

DIVISION OF IMMUNOLOGY

LYMPHOCYTE ACTIVATION AND SURVIVAL

Our interest is to determine how cells decide between living and dying. We focus on the mechanism of action of TNF receptor family members, since these govern such decisions. Lymphocytes are our main cell type of interest, since throughout their existence, they mostly live “on the edge” between life and death. Our work is inspired by the desire to improve cancer immunotherapy. The second aim of our work is to contribute to the design of novel therapies aimed at killing cancer cells by activating apoptotic pathways.

TNF receptor family members and control of the immune response From our work, TNF receptor family member CD27 and its ligand CD70 have emerged as interesting targets to improve anti-tumor immunity. This costimulatory receptor/ligand pair promotes the generation and maintenance of effector CD8 T cells, the formation of memory CD8 T cells and their secondary expansion. CD27 rescues primed T cells from apoptosis, which partially explains its impact on the magnitude of the CD8 T cell response.

T cell survival To determine by which molecular mechanisms CD27 directs the T cell response, we have used genome-wide expression profiling. In this way, we have identified IL-2 as a key CD27-directed gene product in primed CD8 T cells. *In vivo* experiments with reconstituted T cells proved that CD27 promotes the survival of effector CD8 T cells at the tissue site via an autocrine IL-2/IL-2 receptor pathway. However, CD27 promotes survival of clonally expanding CD8 T cells at the priming site via different mechanisms. One of these involves engaging the pro-metabolic and anti-apoptotic Pim-1 serine/threonine kinase that appears to be a direct target of CD27 signaling.

CD4 T cell function We have also determined the array of CD27 target genes in CD4 T cells. CD27 supports the survival of CD4 T cells, but also has qualitative effects on these cells. In the first place, CD4 T cells require CD27 to deliver help for the CD8 memory response. This involves a programming of CD8 cells during the primary response, enabling them to express certain effector genes upon secondary stimulation. One of these is a membrane molecule that we hypothesize to be essential for memory T cell function. Secondly, CD27 target genes in CD4 T cells are diagnostic for a T helper-1 (Th1) effector function, in agreement with data from other researchers that identify CD70 on dendritic cells as a mediator of IL-12-independent Th1 differentiation. Thirdly, we have recently found that CD27 signaling has a dramatic effect on CD4 T cells that are on their way to develop into T helper-17 (Th17) cells. This pro-inflammatory CD4 T cell subset has raised much attention in recent years, because of its causative role in certain auto-immune diseases. Deliberate CD27 stimulation of developing murine Th17 cells by means of an agonistic recombinant CD70 that we produce in our laboratory disables Th17 effector function. This was also apparent *in vivo*, in a model of Th17-dependent auto-immunity, experimental allergic encephalomyelitis (EAE), which is a disease reminiscent of human multiple sclerosis. Absence of CD27-CD70 signaling in CD27^{-/-} and our recently developed CD70^{-/-} mice led to increased severity of disease, concomitant with increased numbers of Th17 cells. Conversely, in our CD70 transgenic mice that constitutively express CD70 on dendritic cells, the disease was less severe than in control mice and fewer Th17 cells developed. CD27 signaling does not affect the master regulators of transcription that determine commitment of CD4 T cells towards the Th17 lineage, but selectively silences expression of the IL-17 gene by histone modification. We are currently looking into the molecular details of this important process.

Hematopoiesis By analysis of CD70 transgenic (tg) mice, we have discovered a novel link between CD27-CD70 and bone homeostasis. CD70tg mice that express CD70 under control of the CD11c promoter develop increased trabecular bone mass, accompanied by decreased cellularity of bone marrow, progressive anemia and extramedullary hematopoiesis. Osteoclast numbers, but not osteoblast



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Verbrugge I, Maas C, Heijkoop M, Verheij M, Borst, J. *Radiation and anti-cancer drugs can facilitate mitochondrial bypass by CD95/Fas via c-FLIP downregulation. Cell Death Differ.* 2010;17:551-61

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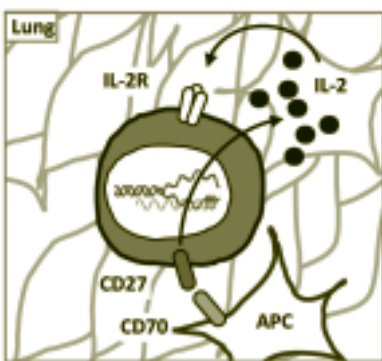


Figure 1: CD27 mediates effector CD8⁺ T cell survival at the tissue site via an autocrine IL-2 pathway. A complementation assay, in which wild-type or CD27^{-/-} T cells were retrovirally transduced to express the *il-2* gene and followed in vivo for their responsiveness to influenza virus infection, proved that CD27 signaling allows effector CD8⁺ T cells to survive in non-lymphoid (lung) tissue via autocrine IL-2/IL-2 receptor signaling (Peperzak V. et al., *J Clin Invest* 2010).

numbers were reduced in CD70tg mice. Strikingly, CD27 was found on a certain cell population in normal bone marrow that showed a strong commitment toward osteoclast formation. We conclude that CD27 hallmarks a newly defined osteoclast progenitor and that sustained engagement of CD27 on these cells inhibits osteoclast development, leading to an increased trabecular bone mass and perturbation of the bone marrow niche. This negative feedback may be provided by CD70-bearing activated immune cells and may underlie bone remodeling observed under such pathological conditions.

Ligand trafficking Deliberate constitutive expression of CD70 at the cell surface has dramatic effects on naïve lymphocyte homeostasis, as demonstrated in CD70tg mice. This can convert immunological tolerance or ignorance into immunity. We have discovered that cell surface expression of CD70 is intricately regulated by its intracellular storage and directed transport, which is governed, surprisingly, by the MHC class II chaperone Invariant chain (CD74).

Conclusion Our novel findings indicate that targeting the CD27-CD70 costimulatory axis may be of interest not only in the context of anti-tumor immunity, but also in auto-immune or inflammatory disease. Expression of CD27 and CD70 in the mature immune system are largely conserved between human and mouse, and CD70 expression hallmarks patients with constitutive immune activation. However, it remains to be shown to which extent the mechanistic details of CD27-CD70 action are conserved. In collaboration with former Organon (now MSD), we are exploring the production of functionally active reagents that target the human molecules and may prove clinically interesting. We have filed a European patent on one such reagent.

Apoptosis signaling and cancer therapy Pro-apoptotic agents are of great interest for cancer therapy, provided that they can act in a tumor-specific fashion. The TRAIL death receptors conform to this condition; they are not toxic on normal tissue and induce apoptotic cell death in many different tumor types. Although the exact mechanisms underlying this tumor-specificity are not known, agonist reagents that target the two TRAIL receptors in human have moved rapidly through preclinical testing and are now in Phase I and II clinical trials. Interestingly, TRAIL receptor agonists act synergistically with conventional and novel therapeutics in many cases, by a variety of mechanisms. One is the p53-dependent upregulation of the TRAIL receptors. We have revealed other sensitization mechanisms, including p53-independent downregulation of c-FLIP molecules that block death receptor-induced inducer caspase activation.

Factors that modulate tumor cell sensitivity to TRAIL receptor agonists are of interest, given the efficacy of this novel type of anti-cancer therapeutic. We have identified two molecules that control the cell surface expression of TRAIL receptor-1, but not TRAIL receptor-2. One of these impacts on intracellular transport of TRAIL receptor-1 in the biosynthetic route, while the other is a ubiquitin ligase that determines receptor turnover by endocytosis at the plasma membrane. These mechanisms attenuate apoptosis sensitivity of the tumor cells and are therefore of clinical relevance.

Apoptosis signaling relies on Bcl-2 family members that act either pro- or anti-apoptotically. BH3 domain-only proteins within this family are essential to convey the apoptotic signal to mitochondria, from where caspase activation is initiated. BH3 domain mimetic drugs are very promising anti-cancer therapeutics that act tumor-specifically according to the principle of “oncogene” addiction. Tumor cells may be more reliant on inhibitory Bcl-2 family members than normal cells, due to their dangerous life style. Inactivating inhibitory Bcl-2 family members by BH3 domain mimetic drugs therefore allows for selective tumor cell killing. We study the mechanism of action of certain BH3 domain-only proteins and their antagonists, with focus on the role of ubiquitination in determining their activity and half life.