Leptin Receptor Expression on Neutrophils

Abstract

Objective/Introduction:

Leptin is a peptide hormone that functions in metabolic control and has been shown to alter the immune response. In neutrophils, leptin appears to serve as both a chemo-attractant and anti-apoptotic. Specifically, in the pulmonary inflammatory response, leptin's anti-apototic and chemo-attractant effects on neutrophils contribute to a prolonged, exaggerated response that can exacerbate lung injury. However, little is known regarding which of the multiple isoforms of the leptin receptor (Ob-R) are relevant to neutrophil activation. We hypothesized that neutrophils express only the 'short form' of the leptin receptor, ObRa, which has fewer intracellular signaling domains than the 'long form' (ObRb)

Design:

Morphologically mature bone marrow neutrophils were isolated by discontinuous density gradient from lean C57Bl/6 mice. Total cellular mRNA was isolated from neutrophil lysates (PrepEase kit, USB), and cDNA was prepared (qscript cDNA SuperMix), before ObRa and ObRb transcripts were assayed using rt-PCR and quantitative PCR with Cyber Green probe.

To evaluate the signaling function of leptin receptors on neutrophils, we next examined two signaling molecules, STAT3 and P38-MAPK, which are downstream of the leptin receptors, for evidence of activation by phosphorylation. Neutrophils were exposed to leptin or positive controls (IL-6 for STAT3-activation or

lipopolysaccharide for P38) for 15, 30, or 60min. Total protein from neutrophil lysates was separated on a 10% SDS-PAGE gel before being transferred onto a nitrocellulose membrane. This was then incubated overnight with antibodies against phospho-STAT3 or phospho-p38, before incubation with fluorescent-labeled secondary antibodies. Detection of leptin receptor proteins was performed similarly.

Main Results:

Neutrophil expression of both ObRa and ObRb mRNA was detected, with ObRa being the predominant form. A similar pattern of protein expression was observed using Western blots.

We found no evidence of STAT3-phosphorylation in neutrophils following leptin exposure, but detected significant increases in phospho-p38.

Conclusions:

We find that neutrophils predominantly express the ObRa isoform of the leptin receptor. Furthermore, leptin induces neutrophil signaling through the MAPK pathway (known to be activated by both ObRa and ObRb) but not STAT3 (which is activated only by ObRb). Taken together, these findings suggest that neutrophil response to leptin is mediated by the short form of the leptin receptor.