# Regulation of Cell-Death and Immune Defense by receptors of the TNF/NGF family

**David Wallach**

**Elena Appel**

**Andrew Kovalenko**

**Inna Kolesnik**

**Tatniana Shalevich**

**Jin Hyuk Jung**

**Tae Bong Kang**

**Yaakov Krelin**

**Gisu Oh**

**Santosh Ramachandran**

**Paramewaran Ramakrishnan**

**Jin Chul Kim**

**Akhil Rajput**

Receptors of the TNF/NGF family control all aspects of immune defense and also play important roles in the regulation of embryonic developmental processes. Our studies are aimed toward elucidating the mechanisms that regulate the induction of two cardinal activities of these receptors: programmed cell death, an effect mediated by members of the caspase cysteine-protease family, which are activated by the receptors in a protein-synthesis independent manner, and the induction of immune defense mechanisms through gene activation, which to a large extent relies on stimulation of transcription factors of the NF-kappa B family. We are applying genetic screens and proteomic approaches to identify the signaling proteins that participate 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

Ours and others' attempts to elucidate the sequence of protein-protein interactions by which the receptor Fas (CD95) induces cell death, led to identification of the signaling proteins from which the, so called, 'extrinsic cell-death pathway' is comprised: the adapter

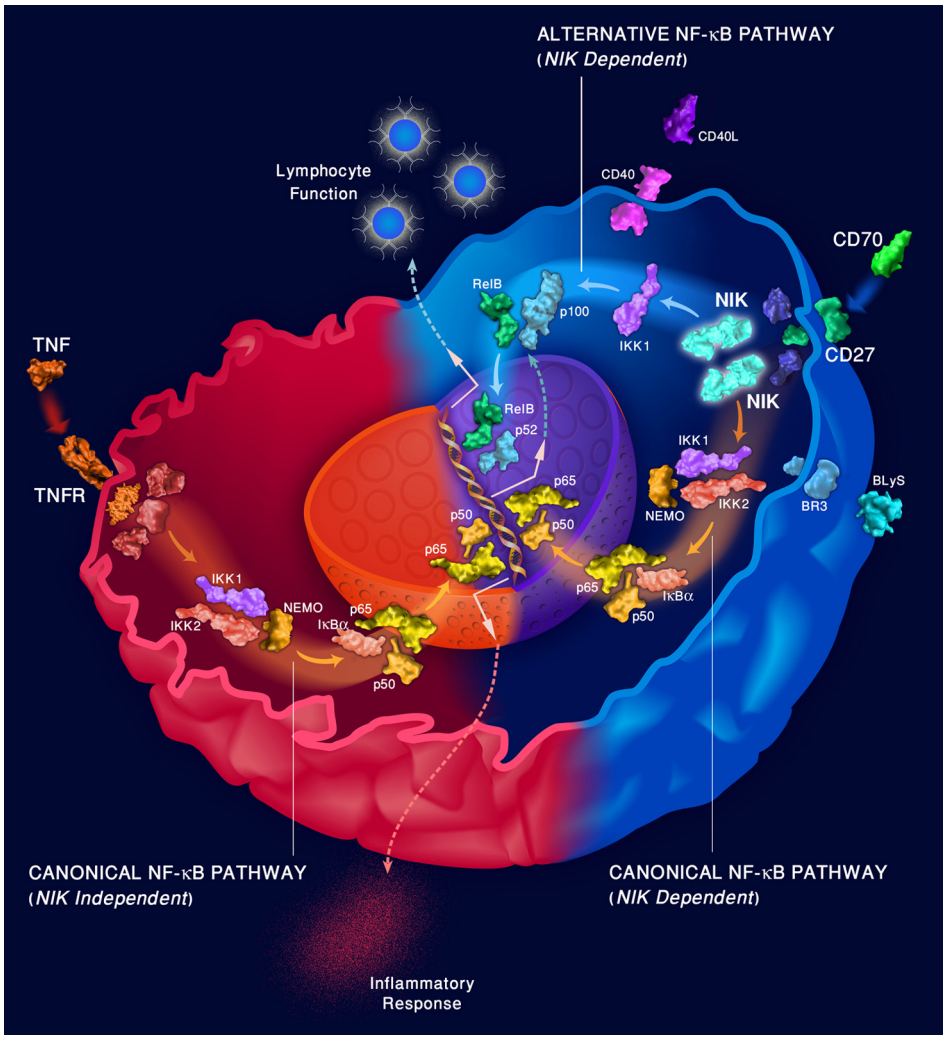


***Fig. 1*** *The role of NIK in signaling for NF-kappa B activation. NIK is shown to mediate activation of a unique pathway (the 'alternative' pathway) for NF-kappa B activation and to be specifically involved in the function of those members of the TNF family that regulate lymphocyte activities which contribute to adaptive immunity (and not to the induction of inflammation).*

**Department of Biological Chemistry**

972 8 934 3941

972 8 934 3165

d.wallach@weizmann.ac.il

www.weizmann.ac.il/Biological_Chemistry/scientist/Wallach/home.html

protein, FADD/Mort1 which associates with death receptors, caspase-8, a member of the caspase cystein-protease family that associates with FADD/Mort1 and plays a crucial role in the initiation of cell death through the extrinsic pathway, and cFLIP/CASH, a caspase-8 homologue that serves as a natural inhibitor of death induction and initiator of non-apoptotic effects of the receptors. Currently, our studies are focused on further elucidation of the functions and mechanisms of action of caspase-8.

Our analysis of the *in vivo* role of caspase-8 by targeted disruption of its gene in mice, and by its conditional knockout using the Cre/loxP recombination system, has confirmed that the enzyme plays a pivotal role in death induction.

Applying Cre mediated ablation of capsase-8 to explore the function of this caspase in specific tissues in mice we and others found that, besides this pro-apoptotic function, caspase-8 also contributes to embryonic development, immune regulation, and tissue homeostasis, through non-apoptotic pathways. Gene expression profiling to assess the impact of caspase-8 modulation within specific tissues and in their vicinity indicated that the functional alterations resulting from such modulation in part reflect impact of caspase-8 on cytokine generation. Thus, deletion of caspase-8 in the basal cell of the epidermis was found to result in development of severe inflammatory skin disorder (Figure 2), associated with enhanced growth of the basal cells, which, as in the case of psoriasis in man, was greatly amplified by generation of TNF.

To explore structure-function relationship in caspase-8 for the various effects that this enzyme exerts *in vivo*, we are employing bacterial artificial chromosome (BAC) mediated transgenesis for complete or partial replacement of the endogenous caspase-8 alleles in mice with either wild-type caspase-8 gene, or various mutants thereof.

To gain further knowledge of the mechanisms controlling the kind and extent of effects that caspase-8 mediates, we employ both genetic screens and protein-purification approaches to isolate regulatory proteins that associate with this enzyme and identify covalent modifications by which its activity is modulated.

**Signaling for activation of transcription factors of the NF-kappa B family**

NF-kappa B 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, but each with different functional consequences. Some promote adaptive immunity, and others, innate immunity; some control embryonic development through NF-kappa B activation, and some employ this transcriptional factor to restrict their own cytotoxic activity. 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-gamma), acts 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 a novel interaction of the receptor-associated adapter protein TRAF2 with a novel protein kinase, NIK, that through phosphorylation of the IKK complex controls the expression of NF-kappa B target genes specifically involved in adaptive immunity. 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. Its activation by these specific receptors leads to activation of several different NF-kappa B proteins (Figure 1).

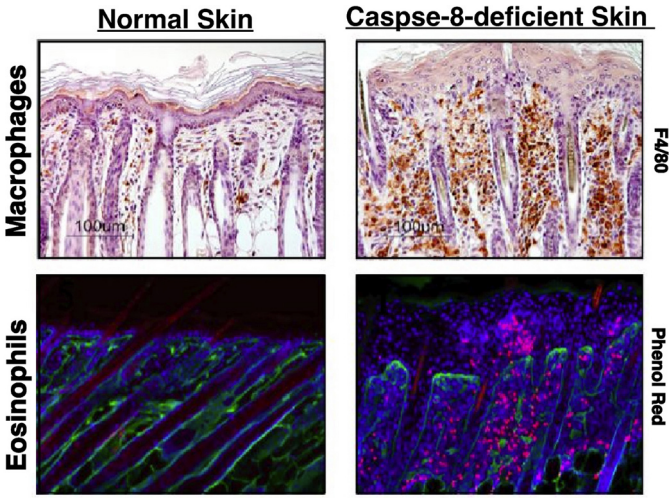


***Fig. 2*** *Deficiency of caspase-8 in the epidermis results in inflammatory skin disorder. Hisotochemial analysis of the skin of normal mice (left) and of mice in which the caspase-8 gene was specifically deleted in the basal cell of the epidermis (right) reveals massive accumulation of macrophages and eosinophils in the caspase-8-deficient skin at the 7th day after birth.*

We employ both genetic and proteomic approaches to explore the mechanisms for this receptor-specific activation of NIK.

We have also discovered a novel interaction of NEMO with CYLD, a protein that possesses de-ubiquitinating activity that is directed towards K63-linked poly-ubiquitin chains. It negatively modulates NF kappa B activation on the part of members of the TNF/NGF family by arresting signaling events that depend on K63-linked poly-ubiquitination of NEMO and of members of the TRAF adapter protein family. CYLD dysfunction leads to excessive activation of NF-kappa B and can trigger cell transformation, apparently through increased resistance to apoptosis or perturbation of the cell cycle.

**Medical implications**

Functions of receptors of the TNF/NGF family and of the signaling mechanisms that they activate are central to the pathology of various diseases. Both deficient activation of cell-death mediating molecules such as caspase-8 and excessive activity of NF kappa B and of signaling molecules like NIK that activate NF kappa B are known to contribute to the emergence of tumors. Excessive NF-kappa B activation also contributes to autoimmune and inflammatory disorders, and so does excessive production and function of TNF, and of other members of the TNF-ligand family. Our discovery, 19 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 chronic inflammatory diseases such as Rheumatoid Arthritis and Psoriasis to which TNF contributes. Elucidation of the intricacies of the signaling mechanisms activated by the TNF/NGF family will form the basis for future development of drugs against autoimmune and chronic inflammatory diseases and against cancers to which the function of this receptor family and its signaling molecules contribute.

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