Developmental Regulation of Airway Epithelial Receptors Involved in Host Defense

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INTRODUCTION

Respiratory diseases are a major cause of death in young children. In fact, it was the fourth leading cause of death in children under 5 years of age in 2000-2012. Adults do not experience this lethality, but it is important for the medical and scientific communities to define and understand this age-related susceptibility.

One potential mediator of this disparity is the respiratory epithelial barrier. These cells act as the first line of defense against pathogens with active secretion of mucus and antimicrobial proteins. Epithelial cells also act in pathogen sensing and immune cell recruitment, suggesting a role in mucosal immune responses. In cell culture, the transcription of these genes was compared in infants and adults and the effect of environmental exposure was also investigated.

IL-22R1, in conjunction with its ligand IL-22, is critically important in lung functions such as wound repair, antimicrobial peptide production, and chemokine secretion at mucosal barriers. While its mechanism of regulation in respiratory epithelium is unclear, it was previously found to have significantly greater mRNA expression and immunofluorescence showed a progressive increase in protein expression over the first 12 months of life (Figure 1).

IL-28R1 (IFNLR), and its ligand interferon lambda, are most associated with viral response and defense. The gene for IL-28R1 is located in close proximity to the IL-22R gene and is also transcribed in the same direction, indicating that the expression of these genes may be co-regulated under certain conditions.

HYPOTHESES

(1) Epithelial expression of interleukin receptors, IL-22R and IL-28R, is dependent on chronicologic age in the rhesus macaque.

(2) Epithelial expression to bacterial lipopolysaccharide will upregulate IL-22R and IL-28R expression.

METHODS AND RESULTS

Primary tracheobronchial epithelial cells (TBE) were isolated from rhesus macaque monkeys using previously published methods and grown until differentiated and polarized (see Fig. 2 and Fig. 4) and grown to confluence. Cells were then collected and RNA was isolated. This isolation was followed by DNase treatment, RNA quantification, and cDNA synthesis. Data was analyzed by Taqman RT-PCR using GAPDH as a reference house-keeping gene.

Baseline Expression of Interleukin Receptors

![Image](Image)

Figure 2: Experimental setup, in vitro study. Animals were raised in outdoor colonies. Both infant and adult explant samples were collected at necropsy. Cells were then grown in culture and then analyzed for mRNA expression.

![Image](Image)

Figure 3: There is a trending difference (p=0.0522) between infant and adult macaque monkeys regarding IL-22R1 mRNA expression in the respiratory lung epithelium. Likewise, there is a significant difference (p<0.05) between infant and adult macaque monkeys regarding IL-28R1 expression in the respiratory lung epithelium. At baseline, adult macaque monkeys tend to express more IL-22R1 mRNA and more IL-28R1 mRNA as compared to infants.

![Image](Image)

Figure 4: Experimental setup, in vitro study. Animals were raised in filtered air. Animals were challenged with exposure to lipopolysaccharide, experiments showed a trending increase in IL-22R1 expression and a significant increase in IL-28R1 expression. These changes in expression pattern could indicate co-regulation of IL-22R1 and IL-28R1 in response to LPS throughout life.

LPS Increases Interleukin Expression in Infants

![Image](Image)

Figure 5: There is a trending difference (p=0.14) between control and LPS-treated macaque monkeys regarding IL-22R1 mRNA expression in the respiratory lung epithelium. Likewise, there is a significant difference (p=0.066) between control and LPS-treated macaque monkeys regarding IL-28R1 expression in the respiratory lung epithelium. After LPS treatment, macaque monkeys tend to express more IL-22R1 mRNA and more IL-28R1 mRNA as compared to control animals.

CONCLUSIONS

With regard to age-related expression of interleukin receptors, these experiments demonstrated a trending difference in IL-22R1 expression and a significant difference in IL-28R1 (IFNLR) expression. These differences could contribute to age-related respiratory susceptibility of younger animals to respiratory diseases.

When animals were challenged with exposure to lipopolysaccharide, experiments showed a trending increase in IL-22R1 expression and a significant increase in IL-28R1 (IFNLR) expression. These changes in expression pattern could indicate co-regulation of IL-22R1 and IL-28R1 in response to LPS throughout life.

FUTURE DIRECTIONS

The next step in this project is to look at protein expression of IL-22R1 and IL-28R1 in these cells. Although quantifying message is the first step, it is important to observe a similar pattern in protein expression. Additionally, it would be interesting to investigate the mechanism of co-regulation for IL-22R1 and IL-28R1. Some promising candidates include NFκB and AHR. Lastly, it is important to understand the mechanism of LPS regulation on IL-22R1 and IL-28R1 in order to understand why and how LPS affects expression of these interleukin receptors.

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