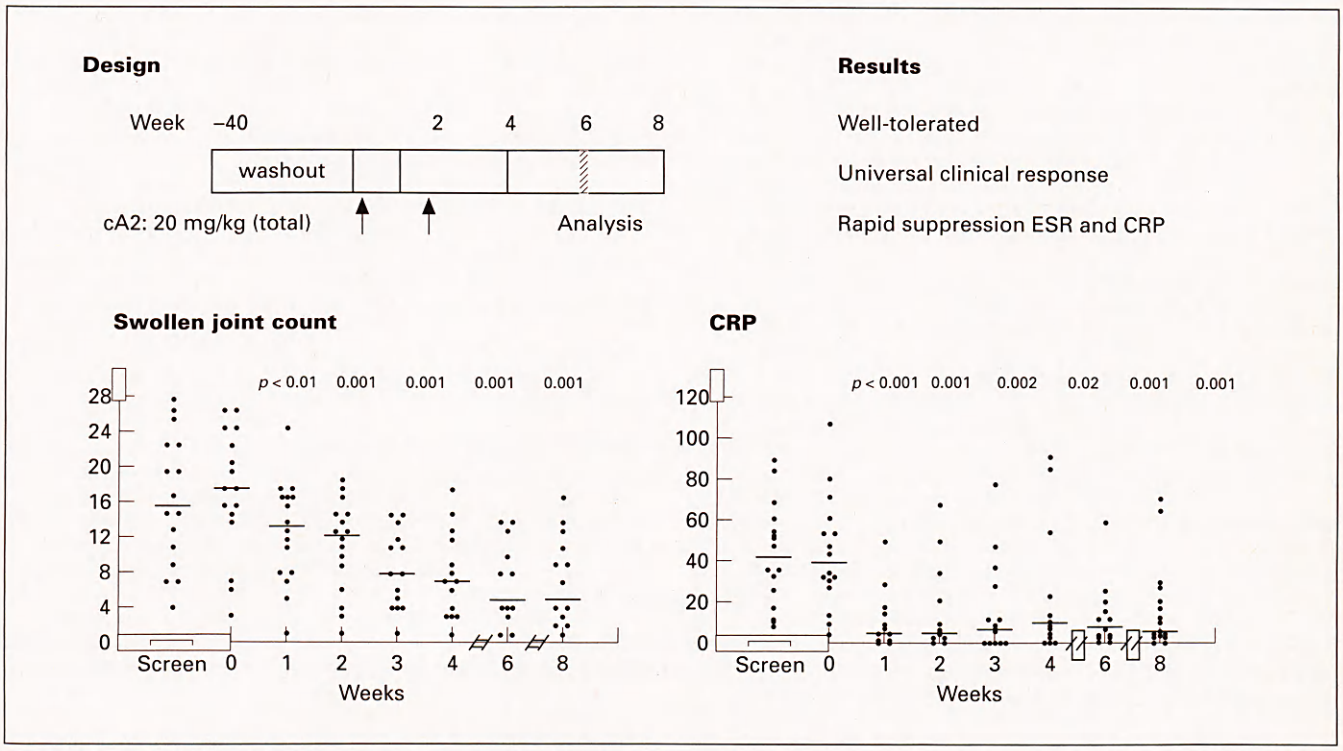


Fig 7. *Open-label clinical trial for anti-tumour necrosis factor in rheumatoid arthritis* (CRP = creatine phosphate; ESR = erythrocyte sedimentation rate) (data drawn and reproduced, with permission, from Elliott *et al* [72])

have much clinical benefit. This line of reasoning of 'cytokine redundancy' led to the premature conclusion that cytokines are poor targets for immunotherapy in inflammatory/autoimmune disease (discussed in Ref 72). This contrasts with the concept of the 'cytokine cascade', in which the expression of multiple pro-inflammatory cytokines are linked and are TNFα- dependent (Fig 4).

One of the most important general conclusions from our work is that cytokines *are* good therapeutic targets, provided that the cytokine chosen is rate-limiting and of major importance in the disease process. Chronic inflammatory diseases have related pathogenesis, so it was to be expected that blocking TNFα would be beneficial in other inflammatory diseases. This prediction has already been successfully tested in Crohn's disease where marked benefit has been shown in severe, often steroid-resistant, Crohn's disease— even with fistulae [79,80]. In view of the capacity of anti-TNFα antibody to prevent or ameliorate experimental allergic encephalomyelitis [81,82] (an animal model of multiple sclerosis), it could be predicted that multiple sclerosis may also be amenable to anti-TNF therapy. But the blood-brain barrier, which excludes antibodies, may prevent this prediction from being successfully tested with an antibody molecule. Drugs such as rolipram, a phosphodiesterase type IV inhibitor, which was effective in experimental allergic encephalomyelitis in rats and monkey [83,84], may be more effective.

The work described here has also convincingly shown that monoclonal antibodies are an efficient tool for testing therapeutic concepts, for 'proof of principle' therapeutic trials, because their target specificity is clearly defined, and unwanted or cross-reactions can be monitored. This is not the case for small organic chemicals which, by diffusing across cell membranes, can access all the approximately 100,000 gene products expressed in the body. Thus, effects noted with small organic chemical drugs need not be due to their already known mechanisms of action, but could be due to other, as yet undefined, interactions. A good example, methotrexate, is a folate antagonist, which is an effective anti-rheumatic drug, but acts by a different (and still unclear mechanism) since it is effective in low dose and even in the presence of dietary folate supplementation.

The specificity of monoclonal antibodies makes it possible to study changes occurring in patients after anti-TNFα antibody treatment, and to conclude with some confidence that the changes noted are *consequences* of TNFα blockade. We have studied the mechanism of action of anti-TNFα therapy and found that this therapy interfered with the cytokine cascade and, most importantly, also with leukocyte recruitment to affected joints [72,75,85].

Our work has clearly shown that blocking TNFα has marked beneficial effects in RA, and thus predicts that other ways of blocking TNFα would also be of benefit in this disease. There is already preliminary and anec-

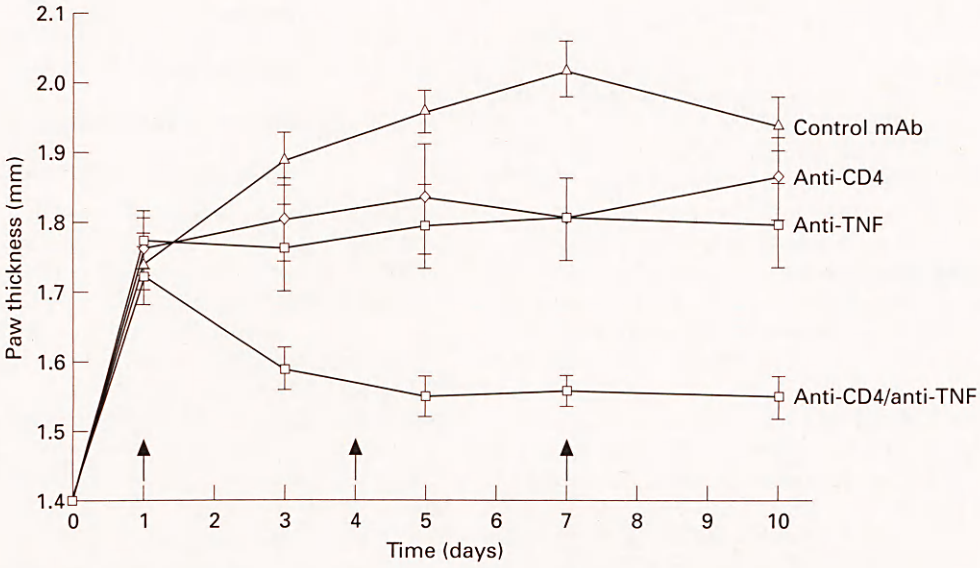


Fig 8. *Synergy of anti-CD4 and anti-tumour necrosis factor (TNF)α antibodies in ameliorating collagen-induced arthritis* (mAb = monoclonal antibody) (reproduced, with permission, from Ref 86)

dotal evidence that this is the case with biological agents (such as fusion proteins of TNF-R and IgG (see above). Orally available drugs which block TNF production or its actions are active in animal models of disease, for example, the phosphodiesterase type IV inhibitors such as rolipram, and more are in clinical development. The pace of this development has hastened considerably since we first reported the clinical benefits of blocking TNF in RA [72], auguring well for the benefit of the patients.

Prospects for the future

Blocking TNFα temporarily, even for several months, does not result in a cure in long-standing RA patients despite significant clinical benefit in essentially all patients. It is thus worth considering what other approaches may be needed to yield 'cures'. Fig 1, our working hypothesis of the pathogenesis of autoimmunity, illustrates the importance of the T cell antigen presenting cell interaction in disease maintenance, and provides one approach to this problem, which we have explored in models of arthritis.

The potential of immune blockade to augment TNF neutralisation was tested by using anti-CD4 monoclonal antibody in conjunction with anti-TNF monoclonal antibody in the collagen-induced DBA/1 mouse model of arthritis after disease onset. Doses of anti-TNFα and anti-CD4 antibodies, which were ineffective if used alone [86], showed marked synergy, as judged by reduction in inflammation (footpad swelling), new limb recruitment or joint erosions. Exploiting such synergies offers a possible way of avoiding complications from the long-term blockade of TNFα (Fig 8). The combination of anti-immune and anti-cytokine therapy may be a productive avenue for the treatment of several immune/inflammatory diseases including RA, Crohn's disease and multiple sclerosis. The challenge, as always, is to maximise efficacy and safety and, in the modern era, cost.

Acknowledgements

The work described here was chiefly funded by the Arthritis and Rheumatism Council (UK), with contributions from the Nuffield Foundation, Wellcome Trust, MRC and Xenova plc. Clinical studies were funded by Centocor Inc. This work would not have been possible without the contributions of many colleagues, in particular, Professor Ravinder Maini, Drs Fionula Brennan, Richard Williams, Michael Elliott and Glenn Buchan (all of the Kennedy or Sunley Institutes), and Dr James N Woody of Centocor Inc.

I thank Drs Brennan, Elliott and Mrs Jane Templeman for preparing the illustrations, and Mandy Wilcox, Philippa Wells and Maria Silvester for their assistance with the manuscript.

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