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# Regulation of cell-death and immune defense by receptors of the TNF/NGF family

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Receptors of the TNF/NGF family control all aspects of immune defense and play important roles in the regulation of developmental processes. Our studies aim at elucidating the mechanisms regulating the induction of two cardinal activities of these receptors: programmed cell death, an effect that occurs in a protein-synthesis independent manner, and activation of transcription factors such as NF-κB that participate in the induction of immune defense mechanisms. We are applying genetic screens and proteomic approaches to identify the signaling proteins participating in the induction of cell death and immune defense, and complement these *in vitro* studies by transgenic approaches for elucidating the *in vivo* role of these proteins.

**Signaling for cell death**

**Studies *in vitro***

Exploring the sequence of protein-protein interaction events initiated by the death receptor Fas (CD95), we have discovered an adapter protein, FADD/Mort1, that associates with death receptors, *caspase-8*, a member of the caspase cystein protease family that plays a crucial role in all apoptotic processes, and cFLIP/CASH, a *caspase-8* homologue that serves as an inhibitor of death induction. Both genetic screens and protein-purification approaches are applied to isolate regulatory proteins that associate with *caspase-8* and cFLIP/CASH.

**Studies *in vivo***

Our analysis of the *in vivo* role of *caspase-8* by targeted disruption of its gene in mouse and by its conditional knockout using the Cre/*loxP* recombination system confirmed that the enzyme plays a pivotal role in death induction. Deletion of *caspase-8* in hepatocytes, for example, protected them from Fas-induced hepatocyte death. In addition, this analysis revealed that *caspase-8* also serves cellular functions that are non-apoptotic. We are currently exploring the mechanisms that determine whether *caspase-8* activation in a cell will lead to its death, or, alternatively, to non-apoptotic changes.

**Signaling for activation of the transcription factor NF-kappa B**

NF-κB is a highly pleiotropic group of transcriptional factors that regulate a wide range of genes, mainly participating in immune defense and development. All members of the TNF/NGF receptor family activate these transcriptional factors, yet with different functional consequences. Receptor-associated adapter proteins of the TRAF family serve to initiate this activation, and a kinase complex composed of two protein kinases, IKK1 and IKK2, as well as a non-enzymatic component, NEMO (IKK-γ), act as the effector element in it. Attempting to elucidate the molecular interactions that impose specificity of action on this common set of signaling molecules, we have discovered

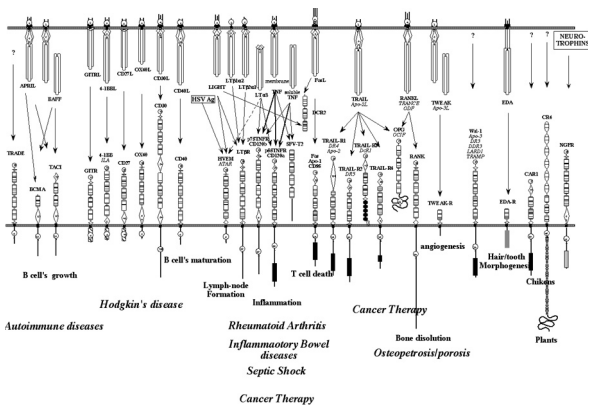


**Fig. 1** *Diagrammatic representation of the known interactions among members of the TNF ligand family and members of the TNF/NGF receptor family and some of the physiological and pathological consequences of their interactions.*

novel interaction of the receptor-associated adapter protein TRAF2 with a novel protein kinase, NIK, that through phosphorylation of the IKK complex enhances expression of NF-κB target genes. Our findings indicate that NIK participates in a unique set of proximal signaling events, initiated by specific members of the TNF/NGF family that regulate adaptive immune responses. We have also discovered a novel interaction of NEMO with CYLD, a protein that has deubiquitinating activity which is directed towards K63-linked polyubiquitin chains. CYLD dysfunction leads to excessive activation of NF-κB and can trigger cell transformation. We currently explore involvement of other NF-κB and cell death regulating proteins in human cancer.

Functions of receptors of the TNF/NGF family are central to the pathology of various diseases. Our discovery, 14 years ago, of the soluble forms of the TNF receptors formed the basis for the current wide application of these soluble receptors for effective treatment of Rheumatoid Arthritis Psoriasis and Crohn's disease. Elucidation of the intricacies of the signaling mechanisms activated by the TNF/NGF family will form the basis for future development of drugs for other diseases to which this receptor family contributes.

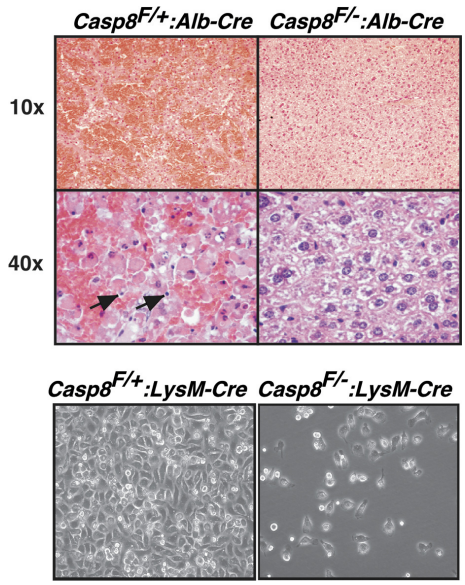


**Fig. 2** *Deletion of the caspase-8 gene in hepatocytes (dictated by Cre expression under control of the albumin, Alb, promoter) endows them with resistance to Fas cytotoxicity while its deletion macrophage precursors (dictated by Cre expression under control of the lysozyme M, LysM, promoter) compromises their differentiation. Top and middle panels: liver histology 6 h after injection of anti-Fas antibody to mice with the indicated genotypes. Note the hemorrhage, and pycnotic nuclei in apoptotic cells (arrows) in the livers of the Casp8F/+:Alb-Cre mice, yet not in those of the Casp8F/-:Alb-Cre mice that are devoid of caspase-8. Bottom panels: macrophage yield after culturing bone marrow cells of mice with the indicated genotypes for 7 days with M-CSF.*

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