The role of cytokines in rheumatoid arthritis

The Croonian Lecture 1995

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FRCP

Genes, immunity, and cytokines in the pathogenesis of RA

When William Croone practised medicine in London in the years between 1660 and 1684, rheumatoid arthritis (RA) was not a recognised disease. It was Garrod, two centuries later, who first described this destructive disorder of joints, with its predilection for women [1]. The relatively recent description of RA has prompted speculation that it is a disease of the modern age [2], perhaps reflecting an encounter with a recently introduced environmental agent of modern civilization. The pros and cons for this proposition continue to be debated, but there can be little doubt that epidemiological data support the case for genetic factors and an environmental agent in the causation of RA. Thus, a concordance rate of only 33% in identical twins, presumably with the same copies of DNA, against a background prevalence of around 1% in the general population, is a forceful argument both for an environmentally triggered disease and a genetic susceptibility [3,4].

Research in the past decade has revealed a link between genes in the human leucocyte antigen (HLA) system and the occurrence of RA. The genetic link is with the hypervariable region of the β chain of HLA-DR molecules encoding a pentapeptide sequence in positions 69 to 74 and expressed by the allelic variants of subtypes of HLA-DR4 (eg DW 4, DW 14 and DW 15) and other types, eg DR 1, DW 16 and DR 10 [5,6]. This region of similarity has given rise to the concept of a *shared epitope*, which equates with the genetic element of susceptibility to RA. The location of the 'susceptibility sequence' within the antigen-binding groove of HLA molecules is consistent with the hypothesis that it might encode a functional epitope that is recognised by T cells (Fig 1). Thus, HLA-restricted antigen presentation to a T cell could provide a rational explanation for the previously recognised features of RA such as activated T and B lymphocytes in the rheumatoid synovium and, via T and B cell co-operation, the occurrence of disease-specific autoantibodies which include rheumatoid factors of all isotypes, as well as immunoglobulin G (IgG) anti-perinuclear, anti-keratin, and anti-RA33 (anti-nuclear) antibodies [7].

Cytokines and chronicity

While disease initiation may be explained by an immune response to an environmental antigen and perpetuation (or chronicity) of disease by autoimmunity, increasing knowledge of the role of cytokines in RA provides a framework for understanding the pathophysiology of localisation of the disease to joints. It illuminates our understanding of the locally destructive potential of the immuno-inflammatory response and the systemic features of the disease [8]. Indeed, it can be argued that in established chronic RA, cytokines become the dominant biological force, with the trimolecular immunological interaction between HLA molecules, peptide antigens, and T cell receptors, which was critical at the initiation of disease, now playing a low-key, but obligatory, role in its perpetuation (Fig 2).

Cytokines are intercellular messenger molecules that principally exert their effects on other cells in the local milieu in which they are produced by binding to their cognate receptors. Many cytokines are produced in the course of a biological response to a stimulus. The interconnections, resulting in agonistic and antagonistic effects, have been referred to as a *cytokine network*. When produced in sufficient quantities to circulate in blood, they can exert effects on distant tissues. At the Kennedy Institute of Rheumatology, we have found that an impressive range of cytokines and natural inhibitors is produced in rheumatoid joints and have demonstrated the importance of 'tumour necrosis factor α' (TNFα) as the controlling element of the 'cytokine network' [9].

An important starting point of our investigations was the demonstration of interleukin-1 (IL-1), TNFα, TNF receptors, and interleukin-6 (IL-6) by immunohistology in the synovial membrane of RA joints [10–14]. Here the cytokines produced by inflammatory cells, immune cells, vascular endothelium and fibroblasts were candidate agents for orchestrating the immuno-inflammatory response. More importantly, TNFα and IL-1 were also found at the cartilage–pannus junction, where they could exert their destructive

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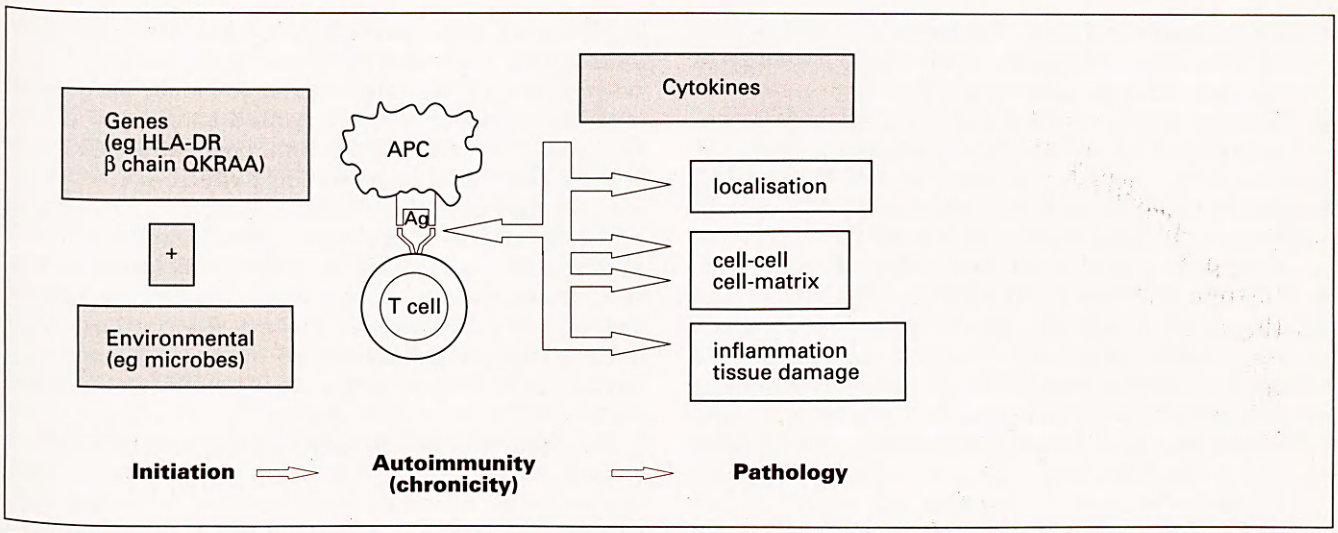


Fig 1. The pathogenesis of rheumatoid arthritis can be viewed as a multi-step process. Environmental factors (eg antigens of micro-organisms) may initiate an immune response by antigen binding to HLA-DR molecules and this bimolecular complex then interacts with a T cell receptor. The genetic susceptibility to RA has been mapped to a 'shared epitope' expressed by some DR4 subtypes and DR1 on the β chain of HLA-DR alleles. Subsequently, the immune response could be perpetuated by autoantigens (for example, by molecular mimicry or 'epitope spreading'). Localisation of the disease process to joints and other tissues involves adhesion of cytokine-activated endothelial cells to ligands on circulating leucocytes. These leucocytes then traverse the vascular barrier under the influence of chemokines. In tissues, cell–cell and cell–matrix interactions in synovium and at the cartilage–pannus junction further perpetuate inflammation and joint damage. Abbreviations: Ag, antigen; APC, antigen-presenting cell

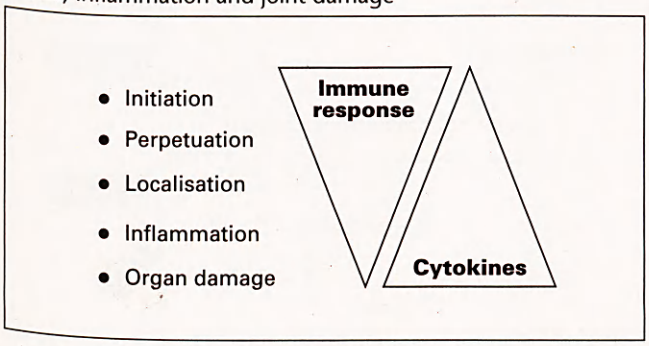
potential. The predominant, but not exclusive, cell type producing IL-l, TNFα and IL-6 appeared to be derived from the macrophage lineage.

When preparations of mononuclear synovial cells (consisting of macrophages, lymphocytes, fibroblasts and other cell types), obtained by enzymic dissociation of surgically excised synovial membranes, were analysed, we detected increased quantities at messenger RNA level of IL-l, TNFα, IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and other cytokines [15–19]. All the cytokines continued to be synthesized for several days by *in vitro* cultures of these mononuclear cells without any added stimulants. Most significantly from the point of view of conceptual developments, their production was suppressed by the addition of neutralising antibodies to TNFα. These *in vitro* experiments provided the first important cue supporting a pivotal role for TNFα in regulating the production of other cytokines [20]. The experiments demonstrated that cytokine production in RA joints behaved more like a cascade, with TNFα at its head, than a network of randomly connected molecules.

From this array of cytokines produced in RA joints, and their known biological activities, it was possible to construct a coherent scheme and hypotheses of their possible clinicopathological significance [8,21]. For example:

- IL-l and TNFα emerged as the most promising candidates involved in cartilage degradation. Singly, and synergistically, these cytokines can induce synthesis and release of inflammatory mediators such as metalloproteinases, prostaglandins and nitric oxide in many cell types, eg synovial fibroblasts, macrophages and chondrocytes. The production of these inflammatory mediators could well explain the destructive effects of pannus on cartilage matrix and of pericellular degradation of the matrix around chondrocytes. TNFα and IL-l can also inhibit synthesis of matrix components,

Fig 2. Steps in the pathogenesis of RA. The triangles show the hypothetical inverse relationship of the importance of an immune response and cytokine-mediated pathophysiology in the steps involved in the pathogenesis of RA. The immune response is more important in initiating and perpetuating chronicity and could play a part in localisation to tissues, while cytokines are more implicated in the regulation of cellular traffic, inflammation and joint damage



thus hindering repair mechanisms. Their effects *in vitro* were consistent with the pathology of cartilage damage observed in RA.

- IL-1 and TNFα mediate additional effects relevant to understanding the pathogenesis of RA. For example, both cytokines induce adhesion molecules such as E-selectin and ICAM-1 on synovial vascular endothelium and simultaneous production of chemokines such as IL-8, MIP-1α, MIP-1β, MCP-1 and RANTES by endothelium and surrounding cells [8]. In a highly vascular tissue the combination of adhesive molecules and chemokines brings about a microenvironment that promotes the adhesion and transmigration of circulating polymorphs, monocytes, and lymphocytes into the extravascular space. Here the cytokine-rich milieu stimulates cell activation and cell–cell interaction, providing an explanation for the immunological and inflammatory phenomena observed. They include the activation of T and B cells, fibroblasts and macrophages.

The RA joint is also a site at which cytokines such as IL-10 and TGFβ, with anti-inflammatory and/or immunosuppressive effects, are produced [22–24]. Natural inhibitors neutralising the activity of IL-1 and TNFα are also produced, such as the soluble shed receptors of these cytokines, and a protein with antagonistic effects, ie interleukin-1 receptor antagonist. However, not enough of these counter-balancing molecules are produced to tip the balance in favour of homoeostasis and termination of the inflammatory reaction [9]. These molecules may, however, explain the timescale of disease progression (years rather than months) and the typical fluctuating course of disease with occasional natural remissions.

Our initial *in vitro* experiments and predictions of the pathogenic role of cytokines, and of TNFα in particular, received support from studies of animal models and set the scene for anti-TNF trials. First, collagen type II-induced arthritis (CIA) in DBA/1 mice, which has similarities with the pathological features of RA and shows similar responses to therapeutic interventions with drugs, was significantly ameliorated by injections of monoclonal anti-TNF antibodies and soluble TNF receptor-immunoglobulin fusion proteins [25,26]. Second, transgenic mice expressing a human TNFα gene developed a rheumatoid-like disorder at 5 to 6 weeks of age, and the disease could be suppressed by monoclonal anti-TNF antibodies administered from birth [27].

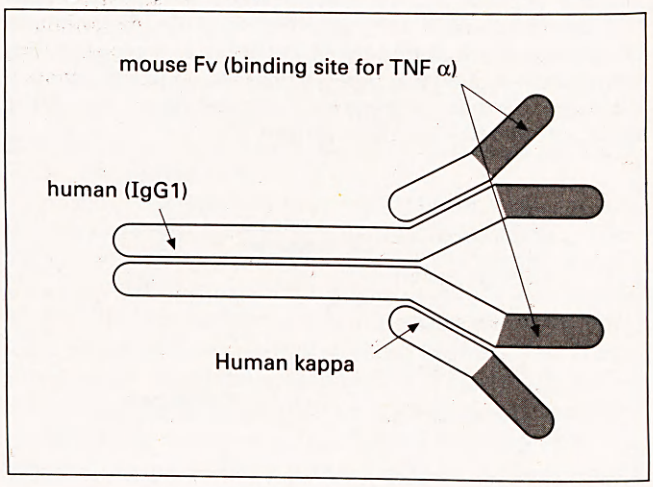
Clinical trials with monoclonal anti-TNFα antibodies: proof of principle and efficacy

By 1992 we had accumulated sufficient evidence to proceed to a preliminary, open-label trial of anti-TNFα therapy of RA. A partially humanised (chimeric) monoclonal anti-TNFα antibody, cA2, manufactured by Centocor Inc., Pennsylvania, USA, was used [28]. This agent, derived from a mouse monoclonal antibody, is genetically engineered to retain its murine variable region and has engrafted to it the constant domains of a human kappa light chain and IgG1 heavy chains (Fig 3). Based on the experience of dose-ranging studies in the murine CIA model, we administered a total of 20mg per kg body weight to RA patients. This was given by intravenous infusion over two hours, in two (or four) divided doses evenly spaced over two weeks. Twenty RA patients with active disease unresponsive to most drugs, many on maintenance therapy with corticosteroids, entered the trial.

We observed an impressive improvement in clinical indices of inflammation (number of swollen and tender joints, duration of morning stiffness and pain score), locomotor function and reduction in C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) in virtually every patient [29]. A mean reduction of 60–70% from baseline was achieved within days, reaching its maximum in four to six weeks. A clinical response was sustained for 12 to 24 weeks, with eventual relapse of disease in all patients.

Although the universality, magnitude and kinetics of the anti-rheumatoid effect exceeded results observed with disease-modifying drugs, a multi-centre randomised placebo-controlled trial was considered to be an essential next step. Accordingly, this was undertaken in 1992/93 on a total of 73 patients with RA. As in the open-label trial, disease-modifying drugs were withdrawn for a period of at least four weeks prior to anti-TNF therapy. The patients were stabilised on a constant dose of non-steroidal anti-inflammatory

Fig 3. The cA2 anti-TNFa monoclonal antibody (mAb) used in clinical trials is derived from a mouse monoclonal antibody. The variable domain of the murine mAb is genetically engineered onto a backbone of constant domains of a human kappa light chain and IgG1 heavy chain. The neutralising mAb has high affinity for TNFα

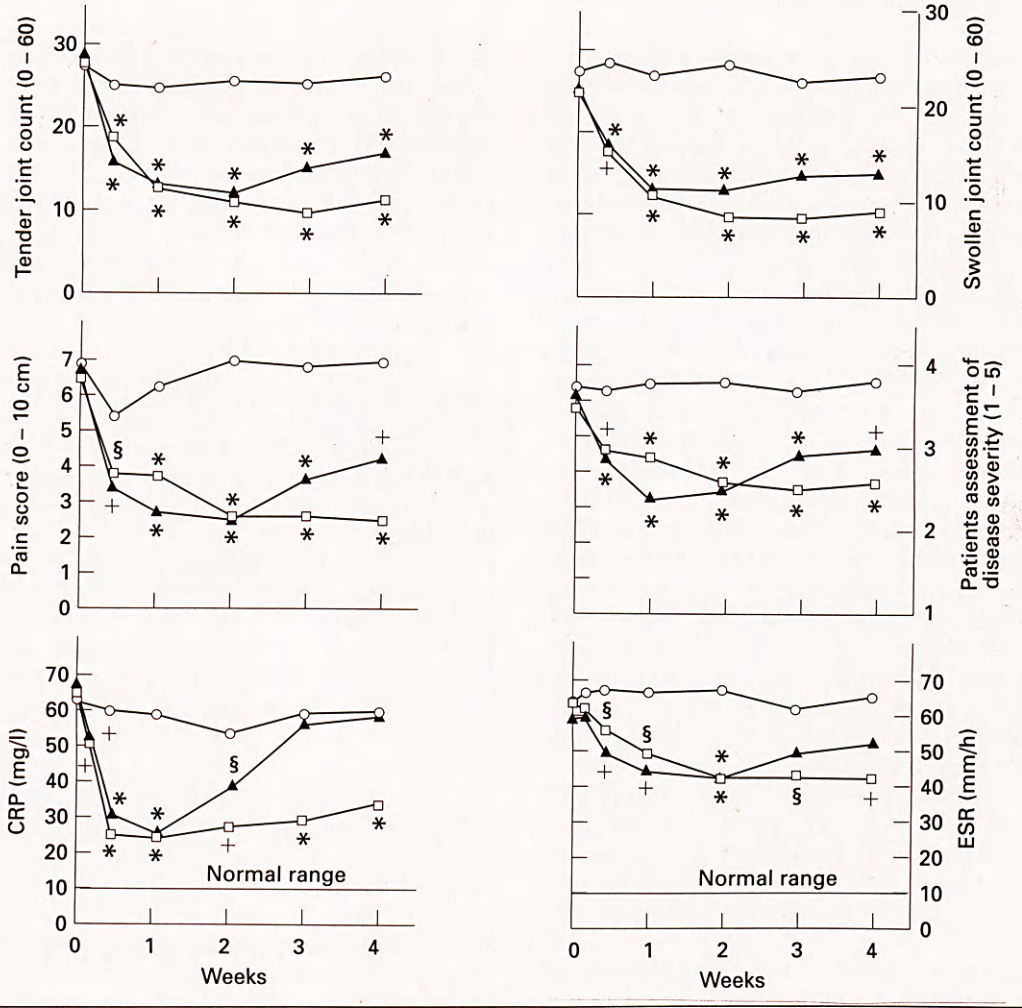


drugs (NSAIDs) and, if already on corticosteroids, on a fixed dose of prednisolone not exceeding 10mg a day. Twenty-four patients received a single intravenous infusion of 10mg per kg, a high dose of anti-TNFα antibody (cA2); twenty-five patients received 1mg per kg (a low dose); and twenty-four patients received a placebo infusion of 0.1% human serum albumin. Over the subsequent days and weeks, patients receiving anti-TNFα antibody showed an improvement in all measures of disease activity [30] (Fig 4).

Using a validated composite index of disease activity (Paulus response) incorporating six clinical measurements at a 20% level of improvement [31], 79% of patients receiving the high dose of anti-TNFα antibody, 58% of patients receiving low dose and 8% of patients receiving placebo gave positive responses at the primary end-point of the study, ie four weeks after the infusion [21,30] (Fig 5).

The duration of the 20% Paulus response was used to monitor benefit and all patients were followed at weekly intervals until disease relapse. After a single dose of 10mg per kg antibody, almost 90% of patients satisfied the response criteria in the first four weeks, 50% of patients maintained benefit for at least six weeks, and 20% of patients for over 20 weeks. Corresponding values for 1mg per kg were three weeks for 50% of patients and eight weeks for 20% of patients. In a group of patients who had previously received a placebo infusion, a 3mg per kg dose of antibody was subsequently administered to establish a dose-response

Fig 4. Placebo-controlled trial of patients taking cA2. Changes in individual disease activity assessments in 73 patients treated with placebo (○), 1 mg/kg (▲) or 10 mg/kg (□) of cA2 in a randomised, double-blind trial. Each of the clinical assessments showed marked down-modulation in both cA2 groups, without significant change in the placebo group. Similar differences were observed for the two acute-phase measurements; ESR and CRP. At week 2 a cA2 dose of 1mg/kg was just as effective as 10mg/kg, but the duration of effect was shorter: see CRP graph especially (*P* values represent significance versus placebo: + $p<0.05$; § $p<0.01$; * $p<0.001$). Reproduced by kind permission from an article by Elliott *et al.* [30]



relationship. The results showed that the main difference in clinical response in the dose range used lay in the duration of the effect, with a marginal difference in the number of responders, and almost none in the maximum degree of change from base line in individual parameters of disease activity. The higher the dose, the higher the frequency of responders and the longer the duration of benefit [32].

From this randomised clinical trial, we concluded that:

- anti-TNFα antibody exerted an impressive anti-inflammatory effect within days, reaching its maximum at three to four weeks, associated with a dramatic reduction in acute-phase proteins and ESR.
- the specificity of the monoclonal antibody for TNFα provided unequivocal evidence of the critical role played by TNFα in regulating the cytokine cascade and secondary effects on inflammatory mediators involved in RA. These clinical and laboratory data pointed to a significant advance in the experimental therapy of RA.

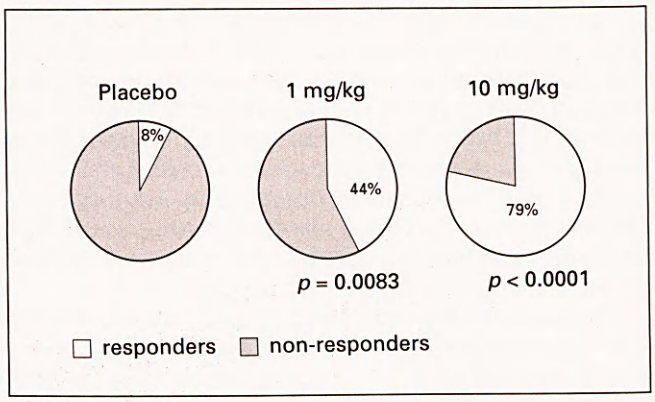


Fig 5. The clinical response of patients treated with cA2 in a placebo-controlled trial. Overall clinical responses (using the Paulus 20% response criteria) in 73 patients treated with placebo, 1mg/kg or 10mg/kg of cA2 in a randomised, double-blind trial. Response states were assessed 4 weeks after treatment. The majority of patients treated with the higher cA2 dose responded, compared with 8% of those receiving placebo, confirming the clinical efficacy of *in vivo* of TNF blockade in the disease (*p* values represent significance versus placebo). Reproduced by kind permission from articles by Elliott *et al.* [30] and Maini *et al.* [21]

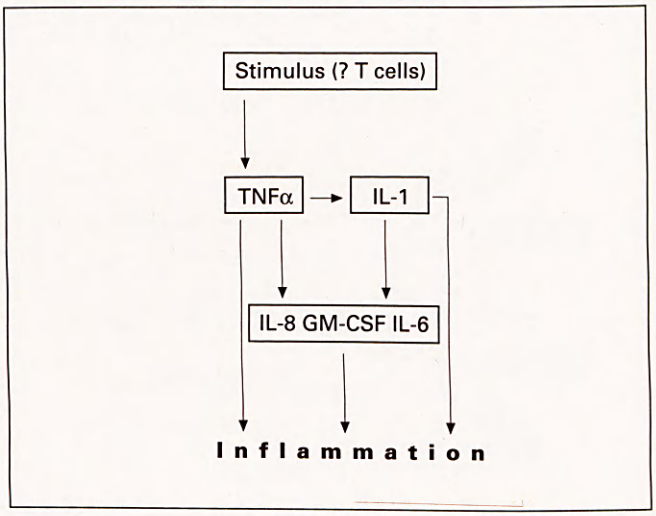
How does anti-TNFα monoclonal antibody work?

Treatment of RA with a monoclonal anti-TNFα antibody provided a unique opportunity to test our fundamental hypothesis that overproduction of TNFα is at the apex of the cytokine cascade and, consequently, of cytokine-inducible secondary molecular pathways of the inflammatory response. If true, we might expect to demonstrate a down-regulation of other cytokines, such as IL-l, IL-6, IL-8 and GM-CSF, which *in vitro* analysis had shown to be regulated by TNFα. However, since most of these cytokines were rapidly cleared from the joint and circulation, joint aspiration and synovial tissue biopsies would have been required to examine the validity of the hypothesis further, but the rapid reduction of joint swelling after therapy constrained our ability to obtain joint fluids. An exception to this rule was IL-6, which was produced in sufficient quantities to give rise to levels that could be measured in blood. Since IL-6 binding to hepatocytes is thought to be the main signal for CRP production, and we had observed a rapid and dramatic reduction in CRP levels following administration of anti-TNFα antibody, it seemed likely that blood levels of IL-6 might give an indication of its regulation by TNFα. Serial measurement of serum IL-6, allowing for diurnal variations, revealed significant reduction of IL-6 blood levels within 12 hours of the administration of anti-TNFα antibody, preceding by 12 to 24 hours a drop in CRP and serum amyloid A levels [21,29,33]. Nevertheless, we are currently seeking more direct evidence of deactivation of the cytokine cascade by examining serial synovial biopsies.

Measuring serum levels of IL-l was unhelpful since it was either undetectable or present in the normal range in the majority of sera. However, serum levels of IL-l receptor antagonist (IL-lra) showed a rapid reduction, in keeping with a downregulation of the cytokine network. At this stage it is not clear how IL-lra production is regulated, but we suspect that TNFα, possibly via IL-l, may be involved in its production, obeying the rules of the cytokine network in RA.

The trials with anti-TNF antibody have clearly demonstrated a dose-dependent effect lasting several

Fig 6. The dominance of TNFα and IL-1 in regulating the cytokine cascade in RA. There is evidence that TNFα regulates IL-1 production in RA but the reverse may not be the case; hence TNFa is regarded as being at the 'apex'



weeks after a single infusion, but since all patients eventually relapse, the intervention does not permanently switch off the immuno-inflammatory response. This is not inconsistent with our current views of the role of TNFα and other pro-inflammatory cytokines in the pathogenesis of established chronic RA (see Fig 2). It is the expected outcome of blocking the cytokine cascade downstream from the factors that induce the production of TNFα (Fig 6). The stimulus for TNFα production is currently not known but probably requires T cell-macrophage interaction involving a ligand–receptor mechanism dependent on cell–cell contact [34]. Whether anti-TNF therapy at earlier stages of RA might exert a longer lasting effect, or even induce remission, will be examined in future trials. It is conceivable that at earlier stages of disease the disequilibrium between cytokine production and the anti-cytokine homoeostatic response, discussed previously, is more amenable to correction. It is certainly likely that earlier intervention would exert the greatest effect in preventing the cumulative effects of joint destruction on functional outcome.

An unexpected finding of the trials

We were surprised that the benefit following a single infusion of a partially humanised (chimeric) antibody lasted for several weeks [28]. Humanised antibodies should offer an advantage over murine antibodies in being less immunogenic in man, but are expected to have a shorter half-life than human immunoglobulin. In fact, it was found that the pharmacokinetics of cA2 (anti-TNFα antibody) are similar to a human immunoglobulin. It persists in the circulation at bioactive levels for about six weeks after a single infusion of l0mg per kg [32]. One explanation is suggested by the results of serial biopsies of synovium from knee joints examined before and after anti-TNFα therapy, which show a significant fall in the numbers of lymphocytes and macrophages per square unit of the histological section [2l,35]. The reduction in synovial cellularity is associated with an increase in the circulating lymphocyte count (still within the normal range) lasting two to four weeks [36]. This suggests that anti-TNFα therapy reduces the flux of lymphocytes into the joints, thereby reducing the immunological signals that sustain synovitis. Other mechanisms, dependent on the complement-fixing property of cA2, may play a part in reduction of macrophages, as might a down-regulation of GM-CSF and other haematopoietic factors. However, when the neutralising capacity of circulating anti-TNF antibody falls below the rate of TNF production, cells gradually re-accumulate in the synovium and inflammatory activity is re-established. Thus benefit of the therapy outlasts the circulating levels of neutralising capacity of the antibody.

The concept that evolved from these observations was that a reduction of cellular traffic may significantly contribute to the mode of action of monoclonal anti-TNFα therapy. In accord with this, we observed a reduction of cell-associated and soluble circulating adhesion molecules such as E-selectin, ICAM-l and VCAM-l [21,35,36], which is entirely consistent with current knowledge of the part that TNFα and IL-l play in regulating their production and release from vascular endothelium [37,38].

Other, as yet uninvestigated, mechanisms could also play an important part in the therapeutic benefits of anti-TNFα antibody. For example, we have observed a significant fall in circulating polymorphonuclear cell count in the first two or three weeks, and a small but significant rise in haemoglobin four weeks after the infusion [30]. The former, we suggest, could result from antibody binding to TNF-expressing polymorphs, leading to their enhanced clearance. The latter could result from removal of the inhibitory effects of TNFα on haemopoiesis. It is also possible that the rapid pain relief and alleviation of fatigue, which treated patients report, is due to blockade of the effects of TNFα on the central nervous system or the hypothalamic–pituitary–adrenal axis.

Future perspectives and conclusions

What of the future of anti-TNFα therapy for RA? Trials with (cA2) anti-TNFα antibody are continuing and further results are expected to define its place in treating RA in the next three or four years. Meanwhile, other TNF blocking agents have confirmed our experience; for example, a murine CDR-3 engrafted anti-TNFα antibody with constant regions of human kappa and IgG4 immunoglobulin [39], and two different soluble TNF-receptors (p55 and p75) linked to immunoglobulin as fusion proteins produced by Immunex [40] and Roche, have all shown an anti-inflammatory effect in RA. Based on the overall experience of research and development, it is possible to come to some interim conclusions and look into the future.

It is clear that a single injection of anti-TNFα antibody controls intractable flares of disease activity in RA, conferring a marked enhancement in the quality of life for some weeks; the control of flares may therefore constitute one indication for the use of the antibody. Since anti-TNFα antibody is not a cure and disease relapse is invariable, repeated therapy will be required [41]. In a proportion of patients monoclonal antibody therapy may prove to be a viable, if expensive, option for long-term control of RA. Repeated anti-TNFα infusions at regular intervals are currently being assessed, as is the possibility that antibody therapy may be effective when used as an adjunct to known disease modifying anti-rheumatoid drugs such as methotrexate.

If continued long-term therapy is possible, will it halt the erosive disease of joints? Protection of joints was observed following anti-TNF therapy in a murine model of arthritis [25]. In that study, experimental

work using a combination of anti-TNF antibody or a soluble TNF receptor immunoglobulin fusion protein with anti-T cell-directed therapy (anti-CD4 antibody) resulted in a marked beneficial effect [42,26]. Such combination therapies could have a similar synergistic benefit in RA, but could also be associated with greater immunosuppression and associated adverse events. Immunosuppression might also occur in patients on anti-TNFα monotherapy, since TNFα has a protective immune function under physiological conditions. Thus it is possible that patients receiving TNF-blocking therapy will be susceptible to intracellular infections similar to those observed in murine models [43]. However, it is also possible that reversing chronic exposure to TNFα, which is itself immunosuppressive, may on balance prove of benefit in RA. The enhancement of lymphocyte proliferative tests observed in patients receiving anti-TNF therapy [44] supports this possibility.

Our trials with monoclonal antibodies have highlighted important principles in understanding the role of cytokines in the pathogenesis of RA and applying the emerging advance in knowledge to refining new therapies [9]. As regards the role of TNFα, it is important to re-emphasise that the clinical benefit and improvement in a panoply of changes in cytokine physiology and cellular activation and kinetics consequent upon anti-TNF therapy should not be taken as evidence that only TNFα is directly involved in mediating pathological effects. Analysis of the effects of TNF by inhibiting the production of other inflammatory molecules on the one hand, and its direct effects on the other, will require careful dissection. A significant anti-inflammatory effect may be achieved by blocking the effects of other molecules (eg metalloproteinases) produced further downstream in the inflammatory reaction. Such selectivity could minimise the possible adverse effects of TNFα blockade on immune defence against bacterial infections. However, our studies have demonstrated added value of TNFα blockade by virtue of its widespread effects and may explain why we found such impressive benefits in clinical trials.

Since it is possible to envisage reduction of TNFα production by interfering with intracellular pathways, eg signal transduction, DNA transcription, stabilisation of messenger RNA and inhibition of enzymic cleavage of TNFα release, one may expect the development of chemical drugs that mimic the results we have seen with anti-TNFα antibody. Such drugs are under development and may offer an alternative for the treatment of RA. However, they are likely to lack the unique molecular specificity of a monoclonal anti-TNFα antibody in unravelling the complexities of disease mechanisms.

Whatever the final outcome of this development, one can safely predict that TNF-directed therapy will preoccupy the agenda of research and development of drugs in the next decade and give new insights into molecular mechanisms of rheumatoid disease.

Acknowledgements

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