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The TNF and TNF Receptor Superfamilies: Integrating Mammalian Biology

Review

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Introduction

Three decades ago, lymphotoxin (LT) and tumor necrosis factor (TNF) were identified as products of lymphocytes and macrophages that caused the lysis of certain types of cells, especially tumor cells (Granger et al., 1969; Carswell et al., 1975). When the cDNAs encoding LTα and TNF were cloned (Gray et al., 1984; Pennica et al., 1984), they were similar to one another and, eventually, it became clear that they were members of a gene superfamily. Not surprisingly, the receptors for these proteins also constitute a TNF receptor (TNFR)-related gene superfamily. Large-scale sequencing of "expressed sequence tags" (ESTs) identified many related proteins, collectively referred to here as TNF- and TNFR-related superfamily proteins (TNF/TNFR SFPs; reviewed in Smith et al., 1994; Ashkenazi and Dixit, 1998; Wallach et al., 1999; Idriss and Naismith, 2000; http://www.gene.UCL.ac.uk/users/hester/tnfinfo.html). The familiar as well as standardized names of these proteins are listed in Table 1, together with their gene locations, phenotypes caused by mutations in these genes, and identified functions.

The discovery that cachectin, a protein known to cause fever and wasting, was identical to TNF provided an early illustration of the importance of members of this family in human disease (Beutler and Cerami, 1986). Though systemic toxicity dashed early hopes of using LTα and TNF as anti-tumor agents, the discovery of new TNF/TNFR SFPs unveiled new lines of investigation into host defense, inflammation, apoptosis, autoimmunity, and organogenesis. The potent biological effects of TNF/TNFR SFPs participate in human diseases and may be harnessed to ameliorate certain illnesses (Siegel et al., 2000). Pharmaceuticals to inhibit TNF have been developed which control previously recalcitrant inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease (Maini and Taylor, 2000; Papadakis and Targan, 2000). Indeed, for reasons we outline below, TNF and other TNF/TNFR SFPs are now being targeted for therapies against widespread human diseases such as atherosclerosis, osteoporosis, autoimmune disorders, allograft rejection, and cancer.

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The receptors and ligands in this superfamily have unique structural attributes that couple them directly to signaling pathways for cell proliferation, survival, and differentiation. Thus, they have assumed prominent roles in the generation of tissues and transient microenvironments. Most TNF/TNFR SFPs are expressed in the immune system, where their rapid and potent signaling capabilities are crucial in coordinating the proliferation and protective functions of pathogen-reactive cells. Here, we review the organization of the TNF/TNFR SF and how these proteins have been adapted for processes as seemingly disparate as host defense and organogenesis. In interpreting this large and highly active area of research, we have focused on common themes that unite the actions of these genes in different tissues. We also discuss the evolutionary success of this superfamily—success that we infer from its expansion across the mammalian genome and from its many indispensable roles in mammalian biology.

Structure/Function Relationships of TNFRs

The normal functions of TNF/TNFR SFPs, as well as certain diseases involving them, depend on the obligatory 3-fold symmetry that defines the essential signaling stoichiometry and structure (Figure 1). The ligands are type 2 proteins that can have both membrane-embedded "pro" as well as cleaved, soluble "mature" forms (for review, see Idriss and Naismith, 2000). Both forms are active as self-assembling noncovalent trimers, whose individual chains fold as compact "jellyroll" β sandwiches and interact at hydrophobic interfaces (Fesik, 2000) (Figure 1A). The 25%–30% amino acid similarity between TNF-like ligands is largely confined to internal aromatic residues responsible for trimer assembly. The external surfaces of ligand trimers show little sequence similarity, which accounts for receptor selectivity (Figure 2). The ligand shape is that of an inverted bell that is embraced on three sides at the base by elongated receptor chains forming a 3:3 symmetric complex (Figures 1A–1C). Certain ligands and receptors in the TNF/TNFR SFP can bind more than one partner with specific high affinity ($K_d = 10^{-9}$–10^{-10} M), thereby enhancing regulatory flexibility and complexity (Idriss and Naismith, 2000). After ligand binding, the receptor cytoplasmic tails form a 3:3 internal complex with signaling proteins such as TRAF2 or FADD (McWhirter et al., 1999) (Figure 1F). Hence, ligand binding and signal complex formation involve stoichiometrically defined protein complexes with 3-fold symmetry. How evolution settled on tri-fold symmetry is unclear. Trimers require more contacts than dimers and may cause an exponential increase in avidity. Trimers could also be necessary to project elongated receptor chains upright on the cell surface. Though few other things in nature occur in threes, trimers provide a unity of design and function for these receptor/ligand superfamilies.

TNFR-like receptors are type 1 transmembrane proteins that adopt elongated structures by a scaffold of disulfide bridges (Figures 1D and 1E). The disulfide

Table 1. Members of the TNF/TNFR Superfamily

	Standardized	Other Names	Accession	Human Chromosome	Mouse Chromosome	Phenotypes associated with mutations	Additional functional observations
Receptor							
NGFR	TNFRSF16	p75	M14764	17q21-q22	11, 55.6 cM	Defective sensory neuron innervation; impaired heat sensitivity	
Troy	TNFRSF19	Taj	AF167555	13q12.11-12.3	14		Expressed in hair follicles and epithelium; the mouse gene is located near the *waved coat* locus.
EDAR			AF130988	2q11-q13	10, 29.0 cM	Hypohydrotic ectodermal dysplasia — abnormal tooth, hair and sweat gland formation	
XEDAR		EDA-A2R		AF298812	X		Likely role in skin, hair and tooth formation
CD40	TNFRSF5	p50, Bp50	X60592	20q12-q13.2	2, 97.0 cM	Defective Ig class switching and GC formation causing immunodeficiency	
DcR3	TNFRSF6B		AF104419	20q13			Secreted decoy receptor for FasL with possible role in tumor evasion
FAS	TNFRSF6	CD95, APO-1, APT1	M67454	10q24.1	19, 23.0 cM	Impaired activation-induced T cell death; lymphoproliferation; autoimmunity (ALPS)	
OX40	TNFRSF4	CD134, ACT35, TXGP1L	X75962	1p36	4, 79.4 cM	Defective T cell responses	
AITR	TNFRSF18	GITR	AF125304	1p36.3	4		Glucocorticoid-induced; inhibits T cell receptor-dependent apoptosis
CD30	TNFRSF8	Ki-1, D1S166E	M83554	1p36	4, 75.5 cM		Marker of Reed-Sternberg cells in Hodgkin's disease
HveA	TNFRSF14	HVEM, ATAR, TR2, LIGHTR	U70321	1p36.3-p36.2			Probable role in T cell proliferation and receptor for herpes simplex virus
4-1BB	TNFRSF9	CD137, ILA	L12964	1p36	4, 75.5 cM		Probable role in T cell responses
TNFR2	TNFRSF1B	CD120b, p75, TNFBR, TNFR80, TNF-R-II	M32315	1p36.3-p36.2	4, 75.5 cM	Increased sensitivity to bacterial pathogens; decreased sensitivity to LPS; reduced antigen-induced T cell apoptosis	
DR3	TNFRSF12	TRAMP, WSL-1, LARD, WSL-LR, DDR3, TR3, APO-3	U72763	1p36.2			A linked, partially duplicated copy of the gene encodes a potential decoy receptor
CD27	TNFRSF7	Tp55, S152	M63928	12p13	6, 60.35 cM	Defective T cell responses	
TNFR1	TNFRSF1A	CD120a p55-R, TNFAR TNFR60 TNF-R-I	M75866	12p13.2	6, 60.55 cM	Impaired clearance of bacterial pathogens; resistance to LPS; LN present; defective GC formation; defective PP formation	
LTβR	TNFRSF3	TNFR2-RP, TNFCR, TNF-R-III	L04270	12p13	6, 60.4 cM	Absence of LN, PP; defective GC formation	
RANK	TNFRSF11A	TRANCE-R	AF018253	18q22.1		Osteopetrosis; absence of osteoclasts; absence of lymph nodes; PP present; abnormal B cell development	Required for lactating mammary gland development
TACI		CAML interactor		AF023614	17p11	11	Probable role in B cell responses
BCMA	TNFRSF17	BCM	Z29574	16p13.1			Probable role in B cell responses
DR6		TR7	NM_014452	6p21.1-12.2			

(*Continued next page*)

Table 1. Continued.

	Standardized	Other Names	Accession	Human Chromosome	Mouse Chromosome	Phenotypes associated with mutations	Additional functional observations
Receptor							
OPG	TNFRSF11B	OCIF, TR1 osteoprotegerin	U94332	8Q24		Osteoporosis; arterial calcification	
DR4	TNFRSF10A	Apo2, TRAILR-1	U90875	8p21			Probable inducer of lymphocyte death and activation
DR5	TNFRSF10B	KILLER, TRICK2A, TRAIL-R2, TRICKB	AF012628	8p22-p21			Probable inducer of lymphocyte death and activation
DcR1	TNFRSF10C	TRAILR3, LIT, TRID	AF012536	8p22-p21			GPI-linked decoy receptor—interferes with TRAIL signaling
DcR2	TNFRSF10D	TRUNDD TRAILR4	AF029761	8p21			Transmembrane decoy receptor—interferes with TRAIL signaling
Ligand							
EDA		EDA1	NM_001399	Xq12-q13.1	X, 37.0 cM	Hypohydrotic ectodermal dysplasia – abnormal tooth, hair and sweat gland formation	
CD40L	TNFSF5	IMD3, HIGM1, TRAP, CD154, gp39	X67878	Xq26	X, 18.0 cM	Defective T cell and IgG responses; hyper IgM syndrome	
FasL	TNFSF6	APT1LG1	U11821	1q23	1, 85.0 cM	Impaired activation-induced T cell death; lymphoproliferation; autoimmunity; ALPS	
OX40L	TNFSF4	gp34 TXGP1	D90224	1q25	1, 84.9 cM	Defective T cell responses	
AITRL	TNFSF18	TL6, hGITRL	AF125303	1q23			Inhibits T cell receptor-dependent apoptosis
CD30L	TNFSF8		L09753	9q33	4, 32.2 cM		Possible role in malignant lymphocyte disorders
VEGI	TNFSF15	TL1	AF039390				Potential vascular endothelial cell growth inhibitor
LIGHT	TNFSF14	LT_, HVEM-L	AF036581	19 (probable)	17		
4-1BBL	TNFSF9		U03398	19p13.3	17	Defective T cell responses	
CD27L	TNFSF7	CD70	L08096	19p13	17, 20.0 cM		
LTα	TNFSF1	TNFB, LT	X01393	6p21.3	17, 19.06 cM	Absence of LN and PP; disorganized splenic microarchitecture; defective GC formation	
TNF	TNFSF2	tumor necrosis factor; cachectin, TNFA, DIF	X01394	6p21.3	17, 19.06	LN present; defective GC formation; increased susceptibility to microbial pathogens	
LTβ	TNFSF3	TNFC, p33	L11015	6p21.3	17, 19.061	Absence of peripheral LN and PP; presence of mesenteric and some cervical LN; defective GC formation	
TWEAK	TNFSF12	DR3L APO3L	AF030099	17p13	11?		Potential role in monocyte and NK cell cytotoxicity
APRIL	TNFSF13		NM_003808	17p13.1	11?		Probable role in B cell responses
BLYS	TNFSF13B	BAFF, THANK, TALL1	AF132600	13q32-34			Probable role in B cell responses
RANKL	TNFSF11	TRANCE, OPGL, ODF	AF013171	13q14	14, 45.0	Osteopetrosis; absence of osteoclasts; absence of lymph nodes; PP present; normal splenic architecture; abnormal B cell and T cell development	Required for lactating mammary gland development
TRAIL	TNFSF10	Apo-2L TL2	U37518	3q26			

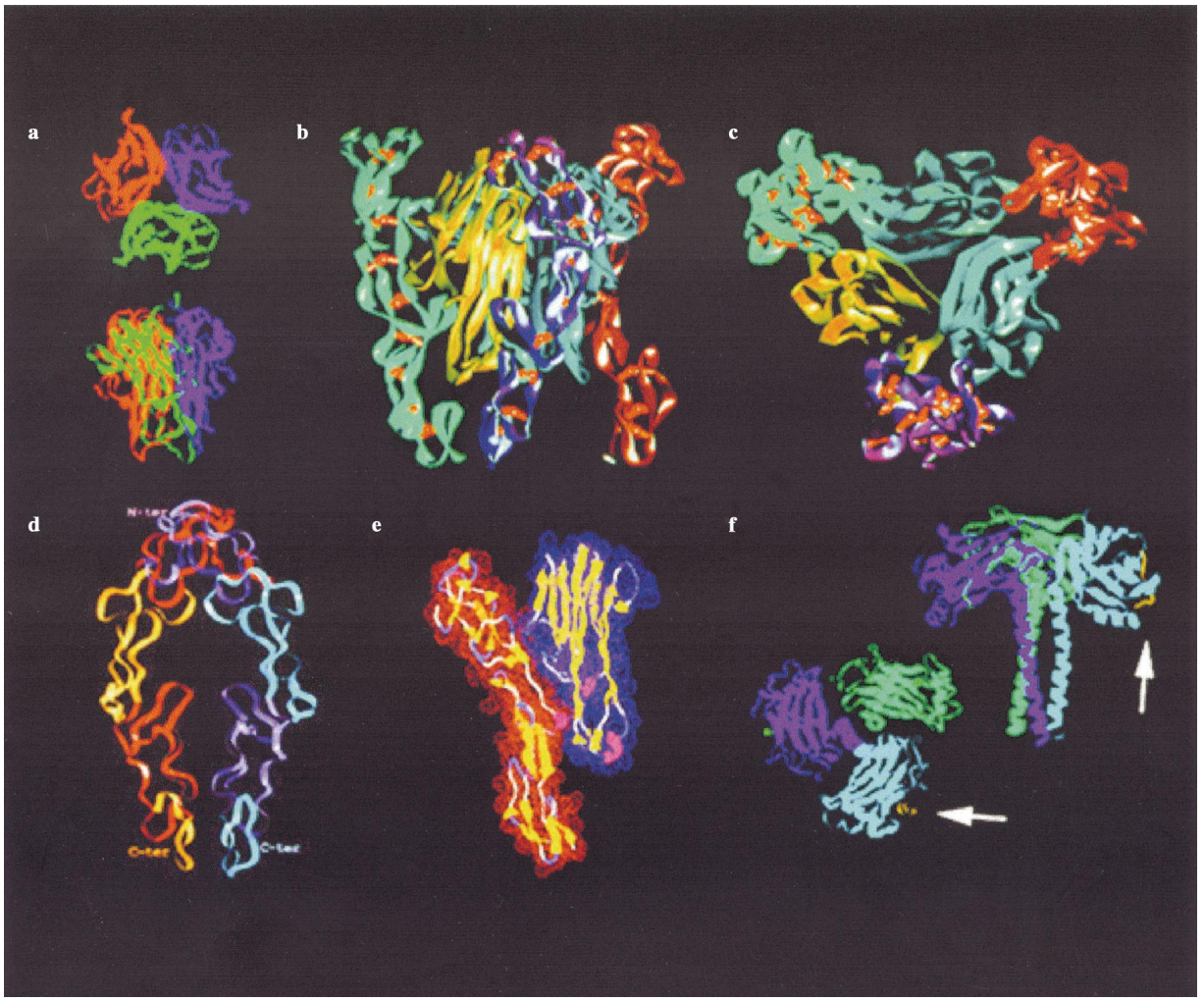

Figure 1. Structure of TNF/TNFR Interaction

(A) Trimeric structure of TNF. Structure of the TNF trimer shown from the top (upper) and side (lower) views with each monomer differently colored. The β-pleated sheets assume a "jellyroll" orientation in each monomer (adapted from Eck and Sprang, 1989).

(B) Trimeric symmetry of the structure of the active domain of TNFR1 complexed with LTα. The elongated ladder of disulfide bridges for the receptor chains (light green, blue, and crimson) is highlighted in red. The predominant contacts occur between the trimeric ligand (chains in dark green and brown) and the middle of the ectodomain in the region of CRD2 (Banner et al., 1993).

(C) LTα/TNFR1 structure from a "top" view looking down at the membrane revealing the tri-fold symmetry in which the receptor chains embrace the ligand at each vertex of a triangle formed by the apposition of ligand monomers.

(D) Neutral pH structure of the unliganded TNFR1 ectodomain. A parallel dimer with extensive contacts in the PLAD region comprising CRD1 is shown. An anti-parallel dimer, not shown, was also observed (Naismith et al., 1995).

(E) Structure of the liganded monomeric complex. Contact structure modeled on the interaction between a monomer of LTα and a monomer of TNFR1 showing that the ligand contacts are made primarily in CRD2 but not CRD1 (Banner et al., 1993).

(F) The trimeric structure of TRAF complexes with CD40 peptides. The three TRAF monomers (light blue, dark blue, and green) extensively contact each other and interact at the tips of the globular N-terminal domains with the cytoplasmic portions of receptor monomers as illustrated by the position of receptor peptides (yellow or gold) indicated by the arrows (McWhirter et al., 1999).

bonds form "cysteine-rich domains" (CRDs) that are the hallmark of the TNFR superfamily (e.g., see alignments of amino-terminal CRDs in Figure 3). These 40 amino acid pseudorepeats are typically defined by 3 intrachain disulfides generated by 6 highly conserved cysteines (Smith et al., 1994). The elongated receptor chains fit in the "grooves" between protomers within the ligand trimer. For Fas and TNFR1, ligand contacts occur mainly in the 2nd and 3rd CRDs (counted from the N terminus). The crystal structure of LTα in complex with the TNFR1 extracellular domain reveals no contact between individual receptor chains (Figures 1C and 1D) (Banner et al., 1993). From this it was inferred that the ligand recruited or "cross-linked" three receptor monomers into the final 3:3 complex. This view has been recently challenged by findings that several receptors in the TNFR family self-assemble in the absence of ligand and signaling involves rearrangement of the preassembled chains (Chan et al., 2000a; Siegel et al., 2000). The structure of TRAIL complexed with its receptor DR5 reveals a remarkable conservation of the same 3-fold ligand-receptor complex as seen for LTα/TNFR1 despite a minimum of primary sequence similarity (Hymowitz et al., 1999).

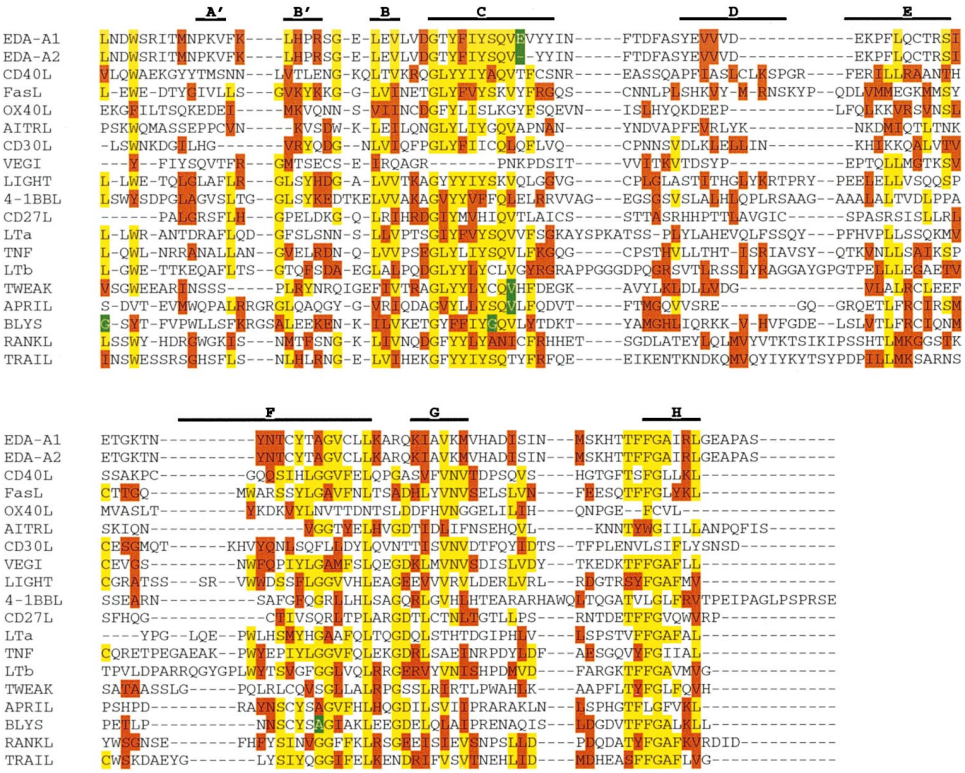

Figure 2. Sequence Alignment of the TNF-Related Proteins

Residues are shaded (yellow for identities, red for conservative substitutions) where they occur in 30% of the grouped sequences. Green shading highlights the corresponding locations of intron excision sites from the mRNAs that encode the proteins. The locations of β strands in the LTα structure are shown at the top of the alignment. Alignments were generated using CLUSTALW.

TNFRs can display noncanonical functions that do not require binding trimer ligands. For example, NGFR binds neurotropins, ligands with no structural resemblance to TNF, and interacts with the Trk family of tyrosine kinase receptors that are unrelated to TNFRs. Thus, the *NGFR* gene, with a dissimilar distribution of introns, may have been formed early in the evolution of the TNFR SF. HveA, formerly HVEM, another TNFR SFP, is a receptor for Herpesvirus, type 1, and binds the TNF-like ligand, LIGHT (Mauri et al., 1998). Similarly, the avian TNFR-like TVB(S1), TVB(S3)(CAR-1), and TVB(T) molecules are receptors for avian leukosis viruses (Adkins et al., 2000). Whether these functions require preassembly and receptor trimers are subjects of ongoing research.

Two Pivotal Modes of Signaling

The cytoplasmic domains of TNFRs are modest in length and function as docking sites for signaling molecules. Signaling occurs through two principal classes of cytoplasmic adaptor proteins: TRAFs (TNF receptor–associated factors) and "death domain" (DD) molecules (reviewed in Fesik, 2000; Inoue et al., 2000). In mammals, at least six TRAF molecules and a number of nonreceptor DD molecules have evolved at locations spread through the genome (Wajant et al., 1999). The signaling adaptor is selected by whether the cytoplasmic domain of the receptor harbors either a DD or a TRAF binding motif. The DD is a roughly 60 amino acid globular bundle of 6 conserved α helices found in the receptor tail and the adaptor that promotes homotypic association (Figure 4). By contrast, the TRAF binding motif is a stretch of amino acids (less than a dozen contact residues) in the receptor tail that is clutched by a pocket in the globular head group of the adaptor through charged residues (Figure 1F). Signaling is extremely rapid and highly specific. For the subset of receptors that have DDs, often called "death receptors" (DR), ligand engagement typically causes the association of adaptors such as Fas-associated DD protein (FADD) and TNFR-associated DD protein (TRADD) that ultimately cause caspase activation and cell death. For Fas, the homotypic association of FADD with the Fas DD leads to the recruitment of caspase-8 or -10 by homotypic interactions between "death effector domains" (DEDs) contained in FADD and the prodomain of these two caspases (Scaffidi et al., 1999).

Why adaptors? An obvious answer is that modularity allows regulatory flexibility. The DD, DED, and "caspase-recruitment domain" (CARD), despite only 10%–20% sequence identity, all share the same overall 6 α-helical structure (Fesik, 2000). This suggests a common origin from a prototype molecule that became specialized for roles at different points in the signaling pathway. For example, whereas DD and DED play essential associative roles in death receptor pathways, the CARD has been diversified for mitochondrial death pathways and inflammatory responses (Fesik, 2000; Humke et al., 2000). The DD also resembles the "ankyrin" repeat, an oligomerization domain common to other signaling sys-

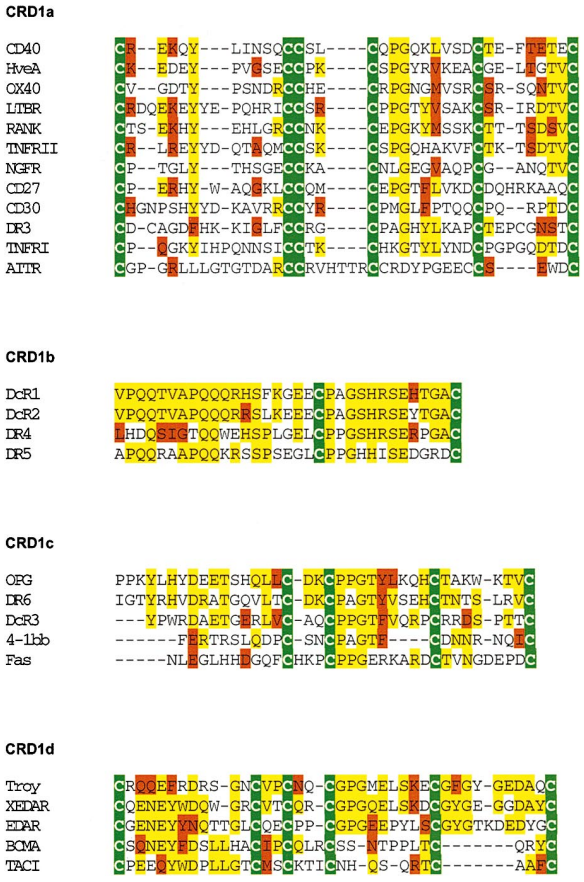

Figure 3. Sequence Alignments of the Amino-Terminal Cysteine-Rich Domains of TNFR-Related Proteins

Residues are shaded (yellow for identities, red for conservative substitutions) where they occur in 30% of the grouped sequences. Sequences are assigned to 4 groups according to the organization of the cysteine residues they contain. Alignments were generated using CLUSTALW.

tems (Feinstein et al., 1995). Typically, these domains are encoded by a single exon, a property that has been conserved from *Drosophila* to mammals, suggesting a genetic unit specialized in evolution. These domains self-associate in a manner which can be blocked by selective binding molecules (Humke et al., 2000; Siegel et al., 2000). How the receptor complex activates downstream signaling pathways is not completely clear. The precise pathways for activation of caspases, NF-κB, and other cellular responses involve a variety of kinases such as p38 and JNK, sphingomyelinase, Ca^{2+}, and other specialized signaling proteins (Wajant et al., 1999; Idriss and Naismith, 2000). Novel functions continue to emerge, such as the recent finding that TRAF6 can function as a nonproteolytic E3-like ubiquitin ligase implicated in NF-κB activation (Deng et al., 2000). Intense investigation is ongoing to clarify these pathways.

Recent evidence has shown that TNFR chains preassemble into complexes on the cell surface prior to ligand binding (Chan et al., 2000a; Siegel et al., 2000). The formation of oligomers, possibly trimers, in the absence of ligand requires the amino terminal end of the receptor including the first CRD of Fas, TNFR1, and TNFR2. This region, termed PLAD for "pre-ligand assembly domain", is necessary and sufficient for the self-assembly. Parallel dimer structures resulting from the crystallization of the unliganded TNFR 1 ectodomain show extensive contacts in the PLAD region (Figure 1D) (Idriss and Naismith, 2000). The PLAD is distinct from the ligand binding domain and the unliganded complex that it promotes is in a "closed" conformation that is distinct from the 3:3 ligand:receptor assembly. The PLAD interactions are highly specific and usually only receptor homotrimers are formed, however, "transplanting" the TNFR1 PLAD onto TNFR2 allows it to enter TNFR1 complexes. Receptor preassembly is essential for ligand binding and signal transmission. The homotypic domain associations that form the essence of receptor function represent key molecular targets for pharmacological modulation either outside the cell (PLAD, ligand-receptor) or inside the cell (DD, TRAF and DED). The ligands can also preassemble into trimers on the cell surface, and several reports suggest that membrane-anchored ligands can send "reverse" signals into the ligand-bearing cells when they contact their receptor, introducing the possibility of two-way signal transmission which warrants further exploration.

TNF/TNFR SFPs Are Cellular Organizers in Metazoans

TNF/TNFR SFPs are communicators between cells and can orchestrate permanent multicellular structures such as lymphoid organs, hair follicles, and bone, as well as impermanent, but long-lived, structures such as the lactating mammary gland. Even more evanescent structures, inflammatory foci, are assembled and disassem-

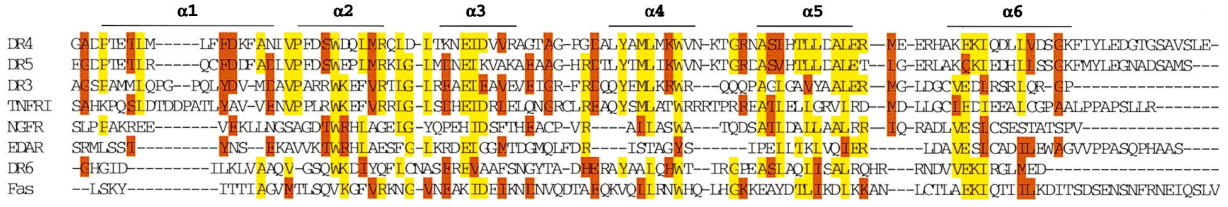

Figure 4. Death Domain-Related Sequences in TNFR SF Proteins

The figure shows an alignment of death domain–related sequences found in the cytoplasmic regions of the indicated TNFR SF proteins. The locations of α helices in the Fas death domain sequence are indicated at the top; these coincide closely with the location of α helices in the NGFR structure. Residues are shaded (yellow for identities, red for conservative substitutions) where they are common to half or more of the sequences. Alignments were generated using CLUSTALW.

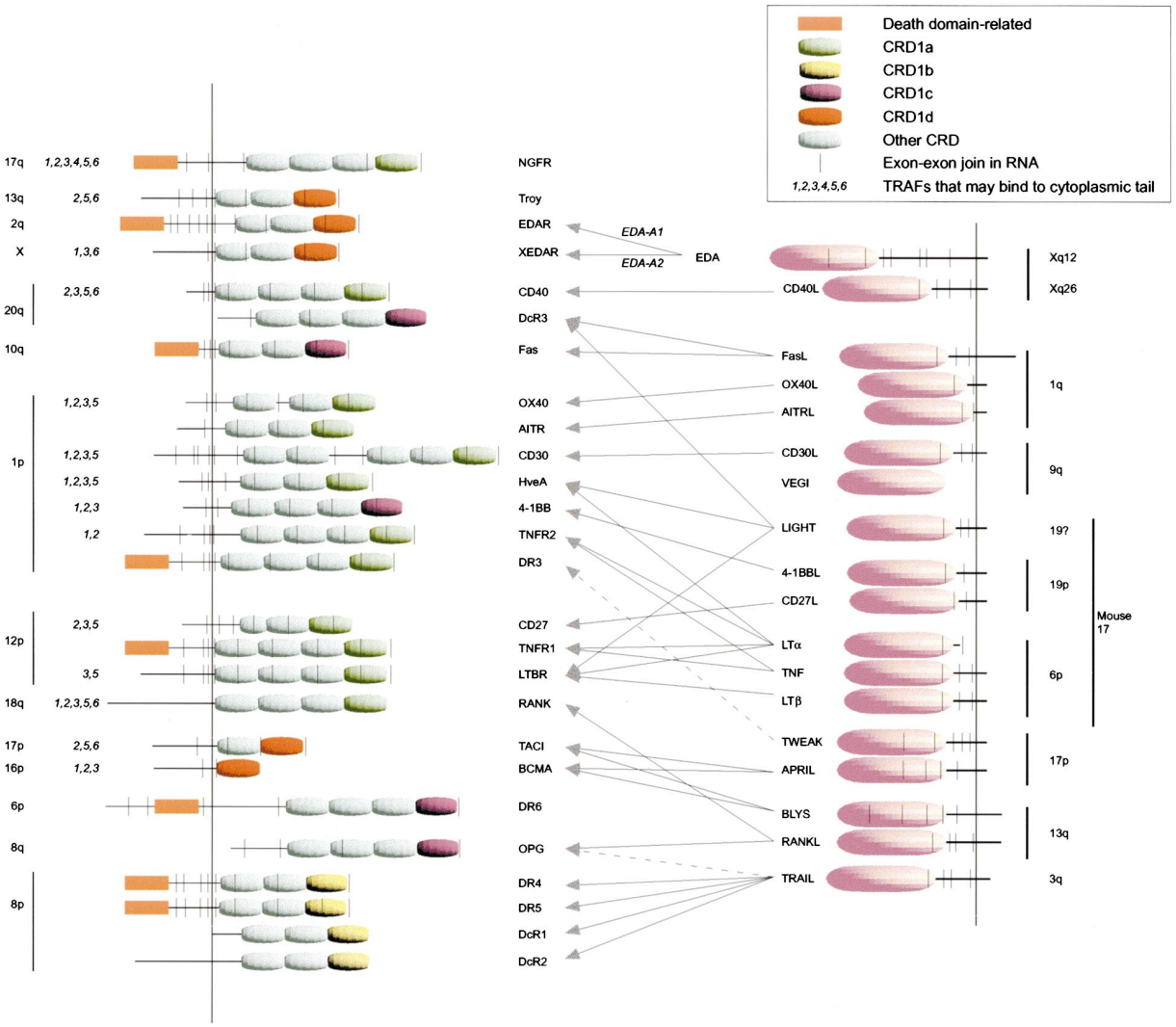

Figure 5. Interacting Proteins of the TNF/TNFR Superfamily

TNFR- and TNF-related proteins are shown at the left and right of the figure, respectively, with arrows connecting ligand–receptor pairs. Cysteine-rich domains (CRDs) are shown as small ovals. The amino-terminal CRDs (CRD1a, 1b, 1c, 1d) are grouped on the basis of sequence similarity (Figure 3) as indicated by the use of colors in the figure. Small vertical lines denote the locations of intron excision sites from the RNAs that encode the proteins (this information was not available for RANK, DcR1, and DcR2). Red boxes mark the locations of death domain–related sequences in the cytoplasmic regions of the TNFR-related proteins. Numbers to the immediate left of the TNFR cytoplasmic regions denote known or inferred interactions with the indicated TRAFs. The locations of the human genes that encode the proteins are provided at the extreme left and right of the figure; the mouse cluster on chromosome 17 is also noted. Potential interactions between TWEAK and DR3, and between TRAIL and OPG, are controversial and are therefore represented by dashed arrows.

bled at limited times and locations in acute responses to infectious stimuli. Broad regulatory patterns involving specific TNF/TNFR SFPs emerge from these examples. The genomic loci and receptor–ligand relationships of the TNF/TNFR SF are depicted in Figure 5.

TNF/TNFR SFPs in the Immune System—Coordinating Structure and Response

The major evolutionary advance represented in the vertebrate immune system is a system of "adaptive" or antigen-directed immunity in which TNF/TNFR SFPs play central roles. This involved acquisition of the two recombinase-activating genes, RAG1 and RAG2, ~450 million years ago, as well as adapting perhaps more ancient somatic mechanisms to mediate recombination and mutation of the combinatorial T cell receptor and B cell receptor genes. Immune receptors thereby diversify during differentiation so that a potentially huge repertoire of B and T lymphocytes (~10^9), each typically bearing only a single receptor, is generated to ensure responsiveness to a large universe of antigens. Powerful as they are, however, lymphocytes cannot act individually. TNF/TNFR SFPs coordinate the social context of cells that enables lymphocytes to maximally respond to pathogens. Cross-species comparisons suggest that the largest expansion of new members of the TNF/TNFR SF may have occurred during the refinement of the adaptive immune system (see below).

Immune responses occur most effectively within secondary lymphoid organs—aggregates of lymphocytes, antigen-presenting cells, and other immune-responsive cells positioned throughout the body with strategic vascular connections to portals of infectious antigen entry. Remarkably, mice that are genetically deficient for LTβ (α1β2) or its receptor LTβR do not develop secondary lymphoid organs, such as lymph nodes or Peyer's patches, and have defective spleen structure and humoral immunity (Fu and Chaplin, 1999). Lymph node anlage, developing at embryonic day (E) 10.5 in the mouse, is formed by invagination of mesenchymal connective tissue constituents expressing LTβR. CD4-expressing hematopoietic cells seed the anlage and express lymphotoxin (LTα1β2) which stimulates colonization by mature lymphocytes. Deletion of RANKL or RANK results in the absence of all peripheral and mesenteric lymph nodes but Peyer's patches remain intact and splenic architecture is unaffected (Dougall et al., 1999; Kong et al., 1999b; Kim et al., 2000a). Requirements for RANKL/RANK and LTα1β2/LTβR do not complement one another. Whereas the latter are distributed on both hematopoietic and stromal cells, RANKL and RANK are expressed mainly on $CD4^+$ hematopoietic precursor cells. This suggests that $CD4^+$ precursors emigrating into the lymph node anlagen use RANKL/RANK as an autocrine survival/differentiative signal until LTα1β2 is produced and allows stromal interactions via LTβR to complete node maturation (Kim et al., 2000a). Plasticity in lymphoid organ boundaries is shown by ectopic expression of LTα3 or LTα1β2—or of B cell chemokines induced by LTα3/LTα1β2 on B cells—which stimulates vascular adhesion molecules typical of specialized lymph node endothelia and generates ectopic lymphoid tissue (Kratz et al., 1996; Cuff et al., 1999; Luther et al., 2000). Conversely, mice deficient in LTβR or its ligands develop perivascular T cell and B cell infiltrates in multiple tissues (Banks et al., 1995; Alimzhanov et al., 1997). Hence, an ordered, sequential process of TNF/TNFR SFPs promotes generative interactions between hematopoietic and mesenchymal cells, and establishes spatial constraints essential for lymphoid organ definition (Nishikawa et al., 2000).

Participation in Acute Immune Responses

Superimposed on the architecture of lymphoid organs is the coalescence of follicles and germinal center (GC) reactions facilitated by TNF/TNFR SFPs during active immune responses. These are cellular aggregates comprising mostly B cells but also T cells and follicular dendritic cells (FDCs) (Cyster et al., 2000). FDCs are specialized mesenchymal cells that collect antigens in draining lymph nodes, interact with clonally expanding B cells, and form networks in the follicle under the influence of TNF, LTα, LTβ, TNFR1, and LTβR (Fu and Chaplin, 1999). In the spleen, 3% of the total volume of the organ can be occupied by B cell–rich GCs during the peak of an antigen response, yet these GCs will largely disappear by 4 weeks. The residue is a collection of memory lymphocytes that respond more efficiently to previously encountered antigens. In GCs, B cells are stimulated and somatically hypermutate their antigen receptor genes; those with better antigen avidity are selected and can undergo heavy chain class switching to produce different antibody subclasses. These processes depend on antigen stimulation followed by engagement of CD40 on the B cells by CD40L on T cells. CD40 or CD40L deficiency impairs $CD4^+$ T cell priming, FDC differentiation, GC formation, and class switching—so IgM-expressing cells cannot undergo isotype conversion to IgG expression, leading to hyper IgM syndrome (see below) (Grewal and Flavell, 1998). Administration of neutralizing anti-CD40L in mature mice causes the abrupt disappearance of existing GCs, revealing the need for tonic CD40/CD40L interactions. Mice deficient for LTα, LTβ, TNF, and TNFR1 have severe defects in follicle and GC formation (Fu and Chaplin, 1999). Recently, BlyS from activated dendritic cells has been found to interact with the TACI and BCMA receptors on B cells and promote their survival (Moore et al., 1999; Laabi and Strasser, 2000) (Table 1). Transgenic mice overexpressing BlyS develop increased B cell numbers and autoimmunity (Mackay et al., 1999). Conversely, TACI blockers inhibit antibody production and GC formation in normal mice as well as abrogate autoantibody formation and fatal immune complex–mediated nephritis in autoimmune-prone mice (Gross et al., 2000; Laabi and Strasser, 2000; Yan et al., 2000a).

T cell activation is also regulated by TNF/TNFR SFPs. Except for a partial block in early thymocyte development in the absence of RANKL (Kong et al.,1999a), effects of TNF/TNFR SFPs in thymic development, CD4/CD8 lineage commitment, or Th1/Th2 differentiation are minimal. However, the initiation of an immune response by sentinel dendritic cells originating in epithelial barriers stimulating naive T cells in draining lymph nodes involves TNF/TNFR SFPs. Both TNFR and Fas can experimentally costimulate T cell activation, but this effect requires further examination in a physiological setting (Siegel et al., 2000). Additional TNF/TNFR SFPs, including OX40, CD27, and 4-1BB, regulate the expansion and survival of $CD4^+$ and $CD8^+$ T cells responding to dendritic cells that express their respective ligands; LIGHT may have a similar role (Tamada et al., 2000). Thus far, targeted deletions of these molecules have less drastic consequences on the immune response than does CD40/CD40L (Table 1). Most TNF/TNFR SFPs are expressed in activated T cells suggesting that critical immune functions for additional family members await discovery.

TNF/TNFR SFPs as Mediators of Cell Death

The capacity to induce cell death is one of the unique properties with great adaptive value that TNF/TNFR SFPs have evolved. Among the 8 homologous death receptors (DRs) in the TNFR superfamily (Figure 4), at least 6 can stimulate apoptosis through activation of a family of cysteine proteases called caspases (Raff, 1998; Screaton and Xu, 2000). Other TNF/TNFR SFPs that lack death domains can potently modulate the response to DRs or directly influence cell survival. For example, TNFR2 markedly enhances TNFR1-induced T cell death and CD40 can augment Fas-induced B cell death (Garrone et al., 1995; Chan et al., 2000b). One major function of DR-induced death is cell-mediated cytotoxicity in response to infectious agents. Fas-mediated cytotoxicity is the major calcium-independent killing mechanism of

$CD8^+$ cytotoxic T cells (Nagata and Golstein, 1995). Another crucial function of DR-mediated death is immune homeostasis to balance recurrent lymphocyte expansion in response to antigen within the limited space of lymphoid organs. Activated cells must be destroyed, sometimes in enormous numbers. High or repeated antigen stimulation of activated T cells induces these death molecules and causes apoptosis in a fraction of the expanding cell population. This negative feedback mechanism, termed propriocidal regulation, prevents the toxic effects of massive lymphocyte expansion (reviewed in Lenardo et al., 1999). Genetic impairment of Fas-induced apoptosis in humans (ALPS) or mice (lpr/gld) causes a dramatic loss of lymphocyte homeostasis and autoimmunity (Rieux-Laucat et al., 1995; Lenardo et al., 1999). Self-tolerance mechanisms also involve TNF and dysregulation of TNF has been associated with human systemic lupus erythematosus and other autoimmune conditions (Jacob et al., 1991).

Much is known about how TNF/TNFR SFPs program cell death, but new concepts are still emerging. Cell death has been conceptually divided into two principal forms: apoptosis (programmed cell death) and necrosis (traumatic cell death) (Raff, 1998). There are key morphological differences—apoptosis causes shrinkage, compaction, and breakdown of the cell into easily phagocytosed bits whereas necrosis involves swelling or bursting of the cell with organelle degeneration and loss of plasma membrane integrity. Molecularly, apoptosis involves evolutionarily conserved pathways of caspase activation, but necrosis is less well defined (Raff, 1998). Death receptors within the TNFR SF are among the best-understood inducers of caspase activation and apoptosis. This appeared ironic given the moniker "tumor *necrosis* factor." However, apoptosis is unable to explain all the death capabilities of these receptors. Death receptor killing in cells without caspase activation and other attributes of apoptosis have now been carefully documented (Kawahara et al., 1998; Vercammen et al., 1998; Villunger et al., 2000). One important example is vaccinia virus. Although the virus encodes a potent caspase inhibitor, infected cells are nonetheless killed by TNF in a manner that does not resemble apoptosis molecularly or morphologically (Li and Beg, 2000). For lack of a better term, these examples have been called necrosis and may be associated with inflammation. Inflammation is not typically found with apoptosis, but may be extremely important for activating the immune system when TNF-induced necrosis is part of an anti-pathogen response. Conversely, purely apoptotic pathways may be involved in tissue remodeling during development, where inflammation could be detrimental. Experiments dissecting TNFR-induced apoptotic and necrotic pathways may uncover fundamental pathways used by these ligand/receptors in development and host defense.

Organizing Reversible Microenvironments in Response to Acute Environmental Perturbations—Acute Inflammation as a Paradigm

The role of TNF/TNFR SFPs in acute inflammation illustrates how they achieve dramatic cellular change and dynamic tissue remodeling. Host defense relies on the rapid recruitment of many inflammatory cell types to the site of an infection. When the infectious danger has been contained, it is essential that inflammation subsides and normal tissue homeostasis is restored. Rapid response and rapid subsidence are crucial to prevent damage to the host by microbial replication or persistent inflammation, respectively.

Of the many TNF/TNFR SFPs that affect inflammation, TNF has a prominent role. TNF secretion can be induced by conserved structural elements common to microbial pathogens, including cell wall moieties such as peptidoglycan, lipopolysaccharide (LPS), and bacterial DNA CpG motifs, that are bound by Toll-like receptors (TLRs) (Aderem and Ulevitch, 2000). TLRs, conserved in protein sequence from *Drosophila* to humans, decorate epithelial cells, tissue macrophages, and dendritic cells, of which the latter two are the sentinel phagocytic and antigen processing cells of the immune system, respectively. TLRs transcriptionally induce proinflammatory cytokines, including TNF, through the convergence of NF-κB and NF-AT activating pathways, and enhance translational efficiency by a mechanism targeting consensus 3′-untranslated AU-rich elements (ARE) in mRNA (Dumitru et al., 2000). IL-1R, a receptor that shares cytoplasmic homology with TLRs, and integrin interactions with extracellular matrix proteins activate similar pathways, thus amplifying the response (Shornick et al., 1996; de Fougerolles et al., 2000). The result is a highly complex biologic cascade—involving chemokines, cytokines, and the induction of endothelial adhesins—that recruits and activates granulocytes, monocyte/macrophages, and lymphocytes at the damaged or infected tissue sites. Local injection of TNF recapitulates these events, and TNF- and TNFR-deficient mice show attenuated contact hypersensitivity to irritants and susceptibility to diverse microbial pathogens (Pfeffer et al., 1993; Rothe et al., 1993; Erickson et al., 1994; Pasparakis et al., 1996).

Studies of how TNF mRNA stability is regulated by the presence of an ARE sequence in the 3′-untranslated region may provide insights into the role of TNF/TNFR SFPs and lymphokines in disease. The ARE is common to many cytokine mRNAs and is bound by tristetraprolin (TTP), a zinc finger–containing protein that accelerates the turnover of ARE-containing mRNAs (Carballo et al., 1998). TTP is induced by TNF as a negative feedback loop that limits TNF activity. Mice lacking TTP develop spontaneous, chronic, TNF-induced inflammation, with cachexia, arthritis, and high-titers of anti-DNA antibodies dependent upon TNFR1 (Taylor et al., 1996). Mice containing TNF genes with a deleted ARE sequence are similar, although inflammatory bowel disease also occurs (Kontoyiannis et al., 1999). Mice deficient in TRAF2 (Nguyen et al., 1999) or the TRAF2-interacting zinc finger protein, A20 (Lee et al., 2000), also develop states of TNF excess, suggesting that TRAF2-dependent pathways are involved in negative feedback, perhaps through activation of p38 and JNK (Kontoyiannis et al., 1999).

Spatial and temporal constraints on inflammation by TNFR/TNF SFPs are imposed by feedback inhibition; regulated expression of receptors; soluble processing of membrane-tethered ligands and receptors into soluble forms; and the induction of nonsignaling decoy recep-

tors. Perhaps the most innovative form of regulation for both ligands and receptors of the TNF/TNFR SFP is the proteolytic release of soluble bioactive oligomers from membrane-bound precursors. For TNF, this is accomplished by TACE, a *cis*-acting membrane protease of the metalloprotease/disintegrin/cysteine-rich family, whose regulation is currently obscure (Blobel, 1997). Release of TNF systemically may promote viral infection and cause other pathogenic effects in viral diseases such as AIDS (Fauci et al., 1991). Cleavage from the membrane likely expands the range over which ligands can act, but may also be responsible for more specific effects. For example, stimulation of TNFR2 for certain functions may occur primarily through membrane-bound, but not soluble, TNF (Idriss and Naismith, 2000). Also, lymph node development is predominantly triggered by membrane-bound, but not soluble, LT (Fu and Chaplin, 1999). TACE and/or related proteases cleave TNFRs, thus generating soluble receptors capable of inhibiting TNF. Early lethality in TACE-deficient mice suggests that proteolytic release is a developmentally crucial process that has been subserved by the TNF/TNFR SFPs (Peschon et al., 1998). Some TNFR members, for example, DcR3 and OPG, have evolved a soluble function by loss of a transmembrane domain, whereas others, for example DcR1 and DcR2, can be membrane-tethered but lack a functional death domain. Although regulation through cleavage of TNF/TNFR SFPs remains a plausible but unproven regulatory mechanism, constitutional inflammation and fever in certain cases of TNF receptor–associated periodic syndrome (TRAPS, see below) may be due to defective proteolytic shedding of TNFR1 (Galon et al., 2000). Genetically engineered soluble forms of TNF/TNFR SFPs have been widely used for both research and clinical applications. Typically, fusions with immunoglobulin or leucine zipper oligomerization domains are used to achieve high-level activity of the soluble ligand or receptor (Haak-Frendscho et al., 1994; Walczak et al., 1999). Inflammatory conditions such as arthritis, inflammatory bowel disease, and TRAPS have been successfully treated with soluble TNFR2-Ig fusion protein constructs (Galon et al., 2000).

Left unregulated, TNF can cause chronic inflammation, generalized wasting and, at high levels, septic shock (Idriss and Naismith, 2000); lymphotoxin can induce acute virus-induced shock (Puglielli et al., 1999). Other TNF/TNFR SFPs may be similarly involved in disease pathogenesis. CD40 is induced on vascular endothelial cells in response to TNF (Phipps, 2000). Ligation of endothelial CD40 by CD40L, either expressed on activated monocytes or T cells, or disgorged from platelet granules after activation, produces various inflammatory cytokines, chemokines such as MCP-1, procoagulant activity, adhesion molecules, matrix-degrading metalloproteinases, and inflammatory lipid mediators. Atherosclerotic plaques formed in this milieu are populated by lipid-laden macrophages derived from MCP-1-recruited blood-borne monocytes. Normally, the plaque microenvironment will dissolve upon loss of chronic CD40-CD40L-mediated signals, exemplifying the inherent reversibility of TNF/TNFR SFP-induced neo-tissues. However, TNF or other inflammatory stimuli may promote plaque extension. Interventions that disrupt CD40-CD40L in experimental models of atherosclerosis both interrupt progression of lesions and change their composition to a more stable, collagen-anchored, character (Lutgens et al., 1999). Patients with unstable angina, a condition associated with risk of plaque rupture and heart attacks, demonstrate high levels of serum CD40L, raising prospects of targeting the TNF/TNFR SFPs in human atherosclerosis (Aukrust et al., 1999).

Regulators of Bone and Mammary Gland Homeostasis

Besides lacking secondary lymph nodes, mice deficient in RANKL or its receptor, RANK, have severe osteopetrosis (increased bone density) (Kong et al., 1999a). Normal skeletal homeostasis precisely balances bone-forming osteoblasts and bone-resorbing osteoclasts, derived from distinct mesenchymal and hematopoietic lineages, respectively. Bone-resorbing agents, including vitamin D3, parathyroid hormone, and proinflammatory cytokines, such as IL-1 and TNF, induce RANKL in stromal cells/osteoblasts. RANKL mediates the differentiation and activation of osteoclasts from a monocyte precursor. RANKL or RANK deficiency ablates osteoclasts, revealing an essential role in normal osteoclast survival/differentiation (Kong et al., 1999b; Kim et al., 2000b; Li et al., 2000). TRAF6-deficient mice also lack osteoclasts, indicating that this adaptor transduces RANK signals (Lomaga et al., 1999). These mutant mice have dense bones and fail to thrive due to loss of tooth eruption, a process that requires bone resorption to allow the passage of teeth through the jawbone. Hypercalcemia induced by bone-resorbing agents such as vitamin D_3, parathyroid hormone, and IL-1 is defective in RANK/RANKL-deficient mice (Li et al., 2000). TNF both synergizes with RANKL to promote osteoclastogenesis (Lam et al., 2000), and acts independently to induce osteoclast development and bone resorption restricted to localized sites of injection in RANK/RANKL-deficient mice (Li et al., 2000). In infectious diseases, RANKL and TNF expressed by activated $CD4^+$ T cells can cause bone erosion (Kong et al., 1999b; Teng et al., 2000). Negative regulation of bone resorption during inflammation may be provided by IFN-γ, which activates TRAF6 degradation, thereby blocking RANK signaling (Takayanagi et al., 2000). Thus, in both mice and humans, TNF/TNFR SFPs have central roles in regulating osteoclast differentiation and activation—and, hence, calcium storage and mobilization.

Osteoporosis—bone thinning—is a major medical problem for postmenopausal women and may be regulated by the production of OPG, a soluble RANKL decoy receptor, made by stromal cells and osteoblasts. Estrogen induces OPG, potentially explaining postmenopausal osteoporosis and the protective effects of estrogens on bone. Estrogen deficiency, at least in experimental animals, is also associated with enhanced RANKL and TNF production by T cells (Cenci et al., 2000).

In addition to their critical function in bone homeostasis, RANKL/RANK signals also govern the terminal differentiation of mammary gland alveolar buds to create lobulo-alveolar structures competent for lactation (Fata et al., 2000). Pregnant RANKL-deficient mice fail to form lactating breast tissues or produce the major milk pro-

tein, β-casein. Without RANK, mammary epithelial precursors undergo accelerated apoptosis because of a failure to activate the antiapoptotic Protein Kinase B (PKB/AKT). FasL/Fas may also play a role in early involution of the mammary gland postpartum (Song et al., 2000). RANKL/RANK involvement in mammary gland development during pregnancy presumably occurred with the appearance of mammals 200 million years ago and may have coordinated the transfer of minerals mobilized from the mother's bone through the milk to the growing skeleton of the newborn. Both bone metabolism and mammary gland maturation illustrate the powerful capabilities of TNF/TNFR SFPs—homeostatic regulation, spatial organization of tissues and inherently reversible effects.

Hair Follicle and Sweat Gland Development

Hair follicle formation in the mouse begins around E16.5–18.5 with intense NGFR expression in the mesenchymal condensation that forms the dermal papillae (Botchkareva et al., 1999). The epidermal–dermal sandwich in skin represents a complex interplay between mesenchymal dermal fibroblasts and epidermal keratinocytes. Cell renewal in hairy skin, especially re-epithelialization after injury, may involve stem cells that arise from a specialized structure, the follicular bulge, in the hair follicle (Taylor et al., 2000). The sebaceous glands develop as an appendage of the hair follicle adjacent to the bulge. During hair follicle development, NGFR expression is attenuated as the ectoderm placodes invaginate at dermal papillae to localize developing follicles. Thereafter, NGFR remains localized to the sensory neurons that innervate the follicular bulge and to cells of the outer root sheath. NGFR-deficient mice demonstrate developmentally accelerated hair follicle development, suggesting a negative regulatory effect important in temporally coordinating mesenchymal-ectodermal events (Botchkareva et al., 1999).

Additional members of the TNF/TNFR SFP—the receptors EDAR, XEDAR, and Troy, as well as the ligand EDA—help form the hair follicle. EDAR and Troy appear in developing and invaginating placodes that originate above the NGFR-rich dermal condensations. By contrast, EDA is expressed ubiquitously in developing skin in cleaved, diffusible form. Thus, spatiotemporal regulation is maintained by receptor expression. Mice deficient in either EDA (tabby) or EDAR (downless) have no primary hair follicles or sweat glands and have malformed teeth (Headon and Overbeek, 1999). Human mutations in EDA and EDAR cause similar effects (Monreal et al., 1999). The Troy locus maps near the mouse Wv (wavy) mutation, an autosomal dominant hair phenotype that is lethal when homozygous, hinting that Troy may be responsible for this phenotype (Kojima et al., 2000). Finally, crinkled is a mouse X-linked mutation that phenocopies EDA/EDAR deficiency, and might represent an XEDAR mutation.

An Unusual Regulator of Neural Development

NGFR is the most divergent member of the TNFR family, with no known TNF ligand and a propensity to dimerize rather than trimerize. NGFR shares cytoplasmic death domain-like sequences (Figure 4) and TRAF binding motifs, and supports the development of sensory neurons by interactions with high-affinity nerve growth factor receptors (Bibel and Barde, 2000). Alone, NFGR can mediate apoptosis. NGFR can associate physically with high-affinity receptors of the Trk family, however, and promote differentiation and survival, presumably reflecting differential displacement of downstream adaptors in the presence or absence of the associating receptors (Salehi et al., 2000). NGFR is critical in mediating the structural plasticity required for development of a sensory cutaneous network responsive to environmental stimuli; NGFR-deficient mice manifest severe cutaneous sensorineural defects (Lee et al., 1992).

Genomic and Sequence Relationships of the TNF/TNFR SFPs

In Figure 5, we show the proteins of the TNF/TNFR SF and their known ligand-receptor interactions. The proteins have been grouped according to sequence comparisons and based on the locations of their genes in the human genome. The groupings represented in the figure suggest relationships that are interesting from an evolutionary standpoint. As the genomes of different species are sequenced, it seems likely that some of these relationships will be solidified such that it may eventually be possible to trace the evolution of the SF. Although speculation along these lines is largely beyond the scope of this review, a few observations deserve brief comment.

Death domains are present in two of the ten proteins located in the clusters on human chromosomes 1 and 12. These clusters include genes that regulate the development and organization of lymphoid tissue (the genes for LT and TNF receptors) and other immunoregulatory genes, including *4-1BB*, *CD27* and *OX40* (Table 1). It seems improbable that the single exon encoding the DD was introduced separately into similar locations within single TNFR-like genes in each cluster. Instead, it is perhaps more likely that the ten genes were derived from a common ancestor that encoded a TNFR-like receptor linked to a cytoplasmic DD. If so, the recent expansion of the TNFR SF apparently favored the replication of genes encoding receptors that could signal not through direct linkage to a DD, but instead by interactions with other adaptors (most notably TRAFs). TRAFs require only a short stretch of amino acids for binding, a characteristic that could allow for rapid functional diversification during gene duplication and divergence (McWhirter et al., 1999; Park et al., 1999; Ye et al., 1999). The genomic organization thus hints at a key role for DD-containing receptors in the evolution of the modern immune system, with the later addition of derivative receptors for more specialized functions.

Troy, EDAR, and XEDAR share protein sequence (Figure 3) and functional similarities, although here again, only EDAR has a DD-related sequence in its cytoplasmic region. EDAR and XEDAR bind to the products of the EDA-A1 and EDA-A2 transcripts, which are alternatively spliced RNAs derived from the X-linked *EDA* gene (Yan et al., 2000b). Striking patches of identity in the DDs of EDAR and NGFR (Figure 4) raise the possibility that *EDAR* could have diverged from the *NGFR* gene and then subsequently given rise to *Troy* and *XEDAR*. As

mentioned above, an early founding role for the NGFR in the evolution of the TNFR SF might also be suggested by the fact that it does not bind a TNF-related ligand, and by the fact that (like the *DR6* and *OPG* genes) its gene lacks the typical distribution of introns in its CRD-encoding region (Figure 5). Interestingly, TACI and BCMA share loose protein sequence similarity with Troy, EDAR, and XEDAR (Figure 3) and the genes encoding their ligands (*APRIL* and *BLYS*) have introns placed in similar locations to those in *EDA*. From such observations, it is possible to suggest that the genes for *TACI* and *BCMA* may have derived from the *NGFR* gene through duplication of an intermediate *EDAR*-related gene. As with the genes on chromosomes 1 and 12, if this hypothesis is accurate, the expansion of the TNFR SF has involved the generation of additional specialized receptors from founder DD-containing receptors.

Mechanisms of Genetic Diseases of TNFRs

There are four well-defined genetic diseases that affect ligand:receptor interactions in the TNFR SF: X-linked hyper IgM syndrome (HIGM-1, involving CD40:CD40L), the autoimmune lymphoproliferative syndrome (ALPS, involving Fas:FasL), TNF receptor-associated periodic syndrome (TRAPS, involving TNFR1:TNF), and anhidrotic ectodermal dysplasia (EDA, involving EDAR/XEDAR:EDA). A fifth disease, familial expansile osteolysis (FEO, involving RANK/RANKL), has been linked with signal peptide mutations in RANK and leads to lytic bone lesions (Hughes et al., 2000). The most common genetic mechanism for most cases of ALPS is autosomal dominance (gain of function), involving heterozygous dominant-interfering alleles. These alleles encode defective Fas proteins that complex with normal Fas proteins, thereby impairing apoptosis signaling and causing marked lymphoid expansion and autoimmunity (Lenardo et al., 1999; Siegel et al., 2000). Afflicted individuals develop pathogenic auto-antibodies—frequently against hematopoietic cells—that cause hemolytic anemia, thrombocytopenia, or neutropenia (Rieux-Laucat et al., 1995). In TRAPS, heterozygous dominant alleles of TNFR1 with amino acid changes in the extracellular domain apparently cause enhancement of the proinflammatory effects of TNF. This may result, at least in part, from a decrease in TNFR1 shedding (Galon et al., 2000). By contrast, HIGM-1 is typically caused by recessive mutations (loss of function) in the X-linked gene encoding CD40L. Hence, this severe immunodeficiency, characterized by defective CD40-mediated antibody class switching in B cells, is found mostly in males and rarely in females with skewed X-chromosome inactivation (Notarangelo and Hayward, 2000). EDA can result from either dominant or recessive mutations, but in both cases, the genetic aberrations cause defective ligand-receptor interactions necessary for the normal differentiation of ectodermal placodes into hair, sweat glands, and teeth (Yan et al., 2000b). FEO, as well as some cases of familial Paget disease of bone (PDB), has been linked with signal peptide mutations in RANK that lead to activating phenotypes (Hughes et al., 2000). Dominant inheritance results in the appearance in young adulthood of expansile osteolytic lesions in the long bones (FEO) or skull and pelvis (PDB). Early deafness and loss of adult teeth due to resorption are common clinical manifestations. Together, these diseases underscore the diverse functions mediated by TNF/TNFR SFP and illustrate the types of genetic mechanisms that could underlie diseases involving other TNF/TNFR SFPs.

Conclusions: Looking to the Past

We have attempted to summarize and conceptualize the structure, function, and genomic organization of the TNF/TNFR SFP. Functional studies based on gene knockout mice and natural human mutations support a role for these proteins in modulating dynamic, multicellular interactions important to diverse developmental and homeostatic needs. The pairing of modular cysteine-rich ectodomains with existing internal death domains and the evolution of TRAF adaptor binding motifs provided a powerful signaling mechanism used repeatedly in different contexts for coupling diverse environmental stimuli to downstream differentiation pathways. These downstream adaptor/signaling modules were previously tested in evolution, with members represented in the genomes of flies and worms that had already been adapted to body plan development and innate immunity. Although no clear TNFR-like homologs have been discovered, FADD-, RIP-, and TRAF-like molecules have been identified in *Drosophila* (Khush and Lemaitre, 2000), and TRAF-like molecules in *C. elegans* (Wajant et al., 1999). Caspases are present in both organisms (Ruvkun and Hobert, 1998; Rubin et al., 2000). Recently, the gene for a death receptor-like molecule was identified in zebrafish (Long et al., 2000). Further studies in birds and fish will be required to trace the process by which TNF/TNFR SFP became peculiarly adapted to mammals. NGFR, like other neurotrophins, is found only in vertebrates, with the earliest genes identified in jawless fish, which arose about 460 million years ago during the period of acquisition of the RAG genes critical for the subsequent development of adaptive immunity (Hallbook, 1999). A TNF homolog in bony (teleost) fish suggests divergence from mammalian TNF long before the appearance of LT—found thus far only in mammals and marsupials—but more extensive comparative analyses will be necessary (Hirono et al., 2000). Intriguingly, the 3-fold jelly-roll structure of TNF is remarkably similar to the capsid proteins of small RNA viruses (Fesik, 2000). Several avian virus receptors are TNFRs, and human HveA supports herpes virus attachment (Mauri et al., 1998; Adkins et al., 2000). The similar intracellular signaling domains of some TNFR and TLRs, which function as germline-encoded receptors for pathogen-encoded structures, raise the possibility that TNF ligands represent descendents from the horizontal capture of a gene encoded by an ancient viral pathogen.

If, as argued above, TNF/TNFR SFP gene diversification radiated from early NGFR-like or TNFR1-like precursors, evolution has apparently found these molecules to be valuable instruments in generating multicellular organs and transient microenvironments, or neo-tissues, that respond to external environmental conditions. These multicellular responses have evolved to be rapid, reversible and ultimately, to achieve equipoise in cellular numbers and activation. To achieve this, signaling via TNF/TNFR SFPs is spatially constrained during the orga-

nization of neo-tissues and then dispersed upon removal of tonic signals. As we hope to have illustrated, these functions have been most elaborately explored in the immune system, but also govern similar adaptive responses in bone, skin, mammary gland, and perhaps other organ systems whose molecular organization awaits discovery.

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