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NOVEL LXRALPHA CROSSTALK WITH THE INTERFERON REGULATORY FACTOR 8 MODULATES MACROPHAGES INFLAMMATORY PATHWAYS

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The Liver X Receptors or LXRs are important modulators of cholesterol homeostasis and immune pathways. Accordingly, LXR activation by synthetic ligands decreases the progression of metabolic diseases with a strong inflammatory component such as atherosclerosis. Typically, LXRs control gene expression by heterodimerizing with the retinoid X receptor (RXR) and binding to LXR response elements or LXREs. We have now uncovered an additional mode of positive target gene regulation by LXR in macrophages that involves the hematopoietic transcription factors PU.1 and interferon regulatory factor 8 (IRF8), which control the development and function of several immune cell types. We previously demonstrated that levels of arginase 1, a metabolic enzyme with immunomodulatory actions, are induced by LXR in cultured macrophages and within atherosclerotic lesions undergoing regression. Moreover, elevated levels of arginase 1 are associated with the regression of atherosclerosis. We have also established that LXR does not regulate arginase 1 expression by directly binding to an LXRE in the arginase 1 gene, but instead induces the levels of IRF8 and promotes the interaction between IRF8 and PU.1 and their binding to the arginase 1 promoter. We have now evidence that in addition to arginase 1, the expression of other LXR target genes are dependent on IRF8 in macrophages. Microarray analysis was performed in differentiated bone marrow-derived macrophages (BMDM) activated with the specific LXR ligand GW3965 or vehicle. To examine whether the ligand response of identified LXR activated genes was dependent on IRF8 expression, IRF8 was selectively knocked down using RNAi. Results obtained were further validated in BMDM from IRF8-deficient mice. Interestingly, one of the IRF8-dependent LXR target genes we have identified this way encodes IL-18 binding protein (IL-18BP), a well-characterized endogenous inhibitor of cytokine signaling that has already been shown to modulate atherosclerosis. Il-18BP induced mRNA and protein expression by LXR ligands is dependent on LXR as demonstrated using LXR-deficient mice. Moreover, LXR ligands induce the promoter activity of IL-18BP as assessed by luciferase reporter assays. However, while LXR does not bind to the IL-18BP promoter, IRF8 binding to this locus is significantly enhanced by LXR ligand activation in BMDM as assessed by chromatin immunoprecipitation assays. In addition, to investigate the mechanism by which LXR ligands modulate IRF8 binding activity, LXR regulation of IRF8 post-translational modifications known to modulate its DNA binding was examined. Ligand-induced LXR enhances IRF8 phosphorylation in macrophages thus providing a potential mechanism underlying the induced IRF8 binding to LXR target genes. Collectively, this work identifies a novel crosstalk between IRF8 and LXR on the control of LXR macrophage gene expression that is likely to be relevant to the development of atherosclerosis.