The TNF-TNFR superfamily: History

- Lymphotoxin and TNF first identified as products of lymphocytes and macrophages that mediate lysis of certain tumor cells (1969)
- cDNA is found to be related and part of a large gene family (1984)
- TNF found to be identical to cachectin, a protein that causes fever and wasting (1986)
- TNF blockade in mice found to ameliorate endotoxic shock (1988)
- TNF antagonists approved for clinical use in rheumatoid arthritis and inflammatory bowel disease (1998)

Genomics of the TNF/TNFR superfamily

- Massive expansion of ligand and receptor genes from drosophila to mammals
- Cysteine-repeat domains in receptors related to other receptor subtypes
- Intracellular signaling domains and downstream molecules more ancient
- Many linked clusters of ligand and receptor genes in both mouse and human suggest gene duplications
  - 1p36: 3 linked clusters TNFRSF4 (OX40) and TNFRSF18 (GITR); TNFRSF8 (CD30) and TNFRSF18 (TNFR2); TNFRSF9 (4-1BB) and TNFRSF25 (DR33); TNFRSF14 (HVEM)
  - 6p21 (in HLA locus): TNF (TNFSF2), LTα (TNFSF1), LTβ (TNFSF3)
  - 9p21: TRAIL Receptors (TNFRSF10A-D)
  - 19p13: TNFSF12 (TWEAK), TNFSF13 (APRIL)
  - Xq12: EDAR, XEDAR
TNF Superfamily Cytokines and Receptors in the Healthy and Diseased Immune System

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The TNF-TNFR superfamily: Overview of main areas of action

- Lymph node and neo-lymphoid tissue development
- Inflammation – TNF (TNFR1), CD40L (CD40 on DC)
- Lymphocyte co-stimulation and homeostasis – both positive and negative effects
  - T cell co-stimulation: LIGHT, TNF(TNFR2), TL1A, OX40L, 4-1BBL, GITR-L
  - B cell co-stimulation: CD40L, Blys, APRIL
  - Lymphocyte cell death: FasL and TRAIL
- Cytotoxic effector molecule
- Non-immune effects: development and survival of osteoclasts, mammary gland cells, sweat gland cells

TNF-receptor superfamily signaling types

<table>
<thead>
<tr>
<th>Activating</th>
<th>Dual signaling</th>
<th>Death receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAF</td>
<td>TRADD</td>
<td>FADD</td>
</tr>
</tbody>
</table>

- Lymphocyte co-stimulation
- Osteoclast activation
- Lymphoid organ formation
- CD30, CD40, 4-1BB
- RANK, RANK-L, etc.

- Mixed apoptosis & inflammation
- TNFR1, DR3

- Apoptosis
- FAS, TRAIL-Rs
- "Death Receptors"
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TNF-SF structure-function

TNF ligands

- Type II transmembrane proteins
- B-jellyroll structure
- Trimeric, stabilized by internal residues
- Receptor binding regions highly variable
- Cleaved from the membrane by specific proteases

TNF-SF structure-function

TNF receptors: extracellular domains

- Cysteine Repeat Domains (CRDs) define TNFR-superfamily
- Scaffold of internal disulfide bonds stabilize structure
- Typical CRD contains two subdomains
- Variants can be classified into subgroups

TNF-SF structure-function

Ligand:Receptor binding

- Ligands crystallize in a 3:3 complex with receptors
- Ligand interdigitates between receptor subunits
- Ligand contact residues usually not at N-terminal
- Unliganded receptors may have a different quaternary arrangement
- TNF/TNFR and TRAIL/DR5 structures remarkably similar
TNF-SF structure-function
Death domain
- 6 alpha-helix knot - unique fold
- Shared by death receptors (Fas, TNFR1, TRAIL-receptors 1, 2, DR6, NGFR, EDAR)
- Bind other death-domain containing proteins such as FADD and TRADD
- Structurally related to downstream intracellular signaling proteins in the apoptosis pathway: DED and CARD

TNF-SF structure-function
TRAF domain
- Binds to a short related consensus sequences in non death-domain containing TNFRSF members:
  - PXQXT: TRAF 1, 2, 3, 5; QXPXEX: TRAF 6
  - TRAF 6 also interacts with TOLL/IL1R family members
- TRAF domain structure
  - is a mushroom-shaped trimer held together by a coiled-coil stalk
  - TRAF binds inside a trimer of receptor tails

Pre-association of Fas and other TNFR family members
- May explain inhibition of Fas and other TNFRSF receptors by some soluble splice variants and decoy receptors
- Preassociation may be regulated in different cell types; This could modulate sensitivity to receptor signaling
- Blocking pre-association of TNF-R family members may be a novel way to disrupt function
  - More receptor-specific
  - May avoid problems seen with anti-ligand antibodies (e.g., CD40L)
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Use of the PLAD by other non-classical ligands for TNF-receptors

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Function</th>
<th>TNF family</th>
<th>Interaction Domains</th>
<th>Net effect on TNFR signaling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ligand association</td>
<td>Homotypic receptor interactions</td>
<td>TNFR1, TNFR2, TRAIL receptors</td>
<td>CRD1 (PLAD)</td>
<td>Positive with full-length receptors, negative with decoy soluble receptor</td>
</tr>
<tr>
<td>BTLA</td>
<td>Activates BTLA</td>
<td>HVEM</td>
<td>CRD1 (PLAD)</td>
<td>Neutral</td>
</tr>
<tr>
<td>HSV glycoprotein-D</td>
<td>Activates</td>
<td>HVEM</td>
<td>CRD1 (PLAD)</td>
<td>Neutral</td>
</tr>
<tr>
<td>13</td>
<td>BTLA HVEM binding activates BTLA signaling</td>
<td>TNFR2-TNFR1</td>
<td>?</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Apoptosis triggered by death domain-containing TNF-SF receptors through recruitment of a death-inducing signaling complex (DISC)

1. Unbound receptors
2. Ligand-bound receptors (Receptor clustering)
3. Activated receptor signaling complex
4. Release of active caspase into cytoplasm

FAS-FADD crystal structure reveals a complex containing at least 5 subunits of each death domain

Wang et al., Nature Structural Biology, 2010
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Fas engagement induces rapid receptor clustering in living cells

Pre-tx Anti-Fas (15 min)

- Receptor Clustering requires the death domain
- Receptor Clustering is upstream of caspase activation


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TNF-SF receptors without a death domains trigger diverse gene expression programs through TNF-receptor associated adapter proteins: TRAF (s)

1. Unbound receptor
2. Bound receptor with signaling complex

TRAF-binding sequences

TRAF (s) (TNF-receptor associated proteins)

Differentiation
Inflammation
Organogenesis

TRAF-binding sequences

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Dual signaling by TNF receptors: Sequential formation of two signaling complexes

New gene synthesis
Cell survival
Inflammation

TNF-R1

FADD
Caspase-8

Complex I

> 2 hrs

Complex II

New gene synthesis
Cell survival
Inflammation

c-FLIP

Cell death

Micheau and Tschopp, Cell 2003
TNF-SF signaling:
Major regulation by dynamic expression of ligands

- Expression of TNF ligands is more highly regulated than receptors
- TNF secretion can be directly triggered by pathogen-derived structures through Toll-like receptors (TLR\(\beta\))
- Cytokine & Integrin signals can amplify and independently activate TNF expression
- TNF transcription activated by NF-\(\kappa\)B and MAP-kinase
- mRNA destabilization important in regulating protein levels
- TNF secretion mediated by TACE, a cysteine metalloproteinase
- Some functions may depend on surface vs. secreted ligand

TNF-SF functions (1)
Lymph node development

- LTa, LTb – produced by lymphoid-tissue inducer cell (LTi);
  Acts on non BM derived lymphoid-tissue organizer (LTo)
  to upregulate adhesion molecules
- RANK or RANK-L essential for LN but not spleen GC or Peyer \(\lambda\) patches
- Ectopic expression of LTb promotes [tertiary] lymphoid tissue at sites of inflammation

TNF-SF functions (2)
TNF: enhancement of innate immunity

- TNF release or injection mediates septic shock and chronic wasting syndromes
- Stabilizing TNF mRNA reproduces wasting syndrome dependent on TNFR1
- TNF or TNFR1-/- mice are deficient in innate immune responses to bacteria but resistant to LPS-mediated endotoxic shock;
  Inflammatory pathology mediated by TNF overexpression is independent of T and B cells
- Expression of TNF1 by non hematopoietic cells also may be important (Hepatocytes, Fibroblasts)
- Same pathways that mediate innate immune protection also mediate immunopathology
- TNFR2 - more important in T cell co-stimulation
TNF-SF functions (3)

- Germinal center formation and B cell class switching is dependent on interactions between CD40L (CD154) on T cells and CD40 on B cells
- BlyS (BAFF) additional survival and differentiation factor for germinal center B cells expressing the BAFF-receptor; BlyS also binds TACI and BCMA
- Overexpression of BlyS can induce systemic autoimmunity and BlyS blockade can inhibit disease
- Anti-BlyS antibodies (Belimumab) approved for the treatment of SLE – reduces autoantibody and disease indices

Dillon et al., Nature Reviews Rheum 2006

TNF-SF functions (4)

- HVEM, GITR, TNFR2, CD30, OX40, CD27, DR3 and 4-1BB can co-stimulate antigen-induced activation of T cells
- Deficiency of each co-stimulatory TNF-receptor has different phenotype in knockout mice
- Differential effects on T cell subsets
- GITR may have a special role in reversing suppression mediated by CD4(+)CD25(+) Treg
- May be good therapeutic targets for T cell mediated autoimmune diseases

TNFR family members in T cell expansion and contraction

- Naive
- Effector
- Memory
- Activation HVEM
- Clonal expansion CD27
- Post-activation survival OX40, 4-1BB, CD30
- Post-activation cell death: not known to be dependent on exogenous ligands
- Re-stimulation induced death FAS
- Effector cell accumulation in target tissue DR3

Adapted from Croft, Nature Reviews Immunology 2003
**TNF Superfamily Cytokines and Receptors in the Healthy and Diseased Immune System**

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**TL1A-DR3 interactions critical for effector T cells at the site of inflammation**

- Both Th2 and Th1/Th17 driven autoimmune disease models depend on DR3
- DR3 required on T cells for local effector T cell expansion and effector responses
- Systemic T cell priming or migration not significantly affected
- OX40 deficient mice protected from lung hypersensitivity and EAE but systemic responses affected as well

Meylan et al., Immunity 2008

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**TNF SF function: Negative regulation through apoptosis induction: FasL-Fas interactions**

- FasL-Fas interactions lead to apoptosis induction
- Memory cell generation and maintenance
- Effector cell death

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**Multiple signaling pathways can lead to programmed cell death**

- Extrinsic pathway: FasL-Fas
- Intrinsic pathway: DNA damage, Cellular stress, Growth factor deprivation

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**TNF-SF function:**

Osteoclast development and activation

- Synergy between TNF and RANK-L
- Osteopetrosis in RANK or RANK-L deficient mice
- In experimental arthritis models, RANK-L and TNF expressed by activated CD4 cells can cause bone erosion (blockable by soluble receptors or anti-cytokine antibodies)
- Anti-RANK-L antibodies (Denosumab) approved for therapy in osteoporosis and other diseases


**Molecular mechanisms of genetic diseases involving TNF-SF & TNFR-SF**

- X-linked Hyper-IgM syndrome (HIGM-1): Loss of function mutations in CD40L gene; Mutations leading to loss of protein expression and mutations in the extracellular region of CD40L have been reported
- Autoimmune Lymphoproliferative Syndrome (ALPS): Heterozygous, most often dominant negative mutations in Fas
- TRAPS: Heterozygous mutations in the extracellular domain of TNFR1: due to decreased shedding of soluble TNFR1 and receptor misfolding leading to TNF-independent intracellular signaling by mutant TNFR1
- Ectodermal dysplasias: Can result from recessive or dominant mutations in EDA/EDAR/XEDAR; All result in dysfunctional ligand/receptor interactions
- Familial expansile osteolysis: Heterozygous mutations in the signal peptide of RANK, apparently leading to spontaneous activation of receptor signaling

**ALPS - Autoimmune Lymphoproliferative Syndrome**

Genetic defects in the Fas pathway

- **Clinical:** Lymphadenopathy, splenomegaly and accumulation of CD4-CD8- T cells; Initial presentation: infancy to 5 yrs
- **Lab:** Hypergammaglobulinemia and autoantibodies; Primarily hematopoietic antibody-mediated autoimmune disease 15-fold increased incidence of lymphomas 15-48 years after initial ALPS symptoms
- **85%** of patients harbor heterozygous mutations in the gene coding for Fas/CD95
Fas death domain mutants interfere with recruitment of FADD and formation of the DISC

Extracellular Fas mutations inhibit signaling through pre-association with wild-type chains

Therapeutic uses of TNF-SF blockade in inflammatory diseases
Other anti-inflammatory agents that target TNF-SF action

<table>
<thead>
<tr>
<th>Agent</th>
<th>Action</th>
<th>In Use</th>
<th>Investigational</th>
</tr>
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<tbody>
<tr>
<td>Pentoxysilnine</td>
<td>Inhibits TNF synthesis</td>
<td>Multiple</td>
<td>Sarcoidosis, others</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Block NF-κB activation</td>
<td>Multiple</td>
<td>ESRD, refractory arthritis</td>
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<td>Block NF-κB activation</td>
<td>Inflammatory Arthritis</td>
<td></td>
</tr>
<tr>
<td>Prostaglandin inhibitors</td>
<td>Block NF-κB activation</td>
<td>Inflammatory Arthritis</td>
<td></td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Block NF-κB transactivation</td>
<td>Multiple</td>
<td></td>
</tr>
</tbody>
</table>

Review papers for further reading

2. Croft, M (2003) "Costimulatory members of the TNFR family: keys to effective T cell immunity?" Nature Reviews Immunology 3: 609-620

Online Chart of TNF family Cytokines and Receptors
http://www.niams.nih.gov/Research/Ongoing_Research/Branch_Lab/Autoimmunity/tnfchart.htm

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