

PPAR-G AND LEPTIN IN PULMONARY INFLAMMATION

Obesity is associated with chronic low-grade systemic inflammation and high levels of the adipokine leptin. Predominantly produced by adipose tissue, circulating leptin induces satiety by acting on the hypothalamus. Emerging data are establishing new roles for leptin in the cardiovascular and immune systems; however the role for leptin in the lung is unknown. The nuclear transcription factor peroxisome proliferator-activated receptor-gamma (PPAR-g) regulates inflammation and lipid metabolism and is constitutively expressed in healthy alveolar macrophages. Obesity is often accompanied by pulmonary inflammatory diseases, including asthma, in which PPAR-g deficiencies in alveolar macrophages are observed. Our lab demonstrated that the deletion of PPAR-g in murine alveolar macrophages of conditional knock out mice (PPAR-KO) results in pulmonary inflammation, making this an appropriate model to investigate the relationship of PPAR-g and leptin to pulmonary inflammation. Reports have indicated that elevated leptin levels result in decreased expression of PPAR-g and leptin receptors in primary macrophages. Based on these findings, we hypothesized that the expression of leptin receptors would be reduced in the alveolar macrophages of PPAR-KO. Alveolar macrophages were obtained from C57/Bl6 wild type mice and PPAR-KO by bronchoalveolar lavage. Analysis by real time-PCR showed a 7.5-fold ($P=.03$) decrease in leptin receptor mRNA expression in the PPAR-KO alveolar macrophages, compared to wild type mice. These results demonstrate the expression of leptin receptors on alveolar macrophages, suggesting an interaction between leptin and PPAR-g in the lung. Future studies will determine leptin levels in the lungs of wild type and PPAR-KO mice and its effects on pulmonary inflammation.

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INTRODUCTION

Obesity is a growing, nationwide epidemic, jeopardizing the health of American society today. According to recent reports, 300,000 obesity-related American deaths were recorded in the early 2000s.¹ In the United States, 31% of the population has a BMI of ≥ 30 and are considered obese.¹ Studies have shown that obesity is associated with high circulating levels of the adipokine leptin.^{2,3} Leptin is a protein secreted by adipose tissue (fat cells) that induces satiety by binding leptin receptors in the hypothalamus.⁴⁻⁷ However, emerging data are establishing new roles for leptin in the cardiovascular and immune systems. Obesity is often associated with chronic low-grade systemic inflammation and an increased incidence of inflammatory lung diseases, such as asthma.^{8,9} The role of leptin in the lung is unknown.

The nuclear transcription factor peroxisome proliferator-activated receptor-gamma (PPAR-g) is a known regulator of inflammation and lipid metabolism and is vital to the maintenance of lung homeostasis.¹⁰⁻¹² PPAR-g is expressed at high levels in the alveolar macrophages of healthy individuals and has been shown to be deficient in the alveolar macrophages of patients suffering certain pulmonary inflammatory diseases including asthma.^{8,9,13} Studies have shown that leptin down regulates the expression of PPAR-g in primary macrophages.^{13,14} Leptin may also negatively regulate the expression of leptin receptors in primary macrophages.¹⁵ Taken together, these observations suggest a role for leptin in the lung, possibly through interactions with

PPAR-g. We hypothesized that the alveolar macrophages from wild type mice will express the leptin receptor and the expression will be reduced in PPAR-g-deficient alveolar macrophages.

METHODS

Animal studies were conducted in conformity with Public Health Service (PHS) policy on humane care and use of laboratory animals and were approved by the institutional animal care committee. Bronchoalveolar lavage (BAL) cells were obtained from 8–12 week-old PPAR-g conditional knock out mice (PPAR-KO) and age- and sex-matched wild type C57/Bl6 controls, as previously described.¹⁶ Differential cell counts were obtained from cytopspins stained with a modified Wright's stain. BAL cell differentials from all animals used in experiments revealed greater than 90% alveolar macrophages. Mean viability of lavage cells was greater than 95%, as determined by Trypan blue dye exclusion. For all experiments, at least 3 sets of pooled BAL cells from 3–5 mice were used.

Total RNA was extracted from BAL cells by RNAeasy protocol (Qiagen, Valencia, Calif.) and samples were reverse transcribed into cDNA using SuperScript II First Strand Synthesis (Invitrogen, Carlsbad, Calif.). Expression of mRNA was determined by RT-PCR using the ABI 7300 Detection System (TaqMan; Applied Biosystem, Inc [ABI], Foster City, Calif.) according to the manufacturer's instructions. Samples were analyzed in duplicate using primer sets for mouse leptin receptor (Mm_01265583_ml, ABI, Foster City,

Calif.). Threshold cycle values for genes of interest were normalized to a house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and used to calculate the relative quantity of mRNA expression in PPAR-KO alveolar macrophages compared to wild type murine controls. Data were analyzed by Student's t-test on Prism software (Graph Pad, Inc, Calif.) and expressed as a fold change in mRNA expression relative to control values.¹⁷

RESULTS

Analysis by RT-PCR demonstrated a 7.5-fold ($P=.03$) decrease in leptin receptor mRNA expression in the alveolar macrophages of the PPAR-KO, compared to wild type controls.

DISCUSSION

Data reported here describe a potential role for leptin in the alveolar macrophage. We demonstrate leptin receptor mRNA expression in alveolar macrophages from wild-type mice. Furthermore, leptin receptor mRNA is down-regulated in alveolar macrophages from PPAR-KO mice. Previous work has demonstrated an inverse relationship between leptin and leptin receptor expression.^{15,18} Future studies are necessary to determine leptin levels in the lung. The present studies represent an initial step in defining the complex

pulmonary relationship between PPAR-g and leptin. We anticipate that future studies will further elucidate mechanisms by which the incidence of lung disease is increased in obese individuals.

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