Mechanism of Anti-Inflammatory Action of 5-ASA on Intestinal Epithelium

Asit Panja. UMDNJ-Robert Wood Johnson Medical School; New Brunswick, NJ USA and
AlfaGene Bioscience, Inc.; Somerset, NJ USA.

BACKGROUND

Ulcerative Colitis (UC) is a chronic inflammatory disease of the large intestine with unknown etiology. 5-ASA has long been used for the treatment of UC. However, its mechanism of action is still poorly understood. Several pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, interferon-γ, and TNF-α, have been found to be increased in intestinal mucosa and are believed to have major roles in the pathogenesis of UC. We have previously shown that epithelial cells produce pro-inflammatory cytokines (IL-6, IL-8, and GM-CSF) and significantly contribute to the increased IL-8 production in active UC mucosa.

AIM

In order to investigate potential anti-inflammatory effects of 5-ASA, we studied the effects of 5-ASA on spontaneous and stimulated (LPS, IFN-γ) IL-8 and IL-6 production on a novel cellular model of human intestinal primary epithelial cells (HIPEC) from patients with active UC (n=3) and normal unaffected controls (NL; n=3).

MATERIALS AND METHODS

HIPECs were cultured in the presence or absence of either LPS (1 mg/ml) or IFN-γ (250 u/ml) for 24 hours. The level of cytokine (IL-6 and IL-8) production was measured by ELISA.

RESULTS

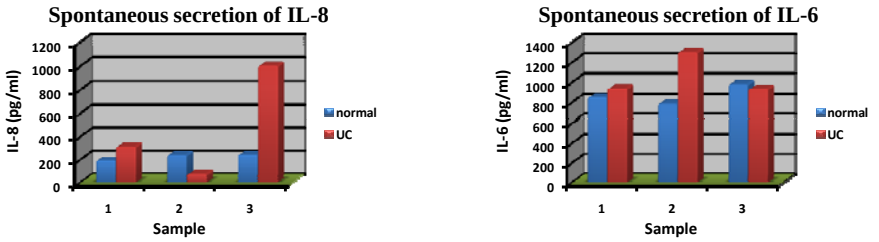

Fig 1. Comparison of spontaneous levels of pro-inflammatory cytokine (IL-6, IL-8) secretion by inflamed (UC) and normal adult human gastrointestinal stem cell derived primary epithelial cells (HIPEC) (n=3). 5 x 10^4 cells/well/ml were cultured in 12 well tissue culture plates for 24 hours. Cell free supernatants were collected and assayed for IL8 and IL6 content by ELISA. A relatively high level of spontaneous IL6 and IL8 secretion were observed (UC>NL) in UC and NL cells.

RESULTS

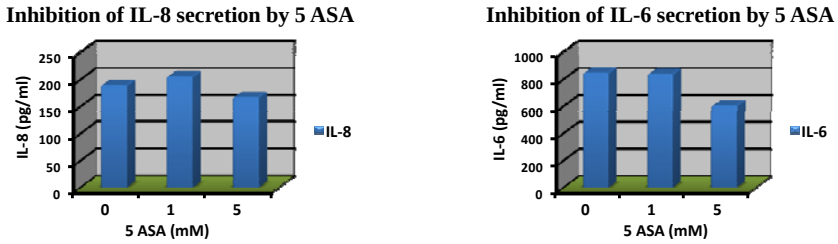

Fig 2. Determination of working concentration of 5 ASA for inhibition of spontaneous IL8 and IL6 secretion by HIPECs. Similar to figure 1, HIPECs were cultured in the presence of varying concentration of 5ASA for 24 hours. Cell free supernatants were assayed for cytokine content by using specific ELISA kits for IL-8 and IL-6. A 5 mM concentration of 5ASA was effective in inhibiting both IL8 and IL6 by ~25%.

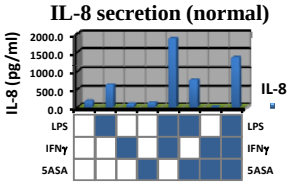

Fig 3. Effect of 5ASA on LPS and/or IFN-γ stimulated IL8 production by normal HIPECs. HIPECS were cultured with or without LPS (1μg/ml), IFN-γ (200 u/ml) or in combination in the presence or absence of 5 ASA (5mM). 5ASA inhibited IFN-γ but not LPS stimulated IL-8 secretion.

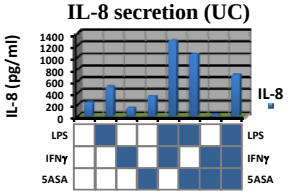

Fig 4. Effect of 5ASA on LPS and/or IFN-γ stimulated IL8 production by HIPECs derived from an actively inflamed tissue from patient with UC. Similar to figure 3, HIPECS were cultured with or without LPS (1μg/ml), IFN-γ (200 u/ml) or in combination in the presence or absence of 5 ASA (5μM). Again, 5ASA inhibited IFN-γ but not LPS stimulated IL-8 secretion.

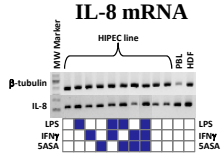

Fig 5. Effect of 5ASA on IL8 mRNA expression of AHGI-SC derived primary epithelial cell line A2J1. RT-PCR result of IL-8 from total RNA isolate of epithelial cell line A2J1. PBL = Peripheral blood lymphocyte control, HDF = Human Dermal Fibroblast control.

SUMMARY AND CONCLUSIONS

These results suggest that the anti-inflammatory action of 5ASA is mediated via inhibition of IFN-γ stimulated IL-8 production in the intestinal epithelium. Although, further study is needed to establish the relationship between IL-8 responsiveness to IFN-γ stimulation and 5-ASA suppression. The results from this study may help in the further understanding of the mechanisms of anti-inflammatory action