The rationale for the current boom in anti-TNF α treatment. Is there an effective means to define therapeutic targets for drugs that provide all the benefits of anti-TNF α and minimise hazards?

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Abstract

Progress in understanding mechanisms of disease are necessary to usher in major changes in treatment. A new era in rheumatoid arthritis (RA) and related chronic autoimmune/inflammatory diseases is now beginning, with a variety of anti-TNF α treatments licensed for use in both RA and Crohn's disease. The rationale for this new treatment lies in an understanding that cytokines are critical, rate limiting molecules lying at the heart of the chronic autoimmune/inflammatory disease process. This understanding was developed from the critical evaluation of a hypothesis that was proposed linking cytokines, antigen presentation and autoimmunity in 1983. Detailed analysis focusing on the major site of the disease, the rheumatoid synovium was essential to developing indications that blockade of TNF α might be efficacious. This clue was validated using anti-TNF α treatment of an animal model of RA, murine collagen induced arthritis, and by immunohistochemical demonstration of upregulated TNF and TNF-R expression in the synovium. With this three pronged rationale, the authors were able to convince Centocor, Inc, which had developed a chimaeric anti-TNF α antibody for use in sepsis, to work with them to test the concept that TNF α blockade would be beneficial in RA. With the success of that first trial, other companies have subsequently tested their anti-TNF strategies successfully. Current interests extend to understanding the processes that regulate TNF production in the rheumatoid joint. Progress in this area is discussed, using adenoviruses to infect normal macrophages and rheumatoid synovium.

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Cytokines are protein mediators that are intimately involved in all biological processes, including cell growth, differentiation, inflammation, immunity, etc. Their biochemical characterisation began in the 1970s and culminated in the cloning of the cDNAs for interferons α , β , γ , IL2, TNF α , and LT between 1981 and 1984.¹⁻³ This provided new tools to evaluate cytokine expression and regulation in rheumatoid synovium.

Rationale for anti-TNF α treatment (table 1)

We rapidly found that essentially all the cytokines were consistently expressed in RA tissue, although in different quantities, regardless of the stage of disease, or its treatment (reviewed by Feldmann *et al*).⁴ This suggested to us that a key aspect of a chronic disease such as RA might be that, unlike experimentally induced cytokine production (for example, with mitogens or antigen), which is transient, diseased induced cytokine production is continuous. To test this idea, we cultured dissociated rheumatoid synovial cultures without extrinsic stimulation, and found that cytokine production in this in vitro model of RA was indeed spontaneous and prolonged, for over six days.⁵ Moreover, we had discovered a system for asking questions about what drives the continuous endogenous cytokine production in RA synovium.

Our approach to this question, as immunologists, was to use neutralising antibodies. Based on the observations that IL1 induced the destruction of cartilage and bone, we studied the regulation of IL1 in the RA synovial cultures. At least half a dozen signals present in the RA synovium were known to regulate IL1, for example, IFN γ , GM-CSF, TNF α , immune complexes as well as IL1 itself, and so it was a surprise when we found that blocking TNF α abolished IL1 bioactivity (fig 1).⁶ This was our first clue that blocking TNF α might be beneficial in RA, as it would also downregulate the equally pro-inflammatory cytokine, IL1. It led us to investigate the ability of anti-TNF α antibody to downregulate the production of other pro-inflammatory cytokines, and we found that GM-CSF,⁷ IL6, IL8⁸ were also downregulated. This has led us to the concept that the pro-inflammatory cytokines, in a diseased tissue, were not independently regulated, but were coordinately regulated. One of the key coordinators was TNF α , to a lesser extent IL1.

The cytokine cascade concept is illustrated in figure 2, cytokine equilibrium in figure 3.

Before the above studies, based on the presence and overlap in properties of the multitude of pro-inflammatory mediators, commonly termed "redundancy", it was thought that cytokines were poor therapeutic targets, based on the premise that if only one was blocked, the others would be sufficient to maintain the pro-inflammatory activities. Clearly it was imprac-

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Figure 1 Inhibition of TNF α in rheumatoid synovium downregulated IL1: the first clue that TNF α was a therapeutic target. Modified and reprinted with permission from Brennan et al. *Lancet* 1989;ii:244–7.

tical to neutralise all the pro-inflammatory cytokines simultaneously.

To validate the concept that TNF α was a prime therapeutic target in RA, two further types of studies were performed. One was to verify by immunohistology that in flash frozen biopsy specimens of RA synovium, TNF and TNF receptors were upregulated.^{9–11} The second was to evaluate anti-TNF α treatment in an animal model of RA, collagen induced arthritis. We and others found that anti-TNF α reduced joint inflammation and joint destruction, even if given in a therapeutic mode, that is after disease onset.^{12–14}

What regulates TNF α synthesis by macrophages and rheumatoid synovium?

The work we pioneered in the rationale for anti-TNF α treatment and its validation in both open 15 and randomised placebo 16 controlled trials with the chimaeric anti-TNF α antibody of Centocor (cA2), now termed Infliximab or Remicade, has led to multiple confirmatory studies with other antibodies (for example, CDP571, Celltech/Bayer¹⁷) TNF-R Fc fusion proteins (Etanercept, Enbrel p75TNF-R Fc, Immunex/AHP¹⁸ or lenercept, p55 TNF-R Fc, Roche¹⁹), and more recently another antibody D2E7 (Knoll/CAT).²⁰ Thus the principle of TNF as an effective therapeutic target is amply confirmed in clinical studies.

However TNF, as a multifunctional cytokine is needed in small amounts at times for normal

immune function. Hence TNF knockouts are at risk of infection with intracellular micro-organisms such as listeria.²¹ Is this the case in anti-TNF α treated patients? Clinical trial data so far suggest that with both Remicade and Enbrel the risk of infection is not higher than in the placebo treated controls. In theory this may not be the case for patients treated in the longer term. It is conceivable that the mechanism of TNF α production in RA tissue may be different from that operating in the immune response to infections, and if so, it would be an intriguing possibility to block the “pathological” TNF α production in RA, but not “physiological” TNF α in an immune response.

Another important reason for seeking to understand what drives TNF α production in macrophages and RA synovium is the fact that most orally available drugs act on intracellular signalling pathways. A key problem is how to study signalling in normal macrophages. These cells, like most normal cells do not transfect easily. Viruses are adapted to inserting their genes into cells, and getting them expressed, and thus we sought to use viruses to deliver inhibitory genes to macrophages.

By using adenoviruses, we have developed conditions that permit us to infect >95% of normal macrophages.²² The TNF α promoter has numerous NF- κ B sites, and so we used an adenovirus overexpressing I κ B α , a natural inhibitor of NF- κ B to evaluate the role of NF- κ B in macrophage cytokine production. The results were interesting. For this single cytokine, for example, TNF α , the role of NF- κ B varied markedly depending on the stimulus. LPS, PMA and UV light were markedly NF- κ B dependent (that is, blocked), but in contrast zymosan and anti-CD45 induced TNF α were not blocked²³ (fig 4). As NF- κ B has been reported to prevent apoptosis,²⁴ the above results with zymosan and anti-CD45 verify that the mechanism of NF- κ B inhibition of TNF production was not by cell death.²⁴ Of course this has been verified in other ways also, by MTT propidium iodide and dye exclusion assays.^{22–25}

These results indicated that the technique of adenoviral infection was suitable to use on RA synovial tissue, at least for analysing the

Figure 2 Cytokine cascade. By a multitude of blocking experiments with anti-TNF α and IL1 receptor antagonist, it was shown that in rheumatoid synovium both the pro-inflammatory and anti-inflammatory mediators are regulated by TNF α and IL1. Modified and reprinted with permission from Feldmann, Brennan and Maini. *Cell* 1996;85:307–10.

Figure 3 Cytokine disequilibrium. Modified and reprinted with permission from Feldmann, Brennan and Maini. *Cell* 1996;85:307–10.

macrophage TNF α production, which is the major, but not the only component of TNF α production. As we wished to study cytokine production in the RA joint in detail, we first evaluated the infectability of some of the critical subsets of cells. Overall, >90% of cells were infected, assessed using an adenovirus expressing β galactosidase and flow cytometry.²⁵ When subsets of cells were analysed by two colour flow cytometry, the results were interesting. As expected CD14⁺CD3⁻ cells, chiefly macro-

phages were infected >95%, CD14⁻CD3⁺ cells, T lymphocytes were also infected at >80%. This was not predicted by prior reports, which reported the low infectability of T lymphocytes. CD14⁻CD3⁺ cells, chiefly fibroblasts, were as anticipated readily infected.

Having established that RA synovial cell mixtures were infectable by adenovirus, we set out to ascertain if the infection with AdvIkB α would change the cytokine profile produced. The results were clear cut. First to be assessed was TNF α , this was reduced by 70%, mean of five synovial samples (fig 5).²² Subsequently other pro-inflammatory mediators were assessed. The results varied. IL6 production was blocked 90%. However, IL1 production was different being reduced by only 40%, and IL8

Table 1 Rationale for anti-TNF α treatment in rheumatoid arthritis

1 Anti-TNF α downregulates production of IL1 and other pro-inflammatory mediators in rheumatoid synovial cultures. ^{6 7 8}
2 TNF α and TNF-receptors are upregulated in rheumatoid synovium. ^{6 9 10 11}
3 Anti-TNF α antibody ameliorates arthritis in collagen induced arthritis. ^{12 13}

Figure 4 The mechanism of TNF α production in macrophages depends on the stimulus. LPS is NF- κ B dependent, zymosan is not. Modified from Bondeson et al. *J Immunol* 1999;162:2939–45.

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