Cytokine research has spawned the introduction of new therapies that have revolutionized the treatment of many important diseases. These therapeutic advances have resulted from two very different strategies. The first therapeutic strategy embodies the administration of purified, recombinant cytokines. The second relies on the administration of therapeutics that inhibit the harmful effects of upregulated, endogenous cytokines. Examples of successful cytokine therapies include hematopoietic growth factors (colony stimulating factors) and interferons. Prime examples of cytokine antagonists that have profoundly altered the treatment of some inflammatory disorders are agents that inhibit the effects of tumor necrosis factor (TNF). In this article, we highlight some of the studies that have been responsible for the introduction of cytokine and anti-cytokine therapies, with emphasis on the development of interferons and anti-TNF agents.

Historical review:
Cytokines as therapeutics and targets of therapeutics

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Cytokines are regulatory proteins that are secreted by white blood cells and several other cell types in the body. Their pleiotropic actions include the regulation of innate and adaptive immune responses and the modulation of inflammatory responses, in addition to many other activities. Some cytokines were first described ~50 years ago, but their molecular characterization occurred much later. Among the factors identified in the 1950s were endogenous pyrogen [1], which is probably identical to the protein now known as interleukin 1 (IL-1), nerve growth factor [2] and interferon (IFN) [3]. In the 1960s, immunologists discovered that lymphocytes produce soluble mediators, termed lymphokines, that affect many functions of the immune system [4–6], including the expression of major histocompatibility antigens and the regulation of the T helper cell 1 (Th1)–Th2 paradigm [7]. Tumor necrosis factor (TNF), another important, pleotropic cytokine, was described originally in the 1970s as a mediator of lipopolysaccharide-induced necrosis of transplantable tumors [8]. Molecular characterization of the cytokines responsible for these actions had to await the purification of the individual proteins and cloning of their genes. Today, the number of known cytokine genes and proteins has reached several hundred, with new ones still being discovered [9,10].

The idea that cytokines could be used as therapeutics is as old as the cytokine field itself. When Isaacs and Lindenmann described IFN [3], they noted that it made cells resistant to virus infection but exerted no adverse effects on uninfected cells. This observation inspired hopes that IFNs could be used to treat viral infections. The demonstration that IFNs can also inhibit cell growth, modulate immune responses and inhibit tumor growth in animals [11] fanned expectations of their potential use in cancer therapy and spurred a race toward the cloning of IFN genes and production of recombinant IFNs. IFNs are, indeed, useful additions to the therapeutic armamentarium, but the over-optimistic expectations of their therapeutic value in cancer and many common virus infections are not borne out by the clinical experience.

The notion that cytokines might contribute to pathology is implicit in the nature of some of the earliest recognized cytokine actions, such as the realization that IL-1 acts as ‘endogenous pyrogen’ [12]. The first clearly documented demonstration that neutralization of a cytokine can alleviate pathology was by Ion Gresser and colleagues, who showed that administration of anti-IFN globulin to suckling mice infected with lymphocytic choriomeningitis virus suppressed disease development [13]. Another milestone of anti-cytokine therapy was the demonstration by Bruce Beutler, Tony Cerami and colleagues that administration of an antibody to TNF protected mice and baboons from bacterial sepsis [14,15]. In humans, Škurkovich and Eremkina [16] reported the detection of IFN in sera from patients with immunological disorders (e.g. systemic lupus erythematosus) and postulated that IFN could have adverse effects in autoimmune disorders. In the 1980s, clinical trials were initiated with anti-Tac monoclonal antibody, which is directed against the α-chain of the human IL-2 receptor [17]. Anti-Tac, which inhibits IL-2-induced T-cell proliferation, has shown promise in the treatment of allograft rejection, graft versus host disease and some lymphomas [18].

It is fair to say that a major advance in anti-cytokine therapy occurred with the successful use of the chimeric human–mouse anti-TNF antibody cA2 in patients with rheumatoid arthritis (RA), a chronic, autoimmune, inflammatory disease that affects ~1% of
the world population [19]. This trial also led to the successful use of the ca2 antibody (now known as Remicade® (infliximab)) in other chronic inflammatory conditions, foremost in Crohn's disease, a form of inflammatory bowel disease [20]. The success of the ca2 antibody has spurred the development of other clinically useful anti-TNF agents, notably the human p75 TNF receptor–IgG fusion protein, Enbrel® (etanercept) [21], and the human anti-TNF monoclonal antibody, Humira® (adalimumab) [22]. Another cytokine inhibitor that has been approved recently for therapeutic use in RA is the soluble IL-1 receptor antagonist, Kineret® (anakinra), which prevents active IL-1 binding to its receptor [23]. Other cytokines that have been targeted in clinical trials by either monoclonal antibodies or soluble receptor constructs include IL-4, IL-5, IL-6, IL-8, IL-12 and IL-15, but it is not known if any of these cytokine inhibitors will satisfy licensing requirements.

In this article we review briefly the therapeutic development of IFNs, particularly IFN-β, as an example of a successful cytokine therapeutic. We also review the development of anti-TNF agents, notably the chimeric antibody, Remicade®, (infliximab), to which we have contributed personally, as an example of an anti-cytokine therapeutic that has revolutionized treatment of RA and some other inflammatory disorders.

Cytokines as therapeutics
IFNs and their properties

The IFNs are a group of inducible cytokines that were identified originally on the basis of their antiviral activity [3] and are known to have many other important functions in innate and adaptive immune responses [24,25]. The IFN-α/β (type I) family comprises numerous genes (Table 1). The IFN-γ (type II) family comprises a single gene in mammals [25]. Another recently identified family of IFN-like cytokines, provisionally termed IFN-λ, is related to type I IFNs [26,27].

As with most cytokines, constitutive production of IFNs is either undetectable or very low. IFN-α/β proteins are inducible in several cells (including specialized dendritic cells, monocytes and macrophages) by viruses, microbial products, double-stranded RNA and some other substances. IFN-γ is produced mainly by T cells and natural killer cells in response to several activating stimuli. Members of the IFN-α/β family, IFN-γ and the recently identified IFN-λ bind to distinct heterodimeric receptors (Table 1).

All IFNs activate the Janus-activated kinase (JAK)–signal transducers and activators of transcription (STAT) signaling pathway [28], which leads to transcriptional activation of genes that contain either an IFN-stimulated response element or an IFN-γ-activated sequence [24,25]. The signaling pathways and the target genes that are activated by IFN-α/β, IFN-γ and IFN-λ overlap partially, so all IFNs share some biological activities. However, the different IFNs also have non-overlapping functions – particularly in the intact organism – because they are produced at different sites, in response to different stimuli and bind to distinct receptors that are expressed differentially.

Clinical uses of IFN-α

Attempts to utilize IFNs therapeutically started in the 1960s but because the IFN preparations available at that time had low potency no clear conclusions about efficacy could be drawn. In the late 1960s and the 1970s, most clinical trials employed IFN preparations that were produced in human peripheral blood leukocytes in culture using methods developed by Stranger, Cantell and colleagues [29]. In fact, much of the world's supply of IFN at that time originated in Kari Cantell's laboratory in Helsinki, Finland. Preparations of natural IFN from human leukocytes contain a mixture of numerous IFN-α subspecies and a small amount of IFN-β. Encouraging results were obtained with this material in clinical trials in some virus infections. However, when animal studies in Gresser's laboratory demonstrated that IFN thwarted some transplantable tumors [30], the focus shifted gradually to the use of IFN in malignancies. Stranger and colleagues in Sweden used leukocyte IFN as an adjuvant therapy in patients with osteosarcoma [31]. The initial, open-label trial indicated efficacy, but the promising findings were not confirmed in controlled trials. Natural human leukocyte IFN was used in many other conditions, but because most of the trials utilized small numbers of patients, few definitive results were generated. Nevertheless, these clinical studies were valuable because they served as the basis for extensive clinical trials with recombinant IFN-α in the 1980s. Today, recombinant

Table 1. Classification and major features of the IFNs

| IFN-α/β family (Type I IFN) | IFN-γ (Type II IFN) | IFN-λ
<table>
<thead>
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<tbody>
<tr>
<td>Genes and proteins</td>
<td></td>
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</tbody>
</table>
| IFN-α (at least 12 functional genes in humans) | IFN-γ | IFN-λ1 (IL-28A)
| IFN-β                        |                     | IFN-λ2 (IL-29B)
| IFN-α                        |                     | IFN-λ3 (IL-29)
| IFN-κ                        |                     |      |
| IFN-τ (only in ruminants)    |                     |      |
| Limitin (identified only in mice) |              |      |
| Location of structural genes |                     |      |
| Chromosome 9 (human)         | Chromosome 12 (human) | Chromosome 19 (human) |
| Chromosome 4 (mouse)         | Chromosome 10 (mouse) |      |
| Receptors                    | Heterodimer of IFNAR1 and IFNAR2 | Heterodimer of IFNGR1 and IFNGR2 | Heterodimer of IFNAR1 and IL-10R2 |

*Abbreviations: IL, interleukin; IFN, interferon; IFNAR1, interferon alpha receptor 1; IFNGR1, interferon gamma receptor 1.

To date, identified only in humans.
IFN-α is approved for several conditions (Table 2) and is used most extensively for the treatment of chronic hepatitis C, often in combination with the nucleoside analog ribavirin.

**Clinical uses of IFN-β**

IFN-β is encoded by a single gene in humans and most other mammalian species (Table 1). IFN-β was initially called fibroblast IFN because it is the predominant IFN produced in human fibroblasts. There is ~30% sequence homology between IFN-β and the IFN-α proteins. IFN-β binds to the same receptor as other members of the IFN-α family. However, the ‘fit’ between IFN-β and the receptor appears to be somewhat different from that of IFN-α subtypes and the same receptor, which results in subtle differences in their actions. In addition, IFN-β is N-glycosylated at a single site, whereas the majority of IFN-α species are not. Finally, IFN-β and IFN-α have different pharmacokinetic properties, with IFN-β being more prone to bind to tissues and less likely to be detected in plasma.

Natural IFN-β, which is produced in culture by human fibroblasts stimulated with the double-stranded RNA poly(I):poly(C) in the presence of metabolic inhibitors [32], is licensed to treat some virus infections in Japan and Germany. However, after the cloning of IFN-β cDNA by Tada Taniguchi [33], the use of natural IFN-β has been largely supplanted by recombinant human IFN-β. In most countries, the only approved clinical application of recombinant IFN-β is for the treatment of patients with relapsing multiple sclerosis (MS), in which it reduces the frequency of clinical exacerbations and slows the accumulation of physical disability. Some other potential clinical applications of recombinant IFN-β are being explored, including RA [34].

The first therapeutic trial in MS by Jacobs and colleagues administered natural human IFN-β intrathecally by lumbar puncture [35]. The main rationale for using IFN-β was the belief that MS might have a viral etiology and that inhibition of the putative etiologic agent might be beneficial. Treated patients experienced a lower rate of exacerbations than untreated patients and the beneficial effect of intrathecally administered IFN-β was confirmed in a double-blind, controlled trial [36]. More recent studies have shown that administration of IFN-β by the much simpler subcutaneous and intramuscular routes is also effective in MS.

One type of recombinant IFN-β that reduces the exacerbation rate in MS is a modified, genetically engineered protein in which a serine at position 17 is replaced with cysteine.

<table>
<thead>
<tr>
<th>Interferon (IFN)</th>
<th>Brand name (generic name)</th>
<th>Approved applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α2α</td>
<td>Roferonα (interferon alfa-2a)</td>
<td>Hairy cell leukemia, malignant melanoma, condylomata accuminata, AIDS-related Kaposi’s sarcoma, chronic hepatitis B, chronic hepatitis C, follicular (non-Hodgkin’s) lymphoma, chronic myelogenous leukemia</td>
</tr>
<tr>
<td>IFN-α2β</td>
<td>Intron A (interferon alfa-2b)</td>
<td></td>
</tr>
<tr>
<td>IFN-α2α, pegylated</td>
<td>Pegasisα (peginterferon alfa-2a)</td>
<td>Chronic hepatitis C</td>
</tr>
<tr>
<td>IFN-α2β, pegylated</td>
<td>PEG-Intron (peginterferon alfa-2b)</td>
<td>Relapsing forms of multiple sclerosis</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Avonexα (interferon beta-1a), Rebifα (interferon beta-1a)</td>
<td>Chronic granulomatous disease, severe, malignant osteopetrosis</td>
</tr>
</tbody>
</table>

*Clinical applications approved in the USA.

1 The sequences of IFN-α2α and IFN-α2β differ by one amino acid.

2 IFN-α is often used in combination with ribavirin for the treatment of chronic hepatitis C.

3 Pegasisα is a covalent conjugate of recombinant interferon alfa-2a with bis-monomethoxypolyethylene glycol (PEG).

4 PEG-Intronα is a covalent conjugate of recombinant interferon alfa-2b with monomethoxypolyethylene glycol (PEG).

5 A modified, genetically engineered form of human IFN-β in which the serine at position 17 is replaced with cysteine.
some pro-inflammatory responses, such as IL-12, chemokines and their receptors. Immunomodulatory activities of IFN-β that might mediate its therapeutic effects in MS are discussed more extensively elsewhere [42,43].

**IFN-γ, colony stimulating factors and IL-2**

Recombinant IFN-γ has been tried in several conditions, but its licensed application is limited to two rare diseases (Table 2). Several hematopoietic growth factors, better known as colony stimulating factors (CSFs), are employed widely in medicine. CSFs are cytokines that stimulate the proliferation, differentiation commitment and some end-cell functional activation in hematopoietic cells [44,45]. The CSFs that are licensed for clinical application are granulocyte CSF (G-CSF), granulocyte–macrophage CSF (GM-CSF) and erythropoietin (Table 3). Recombinant IL-2 [Proleukin® (aldesleukin)] is licensed for the treatment of metastatic renal cancer and metastatic melanoma, and is also used experimentally in AIDS patients to increase CD4+ cell counts [46]. In addition, numerous other cytokines, including IL-10, IL-11 and IL-12, have been used experimentally in animals and in patients, but none are licensed for therapeutic application.

**Cytokines as targets of therapeutics**

*Development and preclinical studies of the monoclonal antibody A2/cA2*

The murine anti-human TNF monoclonal antibody A2 was generated at New York University School of Medicine in 1989–1990 (J. Le and J. Vilček, unpublished), but the origins of this project can be traced to the early 1980s when the main research focus was on IFN-γ [47]. Before recombinant DNA technology became available for the production of human IFN-γ, this cytokine was produced laboriously in cultures of mononuclear leukocytes derived from donated human blood. When stimulated with concanavalin A, phorbol ester and other mitogens, mononuclear leukocytes produce IFN-γ and a plethora of other cytokines, including TNF and lymphotoxin-α [47,48]. This work inspired a long-lasting, fruitful collaboration between the Vilček laboratory and the biotechnology company Centocor (http://www.centocor.com). The collaboration started in 1984 with the immediate goal of generating monoclonal antibodies to human IFN-γ [49,50]. Another aim was to determine if IFN, lymphotoxin and TNF have roles in autoimmune disorders, and to generate monoclonals to these cytokines that would be useful for diagnostic and other medical applications.

Generation of the murine monoclonal A2 antibody had to await the availability of purified, human, recombinant TNF (Table 4). Initial assays indicated that the A2 antibody neutralized human TNF and bound to it with high affinity and specificity, which indicated that it might have useful therapeutic applications. By then, animal experiments had indicated that TNF is a key mediator in the pathogenesis of bacterial sepsis [14,15], which raised hopes that anti-TNF antibodies would be useful in the management of this intractable condition. Mouse monoclonal antibodies were known to be unsuitable for long-term therapeutic application. To reduce immunogenicity and improve pharmacokinetic properties of A2, the cA2 chimeric antibody was generated in collaboration with scientists at Centocor, followed by preclinical evaluations carried out cooperatively at Centocor and New York University [51,52]. The variable region of the original mouse antibody accounts for ~30% of the cA2 antibody, and human IgG1 sequences form the rest.

A Phase I/II trial in patients with septicemia was initiated by Centocor in 1991 (Table 4). Although no significant benefit was demonstrated, this trial provided evidence that cA2 in a single dose of up to 10 mg kg⁻¹ was not associated with adverse events. This data helped in the design of subsequent successful clinical trials in RA and, later, in Crohn’s disease and other conditions. Centocor, Johnson & Johnson (http://www.jnj.com/home.htm), Schering-Plough (http://www.sch-plough.com) and Tanabe (http://www.tanabe.co.jp/english/) now market cA2 as Remicade® (infliximab) (Table 5).

**Rationale for using TNF-blocking agents in RA**

In the 1980s several groups started to examine the possible role of cytokines in RA. IL-1 was the first cytokine detected by bioassay in synovial fluid of patients with RA [53],

### Table 3. Clinical uses of colony stimulating factors

<table>
<thead>
<tr>
<th>CSF</th>
<th>Brand name (generic name)</th>
<th>Approved applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoietin</td>
<td>Epogen® (epoetin alfa), Procrin® (epoetin alfa), Aranesp® (darbepoetin alfa)</td>
<td>Anemia in chronic renal failure; anemia caused by chemotherapy; reduction of allogeneic blood transfusion in surgery patients; anemia in zidovudine-treated, HIV-infected patients</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Neupogen® (filgrastim), Neulasta® (pegfilgrastim)</td>
<td>Cancer patients receiving myelosuppressive chemotherapy; patients with acute myeloid leukemia receiving either induction or consolidation chemotherapy; cancer patients receiving bone marrow transplant; patients undergoing peripheral-blood-progenitor-cell collection and therapy; patients with severe chronic neutropenia Following induction chemotherapy in older adult patients with acute myelogenous leukemia; in mobilization and following transplantation of autologous peripheral-blood-progenitor cells; to accelerate myeloid recovery after autologous bone-marrow transplantation; to accelerate myeloid recovery after allogeneic bone-marrow transplantation</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Leukine® (sargramostim)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CSF, colony stimulating factor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte–macrophage colony stimulating factor. Clinical applications currently approved in the USA. Differs from epoetin alfa by the addition of two N-glycosylation sites that result in increased carbohydrate content and higher molecular weight. Aranesp® is currently approved only for the first two approved applications. Neulasta® is a covalent conjugate of recombinant methionyl human G-CSF (filgrastim) and monomethoxypolyethylene glycol (PEG). It is currently approved only for patients with non-myeloid malignancies who are receiving myelosuppressive anti-cancer drugs and exhibit a clinically significant incidence of febrile neutropenia.
followed by the demonstration of TNF, IL-6, IL-2, GM-CSF and other, chiefly pro-inflammatory, cytokines in cultures of RA synovium [54]. The expression of so many cytokines in the RA synovium raised a dilemma. Could a single cytokine be an effective therapeutic target, or would the blockade of one cytokine be of little consequence because the remaining pro-inflammatory cytokines would continue to drive inflammation?

Rheumatoid synovial cell cultures provided a simple in vitro model for exploring the details of cytokine expression in a disease context [54,55]. Such cultures contain a complex mixture of cells, the most abundant of which are T cells and macrophages. In rheumatoid synovial cell cultures, mRNA that encodes IL-1 and other cytokines was detectable for several days, which contrasts with the transient expression of cytokines during physiological responses. There were indications that IL-1 mediated joint damage in animal models, and that IL-1 was regulated by TNF [56], GM-CSF and immune complexes [57].

Fionula Brennan used cultures of synovial cells to demonstrate that in rheumatoid but not osteoarthritic cells, blockade of TNF by a specific antiserum abrogated the production of IL-1 within 3 days [58]. This finding stimulated investigations of the effects of blocking TNF on other pro-inflammatory cytokines, such as GM-CSF [59], IL-6 and IL-8 [60]. This work led to the concept of the TNF-dependent cytokine cascade in which TNF is a prime mover that coordinates the generation of multiple cytokines in a cytokine cascade. Like most concepts in biology, this is an oversimplification, but it was an important step toward the clinical evaluation of anti-TNF therapy [61,62]. Several groups revealed that expression of TNF and TNF receptors is upregulated in RA synovium. In some cases, they showed TNF expression in situ, in tissues of rheumatoid joints [63–66], thus supporting the concept that TNF might be a suitable therapeutic target.

Animal models provided further evidence of the importance of TNF in RA. In collagen-induced arthritis, a model whose histological features resemble human RA, treatment with a monoclonal antibody against mouse TNF (generously donated by Robert Schreiber) reduced disease severity [67], the cellular infiltrate in the joints and joint destruction. Two other groups demonstrated beneficial effects of TNF blockade in collagen-induced arthritis at about the same time [68,69]. Independently, George Kollias and colleagues reported that overexpression of human TNF in transgenic mice results in spontaneous onset of arthritis that can be prevented by administration of monoclonal antibody to human TNF [70].

Clinical trials in RA

Because of the uncertainty about the safety of TNF blockade, the ca2 antibody was used first in arthritis patients in which all available therapies had failed. The dose administered was derived by extrapolation from the therapeutic effects obtained in the mouse model [67], and safety data from the sepsis trial and volunteers. To maximize the chance of success, a high dose of 20 mg kg−1 was given in 2–4 infusions over a 2-week period. Because the efficacy and safety were unpredictable, a double-blind, randomized protocol was inappropriate.

Table 5. TNF-blocking agents licensed for clinical applicationa,b

<table>
<thead>
<tr>
<th>Year</th>
<th>Progress</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>Polyclonal antibody to TNF shown to protect mice from bacterial sepsis</td>
<td>[14]</td>
</tr>
<tr>
<td>1989</td>
<td>Anti-TNF antibody shown to abrogate IL-1 production in synovial cell cultures from RA joints</td>
<td>[58]</td>
</tr>
<tr>
<td>1990–1991</td>
<td>Generation of chimeric anti-TNF monoclonal antibody ca2 from A2 and its preclinical development at Centocor and NYU</td>
<td>[51,52]</td>
</tr>
<tr>
<td>1990–1992</td>
<td>Anti-TNF antibodies shown to be effective in mouse models of arthritis</td>
<td>[67–70]</td>
</tr>
<tr>
<td>1991–1992</td>
<td>Clinical trial with ca2 antibody in sepsis patients sponsored by Centocor; no significant benefit seen</td>
<td>c</td>
</tr>
<tr>
<td>1992</td>
<td>ca2 antibody found to be effective in small, open-label clinical trial in RA at Charing Cross Hospital</td>
<td>[19]</td>
</tr>
<tr>
<td>1993</td>
<td>Formal proof of efficacy of ca2 antibody in RA in a placebo-controlled study</td>
<td>[71]</td>
</tr>
<tr>
<td>1993–1998</td>
<td>Phase I/II trials of ca2 in Crohn’s disease, which led to FDA approval of Remicade® (infliximab) in 1998</td>
<td>[20]</td>
</tr>
<tr>
<td>1993–1998</td>
<td>Clinical trials of the p75 TNF receptor–IgG fusion protein [Enbrel® (etanercept)] in RA, which led to FDA approval in 1998</td>
<td>[21]</td>
</tr>
<tr>
<td>1993–1999</td>
<td>Phase II/III trials of ca2 in RA, which led to FDA approval of Remicade® in 1999</td>
<td>[74–76]</td>
</tr>
</tbody>
</table>

aAbbreviations: FDA, Food and Drug Administration; IL, interleukin; NYU, New York University; RA, rheumatoid arthritis; TNF, tumor necrosis factor.
bJ. Le and J. Vlcek, unpublished.
cUnpublished.

d Approved in either USA or Europe, or both.
The clinical results of the open study [19] were dramatic (Figure 1). There was a strong symptomatic response with relief of stiffness, pain, and reduced swelling and tenderness. Blood tests demonstrated that the inflammatory markers C-reactive protein and IL-6 were reduced markedly within 1 week of initiating therapy (as was rheumatoid factor after 8 weeks), verifying what had been indicated subjectively by changes in disease symptoms and signs. The response to the 2-week course of treatment averaged 13 weeks before relapse.

The first open trial of 20 patients was performed solely with Ravinder Maini and Marc Feldmann as principal investigators at Charing Cross Hospital in London [19], but the formal proof-of-principle, a double-blind, randomized, placebo-controlled trial, was performed in four European centers, with two doses of cA2 (1 mg kg\(^{-1}\) and 10 mg kg\(^{-1}\)) compared to placebo [71]. The therapeutic response, measured by the validated Paulus criteria, were clear-cut; 8% of placebo-treated patients met the criteria, compared with 44% given 1 mg kg\(^{-1}\) cA2 (\(P = 0.0083\)) and 79% given 10 mg kg\(^{-1}\) cA2 (\(P < 0.0001\)).

Whereas the 4-week, randomized, double-blind, placebo-controlled trial provided the formal proof-of-principle, for the patients the relief was short-lived because only a single dose was administered and all patients relapsed. A key, unanswered question was whether patients could be retreated with cA2. There were concerns that cA2 might be too immunogenic and that, in the absence of TNF, another pro-inflammatory cytokine would drive disease activity. The first indication that a long-term benefit might be achievable came from the retreatment of eight patients from the first open study, in which a therapeutic benefit of a similar magnitude and duration was achieved upon 3–4 repeated administrations of cA2 [72].

In a subsequent Phase II study, the response to 1 mg kg\(^{-1}\), 3 mg kg\(^{-1}\) and 10 mg kg\(^{-1}\) of cA2, without or with methotrexate (MTX) at a low, fixed dose of 7.5 mg week\(^{-1}\), was compared to MTX alone [73]. This trial helped to establish the routine treatment protocol for Remicade\(^{2}\) and, subsequently, other anti-TNF agents. The trial showed that cA2 at 1 mg kg\(^{-1}\) did not provide a long-term benefit in the absence of MTX, whereas 3 mg kg\(^{-1}\) and 10 mg kg\(^{-1}\) were effective. However, at all dose levels, cA2 was more effective when administered with MTX. The mechanism of the synergistic action of cA2 and MTX is not understood fully, but higher blood levels of cA2 were detected in patients given MTX and the anti-idiotype antibody response was also reduced strongly [74]. Therefore, the subsequent Phase III study (the ‘ATTRACT’ trial) of cA2 was performed in RA patients treated concomitantly with MTX. This trial, in a population of long-term RA patients with moderate to severe disease activity despite prior treatment with multiple disease-modifying agents, provided convincing evidence for the benefit of anti-TNF therapy. The large-scale introduction of Remicade\(^{2}\) into clinical practice has confirmed its effectiveness [75, 76], including radiological evidence of improvement after 1 and 2 years. Yet to be understood is why apparently only 1/3 of patients show healing of joint damage, and why ~30% of patients fail to show significant improvement as defined by the American College of Rheumatology criteria, even though some in the latter group show radiological evidence of joint protection.

A role for TNF in protective immunity against infections has been established in experimental models [77–79]. Hence an increased risk of infections was anticipated with anti-TNF therapy. In clinical trials, the incidence of sinusitis and infections requiring antibiotics was increased marginally but serious infections did not increase in frequency compared to MTX-treated controls. Post-marketing reports of tuberculosis, histoplasmosis, listeriosis, coccidiomycosis, pneumocystis and other bacterial infections leave little doubt of the small but definite

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**Figure 1.** Results of an open-label clinical trial of the chimeric anti-tumor necrosis factor monoclonal antibody cA2 in 20 rheumatoid arthritis patients. (a) During washout, patients were not treated with disease-modifying drugs for 4 weeks. cA2 antibody was administered at 0 and 2 weeks following washout. Administration of cA2 antibody resulted in a rapid and dramatic improvement in the clinical signs of disease, as evidenced by a decrease in swollen joint count (b) (determined by palpation of 28 joints; thus, the maximum count is 28), and in laboratory markers of inflammation, as evidenced by decreased levels of C-reactive protein (c) (levels are in mg per liter (normal = 0–10)). Short horizontal lines are median values. ‘Screen’ is the period when patients were examined before the initiation of therapy. Adapted, with permission, from Wiley-Liss [19].
increased risk of some infections. Exclusion of susceptible populations, screening for latent infections and their eradication before therapy, and close monitoring of patients under treatment, allow adequate management of the risk of infection [76,80,81]. Current evidence indicates that the benefits of anti-TNF therapy outweigh risks in the patient population for whom this therapeutic option is indicated [76,81].

**Mechanism of therapeutic action and future prospects**

Several studies indicate that the ‘cytokine cascade’, which was defined originally in studies of cultured RA synoviocytes, also operates in intact organisms. Thus, anti-TNF-induced reductions have been noted in levels of IL-6 [82], IL-1 [83], VEGF [84], IL-8 and other chemokines [85]. Immunohistological studies document that anti-TNF-treatment reduces the expression of adhesion molecules, cellularity [86], angiogenic factors and angiogenesis [84,87]. In an important study, Peter Taylor showed that the influx of radiolabeled granulocytes was reduced by ~50% in the joints 2 weeks after a single dose of Remicade® [85]. Because other leukocytes possess similar chemokines and adhesion molecules as granulocytes, this study indicates that reduced recruitment of leukocytes to joints plays an important part in the benefit of anti-TNF therapy.

In view of our personal involvement, in this review we have focused on the development of cA2-Remicade® as a therapeutic for RA. Remicade® was the first TNF antagonist to be used in patients and shown to be effective in RA. Two other TNF-blocking agents (Enbrel® and Humira®) are now approved for use in RA and several other inflammatory disorders in many countries (Table 5), with other approvals, such as for psoriasis, expected soon. Positive results in glomerulonephritis, amyloidosis, ulcerative colitis, vasculitis and other conditions indicate additional, future indications. With sales of TNF inhibitors in 2003 expected to reach nearly US $4 billion, and with close to a million patients treated to date, this class of therapeutics is already of substantial medical and pharmaceutical significance.

In the future, anti-TNF therapy is unlikely to be limited to the three agents that are licensed currently. Additional agents are already in clinical trials, including a pegylated, soluble, TNF-receptor construct and a pegylated, monoclonal antibody Fab fragment. Anticytokine therapy is also unlikely to be limited to antagonists of TNF and the IL-1 receptor antagonist, Kinere® (anakinra) [23], because other cytokines are being targeted in animal experiments and clinical trials, including IFN-α, IFN-γ, IL-4, IL-6, IL-8, IL-12, IL-15, IL-17 and IL-23.

**Concluding remarks**

Paul Ehrlich, the father of chemotherapy, said that success in medical research and therapeutic drug development requires four ‘G’s. These are: Geduld (patience); Glück (luck); Geschick (skill); and Geld (money). We agree with Paul Ehrlich, but in our experience a fifth ‘G’ is equally important, namely good working relations among investigators that participate in the many stages of research that are required for successful laboratory and clinical development, and particularly the existence of harmonious relations between scientists in academia and in the biotechnology and pharmaceutical industry. Remicade® (infliximab) would not exist if we had not been able to convince the management and scientists at Centocor, first, to invest in the development of an anti-TNF antibody for clinical application and, second, to support what appeared to be a risk-laden clinical trial in patients with RA. Had we not succeeded, it is likely that the development of other anti-TNF agents would not have happened or, at least, have been delayed significantly. We succeeded because of the open-mindedness of our colleagues at Centocor and because we earned their trust through good personal relations. On the basis of our ‘historical experience’, one piece of advice we can give to colleagues who are considering pursuing translational research aimed at drug development is: ‘Do not underestimate the importance of good personal relations with your colleagues and with your industrial partners’.

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**References**

7 Paul, W.E. ed. (2003) Fundamental Immunology, Lippincott Williams & Wilkins


Sheppard, P. et al. (2003) IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat. Immunol. 4, 63–68


Fontana, A. et al. (1982) Interleukin 1 activity in the synovial fluid of patients with rheumatoid arthritis. Rheumatol. Int. 2, 49–53


68 Piguet, F.P. et al. (1992) Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. Immunology 77, 510–514
71 Elliott, M.J. et al. (1994) Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 344, 1105–1110
72 Elliott, M.J. et al. (1994) Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. Lancet 344, 1125–1127

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