

Meeting report

Advances in Targeted Therapies III, Nassau, Bahamas, 27 April–1 May 2001

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Abstract

This conference was the third in a series focusing on developments in the therapy of rheumatoid arthritis (RA) and other rheumatic conditions with biologicals; in many ways, it was perhaps the best one so far. One strength of the meeting was the mix of scientists from academia and from industry, and of workers in basic science as well as clinical investigators. The risks of inhibition of tumor necrosis factor (TNF) were covered in depth for the first time. A number of putative and actual new targets were presented. An updated consensus document on the use of TNF inhibitors will appear in the near future in the *Annals of Rheumatic Diseases*, authored by Dan Furst *et al.*

Keywords: cytokine biology, cytokine blockade, joint damage, regulatory agents, rheumatic diseases

Setting the stage

We are all excited over the continued and strengthened evidence of success for the inhibition of tumor necrosis factor (TNF) in patients with rheumatoid arthritis (RA). However, much remains to be learnt about mechanisms and potential risks of this approach. George Kollias (Athens, Greece) started the meeting by reporting on his new data pertaining to control of TNF synthesis. As he and his colleagues showed in 1999 [1], the AU-rich element in the TNF gene controls mRNA stability for TNF, and deletion of this element leads to prolonged persistence of TNF after, for example, stimulation with lipopolysaccharide. Tristetraprolin, a product of an immediate-early response gene, binds to the AU-rich element on the TNF gene and helps to destabilize mRNA. Consequently, mutations of the genes for either tristetraprolin or the AU-rich elements leads to impaired abrogation of the TNF formation after, for example, lipopolysaccharide stimulation, resulting in

disease such as arthritis or colitis. IL-10 is protective in these models, and the protection is mediated through influence on the AU-rich element via the p38 mitogen-activated protein (MAP) kinase pathway. These results are interesting also because they point to potential new therapeutic targets.

Matthew Fenton (Boston, MA, USA) then talked on the newly identified Toll-like receptors (TLRs), which we inherited from *Drosophila* and which share intracellular sequences with the IL-1 and IL-18 receptors. Dimerization is needed for receptor signaling, and stress or tissue damage leads to dimerization. Lipopolysaccharide also probably targets TLRs. NF- κ B is involved downstream. Knockout experiments have shown that tubercle bacilli (Tbc) signal through TLR2 and TLR4. TLR4-deficient mice are highly susceptible to tuberculosis, showing the protective importance of this receptor. The intracellular events lead to an

inhibition of NF- κ B like that of lipopolysaccharide, but cellular responses are different. TLR proteins mediate the production of Tbc-induced TNF but not of NO [2].

Endpoints – do we need new ones?

This evening discussion was opened by Claire Bombardier (Toronto, Canada), who stressed the need to learn more about relations between radiographic signs and functional outcome and impairment of quality of life. Ted Pincus (Nashville, TN, USA) stressed the need to characterize (and target) nonresponders, regardless of disease duration, and indicated that he was involved in such a study. Désirée van der Heijde (Maastricht, the Netherlands) emphasized that the OMERACT (outcome measures in rheumatology) core set of variables perform well but that we need measures of the quality of life. She pointed out that the DAS disease activity index is better than the American College of Rheumatology (ACR) response criteria, because it is an index of absolute activity whereas the ACR response measures before/after changes. Lee Simon (Boston, MA, USA) defended the ACR response criteria by pointing out that they were specifically constructed to be useful in trials, which they have proven to be. Responding to Désirée van der Heijde's comment, Marc Hochberg (Baltimore, MD, USA) was of the opinion that the EURO QUAL instrument performs well and is sensitive to change.

New targets for intervention

IL-18 was the theme of the talk by Charles Dinarello (Denver, CO, USA). This cytokine was cloned in 1995 and shown to be a strong inducer of IFN- γ in Th1 lymphocytes. In contrast to IL-1 β , pro-IL-18 shows constitutive expression. The two cytokines share several biologic effects, but IL-18, in contrast to IL-1 (and TNF), does not activate cyclo-oxygenase 2. Knockout experiments have shown that, like IL-1 β , IL-18 needs to be activated through splitting by caspase-1. It also needs to act in concert with IL-12 on Th1 cells for production of IFN- γ . Pro-IL-1 β and pro-IL-18 are cleaved at amino acids 116 and 37, respectively. The receptors for IL-18 and IL-1 are also homologous, and consist of an α and a β chain, which form a high-affinity signaling heterodimer. An IL-18-binding protein has also been isolated, using similar techniques to those involved in the discovery of TNF-binding proteins. It is a decoy receptor that is typically induced by virus infection and inhibits T-cell activation. The normal ratio is 34 molecules of binding protein for each molecule of IL-18 [3]. IL-18 has other actions besides inducing IFN- γ , as has been shown in IFN- γ -null mice. IL-18 causes disease similar to TNF and IL-1, which can be inhibited by IL-18-binding protein.

Ischemia and reperfusion causes upregulation of IL-18 and distorts the balance between IL-18 and its binding protein. All these interesting data point to a possible new

therapeutic approach to anti-inflammatory therapy, involving, for example, caspase-1 inhibition or administration of IL-18-binding protein.

Sander van Deventer (Amsterdam, The Netherlands) reported on developments regarding inhibition of p38 MAP kinase, in particular using the compound named SB203580. The desired effect is diminished synthesis and release of TNF and related cytokines. One caveat, however, is that not all cells react by reduced output; macrophages in one study actually increased their release of TNF [4]. The mice studied also showed lowered resistance to tuberculosis and worsened inflammatory bowel disease. However there are other inhibitors of MAP kinase, including one from Boehringer Ingelheim (Ingelheim, Germany) that prevents phosphorylation and inhibits TNF release in all cells. Another interesting compound is CN1493, which inhibits stress-activated protein kinase and reduces TNF release. This compound is only available for intravenous administration, however. In preliminary tests in patients whose Crohn's disease does not respond to infliximab, it showed efficacy lasting several months. Liver toxicity is reported, and this seems to be a class effect.

TNF- α -converting enzyme is a member of the ADAM (a disintegrin and metalloproteinase) family (ADAM 17) and converts 26-kDa pro-TNF to soluble, 17-kDa active TNF. It is another promising target for inhibition, that was addressed by Robert Newton of Dupont (Wilmington, DE, USA). He mentioned that the inhibitor can be administered orally and can inhibit lipopolysaccharide-stimulated TNF production in human monocytes by 95%. Apparently, processing of TNF- α -converting enzyme is mainly intracellular. In animal models of TNF-induced arthritis, the enzyme's inhibitor was more potent than etanercept, probably due to better cell penetration.

Peter Krammer (Heidelberg Germany) delivered an overview of the two different types of apoptosis and their essential role in the maintenance of health, in tumor growth, in sepsis, and in stroke as studied in a rodent model. This presentation was a real *tour de force*, delivered at express speed by a man who has initiated and been in the forefront of CD95-related research throughout his life. Interested readers are referred to a recent review by his hand [5]. Sentrin expression may lead to impaired apoptosis in RA synovium, and stimulation of apoptosis may therefore be a possible therapeutic target [6].

Lionel Ivashkiv (New York, NY, USA) reviewed cytokine signaling via the Jak/STAT pathways and not only indicated their central role in inflammation and its control but also hinted at possible targets for intervention. One interesting observation was evidence that IL-6 and IL-10 fail to function in macrophages in RA synovitis but not in reactive synovitis. The inhibition is apparently mediated through

MKK/p38 kinases and occurs upstream of STAT. Disturbed regulation of cytokine signaling in RA is clearly another possible target for future interventions.

Inhibition of tumor necrosis factor in conditions other than rheumatoid arthritis

Eric Veys (Ghent, Belgium) presented good results in 21 patients in an open study with infliximab confirmed by a 12-week placebo-controlled study on 40 patients. The three adverse reactions consisted of one case of tuberculosis, one of pyelonephritis, and one of otitis and tooth abscess. Histology for eight patients showed diminished synovial proliferation and vascularity and lower staining for vascular cell adhesion molecule (VCAM)-1, neutrophils, and macrophages (CD68). On the other hand, CD20, plasma cells, and IgG increased.

Philip Mease (Seattle, WA, USA) presented a single-center, placebo-controlled study of etanercept in psoriatic arthritis and psoriasis [7]. Radiographic data were not available, but clinical efficacy was evident.

Peter Greenberg (Stanford, CA, USA) reported experiences in an open phase I/II study of patients suffering from fairly advanced myelodysplastic syndromes, using etanercept (25 mg twice weekly). This was based on observations of increased serum levels of TNF in such patients. Some hematological improvement was likely in five of the patients, whereas four developed infections. The number of blast cells in bone marrow remained unchanged.

Christian Antony (Erlangen, Germany) reported on infliximab treatment of six cases of Still's disease in adults. The observation time was between 3 and 30 months and the clinical impression very favorable: after five infusions of 5 mg/kg, most of the systemic manifestations had disappeared. This is a condition that is difficult to control with conventional treatment. One patient developed a serious infection.

Jochen Sieper (Berlin, Germany) reported on 12 patients with ankylosing spondylitis treated with infliximab without methotrexate and monitored by MRI. The clinical response appeared promising, but he encountered one case of Tbc. Subsequently, a placebo-controlled trial was performed on 70 patients, using the Bath ankylosing spondylitis disease activity index (BASDAI) 50% improvement as endpoint. The outcome showed that 53% of patients on infliximab reached the endpoint, versus only 6% of the patients given the placebo.

Muhamad Hussein (Cleveland, OH, USA) had given etanercept to nine male and five female patients with amyloidosis of the light chain related type, assuming a possible role of TNF in this severe condition. Of the ten patients with heart involvement, two died early in the trial. The only therapy besides etanercept was directed against

heart failure. The median follow-up was 14 months. The study is still not completed, but the open study indicated clear improvement in, e.g., macroglossia and gastrointestinal symptoms in some patients.

Update on safety of cytokine-blocking agents

This session was of special interest, since one criticism of last year's meeting was lack of information on risks involved in connection with TNF inhibition. Martin Röllinghoff (Erlangen, Germany) had previously reported that a leishmaniasis-resistant BALB/c mouse strain lacking both TNF receptor genes was still resistant to infection. The new data with this strain (C57BL/6TNF mutation) showed unexpected fatal disease. This seemed related to uncontrolled dissemination from the primary site of inoculation, which seemed related to an altered microarchitecture in secondary lymphoid organs, with an absence of germinal centers. Interestingly, the animals could be rescued by transplantation with TNF-producing bone marrow cells [8]. It is not clear how these results can be extrapolated to the human situation.

Gregory Harriman (Centocor; Malvern, PA, USA) surveyed serious adverse events from analysis of 1900 infliximab patients and some 200 controls. Three cases of Tbc, eight of sepsis, and one of transient pancytopenia, but no instance of aplastic anemia, had been identified. Other hematological changes were benign neutropenia and lymphocytosis; this is related to altered trafficking and considered to be part of the drug's pharmacodynamic effect. Two patients with pre-existing multiple sclerosis showed worsening of lesions detected by MRI; in one patient treated for Crohn's disease, multiple sclerosis developed *de novo*; and one patient with RA developed Guillain-Barré syndrome. Antinuclear antibodies (ANAs) arose in 16% of the patients, and three cases of transient systemic lupus erythematosus are on file. Malignancies were, if anything, less prevalent than expected. 'Serious' infusion reactions were rare. This account of patients included in trials did not address the several-fold-larger number of patients treated since the drug was licensed and must still be considered to represent a fairly short term. In postmarketing analysis, 55 cases of Tbc have been noted, 11 in North America and 44 in Europe. Of these, 17 were severe – that is, disseminating – and 7 were fatal. The onset was usually after three or fewer infusions, which makes drug causation more likely, but also easier to monitor. Other infections included 10 cases of histoplasmosis, 6 of *Pneumocystis carinii* infection, 6 of listeriosis, and 5 of aspergillosis. Infliximab antibodies developed in 7–8% of the patients, and this immunization doubled the risk of infusion reactions. However, it did not impair clinical effects or radiographic progression.

Wayne Wallis (Immunex; Seattle, WA, USA) presented data from 2500 trial patients and more than 100,000

postmarketing exposures to etanercept. In these patients, eight cases of Tbc (= expected US rate) were observed. Of these, two developed the miliary form. Mortality-related infections accounted for 18% of fatalities, the expected US rate being 15%. Malignancies occurred in 9 patients; the expected rate was 12.7. Definite multiple sclerosis developed in one patient and four further patients showed probable multiple sclerosis. Aplastic anemia had occurred in three patients; the 'expected' prevalence was quoted as two cases. The figure was based on a 5.8- to 7.6-fold increased relative risk in RA.

Immunogenicity to etanercept was observed only in RA patients, and the antibodies were not neutralizing in nature.

Steven Fishkoff (Abbott; Mount Olive, NJ, USA) reported experience from some 2000 patients exposed to adalimumab (= D2E7), corresponding to more than 2,800 patient years. The cases of Tbc observed with this all-human anti-TNF antibody have with one exception occurred between 3 and 8 months. All but one case occurred at doses given in earlier studies, which were distinctly higher than those that are used in ongoing studies. Only one case was observed among 1800 recent patients, 11 of whom were treated with the proposed 'market' dose of 0.25 mg/kg/week. Furthermore, all patients seem to have recovered. Most cases have occurred in endemic areas such as Mexico and Germany. This fairly encouraging presentation was criticized by Charles Dinarello for not providing raw data – "propaganda rather than data".

Carl Edwards (Amgen; Thousand Oaks, CA, USA) recalled a recent experimental study showing that IL-1RA mutation in BALB/c mice caused spontaneous arthritis. Close to 3000 patients have now been exposed within randomized controlled trials. The safety record is said to be better than that for TNF inhibitors. Effects of combined therapy with IL-1 receptor antagonist and a PEGylated TNF-receptor-type-I compound were at least additive in a recent study [9]. Data from human studies with this combined therapy are eagerly awaited. The primary outcome is set at the ambitious level of ACR50 response.

Dr Z Swieterman, FDA (Rockville, MD, USA), could not attend but joined the conference by telephone link. He stressed that safety data for TNF inhibition still are based on short-term exposure. Tbc has now been observed in 70 patients exposed to infliximab and 3 patients on etanercept. Many cases have been extrapulmonary and three were fatal. Demyelinating conditions have been reported in 17 patients, 15 of whom had been exposed to etanercept. It has previously been reported that lernercept also causes worsening of multiple sclerosis [10]. All events were temporally related to exposure, and symptoms resolved partly or completely after the drug was stopped. Two cases of

aplastic anemia and seven of pancytopenia had occurred during treatment with etanercept. The denominator for all these cases was, however, not well defined.

Radiography as outcome measure in rheumatoid arthritis

Désirée van der Heijde (Maastricht, the Netherlands) talked on radiography as an outcome measure and stressed the difficulties of group analysis in a disease as heterogeneous and irregular as RA. Nevertheless, in most therapeutic studies, one finds a correlation between disease activity function and, in most recent studies using validated techniques, between effects on disease activity and on radiographic progression. Correlation between radiographic damage and disability becomes stronger with time. Also, van der Heijde and others find a strong correlation between local activity and local radiographic progression. Therefore, despite its limitations, radiography is still far from obsolete as an outcome measure.

New approaches

Fionula Brennan (London, UK) had posed the question whether it was possible to suppress pathologic TNF selectively. Support for such a possibility was provided from cell-to-cell experiments with glutaraldehyde-treated T-lymphocyte membranes and macrophages, using a method described by J-M Dayer's laboratory in Geneva [11]. Using T-cells, obtained from normal peripheral blood and stimulated with CD3, the macrophages secreted both TNF- α and IL-10. In contrast, T cells recovered from RA synovium, without further stimulation, only induced secretion of TRNF- α and not IL-10. This clearly indicates a new role for pathological T cells to support persistent inflammation. Such T cells perhaps would be an important candidate to be targeted in order to abrogate rheumatoid inflammation upstream from macrophage effector cells.

Satwant Narula (Schering-Plough, Kenilworth, NJ, USA) highlighted efforts in progress to find a suitable chemokine target among the 40 chemokines and 20 chemokine receptors. Cell activation, growth, and development; angiogenesis; and in particular cell trafficking are controlled by chemokines and chemokine receptors. The phenotype of the CCR6-null model [12] shows impaired mucosal immunity. This is characterized by lack of myeloid dendritic cells in the Peyer's patches. Work is now in progress with a specific CCR5 inhibitor (Sh-c) that is in clinical development. CCR5 is a coreceptor for HIV, and individuals homozygous for nonfunctional CCR5 Δ 32 are highly resistant to AIDS, and RA is also probably rare and/or benign in such individuals. Apparent redundancy is intriguingly prominent in the chemokine system, perhaps pointing to recent phylogenetic development [13]. Among the ligands of CCR5 are RANTES, MIP-1 α , and MCP-1, and their activity is abolished by the Schering compound. CCR5 is upregulated on T lymphocytes in RA synovium. Lack of penetra-

tions into CNS and oral administration ($T_{1/2}$ 20 hours) are other attractive features. One problem is that Sh-c is specific for man and rhesus monkeys. Experiments with monkeys in Rijswijk, the Netherlands, showed resistance to collagen-induced arthritis in five treated animals, whereas four of five untreated control animals developed arthritis.

David Close (Roche; Welwyn Garden City, UK) addressed the question of whether there is any future for protease inhibitors. This question is indeed relevant after the many failures in this field, the most recent one being that of Trocade, from the company Dr Close works for. Perhaps the goal of finding the right degree of specificity or potency of inhibition is unattainable. Licensing authorities may offer another obstacle. Even if one could document damage protection, one needs proof that this also is of benefit to patients. This seems to be a field for brave and incurably optimistic investors and investigators.

Dennis Carson (La Jolla, CA, USA) reminded the meeting of DNA's capability as an immune stimulator. It accounts for 50% of the effect in Freund's adjuvant, judging from experiments with DNAase-treated adjuvant and IFN- γ formation as read out. The immune stimulatory effect can be linked to specific immune stimulatory sequences. These are believed to play a role in innate immunity. DNA vaccination shows promise in cancer immunotherapy, allergy, and infectious diseases, and perhaps should not be excluded from the minds of rheumatologists.

Alexandra Kiemer (Munich, Germany) focused on another player, atrial natriuretic peptide, and its potency to inhibit formation of lipopolysaccharide-stimulated inducible NO synthase (iNOS). This is mediated through destabilization of mRNA for iNOS, and the reduced expression of transcription factors NF- κ B and activator protein 1. This author also has interesting evidence that TNF- α and IL-1 β are inhibited by a similar mechanism [14]. Importantly, IL-10 formation is not affected. One additional effect was reduced expression of cytochrome-c oxidase 2, in both murine and human cells. Atrial natriuretic peptide may become of rheumatologic interest as yet another new way of reducing TNF production.

Synovial tissue is rich in angiogenic factors, and inhibition of angiogenesis as a therapeutic principle was the subject of Paul Brenchley (Manchester, UK). Vascular endothelial growth factor (VEGF) was proposed as a putative target, in combination with anti-TNF therapy. VEGF-A, as it is now termed, is formed in neutrophils, and synovial fluid levels correlate with neutrophil counts. TNF- α can induce VEGF formation in mononuclear cells, but on the other hand TNF downregulates VEGF receptors, so its influence on angiogenesis is a double-edged sword [15]. VEGF is genetically polymorphic and the genes are located close to HLA on chromosome 6. Recent work indicates an association

of 1 of some 15 polymorphic variants with RA [15]. Heparanase, which is found in rheumatoid synovium, liberates VEGF from extracellular matrix. Nonsteroidal anti-inflammatory drugs, bucillamine, and dexamethasone have been shown to inhibit VEGF. Anti-VEGF drugs developed for use in oncology may prove useful in RA.

Alan Wahl (Seattle Genetics, Bothell, WA, USA) presented oncostatin M as a putative therapeutic agent in RA. This proposal is based on evidence that this member of the IL-6 superfamily interacts with various cytokines; the results can be summarized as inducing net anti-inflammatory effects without immune suppression. TIMP (tissue inhibitor of metalloproteinases) is upregulated by oncostatin M. Survival of lethal stimulation with lipopolysaccharide is improved and radiation resistance is augmented in experimental animals. Oncostatin M was also beneficial in experimental allergic encephalitis, a model related to multiple sclerosis.

Ulrich Feige (Amgen; Thousand Oaks, CA, USA) discussed osteoprotegerin (OPG). This is an inhibitor of osteoclast activation and acts antagonistically to its ligand (OPGL, or RANKL). OPG-null mice, not surprisingly, suffer from brittle bones. OPG treatment reduces bone erosions in adjuvant arthritis without influencing inflammation. The literature contains reports of beneficial effects of this inhibitor on osteolytic metastases, and the prospect for rheumatology is that OPG may be an agent that inhibits damage without affecting inflammation.

The final talk, entitled 'Can a pill compete with anticytokine therapy?', was given by Richard Griffiths (Pfizer; Groton, CT, USA). He envisaged three ways in which this could be possible: ease of administration, efficacy-safety profile, and cost. This talk covered a number of issues and developments in several companies, demonstrating the profound impact that the success of TNF inhibition has had on industrial research. Attempts to construct smaller peptides that could replace etanercept or IL-1 receptor antagonist have so far not been successful, although some results have been seen with constructs 16 amino acids in length. TNF- α -converting enzyme is a possible target, and inhibitors of this enzyme, Ro-32-7315 and GI 5402, are in phase I development. A problem with this method of inhibiting the availability of circulating TNF is that the factor will still be present on cell membranes. Also the enzyme may have other functions than releasing TNF, and inhibition could lead to unexpected toxicity. However, beneficial effects have been seen in a model of arthritis in the rat streptococcal cell wall. GI 5402 also inhibited the rise in circulating TNF after injection of lipopolysaccharide into healthy volunteers.

Caspase-1 is a cysteine protease that cleaves pro-IL-1 β and pro-IL-18, and Vertex (Cambridge, MA, USA) has an inhibitor, VX-740, in phase II development. It has a good effect on collagen-induced arthritis. A final example was

inhibition of p38 MAP kinase. This enzyme has important functions both in embryonic development and in adult life, and mutation of the gene is lethal. Nevertheless, several companies have explored p38 inhibitors, and at least one inhibitor, VX-745, is in phase II clinical development. Thus, it can be concluded that several approaches are in progress in an attempt to find small molecules that are competitive with etanercept and infliximab.

Final comment

The organizing committee – Ferdinand Breedveld, Joachim Kalden, and Josef Smolen – are to be congratulated on creating and maintaining this forum for unrestricted discussions and presentations of cutting-edge science in a dynamically developing field. There is plenty of potential for another meeting on Advances in Targeted Therapies. I would be surprised if ATT IV is not already in advanced planning by the troika Breedveld–Kalden–Smolen. Those participating in developing and following this dynamic field are very grateful for their initiative.

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