MODELLING GOBLET CELL RESPONSES IN ALLERGY AND PARASITISM

Jeremy K. Brown, Steven H. Wright, Julie A. Bethune, Pamela A. Knight and Alan D. Pemberton

Division of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, UK

INTRODUCTION: Th2 mediated epithelial effector mechanisms targeted at parasites and allergens contribute to the pathology associated with these responses, including goblet cell metaplasia and the release of mucins and other effector molecules. In this study, the human goblet cell-like LS174T colon adenocarcinoma cell line was used to model the goblet cell response to Th2 cytokines.

TRANSCRIPT ANALYSIS: Microarrray analysis of LS174T cells grown in control medium and medium supplemented with 1ng/ml of Th2 cytokines IL-4 or IL-13 revealed a small number of transcripts highly upregulated (>10-fold) by both cytokines, (Table 1) [1] namely intelectin-1 (ITLN1), chemokine CCL26, matrix metalloproteinase-1 (MMP1), calcium activated chloride channel 1 (CLCA1) and transmembrane protein 16E (TMEM16E). PCR analysis (Figure 1) indicated that ITLN1 and CLCA1 were significantly upregulated by as little as 25pg/ml IL-4.

Gene	Identity	Function	Fold change +IL-4 (1ng/ml)	Fold change +IL-13 (1ng/ml)
Itln1	Intelectin-1	Galactofuranose binding, Lactoferrin receptor	78.9	43.1
Ccl26	Eotaxin-3	Eosinophil recruitment	36.4	11.9
Mmp1	Matrix metalloproteinase-1	Tissue remodelling	31.2	15.4
Tmem16e	Transmembrane protein 16E	unknown	29.6	20.1
Clca1	Chloride channel, calcium activated, 1	? secreted in mucus	18.3	15.1

Table 1. Preliminary microarray analysis (Agilent Whole Human Array) of LS174T cells treated with Th2 cytokines. Only genes upregulated >10-fold by both cytokines are shown.

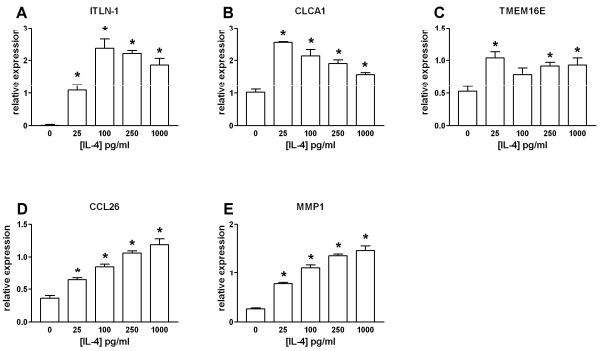


Figure 1. Confirmatory semi-quantitative RT-PCR. LS174T cells were treated with IL-4 at 0, 25, 100, 250 and 1000 pg/ml. Semi-quantitative RT-PCR was performed for the five main Th2 responsive genes and results expressed relative to housekeeping gene RPL19. Significant up-regulation of expression (*, p<0.05) was observed at 25pg/ml IL-4.

GOBLET CELL DEGRANULATION: Incubation of LS174T cells with IL-4 significantly increased responsiveness to the goblet cell secretagogue, tauroursodeoxycholate (TUDC) (Figures 4, 5).

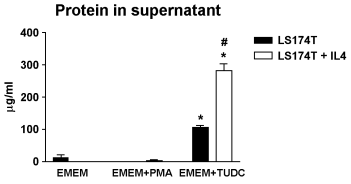


Figure 4. (left) Release of goblet cell contents stimulated by PMA and TUDC. Significantly more protein was secreted when cells were treated with TUDC (*, p<0.05). TUDC induced significantly more protein secretion in cells pre-treated with IL-4 (#, p<0.05). Neither PMA nor TUDC had any affect on viability.

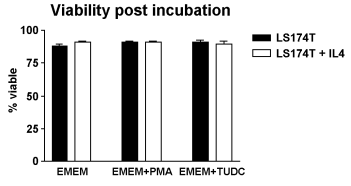
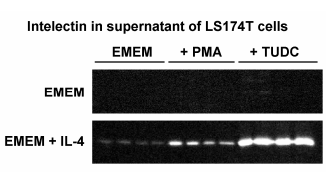


Figure 5. (right) Anti-intelectin Western blot of LS174T goblet cell secretions, in the presence / absence of IL-4 and secretagogues PMA and TUDC. This shows that increased expression of Itln1 transcripts by Th2 cytokines also results in increased levels of Itln1 protein secretion by goblet cells.



CELL MORPHOLOGY: Th2 cytokine-treated goblet cells grew in colonies with a distinct rounded-up morphology and reduced mean colony area. We investigated the potential roles of ITLN1 and CLCA1 in this morphology change. Use of ITLN1-specific siRNA reduced ITLN1 expression by >70% but did not affect colony morphology. However, the CLCA1 inhibitor, niflumic acid, reversed the IL-4-induced morphology change when applied at 100µM. The use of niflumic acid also downregulated ITLN1 and CLCA1 expression, indicating that it suppresses Th2 responses in general [2].

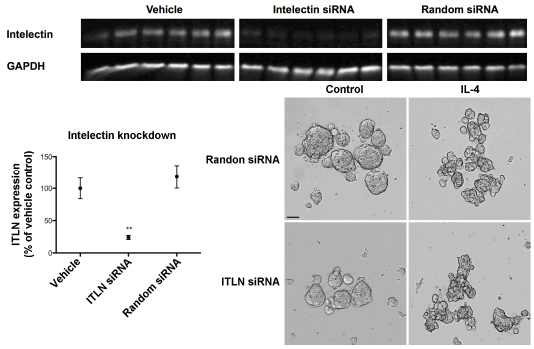


Figure 2. siRNA mediated knockdown of intelectin protein expression does not reverse IL-4 induced morphological changes in LS174T cells. LS174T cells were cultured for 72 hours +/- 1 ng/ml of IL-4, and with:1) siRNA transfection reagent alone (Vehicle); 2) Itln specific siRNA; or 3) random siRNA. Itln protein levels were reduced by 76% in IL-4 cultures treated with specific siRNA but this had no effect on IL-4 mediated morphology changes.

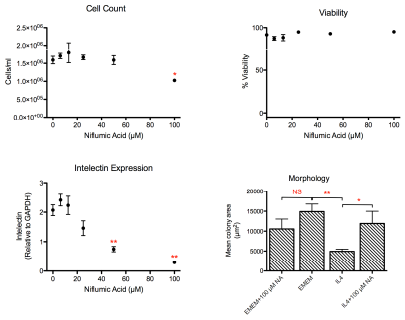


Figure 3. Niflumic acid inhibits the effects of IL-4 in LS174T cells. LS174T cells were cultured +/- 1 ng/ml of IL-4 plus various concentrations of niflumic acid. Cell numbers and viability were assessed. Intelectin expression was quantified by western blot. IL-4 dependent morphological changes were quantified using an automated image-analysis routine. Niflumic acid blocked Itln expression in a dose dependent manner and reversed morphological changes induced by IL-4. Note that niflumic acid significantly increased LS174T colony sizes despite reducing the number of cells present in the cultures.

CONCLUSION: This study further defines the responses of goblet cells to Th2–induced mucosal responses, highlighting key effector molecules for further investigation.

REFERENCES

1) Knight, P.A. et al (2008) *Parasitology*, in press

2) Nakano, T. et al. (2006) Am J Respir Crit Care Med, 173, 1216-1221

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