

symbionts, highlighting pitfalls in phylogenetic tree construction and arguing that many statements about horizontal gene transfer might be wrong because of artifacts.

Although the genome is an organism's blueprint, the biologist's main interest is to understand proteins, the actual building blocks of life. More and more scientists try to shift into this direction, and following this trend there were three invited, and a large number of contributed, talks focusing on this subject. Douglas Brutlag (Stanford University; <http://www.stanford.edu>) described a new approach that uses robotic motion and roadmap planning techniques for protein folding and ligand docking.

Sarah Teichmann (MRC Laboratory of Molecular Biology, Cambridge; <http://www.mrc-lmb.cam.ac.uk>) investigated principles and types of protein-protein interactions and their evolutionary conservation. Peer Bork (Head of the Comparative Sequence

Analysis Group, EMBL Heidelberg) focused on the analysis of protein interaction networks and described STRING (<http://www.bork.embl-heidelberg.de/STRING/>), a search tool for retrieval of interacting genes or proteins. He warned that there is no solid ground to build on – our knowledge of gene prediction remains provisional. He then reviewed different approaches to infer functional association and pointed out that, to build protein interaction networks, we have to combine results of different methods because no single approach could reliably cover the complete set of interactions. This combination of methods requires careful benchmarking and quantification, because the error rates of these methods might be quite different. Surprisingly, the result of this benchmarking showed that the performance of *in silico* methods (e.g. phylogenetic profiling, the Rosetta Stone approach, gene order comparison) is comparable to wet lab

approaches, an encouraging result for further research.

### Summary

ECCB 2002 was a great success! The conference was well-organized and provided an ideal environment in which to interact with the main European players. The talks and invited lectures were well-balanced, but a surprising trend towards sequence analysis and (less surprising) towards whole genome applications and systems biology was noticeable. We are looking forward to next year's ECCB, which will be held in conjunction with the French National Bioinformatics conference, Journée Ouvertes Biologie Informatique Mathématiques (JOBIM) (27 September–1 October 2003, Paris).

### Reference

- 1 Lengauer, T. and Lenhof, H.P., eds (2002) ECCB 2002 – Proceedings of the European Conference on Computational Biology 2002 in conjunction with the German Conference on Bioinformatics (GCB 2002). Bioinformatics 18, Suppl. 2, Oxford University Press

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# The TNF superfamily is on the TRAIL to BlyS

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The 9th (Biennial) International Congress on TNF-Related Cytokines (30 October–2 November 2002; The Manchester Grand Hyatt, San Diego, CA, USA) covered recent advances in the understanding of these biologically and clinically important molecules. Organized by Carl Ware (La Jolla Institute for Allergy and Immunology; <http://www.liai.org>), this conference series dates back nearly two decades.

The plethora of new discoveries highlighted in 76 presentations over three days testifies to the complexity of the TNF ligand and receptor families and their biological and clinical relevance. The conference further revealed the history of this growing family of proteins by recapitulating scientific progress from clinical trials and therapeutic developments from transgenic mouse models, cell biology, biochemistry and

the genetics/genomics of the tumor necrosis factor (TNF) superfamily.

### Core TNF superfamily members

TNF has been clinically validated as an important driver of both acute and chronic inflammation, therefore, there was a refocus on the role of TNF in the innate immune system toward the effects of TNF on the adaptive immune response and dissection of the dual

ligand (soluble versus membrane-bound TNF)/dual receptor axis.

### *Probing TNF biology*

George Kollias (Institute for Immunology, Biomedical Sciences Research Center; <http://www.fleming.gr>) initiated such presentations by discussing the specific contributions of soluble versus membrane-bound TNF and the p55 (TNFR1) versus p75 (TNFR2) TNF receptors. Using receptor null and TNF transgenic alleles with modified TNF proteins, Kollias presented an argument for a bifurcation role of TNF where the p55 and soluble TNF are primarily involved in pro-inflammatory processes, while membrane-bound TNF and p75 are primarily involved with immune regulation and disease suppression. This implies that selective p55/soluble TNF inhibition could provide the therapeutic benefit, while retaining favorable immunological effects of TNF.

Exploring another aspect of TNF biology, Fionula Brennan (Imperial College London; <http://www.ic.ac.uk>), presented data indicating that macrophage production of TNF elicited by T cells derived from rheumatoid arthritis (RA) synovium is uniquely regulated relative to that induced by other T cell populations, suggesting that more selective TNF inhibitors might be feasible.

### *Lymphotoxins and other ligands*

Several presentations focused on lymphotoxins (LT)- $\alpha\beta$  (previously known as TNF $\beta$ ) in lymphocyte biology and lymph tissue development. Nancy Ruddle (Yale University; <http://www.yale.edu>) discussed data supporting the hypothesis that LT regulates lymphoid organogenesis by affecting the L-selectin ligand PNAd via post-translational modification by a sulfotransferase. Regulation of tertiary lymphoid neogenesis is relevant in RA, type-1 diabetes and other chronic inflammatory or autoimmune conditions.

Similarly, Yang-Xin Fu (University of Chicago; <http://www.uchicago.edu>) dissected the role of LT in airway inflammation and Jeffrey Browning (Biogen; <http://www.biogen.com>) described the efficacy of a LT $\beta$ R-Ig soluble receptor in several animal models of inflammation, along with evidence that these effects are probably a result of, at least in part, the attenuation of the other ligand for LT $\beta$ R, LIGHT. Furthermore, Theresa Banks (La Jolla Institute for Allergy and Immunology) presented evidence for a role for LT and LIGHT as anti-viral ligands in herpes virus infections.

### **Other well-studied TNF superfamily members**

Several of the more recently identified TNF superfamily members received a great deal of attention because of their unique properties. Numerous researchers presented data supporting a role for BAFF (B-cell activating factor, also called BlyS and TALL-1) and its receptors TACI, BCMA and BAFFR, in B-cell survival and autoimmune disease.

Fabienne Mackay (Garvan Institute of Medical Research; <http://www.garvan.org.au>) presented data from transgenic mouse studies indicating that overexpression of BAFF results in a phenotype similar to systemic lupus erythematosus (SLE) as a result of the presence of large numbers of auto-reactive B cells. Pascal Schneider (University of Lausanne; <http://www.unil.ch>) described how this probably occurs by BAFF effects on the anti-apoptotic regulator Bcl2. In addition, Bertrand Huard (Medical University, Geneva; <http://www.unige.ch>), demonstrated that BAFF is expressed by numerous antigen presenting cells, while BAFFR – but not TACI or BCMA – is expressed by T cells and this could represent an autocrine T cell stimulation system.

Both OX40 (CD134) and 4-1BB (CD137) have emerged as TNF superfamily members involved in T cell

stimulation. Michael Croft (La Jolla Institute for Allergy and Immunology) presented data indicating that both of these molecules regulate the survival of T cells that have divided numerous times enabling them to survive the selection process. This is mediated via effects on the anti-apoptotic Bcl2 pathway. These findings implicate OX40 and 4-1BB in lung inflammation, graft versus host disease and multiple sclerosis (MS).

### **Experimental therapeutics derived from the TNF superfamily**

The TNF superfamilies of ligands and receptors have important roles in inflammation oncology, autoimmunity, osteoporosis and diabetes. Experimental therapeutics based on the TNF superfamily were described as having modalities comprising recombinant ligands, soluble receptors, monoclonal antibodies and small molecules. Of note are the therapeutics based on TNF, TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), and the bone attenuators, OPG, GITRL, and DcR3.

### *TNF*

The thorough knowledge of the biology, signaling pathways and strong disease associations of TNF is leading to the development of therapeutic antagonists: recombinant mutant TNF dominant-negative ligand (DN-TNF; Xencor, <http://www.xencor.com>), monoclonal antibody (mAb) against TNF ligand (infliximab, Remicade; Centocor, <http://www.remicade.com>), and soluble TNFRII receptor (etanercept, Enbrel; Immunex, <http://www.enbrel.com>). DN-TNF is in pre-clinical development and represents a novel class of TNF superfamily inhibitors, while Remicade is approved for RA and Enbrel is used for the treatment of RA and inflammatory bowel disease (IBD). In addition to these FDA approved indications, the TNF therapeutic antagonists have potential for the treatment of SLE, MS, ankylosing spondylitis (AS) and diabetes. An

**Table 1. Tumor necrosis factor (TNF) superfamily derived experimental therapeutic agents**

Agent	Modality	Indication	Mechanism of action
TNF/IFN $\gamma$	Soluble recombinant protein	Oncology	Activates apoptosis when locally administered to tumor cells
TNF-mAb (Remicade)	mAb to TNF	RA, SLE, IBD, MS, AS, diabetes	Antagonizes TNF signaling by neutralizing TNF
TNF-RII (Enbrel)	Soluble recombinant receptor -Fc fusion	RA, SLE, IBD, MS, AS, diabetes	Antagonizes TNF signaling by neutralizing TNF
TNF-DN	Soluble recombinant mutant TNF-ligand	RA, SLE, IBD, MS, diabetes	Dominant-negative ligand antagonizes TNF signaling
LT $\beta$ -R	Soluble recombinant LT $\beta$ -R-Fc fusion	RA, SLE, IBD, MS, diabetes	Antagonizes LT $\alpha$ / $\beta_2$ signaling by neutralizing LIGHT
LT $\beta$ -RA	Receptor agonist not specified	Autoimmunity and chronic inflammation	Activation of the BLC/CXCR5 chemokine system
TRAIL	Soluble recombinant ligand	Oncology, MS CNS autoimmunity	Activates apoptosis in tumor cells; anti-inflammatory
TRAIL/ Zoledronate	Recombinant ligand/ bisphosphonate	Osteosarcoma	Synergize to activate apoptosis in tumor cells
TRAIL/ PS-341	Recombinant ligand/ proteasome inhibitor	Oncology	Both activate apoptosis in tumor cells
TRAIL-DR5 mAb	Activating mAb to DR5 receptor	Oncology	Activates apoptosis in tumor cells with little hepatotoxicity
CD40	Soluble recombinant ligand	SS	Corrects defect in endogenous CD40L
CD40-R mAb	Activating mAb to CD40 receptor	Oncology, infectious diseases	Activates T cells co-stimulation
CD40-R mAb	Antagonizing mAb to CD40 receptor	SLE, SS, IBD, RA, EAE, tissue transplantation	Suppresses T cell co-stimulation
OPG	Soluble recombinant OPG-Fc	Bone resorption disorders RA, prevention of arterial calcification	Anti-osteoclastogenesis by neutralizing RANKL
GITRL (Osteostat)	Soluble recombinant ligand	Osteoporosis, bone cancer and metastasis	Interferes with osteoclastogenesis
DcR3	Soluble recombinant receptor	Osteopetrosis	Induces osteoclastogenesis and bone resorption
DcR3	Soluble recombinant receptor	Lung inflammation	Neutralization of FasL in the lung
TWEAK/IFN $\gamma$	Soluble recombinant ligand	Oncology	Induces apoptosis in presence of IFN $\gamma$
TWEAK-R mAb	mAb to receptor Fn14	Oncology	Antagonizes TWEAK signaling and angiogenesis
BAFF-R	Soluble recombinant receptor	SLE, SS, RA	Antagonizes T cell activation by neutralizing BAFF
EDA1	Soluble recombinant ligand	XL-HED	Corrects congenital defect <i>in utero</i>
DMXAA	Small molecule	Oncology	Upregulates TNF, IP-10 and IFNs

The experimental therapeutic agents detailed in the table were those highlighted at the 9th (Biennial) International Congress on TNF-Related Cytokines (further information can be found at: <http://www.liai.org/tnf2002>). Abbreviations: RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; IBD, inflammatory bowel disease; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; AS, ankylosing spondylitis; XL-HED, X-linked hypohidrotic ectodermal dysplasia; mAb, monoclonal antibody.

elegant dissection of the clinical data on anti-TNF therapies was presented by Stephan Targan (Cedars-Sinai; <http://www.csmc.edu>) addressing the underlying biology behind the differential efficacy of these therapies in Crohn's disease (see Table 1 for therapeutic agents highlighted during the conference).

#### TRAIL

Soon after the discovery of TRAIL signaling agonists, much hope was placed on them for their suitability for oncology indications. This was based on recombinant TRAIL ligand inducing apoptosis in many cancer cell lines but not in normal hepatocytes. There has been some controversy over whether

TRAIL causes *in vivo* hepatotoxicity. To avoid hepatotoxicity, a clever approach was presented by Eiji Mori (Kirin Brewery, Takasaki, Japan; <http://www.kirin.co.jp>) using fully human agonizing mAb specifically directed against each of the two TRAIL receptors (TRAIL-R1 and TRAIL-R2). The mAb against TRAIL-R2 induces cancer

cell apoptosis with minimal hepatotoxicity, while the mAb against TRAIL-R1 caused apoptosis in both cancer and normal cells.

Another approach to reducing side effects involves combination therapies consisting of a small molecule drug in addition to TRAIL. Andreas Evdokia (The University of Adelaide; <http://www.adelaide.edu.au>) showed that zoledronic acid, a potent bisphosphonate, enhanced TRAIL cytotoxicity of human osteogenic sarcoma cells without normal cell toxicity. Thomas Sayers (National Cancer Institute at Frederic; <http://nci.nih.gov>) presented another combination therapeutic using the proteasome inhibitor PS-341 to sensitize murine myeloid leukemia and renal cancer cells to TRAIL-mediated apoptosis.

#### OPG, RANKL, GITRL and DcR3

Many presentations and posters collectively demonstrated molecular

links for the role that vascular endothelium has in bone remodeling. In the bone, there is a close physical association between vascular endothelial cells, osteoblasts and osteoclasts. In addition, arterial calcification links bone and vascular physiology. The TNF superfamily members osteostat (GITRL), OPG, RANKL and DcR3 are all expressed in vascular endothelial cells and each is an important modulator of bone remodeling.

Bernadetta Nardelli (HGS, Rockville; <http://www.hgsi.com>) presented a poster showing, for the first time, that recombinant and membrane bound forms of GITRL inhibited resorption of artificial bone discs by preventing the differentiation of osteoclasts. Another first was presented in a poster by Chia-Ron Yang (Taiwan University Hospital; <http://www.mc.nfu.edu.tw>) showing that soluble decoy receptor DcR3 induces osteoclast formation in the murine monocytic cell line RAW264.7.

#### Future directions

Although our understanding of many TNF superfamily ligands and TNFR superfamily receptors has evolved a great deal in the two years since the last conference, many of these ligand/receptor axes need clarification. This is especially true for family members such as CD27/CD27L and CD30/CD30L, which have only recently gained the attention of the community. Undoubtedly, the TNF/TNFR superfamilies will remain fascinating areas of research as we anticipate the presentation of new findings at the 10<sup>th</sup> conference in September 2004 in Montreaux, Switzerland organized by Jurg Tschopp, Institut de Biochimie, University of Lausanne.

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Conference abstracts are available at <http://www.liai.org/tnf2002>

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