

LABORATORY OF INFLAMMATION AND CANCER

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BACKGROUND

Inflammation and inflammatory diseases are complex diseases implicating various cell types and molecules including neuroendocrine factors. Stress is known to affect the development of inflammation and it has been demonstrated that CRF peptides are key molecules in this crosstalk. Moreover, adipokines, endocrine factors secreted by adipocytes, are also implicated in the inflammatory process.

Modulation of the innate immune response is largely regulated by intracellular signaling molecules. The magnitude of Toll-like receptor signals is affected both by the expression levels of the receptors and by intracellular molecules that affect TLR signaling, including IRAK-M, Akt kinases and Tpl2 kinase.

CRF is a major neuroendocrine component of the crosstalk between the stress-response axis and the immune system. CRF also affects the immune system at a peripheral (paracrine and/or autocrine) level, possessing pro- or anti-inflammatory properties depending on the cell type and environment. Earlier studies from our laboratory have shown that CRF peptides directly affect macrophage activation and homeostasis. CRF augments pro-inflammatory cytokine secretion in LPS-activated macrophages via upregulation of TLR4, thus increasing macrophage sensitivity to pro-inflammatory agents.

The innate and adaptive immune systems are also important in combating cancer. Tumours secrete substances that modulate the immune response. Several types of tumours secrete neuropeptides of the CRF family including CRF and UCN1. CRF peptides directly affect at least one component of immune response, macrophages. Dendritic Cells (DCs) share common characteristics with macrophages. They are responsible for the presentation of tumour antigens to facilitate an effective immune response and eliminate the tumour. Another important modulator of the immune response against cancer are the Regulatory T-cells (Treg). Regulatory T-cell number is increased in the vicinity of tumours and suppresses cytotoxic T-cells. Our group is investigating the role of CRF peptides on dendritic and regulatory T-cells function.

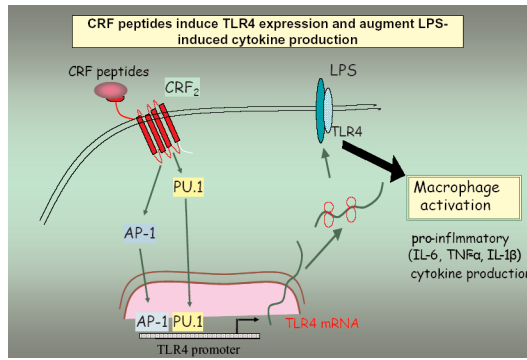
Tumor-produced factors also affect their motility and invasive potential. Tumor cell invasiveness is regulated through cytoskeletal changes and production of prostaglandins and/or metalloproteinases increasing vascular permeability and degrading the connective tissue allowing the tumor cell to escape. CRF peptides promote PGE2 production and potentially affect cancer cell metastasis.

RESEARCH

The goal of our research is to investigate the role of neuroendocrine factors in inflammatory diseases and cancer, focusing on the molecular mechanisms involved.

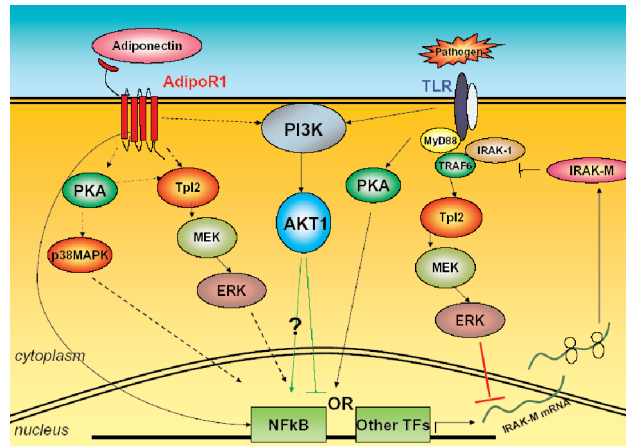
a) Signaling molecules controlling macrophage sensitivity and tolerance (A. Androulidaki, A. Arranz de Miguel, V. Zacharioudaki)

Neuroendocrine factors such as CRF and the Vasoactive Intestinal Peptide (VIP) affect macrophage sensitivity to bacterial LPS by modulating TLR4 expression. We are currently investigating the signaling mechanism involved as well as the significance of key molecules in the TLR signaling being Akt kinases, Tpl2 kinase and the inactive IRAK isoform IRAK-M.



Studies are being conducted in cell culture using cell lines and primary cells isolated by wild type and Tpl2^{-/-}, Akt1^{-/-} or IRAK-M^{-/-} mice, as well as in vivo using the same mouse strains. In addition, we study the role of CD40, a co-stimulatory molecule present on macrophages, in inflammatory bowel disease in mice, using liposome encapsulated anti-sense oligonucleotides in vivo.

b) The role of Adiponectin in regulating macrophage tolerance to pro-inflammatory stimuli (V. Zacharioudaki)



Adiponectin is an adipokine abundant in the plasma. The levels of adiponectin decrease when the body fat mass increases and has been reversely associated with development of the metabolic syndrome and coronary disease. We have previously shown that Adiponectin can be pro-inflammatory in vitro but induces tolerance to itself in vivo, being a major participant in controlling macrophage

sensitivity to pro-inflammatory stimuli. We are currently investigating the molecular mechanism that underlines the induction of tolerance using cell culture and in vivo models of knock-out animals such as Akt1^{-/-}, IRAK-M^{-/-} and Tpl2^{-/-} mice.

c) The role of CRF peptides in immunomodulation against cancer (A. Androulidaki)

Earlier work from our laboratory has shown that CRF peptides modulate macrophage activation by controlling the expression of TLR4 and the induction of PGE₂ in macrophages, a major component of the innate immune system. We are currently investigating the role of CRF peptides on Dendritic Cell (DC) differentiation, maturation and function as a potential way for the tumor derived CRF to alter the immune response against cancer. Regulatory T-cells are also important in modulating the immune response against cancer and several tumors utilize Tregs to suppress T-cell mediated responses. We are, therefore, examining the role of neuroendocrine factors on Treg function. The intracellular signaling mediators of these effects are analyzed.

d) The role of peripheral CRF in tumor growth and invasiveness; use of CRF agonists and antagonists in combating tumor growth (A. Arranz de Miguel)

Early studies indicated an association of stress with tumor growth and metastasis. The effect was partly attributed to impairment of the immune response. Recent evidence from our laboratory indicated that CRF promotes cancer cell motility in culture, suggesting that peripheral CRF has an impact on tumor growth. We are currently investigating the impact of stress and peripheral CRF on breast cancer cell motility and invasiveness using cell culture and in vivo studies, such as mice orthotopically transplanted with breast cancer cells. Studies utilize CRF and CRF receptor deficient animals as well as in vivo use of CRF receptor agonists and antagonists.

REPRESENTATIVE PUBLICATIONS

1. C. D. Dumitru, J.D. Ceci, C. Tsatsanis, D. Kontoyiannis, K. Stamatakis, J-H Lin, C. Patriotis, G. Kollias, N.A. Jenkins, N.G. Copeland and P.N. Tschlis. TNF- α induction by LPS is regulated posttranscriptionally via a Tpl-2/ERK-dependent pathway. (2000) *Cell* 103:1071-1083
2. S. Agelaki, C. Tsatsanis, A. Gravanis and A.N. Margioris Corticotropin-Releasing Hormone (CRH) augments proinflammatory cytokine production from macrophages in vitro and in LPS-induced endotoxin shock in mice. *Infect. Immun.* (2002) 70:6068-74
3. Tsatsanis C, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, Gravanis A, Margioris AN. Adiponectin induces TNF-alpha and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. *Biochem Biophys Res Commun.* (2005) 335(4):1254-63
4. Tsatsanis C, Androulidaki A, Alissafi T, Charalampopoulos I, Dermitzaki E, Roger T, Gravanis A, Margioris AN. Corticotropin-releasing factor and the urocortins induce the expression of TLR4 in macrophages via activation of the transcription factors PU.1 and AP-1. *J Immunol.* (2006) 176(3):1869-77
5. Tsatsanis C, Androulidaki A, Dermitzaki E, Gravanis A, Margioris AN. Corticotropin releasing factor receptor 1 (CRF1) and CRF2 agonists exert an anti-inflammatory effect during the early phase of inflammation suppressing LPS-induced TNF-alpha release from macrophages via induction of COX-2 and PGE2. *J Cell Physiol.* (2007) 210(3):774-83
6. Tsatsanis C, Vaporidi K, Zacharioudaki V, Androulidaki A, Sykulev Y, Margioris AN and Tschlis PN. Tpl2 and ERK transduce anti-proliferative T cell receptor signals and inhibit transformation of chronically-stimulated T cells. *PNAS* (2008) In press

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