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The TNF superfamily in 2009: new pathways, new indications, and new drugs

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Today's most successful class of biologics targets the inflammatory cytokine tumor necrosis factor in autoimmune diseases including rheumatoid arthritis, psoriasis and Crohn's. With five anti-TNF biologics now on the market, attention has turned toward novel strategies to improve the safety and efficacy of next-generation TNF inhibitors. Beyond TNF, drugs are under development that modulate many other ligands and receptors of the TNF superfamily. Biologics targeting at least 16 of the approximately 22 known ligand–receptor pairs are now in clinical development for autoimmune diseases, cancers and osteoporosis. A deeper understanding of intracellular signaling has also facilitated small-molecule interventions, opening the door to oral therapies. This report summarizes recent developments in this highly druggable superfamily, including highlights of the latest international TNF conference.

From the time TNF was cloned and characterized in 1984 [1], a relatively rapid 15 years transpired before the first two drugs rationally designed to target this cytokine reached the market. These biologics (the antibody Fc–decoy receptor fusion Enbrel in 1998, and the chimeric antibody Remicade in 1999), along with the fully human antibody Humira (launched in 2003) have become phenomenally successful blockbuster drugs for inflammatory autoimmune diseases. From the scientific, clinical and commercial perspectives, TNF is undoubtedly one of the major successes of rational drug design, and TNF blockers are now the best-selling class of biologics. The three established anti-TNF biologics (Enbrel, Remicade and Humira) combined for over US$ 16 billion in sales in 2008 [2], and in contrast to other biologic blockbusters such as the erythropoietins, the TNF market is among the fastest growing [3]. Ironically, given the cytokine's initial identification as a tumor necrosis factor, the greatest value has not been TNF agonists in cancers, but antagonists in inflammatory and autoimmune diseases, with seven such indications approved for TNF blockers. By contrast, owing to its extreme toxicity, the approved uses of TNF (Beromun, recombinant human TNF) in oncology have been severely limited to isolated limb perfusion chemotherapy [4]. First-generation nonselective TNF blockers have also failed clinical trials in diseases including multiple sclerosis (MS) and sepsis [5,6], and labels of approved drugs now list both demyelinating disease and sepsis as risk factors. However, a growing understanding of the key roles of different forms of TNF in inflammation beyond the joints and the gut (e.g. in the CNS) is opening up additional indications for next-generation selective TNF blockers, including neurodegenerative diseases such as MS, Alzheimer's and Parkinson's disease.

The TNF superfamily – a rich source of drug targets

Building on TNF as a prototype, the past 20 years have seen the identification and exploration of an entire superfamily of TNF- and TNF receptor-like molecules. All are similar in structure to TNF, with both ligands and receptors functioning as trimers (the vast majority as homotrimers). Receptors are largely membrane-bound signaling molecules (with the occasional soluble decoy receptor), while ligands can be either membrane or soluble forms; often both forms are active.

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2009.10.002

There are roughly 20 ligand–receptor pairings now recognized for the TNF superfamily, with a modest degree of crosstalk (i.e. multiple ligands signaling through one receptor, or a single ligand signaling through multiple receptors) (Fig. 1). TNF itself illustrates such crosstalk, with two ligands (soluble and transmembrane TNF) signaling through two receptors (TNFR1 and TNFR2), with markedly different functional endpoints. This complex group of ligands and receptors has served as a rich source of drug targets not only in inflammation and autoimmunity, but also in oncology and bone diseases. Remarkably, every well-characterized TNF superfamily pathway is now being interrogated in clinical trials of biologic agonists or antagonists (Table 1). Several of these family members may be clinically and commercially validated in the near future, with the RANKL and BAFF pathways perhaps closest to approval, as discussed below.

This cornucopia of drugs and drug targets was discussed at the field's most recent international conference, '*The TNF superfamily and its interactions with other signaling proteins in infection, autoimmunity, cancer and therapy*', held April 26–29, 2009 in El Escorial, Spain. With the robust growth of interest in the superfamily, this meeting (the 12th in a biennial series) has expanded from a small gathering to include roughly 350 attendees, with over 50 from industry. To facilitate coverage of new topics, the organizers ambitiously compressed a staggering ~130 speakers and 110 posters into three long days. The particular strength of a select few companies in this field was evident in the agenda; of the 13 industry talks, five speakers were from Roche/Genentech, with two each from Amgen and Biogen-Idec. As it would be impossible for this short review to do justice to the 14 session topics and 9 roundtable discussions, we have instead attempted to highlight topics with the most relevance to drug discovery. We particularly emphasize promising superfamily targets as well as next-generation approaches to TNF itself, which remains the only clinically validated target in the superfamily, at least for the near future.

Next-generation strategies for TNF blockers

Many years after the first anti-TNF product launches, this cytokine still remains an exceedingly attractive target to drug developers, with two more anti-TNF biologics launched in 2008 and 2009. The first is Cimzia (UCB), a unique anti-TNF biologic approved for Crohn's disease in 2008 and for rheumatoid arthritis in 2009. Consisting of an Fab' fragment of an anti-TNF antibody, Cimzia is the only approved anti-TNF lacking an antibody Fc domain; it is therefore devoid of any Fc receptor-mediated immune effector cell activities. Cimzia is also pegylated for longer serum half-life, binds TNF monovalently rather than divalently, and is produced by *E. coli* rather than by mammalian cell culture. The second new biologic is Simponi (Centocor Ortho Biotech). Like Humira, it is a fully human IgG1κ anti-TNF antibody. Although Simponi and

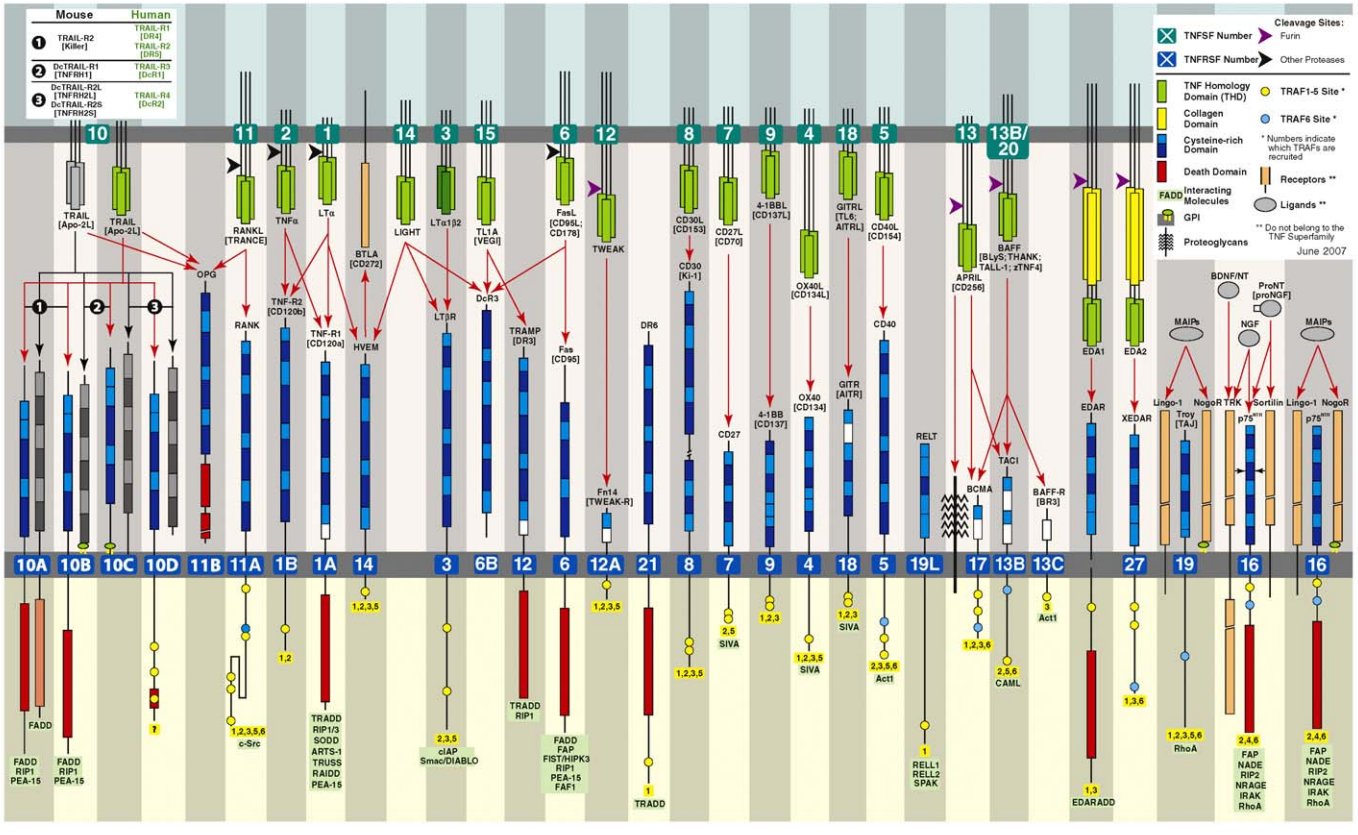

FIGURE 1

Interactions of the TNF superfamily. Top, TNF ligand superfamily (TNFSF), and bottom, TNF receptor superfamily (TNFRSF) members, including common names and standard nomenclature. Arrows on ligands indicate cleavage of membrane-bound forms to release soluble cytokines. Numbering refers to standard nomenclature (www.genenames.org). Reprinted with generous permission from Enzo Life Sciences; detailed figure available online at www.enzolifesciences.com.

TABLE 1

Marketed drugs and selected drugs in development targeting the TNF superfamily[a]

TNFSF ligand	Ligand common names	TNFRSF receptor	Receptor common names	Biologic targeting receptor–ligand interaction	Company	General indications	Status
TNFSF1	Lymphotoxin α/TNFβ	TNFRSF1A	TNFR1	Enbrel (etanercept)	Amgen/Wyeth	I&A[b]	Approved
		TNFRSF1B	TNFR2				
		TNFRSF14	HVEM				
TNFSF2	TNF/TNFα	TNFRSF1A	TNFR1	Remicade (infliximab)	Centocor	I&A	Approved
		TNFRSF1B	TNFR2	Enbrel (etanercept)	Amgen/Wyeth	I&A	Approved
				Humira (adalimumab)	Abbott	I&A	Approved
				Cimzia (certolizumab pegol)	UCB	I&A	Approved
				Symponi (golimumab)	Medarex/Centocor	I&A	Approved
				Beromun (tasonermin)	Boerhinger-Ingelheim	Oncology	Approved
				TNFerade	Genevec	Oncology	Clinical
				TNFα kinoid	Neovacs	I&A	Clinical
				ESBA105	ESBATech	I&A	Clinical
				XPro®1595	Xencor	I&A	Preclinical
TNFSF3	Lymphotoxin α1β2	TNFRSF3	LTβR	Baminercept alfa (LTβR-Ig)	Biogen-Idec	I&A	Clinical
TNFSF4	OX40L	TNFRSF4	OX40/CD134	huMAb OX40L	Genentech	I&A	Clinical
TNFSF5	CD40L/CD154	TNFRSF5	CD40	Dacetuzumab	Seattle Genetics/Genentech	I&A, Oncology	Clinical
				PG102	PanGenetics	I&A	Clinical
				CP-870893	Pfizer	Oncology	Clinical
				HCD122	Xoma/Novartis	Oncology	Clinical
				Seattle Genetics-40	Seattle Genetics	Oncology	Clinical
				XmAb5485	Xencor	Oncology	Preclinical
TNFSF6	FasL	TNFRSF6	Fas	APO010	Topo Target	Oncology	Clinical
		TNFRSF6B	DcR3				
TNFSF7	CD27L/CD70	TNFRSF7	CD27	Seattle Genetics-70	Seattle Genetics	I&A	Clinical
				MDX-1411	Medarex	Oncology	Clinical
				MDX-1203	Medarex	Oncology	Clinical
TNFSF8	CD30L/CD153	TNFRSF8	CD30	XmAb2513	Xencor	Oncology	Clinical
				Seattle Genetics-35	Seattle Genetics	Oncology	Clinical
				MDX-1401	Medarex	Oncology	Clinical
TNFSF9	4-1BBL	TNFRSF9	4-1BB/CD137	BMS-663513 (anti-CD137)	Bristol Myers Squibb	Oncology	Clinical
TNFSF10	TRAIL/Apo2-L	TNFRSF10A	TRAILR1/DR4	Apomab (TRAILR2)	Genentech	Oncology	Clinical
		TNFRSF10B	TRAILR2/DR5	Mapatumumab (TRAILR1)	Human Genome Sciences	Oncology	Clinical
		TNFRSF10C	TRAILR3	Lexatumumab (TRAILR2)	Human Genome Sciences	Oncology	Clinical
		TNFRSF10D	TRAILR4	AMG655 (TRAILR2)	Amgen	Oncology	Clinical
		TNFRSF11B	OPG	Dulanermin (TRAILR1/R2)	Amgen/Genentech	Oncology	Clinical
				LBY-135 (TRAILR2)	Novartis	Oncology	Clinical
				CS-1008 (TRAILR2)	Daiichi Sankyo	Oncology	Clinical
TNFSF11	RANKL	TNFRSF11A	RANK	Denosumab	Amgen	Osteoporosis, oncology	BLA filed
		TNFRSF11B	OPG	AMGN-0007 (Fc-OPG)	Amgen		Clinical
TNFSF12	TWEAK	TNFRSF12A	TWEAKR/Fn14	BIIB023	Biogen-Idec	I&A	Clinical
TNFSF13	APRIL	TNFRSF13B	TACI	Atacicept (TACI-Ig)	Zymogenetics/Merck Serono	I&A	Clinical
		TNFRSF17	BCMA				
TNFSF13B TNFSF20	BAFF/BLys	TNFRSF13B	TACI	Benlysta (anti-BAFF)	Human Genome Sciences	I&A	Clinical
		TNFRSF13C	BAFFR	LY2127399 (anti-BAFF)	Lilly	I&A	Clinical
		TNFRSF17	BCMA	Atacicept (TACI-Ig)	Zymogenetics/Merck Serono	I&A	Clinical
				Briobacept (BR3-Fc)	Genentech/Biogen-Idec	I&A	Clinical
TNFSF14	LIGHT	TNFRSF3	LTβR	Baminercept alfa (LTβR-Ig)	Biogen-Idec	I&A	Clinical
		TNFRSF6B	DcR3				
		TNFRSF14	HVEM				
TNFSF15	VEGI/TL1A	TNFRSF6B	DcR3				
		TNFRSF12	TRAMP/DR3				
TNFSF18	GITRL/AITRL	TNFRSF18	GITR/AITR				
None	EDA1	None	EDAR				
	EDA2	TNFRSF27	XEDAR				

TABLE 1 (*Continued*)

TNFSF ligand	Ligand common names	TNFRSF receptor	Receptor common names	Biologic targeting receptor–ligand interaction	Company	General indications	Status
None	NGF (not TNFSF) proNGF (not TNFSF)	TNFRSF16	NGFR/p75NTR	Tanezumab	Pfizer	Osteoarthritis	
None	Unknown	TNFRSF19	TROY				
None	Unknown	TNFRSF19L	RELT				
None	Unknown	TNFRSF21	DR6				

[a] Drug data from www.clinicaltrials.gov and company web sites (7/09), TNF nomenclature is from the HUGO Gene Nomenclature Committee (www.genenames.org).
[b] I&A = Inflammation and autoimmunity indications.

Humira are reported to have equivalent serum half-lives of approximately two weeks, Simponi is approved for monthly dosing as opposed to the biweekly dosing of Humira. Perhaps owing to such market pressures, Abbott recently conducted a clinical trial of Humira to investigate noninferiority of monthly dosing as compared to biweekly dosing [7], so Humira will potentially soon match Simponi in dosing frequency.

Regardless of differences in structural class, the five marketed anti-TNF drugs are arguably 'me-too' biologics because they all hit the same target, and in general have similar side effect and efficacy profiles (with the notable exception that Enbrel is not effective in Crohn's disease). Most significantly, all anti-TNFs carry black-box warning labels regarding the increased risk of serious infections (such as tuberculosis) associated with the immunosuppressive activity of these drugs. Each drug also carries a warning label regarding an increased risk of CNS demyelinating disorders. Clearly, it would be highly desirable to improve the safety profile of next-generation TNF inhibitors. Toward that goal, several groups at the conference discussed the design of more selective and therefore potentially safer and more efficacious anti-TNF biologics. For example, since the discovery of TNF, it has been recognized that this cytokine is present in both soluble (solTNF) and transmembrane (tmTNF) forms. Much recent work has demonstrated that solTNF (signaling primarily through TNFR1) and tmTNF (signaling through both TNFR1 and TNFR2) have distinctly different, and potentially opposing, functions in inflammation and immunity [8–12]. This new mechanistic understanding has developed through observing inflammation and infection in mice expressing both forms of TNF (i.e., normal wildtype mice), compared to mice lacking both solTNF and tmTNF (total TNF knockouts) and mice lacking solTNF but expressing transmembrane TNF (tmTNF knockins). Such knockout and knockin mice have been widely used to show that tmTNF is crucial in maintaining a normal innate immune response to infections including listeria [8,13,14], leishmania [9] and tuberculosis [10–12]. The crucial role of tmTNF in maintaining resistance to acute tuberculosis infection was presented at the meeting by Nasiema Allie (in the lab of Muazzam Jacobs, Cape Town University) [15,16]. In addition, a similar argument has been made regarding the tolerizing and protective roles for TNF in mouse experimental autoimmune encephalomyelitis (EAE) models of demyelinating disease [8,17–19]. Taken together, results from genetic models suggest that soluble TNF (probably signaling through TNFR1) may be necessary and sufficient to drive inflammation, while by contrast tmTNF (possibly signaling through TNFR2) may be essential to maintain immunity to infections, and to tolerize autoantigens.

Such results in mouse inflammation and infection models support the therapeutic hypothesis that pharmacologic inhibition of soluble TNF is sufficient to suppress inflammation, while the inhibition of tmTNF may suppress immunity to infection. This result may be of clinical relevance, because existing biologics efficiently block both soluble and transmembrane forms of TNF [20,21]. Given the lack of ligand selectivity of the approved TNF blockers, it is not unexpected that the clinical pharmacology of these biologics recapitulates to some extent the genetic phenotype of the solTNF/tmTNF knockout mouse (i.e. suppression of inflammation, immunity and tolerance). Because mouse models show that inhibition of solTNF is anti-inflammatory, while inhibition of tmTNF sensitizes to infection and exacerbates demyelinating diseases, multiple groups are attempting to develop soluble TNF-selective antagonists. For example, Irène Garcia-Gabay (Centre Médical Universitaire, Geneva) presented data showing that a solTNF-selective 'dominant-negative' TNF biologic (XPro®1595) was as anti-inflammatory as Enbrel in a mouse arthritis model [13], without suppressing normal innate immunity to listeriosis or tuberculosis [22]. In addition, Shin-ichi Tsunoda (from the Yasuo Tsutsumi group at the National Institute of Biomedical Innovation, Osaka) described development of selective antagonists of the TNFR1 receptor [23], including 'R1antTNF', a pegylated variant of TNF which blocks TNFR1 signaling but preserves signaling of tmTNF through TNFR2. Tsunoda showed that R1antTNF is as efficacious as Enbrel in a mouse arthritis model, yet safer than Enbrel in mice challenged with adenovirus infection. As further evidence of the protective role of the tmTNF–TNFR2 signaling axis, David Goukassian (Tufts University) showed that TNFR2 is important for myocardium repair after infarction [24], and Ulrich Eisel (University of Groningen) showed the same for the maintenance of CNS neurons [25]. Daniela Männel (from the Joost Oppenheim group at University of Regensburg) also showed that TNFR2 is important in the function of regulatory T cells [26], suggesting a role for tmTNF–TNFR2 signaling in anti-inflammatory effects. Finally, Marcos Milla described Roche Pharmaceuticals' efforts to block soluble TNF release using prodomain-based inhibitors of the TNF Alpha Converting Enzyme (TACE/Adam17) [27]. This small-molecule approach to inhibit TACE cleavage of tmTNF is analogous to a solTNF-selective biologic, as both strategies aim to maintain or amplify tmTNF signaling while blocking inflammation by solTNF.

RANKL inhibition – a promising approach in osteoporosis and cancer

After anti-TNFs, the drug most likely to reach the market targets the RANKL–RANK axis associated with osteoclast-mediated bone resorption. Amgen's antibody Prolia (denosumab), for the treatment and prevention of postmenopausal osteoporosis, is the subject of a BLA submitted to the FDA in December 2008. Amgen and Immunex (before its acquisition by Amgen) have long had an interest in the RANK pathway, and

their previous clinical trials targeted RANKL using the decoy receptor Fc–OPG (an antibody Fc domain fusion with osteoprotegerin, another TNF superfamily member) [28]. The Prolia BLA is the culmination of a large clinical development effort consisting of >11,000 patients in six Phase III trials, and a decision from the FDA is expected in October 2009. Bill Dougall described the rationale for the use of denosumab to prevent bone loss in multiple myeloma, breast and prostate cancers, and showed promising results from recent clinical trials. As Josef Penninger (Institute of Molecular Biotechnology, Vienna) highlighted in his overview talk 'Beyond Bones', the RANK–RANKL pathway appears to be crucially involved in many other processes including lymph node and thymus development, proliferation of mammary epithelium (e.g. in breast cancer), and surprisingly, in regulation of fever.

BAFF and APRIL – potential in SLE and other autoimmune diseases

After the TNF and RANKL pathways, the B cell activating factor BAFF has attracted significant pharmaceutical attention. Even more so than the TNF pathway, BAFF signaling is complicated, consisting of two ligands (BAFF and APRIL) interacting with three receptors (BAFFR, BCMA and TACI). Intriguingly, BAFFR and BCMA appear to be B cell activating receptors, while TACI appears to be an inhibitory receptor. This plurality of receptor functions for the same ligand raises the possibility (as noted with solTNF and tmTNF) that nonselective blockade may lead to opposing consequences. Several interactions among these two ligands and three receptors have been targeted by biologics in clinical development. The most advanced biologic is Benlysta (belimumab, Human Genome Sciences/GSK), an anti-BAFF antibody that blocks BAFF's interactions with both activating receptors BCMA and BAFFR and the inhibitory receptor TACI. Positive results from one of the two large Phase III trials of Benlysta in lupus were announced on July 20; these results were particularly noteworthy because other targeted biologics (e.g. Rituxan, rituximab, an anti-CD20 antibody) have failed in previous large Phase III lupus trials. Little was said about BAFF clinical development at the meeting; however, its coligand APRIL (which shares receptors BCMA and TACI but not BAFFR with BAFF) was the topic of several presentations. For example, Jan Paul Medema (University of Amsterdam) described APRIL as a key modulator of B cell survival, and discussed an anti-APRIL antibody with potential in both B cell lymphomas and in autoimmunity [29].

Lymphotoxin α remains an unvalidated target

The lymphotoxin signaling axis, consisting of LIGHT and LTα1β2 ligands interacting with HVEM and LTβR receptors, has strong support as an inflammatory pathway in animal models [30]. However, efforts to interfere with this pathway in human disease have been disappointing. For example, Jeff Browning discussed Biogen-Idec's termination of clinical development of baminercept alfa, a LTβR–Ig Fc fusion that binds both LIGHT and LTα1β2, in rheumatoid arthritis. As announced by Biogen-Idec in October 2008, no primary or secondary endpoints were met in a large 380 patient Phase II trial. In a complementary approach, Jane Grogan described Genentech's development of anti-LTα antibodies that deplete membrane LTα-expressing Th1 and Th17 cells without blocking LTβR signaling. Grogan also showed that Fc-mediated interactions with Fc receptor-expressing immune cells were crucial to *in vivo* activity in mouse arthritis and EAE models [31], suggesting that Fc-engineered antibodies with enhanced effector functions may be more potent in the clinic than native antibodies.

TRAIL and its death receptors in cancer

Moving beyond inflammatory diseases and osteoporosis targets, the TRAIL (TNF-related apoptosis-inducing ligand) pathway [32] was a major focus of the conference. TRAIL ligand interacts with death receptors DR4 (TRAILR1) and DR5 (TRAILR2) to stimulate apoptosis, with obvious potential in oncology. However, TRAIL also interacts with anti-apoptotic decoy receptors DcR1 (TRAILR3) and DcR2 (TRAILR4) lacking death domains, and with OPG, the decoy receptor for RANKL. As Table 1 illustrates, TRAIL signaling is an area of intense interest for pharmaceutical companies, due to its potential to selectively destroy cancer cells overexpressing DR4 and DR5. One class of biologics targeting this pathway is referred to as 'PARAs' (proapoptotic receptor agonists) and includes agonizing antibodies and recombinant TRAIL itself. For example, Avi Ashkenazi discussed Amgen/Genentech's continuing clinical trials of dulanermin, a recombinant human TRAIL. Dulanermin (AMG 951) is classified as a 'dual PARA' in that it agonizes both DR4 (TRAILR1) and DR5 (TRAILR2) death receptors. It is in clinical trials in combination with other agents for cancers including non-Hodgkin's lymphoma, colorectal cancer and nonsmall cell lung cancer. In another Genentech TRAIL program, Andreas Evdokiou (University of Adelaide) described compelling *in vivo* efficacy of a fully human anti-DR5 antibody (apomab) in breast cancer xenografts [33]. Apomab also blocked bone destruction induced by human cancer cell lines implanted directly into mouse tibias. Finally, Wim Quax (University of Groningen) reported on a strategy to create discrete DR4- and DR5-selective agonists using rational structure-based protein engineering coupled with screening [34,35]. His hypothesis is that selective agents may be more potent and potentially safer against DR4- or DR5-overexpressing tumors than nonselective PARAs. Other work challenged the supposition that apoptosis via the TRAIL pathway is highly selective for cancer cells. For example, Ko Okumura (Juntendo University, Tokyo) showed that apoptosis of normal cholangiocytes (bile duct epithelial cells) in mice can be triggered by an agonizing antibody targeting TRAIL receptor DR5, leading to a phenotype resembling the human liver disease, primary sclerosing choloangitis [36]. Okumura cautioned that DR5 agonists may have similar side effects in the clinic. As a strategy to avoid such on-target but unwanted effects, Klaus Pfizenmaier (University of Stuttgart) described efforts to enhance the selectivity of TRAIL-mediated apoptosis by creating a fusion protein of TRAIL with a single-chain Fv antibody fragment targeting erb2 [37]. This fusion protein was more effective than TRAIL itself against a human colon carcinoma cell line in a mouse xenograft model.

TWEAK – an established role in inflammation

The TWEAK–TWEAKR pathway, one of the lesser characterized TNF superfamily signaling axes, is proinflammatory in mouse arthritis models [38]. TWEAK was first reported as a new TNF superfamily member by Biogen-Idec [39], and the company is developing an anti-TWEAK antagonizing antibody (BIIB023) that is currently in early clinical trials for rheumatoid arthritis [40]. In a novel approach to block TWEAK activity, Marjaneh Razmara (in the lab of Mark Tykocinski, University of Pennsylvania) presented her recently published work on a TWEAKR–TRAIL fusion protein for potential use in MS [41]. In this molecule, the soluble TWEAKR (Fn14) domain serves as a decoy receptor for TWEAK, while the TRAIL domain stimulates apoptosis of activated T cells, thus generating pleiotropic anti-inflammatory effects. Razmara showed that this chimera, given by gene therapy, was effective at suppressing inflammatory T cells in a mouse EAE model.

Beyond ligands and receptors – targeting intracellular signaling

Because the TNF superfamily consists of cell surface receptors and their large protein ligands,

drug development has logically favored biologics (decoy receptors, antibodies and recombinant ligands) over small-molecule agonists and antagonists. However, for those willing to venture inside the cell with small molecules, a rich network of signaling pathways is available for potential therapeutic modulation. As one of the few such strategies presented, Lih-Ling Lin reported on efforts by Wyeth to develop a small-molecule inhibitor of the MAP kinase kinase kinase (MAPKKK) Tpl2, in the ERK pathway [42]. Tpl2 is involved in the production of TNF, and mice lacking this kinase showed a reduced disease severity in the classic collagen-induced arthritis model. Such small-molecule approaches offer the possibility of oral dosing with lower cost of goods and improved patient convenience; however, inhibiting such pleiotropic targets may also increase the risk of side effects as compared to blocking a specific ligand–receptor interaction with a biologic.

New roles for TNF in the central nervous system

Beyond new biologics developed for classical autoimmune and inflammatory diseases such as rheumatoid arthritis, the conference organizers recognized several new therapy areas relevant to the TNF superfamily, including the role(s) of TNF and other family members in the central and peripheral nervous systems. There is a growing understanding that inflammation may be a common early step in neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases [43–45]. For example, Tony Wyss-Coray discussed his recent discovery that plasma levels of several biomarkers (including TNF and TRAILR4) undergo characteristic changes in patients with Mild Cognitive Impairment (MCI), a prodrome of Alzheimer's disease, suggesting a link between early immune system abnormalities and the etiology of Alzheimer's [46]. In addition, in many neurodegenerative diseases, activation of microglia (the resident macrophages of the CNS) is associated with the overproduction of inflammatory mediators including TNF, implying that TNF blockers and other anti-inflammatory biologics may be clinically effective. For example, Malú Tansey discussed her efforts at University of Texas Southwestern to target soluble TNF using a dominant-negative TNF protein delivered either as a biologic or as lentiviral gene therapy. Both anti-solTNF therapies rescued dopaminergic neurons from neurotoxin damage in rodent models of PD [47,48], suggesting that targeted delivery of soluble TNF-selective drugs may slow progression of the human disease. Era Taoufik (Hellenic Pasteur Institute) described her studies using knockout mice to show that caspase-8 (a key executor of apoptosis induced by Fas and TNF) is involved in ischemia-mediated neuronal cell death in the CNS [49], suggesting that anti-TNFs may be of value in stroke. Additionally, Mary Emmanouil (in the lab of Lesley Probert at the Hellenic Pasteur Institute) used IKKβ knockout mice to demonstrate that neuronal NF-κB, usually considered a proinflammatory factor, suppresses the severity of EAE in mice by enhancing neuroprotection and suppressing CNS immune responses [50]. Finally, Yoshinori Takei (in the lab of Ronald Laskey, Hutchison/MRC Research Centre, Cambridge) showed that Nerve Growth Factor (NGF) both promotes TNF synthesis and induces differentiation of neuroblastoma cells; however, the ability of NGF to induce tumor cell differentiation could be blocked by TNF [51]. The clinical implications are that NGF may contribute to the poor outcome of advanced neuroblastoma by stimulating TNF, and that NGF cotherapy with an anti-TNF biologic may induce differentiation of neuroblastoma cells and subsequent tumor regression. Taken together, these and many other studies are generating enthusiasm for the targeted use of anti-TNF and other anti-inflammatory agents in the earliest stages of neurodegenerative diseases.

Surprisingly, TNF plays a role not only in the immune system within the CNS, but may also modulate normal synaptic transmission, as discussed by David Stellwagen (McGill University). His studies show that glial-derived TNF increases synaptic excitation (probably through TNFR1), and is involved in the plasticity that is essential to memory [52]. A TNF neutralizing antibody prevents such plasticity, which is also absent in tissue from TNF-deficient mice [53]. Intriguingly, Stellwagen said that such TNF-deficient mice also lack the normal behavioral response to chronic administration of anti-depressants, suggesting a role for such signaling in human clinical depression.

Finally, evoking the TRAIL results in oncology, Anatoly Nikolaev reported on recently published work from Genentech showing that the TRAIL death receptor DR6 (an orphan receptor) may have a crucial role in normal 'axon pruning' during brain development, and potentially in the axon destruction that occurs in neurodegenerative disorders [54]. Nikolaev suggested that axon degeneration occurring in Alzheimer's may be controlled by DR6, implying that the use of TRAIL antagonists (as opposed to TRAIL agonists as used in cancer) may be a logical therapeutic strategy in this neurodegenerative disease.

Conclusions

In the 22 years since the first biennial meeting, the TNF conference has grown from a small informal gathering to a large international meeting with healthy representation from biotech, biopharma and big pharma. Building on the great success of biologics targeting the eponymous prototype, academic and industry labs are aggressively pursuing agonizing and antagonizing biologics targeting many other superfamily members. Major themes highlighted at the meeting are that these signaling pathways are exceedingly complex, and that their activities extend far beyond their classical inflammatory functions. For example, although TNF is the oldest and best understood member, the roles in inflammation and immunity of soluble versus transmembrane TNF ligands, and TNFR1 versus TNFR2 receptors, are just beginning to be explored. There is also still much to be learned about the biology of other well-established family members such as RANKL, BAFF and TRAIL, all of which interact with multiple receptors, often with opposing functions. The numerous active clinical trials assessing modulators of almost every TNF superfamily receptor–ligand pairing will shed light on the importance of particular pathways in human disease. Undoubtedly, there will be many new insights and surprises to come before the next TNF superfamily meeting, scheduled for May 15–18, 2011 in Awaji, Japan.

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