Anti-TNF therapy from the bench to the clinic: a paradigm of translational research

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Introduction

In the 1980s emerging recombinant DNA and monoclonal antibody technology stimulated research into the molecular concepts of pathogenesis of disease. A number of cytokines were being identified and their biological properties, such as their role in activation, proliferation, and cell death of immune, inflammatory and mesenchymal cells by autocrine and paracrine action in the tissue micro-environment, made a compelling case for their involvement in rheumatoid arthritis (RA). In the mid-80s investigations had focused on the activities of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). For example, it was demonstrated in *in vitro* experiments that IL-1 and TNF-α degraded cartilage in tissue culture and TNF-α stimulated the production of connective tissue degrading collagenase and proinflammatory prostaglandin E2.[1,2]

In 1985, Marc Feldmann and I initiated laboratory investigations into the role of cytokines in RA which culminated in the identification of TNF-α as a therapeutic target and the first proof of concept trials.[3]

Laboratory studies generate a hypothesis

In the initial studies an experimental model was established to reflect the expression and regulation of cytokines in diseased joints by obtaining a mixed population of cells from synovial tissues of RA patients by enzymatic digestion and maintaining the derived mixed population of cells in tissue culture medium. Two significant observations that emerged from this model were that:

- IL-1 and TNF-α were expressed by cultured cells over several days without an exogenous stimulant, suggesting a dysregulated production of cytokines in RA[4]
- adding a neutralising antibody to TNF-α inhibited production of IL-1 and, as shown in subsequent experiments, of IL-6, IL-8 and GM-CSF.[5]

Thus TNF-α appeared to be a master regulator of several potentially important pro-inflammatory cytokines. Furthermore, in synovial and cartilage-pannus junction tissue from rheumatoid joints examined by immunohistochemistry, TNF was expressed predominantly by macrophages in proximity to the two TNF receptor bearing cells, including fibroblastic and lymphoid cells.[6,7] Thus one could envision a tissue micro-architecture environment that would permit biologically important cellular interactions mediated by TNF-α to take place in promoting immune-mediated inflammation and tissue destruction.

The hypothesis that TNF was a candidate molecular therapeutic target was further strengthened by the demonstration *in vivo* of suppression of arthritis and tissue destruction by treating murine collagen-induced arthritis, a model of RA, with a hamster monoclonal and specific antibody directed against TNF-α.[8]

Proof of concept clinical trials and back to the laboratory for mechanism of action studies

In the late 1980s Centocor Inc had developed cA2, a mouse x human chimaeric anti-TNF-α specific neutralising antibody, subsequently known as infliximab (Remicade®) for the treatment of septic shock, and agreed to support a proof concept study in DMARD-recalcitrant RA. In 1992, 20 patients were treated and impressive therapeutic efficacy with excellent tolerability was documented.[9] Continuing studies established pharmacokinetics and the need for repeated therapy.[10,11]

Next in patients with active disease despite prior therapy with low dose methotrexate (MTX) it was demonstrated that the addition of infliximab was more effective in suppressing disease activity than infliximab or MTX plus placebo infusions alone.[12] Subsequently, this surprising finding was confirmed with other anti-TNF drugs etanercept and adalimumab.[13,14] Clinical trials also demonstrated the marked benefit of the concomitant use of MTX and anti-TNF in inhibiting structural damage and restoring quality of life. This approach is now the standard regimen for treating recalcitrant RA.

Subsequent clinical studies have focused on the biological effects of TNF blockade.[15] An early finding was the impressive and rapid reduction in C-reactive protein concentrations associated with a reduction in its inducer IL-6. The reduction in swollen and tender joints was associated on synovial biopsies performed before and after TNF blockade with a reduction in the infiltrating immune-inflammatory cells, adhesion molecules E-selectin and VCAM-1, and angiogenesis. These findings suggested a reduction in cell recruitment into inflamed joints, a hypothesis supported by gamma-camera imaging of joints using 121-indium labelled polymorphonuclear cells before and after therapy. An observed reduction in matrix metalloproteinases may, in part, explain the inhibition of structural damage; other molecular pathways have been proposed for the marked inhibition of bone damage. Normalisation of adaptive immune responses and regulatory cell function is being actively investigated by a number of groups.

Translation into the clinic

Phase 2 and 3 clinical trials progressed quickly and led to the licensing of infliximab and etanercept, and later of adalimumab. Adverse events that have raised concern include the occurrence of re-activation of tuberculosis, serious infections, lymphomas, allergic reactions and the induction of autoimmunity and these mandate careful selection and monitoring of

patients. The evaluation of benefit and harm continues to be investigated in the post-marketing era. In 2002, the National Institute for Health and Clinical Excellence approved the use of anti-TNF drugs for the treatment of RA not responding to two disease modifying drugs. Today, anti-TNF therapy, most frequently added to prior MTX, is a well-established option for aggressive RA. Over one million patients with RA have been exposed to anti-TNF biological drugs worldwide, with long-term benefit in 50–60%. Excellent responses are seen especially in patients with intervention early in the disease course. Treatment indications have extended to psoriasis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease. However, the high cost of treatment limits access and there remains an unmet need.

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Human leukocyte antigen B27

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The year 1973 was quite an eventful one with key important milestones occurring in the political and scientific domains. It was the year when American troops finally withdrew from Vietnam, Pink Floyd released *Dark side of the moon*, and the UK joined the European Economic Community. The first US space station, Skylab, was launched and thalidomide victims finally won compensation from the Distillers drug company.

I was working as a research registrar in rheumatology with Frank Dudley Hart at the Westminster Hospital and, largely due to his encouragement, had developed a clinical and research interest in ankylosing spondylitis (AS) – a specialist area in rheumatic diseases for which Dudley Hart had an international reputation.

At that time there was a burgeoning interest in the major human histocompatibility system (HLA) and the possibility of HLA-linked diseases. The equivalent H2 system in the mouse had already been shown to be linked with immune response genes and reports had appeared in the literature linking autoimmune diseases, such as myasthenia gravis, with specific HLA antigens.[1,2] These reports had interested David James who was working as a consultant haematologist in the blood transfusion laboratories at St Mary Abbott's Hospital. He had a chance conversation with Derrick Brewerton, a consultant in rheumatology and rehabilitation at the Westminster Hospital, and asked him if there were any rheumatic diseases that had a strong familial tendency. Brewerton had the remarkable intuition to reply 'ankylosing spondylitis' and from this serendipitous encounter an exciting research project was born.

I was then running a dedicated AS clinic at the Westminster Hospital and was approached to collaborate in a research project to explore any possible HLA association with the disease. Together with Maeve Caffrey, David James' technician, blood samples were taken from 75 AS patients and 60 first-degree relatives of 25 of them. At that time, the blood samples had to be defibrinated by hand using orange sticks prior to being sent to the lab for HLA analysis.

The results were astonishing in that 96% of patients, but only 4% of the controls, were positive for the HLA-B27 antigen. Moreover, 50% of the patients first-degree relatives