

Regulation of type 2 cytokine release by epithelial cells: characterisation of soluble factor pathways and characterisation of potential mediators.

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**Introduction:** Type 2 cytokines such as IL-13, IL-4 and IL-5 have been shown to play important roles in the pathogenesis of asthma. One source of these cytokines is type 2 CD4+ or CD8+ T cells. We have shown that epithelial cells have inhibitory effects on these T cells and that this regulatory effect could be defective in asthma. We have previously shown that type 2 T cell lines release less IL-13 in the presence of epithelial cells and others have shown that epithelial cells are able to reduce division of CD4+ T cells. We wished to extend these studies to determine whether bulk cultures of PBMC were able to release IL-13 and whether this IL-13 was regulated by epithelial cells and whether this was mediated by direct cell contact.

**Methods:** We used PBMC from healthy donors and cultured cells in the presence and absence of epithelial cells with titrated doses of IL-2. We used transwells and epithelial cell supernatants to determine whether supernatants were also able to reduce type 2 cytokine secretion. We used size exclusion centrifugation to split supernatants into different fractions.

**Results:** After culture of PBMC for 5 days in IL-2, IL-13 release (pg/10<sup>6</sup> cells +/- SD) was 509.95 +/- 84.95 and was reduced to 37.3 +/- 7.4 by A549 epithelial cells separated by a transwell. Titration of A549 cells established that inhibition was cell number dependent. Inhibition was not due to scavenging of IL-13 by epithelial cells during co-culture. Less IL-13 was secreted by IL-2 treated PBMC (pg /10<sup>6</sup> cells +/- SD) in the presence of 50% v/v supernatant from healthy HBEC 49+/-7 or asthma HBEC 90+/-21 p=0.0023. IL-5: HHBEC 14+/-5 AHBEC 26+/- 12 not significant. Splitting the HBEC supernatant into different size fractions showed that the fraction over 3kD was less inhibitory than the fraction under 3kD.

**Conclusion:** There may be a soluble mediator secreted by epithelial cells that is less than 3kDa in mass that is able to inhibit type 2 cytokine release from PBMC. These inhibitory factor(s) could contribute to the regulation of type 2 cells that and affect asthma pathogenesis.