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TNF is here to stay!

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A recent conference on tumour necrosis factor (TNF) and related molecules highlighted the many recent advances, from intracellular signalling to clinical applications, in our knowledge of this fascinating molecule.*

Why is an international congress on tumour necrosis factor (TNF) still successful and well attended? TNF and related molecules are certainly important to biomedical research. But it is the consistent high quality of the presentations, and the stimulating blend of signalling pathway minutiae, transcriptional regulation, molecular genetics, human disease and effective new therapies, that make these meetings so exceptional. Indeed, at the recent meeting held in Trondheim, all 72 oral presentations and over 100 posters were worth hearing and seeing. This report will highlight several of the areas covered.

Signalling pathways

Our understanding of signalling pathways is certainly more complex than it was at the last international congress on TNF, but it is also more 'real', with physiological conditions being used to confirm (or refute) observations made with techniques such as overexpression of dominant-negative constructs (D. Wallach, Rehovot). For instance, preliminary data presented by Z. Cao (Washington University) on the NIK-knockout mouse indicated that this kinase is not essential for the activation of NF-κB. Instead, the phenotype of the mouse was similar to that reported for the aly/aly (alymphoplasia) mouse (T. Honjo, Kyoto). These data concur with information from Brian Foxwell (London), who reported that a NIK-kinase-dead (dominant-negative) transgene failed to inhibit lipopolysaccharide (LPS)- or TNF-induced cytokine expression in macrophages. Thus, utilization of signalling pathways can be cell specific, and observations in transformed cells may not translate to their primary counterparts.

Fas ligand, TNF and TRAIL signal death through similar pathways (A. Ashkenazi, San Francisco, CA; H. Walczak, Heidelberg), but there is now a 'FLIP' side to Fas signalling, which triggers gene activation pathways and cell proliferation (J. Tschopp, Lausanne). Another aspect of the regulation of TNF signalling involves the TNF convertase enzyme (TACE) (R. Black, Seattle, WA). Following activation of cells and release of TNF, the TACE enzyme is internalized from the membrane.

TLRs and HMG-1

The session on Toll-like receptors (TLRs) indicated that TLR4 acts as the main receptor for LPS (A. Poltrak, Dallas, TX; D. Golenbock, Boston, MA), whereas TLR2 binds bacterial lipoproteins and mediates signalling by *Mycobacterium tuberculosis*. LPS-induced toxic shock is mediated by the high-mobility group 1 (HMG-1) protein that is released by monocytes in response to LPS (K. Tracey, Long Island, NY). Exogenous HMG-1 induces LPS-like lethality in mice, and anti-HMG-1 inhibits endotoxin shock. Tracey also described an interesting link between the neural and inflammatory systems: production of acetylcholine from the vagus nerve is a potent inhibitor of TNF production by macrophages.

Clinical implications

EAE and SLE

There is no doubt that the TNF system is implicated in many different pathologies, such as rheumatoid arthritis, Crohn's disease and diabetes (C. Kollias, Athens; R. Flavell, Yale, CT). One new story this year (G. Kassioutis, Athens) was the importance of TNF in the regulation of autoreactive T cells. In TNF-knockout mice on a resistant genetic background, the onset of experimental allergic encephalomyelitis (EAE) is delayed in response to autoantigen challenge (also found by J. Sedgwick, Palo Alto, CA). Controls go into disease remission, whereas TNF-knockout mice continue to develop disease. If the TNF-knockout mice are backcrossed onto p55 (but not p75) TNFR-knockout mice, this exacerbation does not occur. This suggests that TNF is involved not only in inflammation, but also in regulation of autoreactive T cells, which emerge in the absence of TNF by epitope spreading.

The importance of TNF in tolerance induction is also reflected in the well-recognized observation that DNA autoantibodies develop in a proportion of patients treated with anti-TNF therapies. R. Ettinger (Basel) reported that over 75% of TNF-deficient mice also develop anti-nuclear antibodies by 23 weeks of age. Of interest in this setting may be the role of a newly discovered TNF ligand, zTNF4, and its receptors TACI/BCMA (J. Gross, ZymoGenetics, Seattle, WA). zTNF4 (also known as THANK, BAFF and TALL-1) stimulates B-cell proliferation, especially of autoantibody-producing $B220^+$ B cells. Transgenic mice overexpressing zTNF4 develop a systemic lupus erythematosus (SLE)-like syndrome, and the development of renal disease is prevented in lupus-prone mice treated with a zTNF4–TNFR fusion protein.

TRAPS: a disease caused by mutated TNFR

A human genetic disease that involves the TNF system has recently been identified. D. Kastner (Bethesda, MD) reported on a hereditary periodic fever syndrome (TNFR-associated periodic syndrome, TRAPS) that involves serosal surfaces, synovium and skin, with systemic amyloidosis in some individuals. The disease is caused by mutations in exons 2–4 of the p55 TNFR. Patients have decreased soluble p55 levels and increased membrane p55 TNFR, and may respond to anti-TNF therapy. Of interest is the fact that some of these mutations fall within

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the exon encoding the pre-ligand assembly domain (PLAD) (F. Chan, Bethesda, MD). This novel conserved domain is found on both the p55 and p75 TNFR and mediates pre-ligand receptor assembly and signalling. Conventional wisdom dictates that TNF receptors are trimerized by ligand binding. However, it now seems likely that these receptors (and possibly other types of cytokine receptor) may oligomerize before ligand binding, and that ligand stabilizes the interaction between the receptor subunits.

Anti-TNF therapy

It was clear that anti-TNF therapy had finally come of age with two products (Infliximab, a humanized anti-TNF antibody; and Etanercept, a soluble fusion protein of receptor antagonist and immunoglobulin), which are now licensed for use in rheumatoid arthritis and Crohn's disease. Over 100 000 patients have now been treated with these inhibitors. The new data to emerge from long-term trials with Infliximab showed that treatment prevents ongoing joint destruction (M. Feldmann, London).

The next target for these successful treatments may be chronic heart failure, since myocytes produce TNF following heart injury (failing hearts produce 100–200 pg TNF per mg of tissue) (D. Mann, Houston). This 'maladaptive' response of TNF induces cardiac hypertrophy, matrix remodelling and increased rates of cardiac myocyte apoptosis. Transgenic animals with cardiac-restricted TNF expression develop signs and symptoms of heart failure. In a placebo-controlled randomized trial, the TNF antagonist Etanercept improved ejected volume and decreased left ventricle size by about 15%, whereas hearts of placebo patients declined during the same time.

Cancer

The 'holy grail' of the TNF field has always been a treatment for cancer, and while TNF itself is now a licensed drug, it is currently restricted in its use to treatment of irresectable soft tissue sarcoma by isolated limb perfusion together with high dose melphalan and mild hyperthermia. Future applications may exploit the ability of lower doses of TNF to increase permeability of tumour blood vessels and thus local concentrations of chemotherapy (A. Eggermont, Rotterdam). However, since many tumours express TNF and all tumours express TNFRs, local TNF production may be a bad prognostic sign. Indeed, there is evidence that TNF may regulate the tumour microenvironment in the same way that it does the rheumatoid joint. Furthermore, because TNF-knockout mice are resistant to skin carcinogenesis (F. Balkwill, London), it is possible that TNF blockade will have a role in cancer.

Jeff Browning (Biogen) suggested an alternative way to target tumours by stimulating the LT-β receptor. This receptor is overexpressed in epithelial cells from many solid tumours, and activation with an injected monoclonal antibody [or LIGHT (a recently described member of the TNF superfamily) or LT-β heterodimers] inhibited tumour cell growth *in vitro* and *in vivo*. Intriguingly, ligation of this receptor in experimental tumours induced differentiation of malignant cells to ductal-like structures. As there are high levels of LT-βR in the fetal gut, this might indicate that transformed cells have become sensitive to a programme that was used during development.

Box 1. Key outcomes

- Different cells may use different pathways to activate nuclear factor κB (NF-κB) after tumour necrosis factor (TNF)–TNFR interactions
- Fas may have a FLIP side – death receptor signalling does more than kill
- TACE internalization is another way to regulate TNF
- TNF is important in the control of self-reactive T cells
- A newly discovered TNF ligand, zTNF4, and its receptor, TACI/BCMA, stimulates B-cell proliferation, especially of autoantibody-producing $B220^{+}$ B cells
- Genetic lesions in a TNFR lead to human disease (TRAPS)
- TNFR may be trimerized before ligand binding
- TNF antibodies and receptor antagonists go from strength to strength; new disease applications beckon (TRAPS and heart disease)
- TNF is (finally) a licensed drug for treatment of sarcoma, but should it be called tumour promoting factor?

Concluding remarks

This report highlights just a few presentations from a packed, stimulating and high-quality meeting (Box 1). It would take a whole issue of *Immunology Today* to do the meeting justice. In the absence of this luxury, the reader is directed to the abstracts published in the *Scandinavian Journal of Immunology* 51 (1) 2000, which describe in detail the excellent science that was presented in Norway.

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