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Regulation and function of cytokines and their binding proteins

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Cell to cell communication is mediated by polypeptide hormones, cytokines, and their receptors. These systems regulate many physiological and pathological pathways. Currently, our group is studying two cytokine systems, leptin and interleukin-18 (IL-18). Leptin regulates food intake by acting on its hypothalamic receptor. We found that it also regulates angiogenesis in the adipose tissue and further studied its role in ovulation. Interleukin-18 is an early mediator of the Th1 response. We have cloned an inhibitor of IL-18 and are studying its physiology and regulation of its expression.

Novel peripheral activities of leptin

Changes in tissue mass require a concerted adaptation of blood supply, adjusted by the progression or regression of blood vessels. The adipose tissue vasculature is unique because of the capacity of this tissue to grow throughout most of an adult's life. Angiogenesis is promoted by the hypoxia-induced VEGF and by the Tie-2 ligands angiopoietin-1 and 2 (Ang-1 and Ang-2), which act as an agonist and antagonist, respectively. Recently, leptin was reported to act as an angiogenic factor in several model systems. Yet, its effect at its site of production - the adipose tissue, has not been studied. We find that leptin injection triggers extensive apoptosis in adipose endothelial cells in leptin-deficient (*ob/ob*) mice. We further find that leptin induces the expression of Ang-2 in adipocytes, possibly triggering the apoptosis of adjacent endothelial cells. We are now studying the signaling pathway by which leptin induces Ang-2.

The leptin-deficient *ob/ob* female mice have a reduced GnRH secretion, leading to hypogonadism and unovulation. We studied the possible existence of leptin-mediated effects on ovarian development and ovulation. We find that leptin induced basal as well as terminal follicular growth in *ob/ob* mice and in immature normal mice. Our results suggest a leptin-mediated mechanism of ovarian development and ovulation.

IL-18-binding protein, a novel modulator of the TH1 response.

One of the common features of most hormone and cytokine systems is the existence of soluble receptors. Reported already in the 1970's, these variants of the cell surface receptors bind their respective ligand and modulate its activity. Review of recent literature suggests that most soluble receptors serve as ligand carriers.

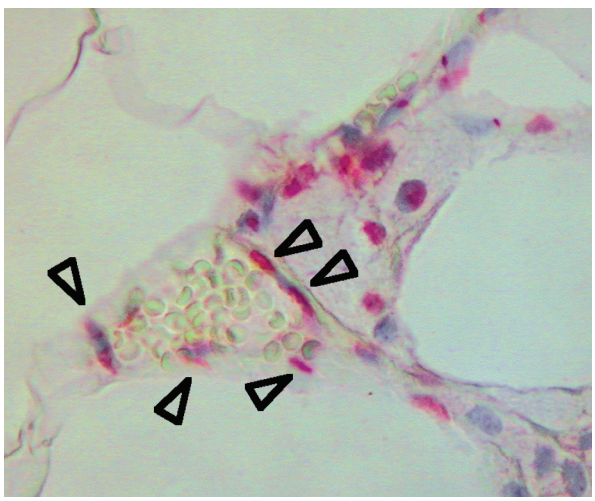

Fig. 1 *Leptin induces apoptosis in adipose tissues of C57Bl-ob/ob mice. Mice were injected with murine leptin (2x1 ug/g weight), adipose tissues were removed after 48 hr, and fixed with formaldehyde. Apoptotic nuclei (arrowheads) were visualized by TUNEL staining (red) and counter-stained with HE.*

IL-18 is an early signal in the development of T-lymphocyte helper type 1 (Th1) responses. It acts together with e.g. IL-12 to induce IFN-γ and several other cytokines and to activate Th1 and NK cells. Also, it was identified in tissues involved in autoimmune diseases, such as type I diabetes and Crohn's disease. We have isolated and cloned an IL-18 binding protein (IL-18BP). Unlike other soluble receptors, IL-18BP is not a splice variant of a cell-surface receptor; rather, it is expressed only as a soluble protein. IL-18BP specifically binds IL-18 and neutralizes its biological activity in vitro and in vivo. The affinity of IL-18BP to IL-18 is very high (Kd=0.4 nM), suggesting that its physiological role is to block IL-18. IL-18BP is currently being developed as a potential therapeutic agent for various inflammatory and

autoimmune diseases.

IL-18BP is potently induced by IFN-γ, as determined both at the mRNA and protein level. Other cytokines, including IL-18, IFN-β, IFN-α, TNF-α, IL-1 and IL-2 do not induce IL-18BP. However, in cultured hepatocytes, TNF-α synergizes with IFN-γ to induce significantly higher levels of IL-18BP. Maximal induction by IFN-γ was obtained at 24 h and the induction was sensitive to cycloheximide, suggesting involvement of an intermediate IFN-γ-induced transcription factor(s). We have localized a promoter and an enhancer element within a 1.4 kb genomic sequence upstream of the first exon of the human IL-18BP gene. Within the promoter, we have identified and IRF-1,2 binding element and demonstrated that the IFN-γ inducible an IRF-1 participates in the induction of IL-18BP. These results further show that induction of IL-18BP by IFN-γ represents a negative feedback loop, curbing extended IL-18 activity.

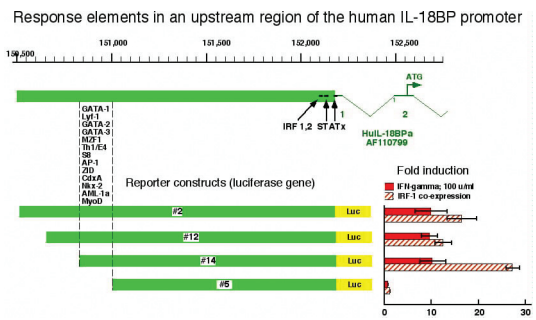

Fig. 2 *Response elements in the IL-18BP promoter region. Two elements were identified by luciferase constructs. An IRF1,2 response element, proximal to the transcription start site, whose mutation abolished the basal promoter activity. A second region 1.4 kb upstream to the transcription start site is required for induction with both INF-γ and IRF-1.*

In another study, we have developed an ELISA for serum IL-18BP and determined the levels of IL-18 and IL-18BP in health and disease. We find that both of these proteins are elevated in various inflammatory conditions. In all cases, a significant portion of the IL-18 is in fact blocked by IL-18BP. These results demonstrate the physiological role of IL-18BP as an inhibitor of its ligand *in vivo*.

Selected Publications

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Acknowledgements

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For additional information see:

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