The Effect of Th17 Cytokines and Environmental Pollutants on Epithelial Polymeric Immunoglobulin Receptor Expression

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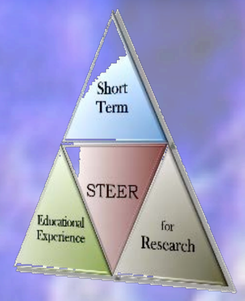

Abstract

The polymeric immunoglobulin receptor (pIgR) is a receptor expressed by epithelial cells that promotes the transcytosis of polymeric IgA and IgM across mucosal epithelia. The effect of Th17 cytokines (IL-17, IL-21 and IL-22), Th17 culture supernates and environmental pollutants on pIgR expression by the mouse epithelial cell lines CMT 64 and CMT 93 was examined. Epithelial cells were cultured in the presence of cytokines and the effect on the level of pIgR mRNA expression (determined by 'real-time' RT PCR) and pIgR protein levels (determined by flow cytometry) was evaluated. Flow cytometry revealed that after 48 hours of stimulation, the Th17 product interleukin-17 (IL-17) induced pIgR expression while IL-21 and IL-22 were less effective. Moreover, neutrophil myeloperoxidase (MPO), an enzyme that creates ozone-like oxidizing products, after 24 hours of stimulation, induced pIgR expression and promoted proliferation of the epithelial cells. These findings demonstrate pIgR expression by epithelial cells is highly responsive to inflammatory Th17 cytokines and the action of MPO. We are currently examining whether the oxidant gas, ozone, also mediates any of these effects.

Introduction

T helper 17 cells (Th17)

T helper 17 (Th17) cells are differentiated CD4+ T cells that are characterized by their production of IL-17, IL-21, and IL-22. Only recently discovered, they are thought to be involved in autoimmune disorders and stimulation of the immune system in response to pathogens[1]. In vivo studies have shown that IL-17 increases expression of the polymeric immunoglobulin receptor (pIgR) by airway epithelial cells[2].

Polymeric Immunoglobulin Receptor (pIgR)

The pIgR is expressed by mucosal epithelial cells. Its primary function is to transcytose polymeric IgA and IgM across epithelium. In addition, proteolytic degradation of pIgR generates a secretory component (SC) which either remains associated with antibody or released in a free form that has anti-microbial properties. After transcytosis, the SC remains bound to IgA, protecting it from proteolytic degredation. This action results in a 1:1 ratio of pIgR to IgA giving significance to the regulation of pIgR[3].

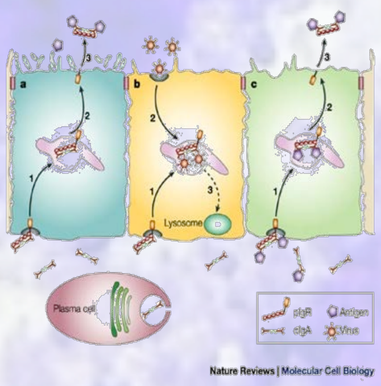

Figure 1: pIgR Transcytosis Model[4]

Ozone model: Myeloperoxidase

In order to model ozone in an in vitro liquid setting, the neutrophil enzyme myeloperoxidase was used. Ozone causes the neutrophil infiltration into the airways, therefore modeling the effect of MPO on the immune system is important in understanding the role of ozone in respiratory inflammation and health[5].

Methods

Cell Culture and Epithelial Cell Treatment:

Two mouse epithelial tumor cell lines CMT 64 (lung) and CMT 93 (colon) obtained from ATCC were cultured in 5% CO2 at 37°C and passaged every 7 days. For the experiments, $1x10^6$ cells were grown in 50ml tissue culture flasks and stimulated for 48 h with the following: Th17 cytokines IL-17, IL-21 and IL-22, supernatants from activated Th17 cells, IL-4 (Th2 cytokine), IFN-Ɣ (Th1 cytokine) and MPO.

ELISA:

CD4+ Th17 were generated from BALB/c lymph nodes in presence OVA323-339, TGF-β, IL-6 and IL-23. After 8 days the Th17 cells were stimulated with anti-CD3 (0.2 μg/ml and 2.0 μg/ml) and the culture supernatant harvested after 24h and used to stimulate epithelial cell lines. ELISA assays were performed to determine the amount of IL-17 and IL-21 in the supernatant.

FACS:

Cells were stained with the secondary antibody Dylight 488 donkey anti-goat (Jackson Immuno Research Westgrove, PA) and anti-mouse pIgR antibody (R&D Systems, Minneapolis, MN) and then analyzed by flow cytometry for pIgR expression by FACS Aria (BD Biosciences).

Real Time (RT)-PCR:

Cells were harvested after the experiments, RNA was extracted by Trizol method and analyzed by RT-PCR to determine the expression of pIgR mRNA (and house-keeping gene, b-actin).

Results

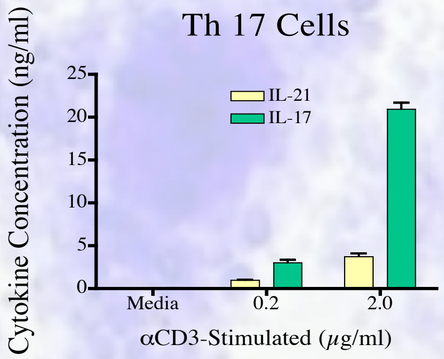

Figure 3. Concentration of IL-21 and IL-17 in Th17 Supernatant.
After anti-CD3 stimulation of Th17 cells the supernatants were removed and analyzed by ELISA for IL-17 and IL-21 concentration.

References

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4. Rojas, R., G. Apodaca, 2002, Immunoglobulin transport across polarized epithelial cells. *Nat Rev Mol Cell Biol*. 3: 12: 944-946
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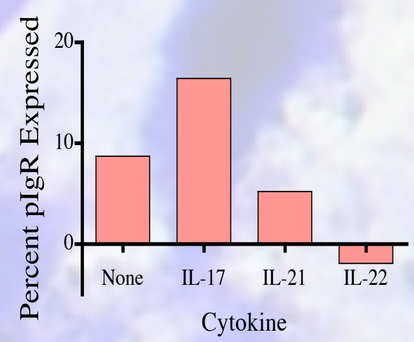
Background from http://www.rajivsaxena.info/Lung_Epithelial_Cells.asp

Results

a. Epithelial line CMT 64 (FACS)



b. Epithelial line CMT 93 (PCR)

c. Epithelial line CMT 64 (PCR)

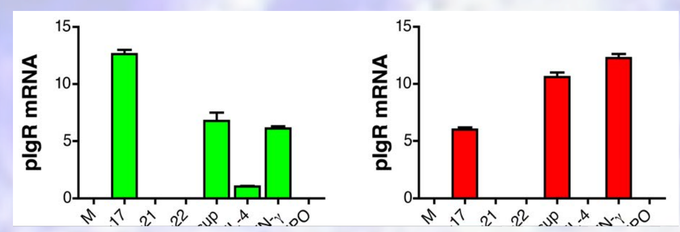


Figure 2: pIgR Expression following stimulation with Th17 cytokines and MPO.
(a) Flow cytometry results after 48 hours of stimulation. Percent pIgR expressed as determined by the difference between the percentage of cells showing pIgR with the secondary antibody, and the percentage of cells showing pIgR with the fluorescent label, Alexa Fluor 488-A. Relative amounts of pIgR mRNA found after stimulation of CMT 93 (b) and CMT 64 (c) with cytokines and MPO.

Conclusions

- FACS analysis showed that small concentrations of MPO (20nm/mL) cause epithelial cell proliferation and promote expression of pIgR suggesting that oxidizing effects of neutrophils may influence epithelial cells. However, the PCR results did not see the same induced expression of pIgR.
- Cells incubated with IL-17 show increased expression of pIgR after 48 hours suggesting that this Th17 cytokine increases polymeric IgA and IgM transcytosis. These results were supported by FACS and PCR.
- Th17 culture supernatant promoted pIgR expression by epithelial cells. The relative contributions of the Th17 cytokines will be resolved using specific blocking antibodies.

Acknowledgements

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