CD6 is highly expressed in fatal asthma patients and may modulate bronchomotor tone

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Abstract

Rationale: Th2 and Th17 cells contribute to airway remodeling, facilitate mucus secretion and increase airway hyperresponsiveness. CD6, a T cell co-stimulatory receptor, interacts with CD166 (ALCAM) and CD318 to modulate activation. We examined whether severe asthma subjects have increased CD6+ cells, the lung inflammatory environment alters CD166 or CD318 expression, and CD6 exposure of airway smooth muscle (ASM) modulates bronchomotor tone.

Methods: Lungs (asthma, non-asthma, n=4/group) were lavaged and lymphocytes examined for CD6 by flow cytometry. Tissue was stained for CD166, CD318, CD3, and CD6. ASM stimulated with carbachol (Cch) or CD6-beads was examined for phosphorylation of myosin light chain (pMLC), paxillin (pPax), or cofilin (pCof). ASM were co-cultured with T cells ± IL-33 with CD166 and CD318 expression assessed.

Results: Flow analysis showed no difference in CD6+ lavage cells from asthma vs. non-asthma donors. Tissue sections had large differences in lymphocyte number and CD3+/CD6+ cells, with CD166 and CD318 expression on ciliated and basal cells. *ALCAM*, but not *cd318*, expression increased in asthma ASM. CD6-coated beads increased pCof and pPax, but not pMLC, to similar levels to Cch. ASM co-cultured with differentiated Th1 cells showed decreased CD166, but not CD318 expression. Co-stimulation with Th1 cells + IL-33 markedly decreased ASM CD166 and CD318 expression.

Conclusions: We demonstrate that CD6+ lymphocytes are significantly increased in fatal asthma lung tissue. Furthermore, CD6+ lymphocytes may modulate bronchomotor tone through actin cytoskeletal rearrangements in HASM, and that the inflammatory environment may influence CD6-dependent changes in bronchomotor tone.

CD6 in asthma

- CD6 is a co-stimulatory molecule that promotes T cell activation and proliferation. CD6 binds to CD166 (ALCAM) and CD318 (CDCP1).
- Identification of 3 single nucleotide polymorphisms in ALCAM (CD166, CD6 receptor) found in active adult onset non-allergic asthma.
- Inflammatory responses decreased in ALCAM-/- mice.
- Sputum and serum ALCAM concentrations increased in asthmatic children.
- Increased CD318 serum levels were associated with poor asthma control.

References

1. Siroux V *et al* (2014) Genetic Heterogeneity of asthma phenotypes identified by a clustering approach. Eur Respir J; 43(2):439-52.
2. Kim M et al (2018) Activated Leukocyte Cell Adhesion Molecule Stimulates the T cell Response in Allergic Asthma. Am J Respir Crit Care Med; 197(8) 944-1008.
3. Kasaian M *et al* (2018) Proteomic analysis of serum and sputum analytes distinguishes controlled and poorly controlled asthmatics. Clin Exp Allergy; 48(7):814-24.

Hypothesis

Upregulation of CD6 and its cognate receptors will modulate airway function in the context of severe asthma and exacerbations of asthma.

Numbers of CD6+ lymphocytes are increased in the airways of severe asthma patients

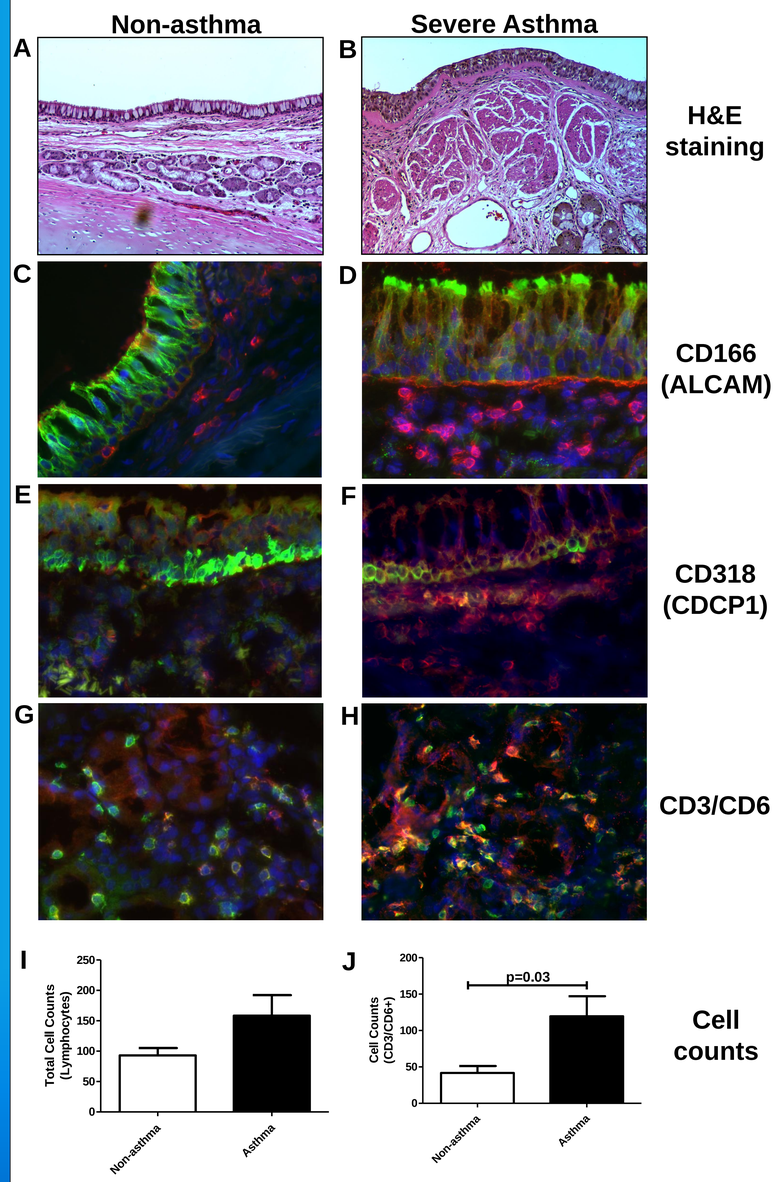

Figure 1 - CD6 and cognate receptor expression in human bronchi. Bronchi isolated from non-asthma and severe asthma donors were H&E stained (A,B), stained for CD166 (green=CD166, red=CD6 - C,D), CD318 (green=CD318, red=CD6 E,F), and co-stained for CD3 and CD6 (green=CD3, red=CD6 - G,H). Total counts of lymphocytes (CD3+, I) and quantification of CD3+/CD6+ cells (J) were performed. Images represent n=4 distinct non-asthma and severe asthma donors, showing mean + standard error with significant evaluated by t-test.

BAL cells derived from non-asthma and severe asthma donor lungs show little difference in lymphocyte subsets

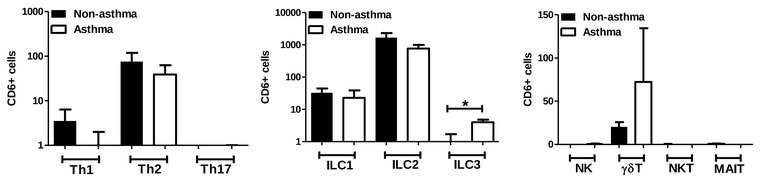

Figure 2 - BAL lymphocyte immunophenotyping shows little difference in CD6+ numbers. BAL cells isolated from non-asthma and severe asthma donor lungs were stained for markers of Th1, Th2, Th17, ILC1, ILC2, ILC3, NK, γδT, NKT, and MAIT cells and examined by flow cytometry. Data is representative of n=4 distinct non-asthma and severe asthma donors, *p ≤0.05.

Bead-bound CD6 elicits phosphorylation of cofilin and paxillin, but not phosphorylation of myosin light chain in human airway smooth muscle (HASM)

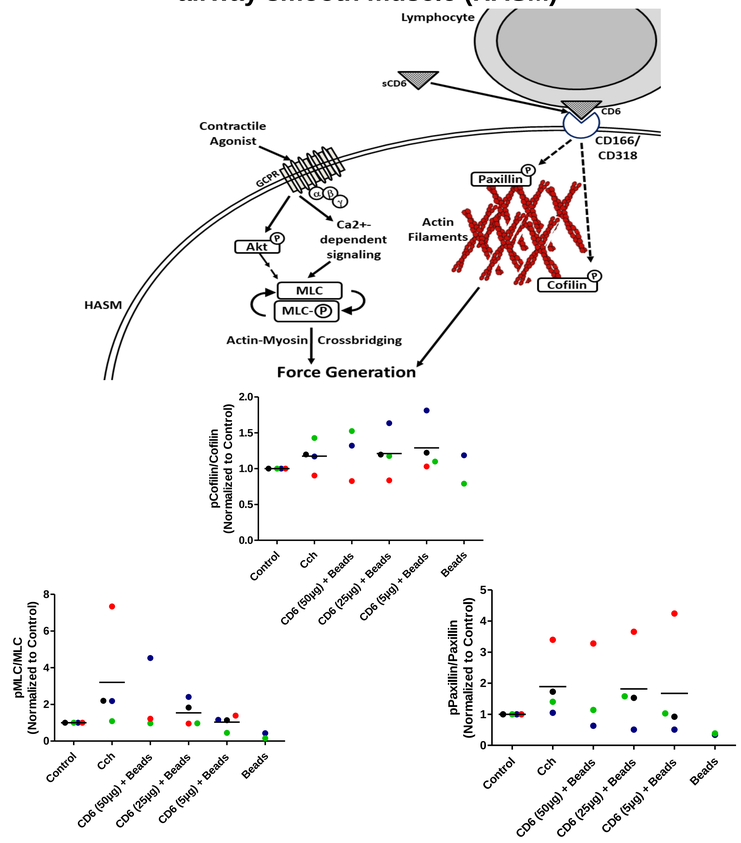

Figure 3 - CD6 elicits phosphorylation of actin-associated proteins, but not myosin light chain. HASM were stimulated with CD6 (5, 25, 50 µg - 10 min) or carbachol (Cch, 20 µM, 10 min), lysed and phosphorylation of cofilin, paxillin, or myosin light chain (MLC). All data was normalized to the non-phosphorylated form of each protein. N=3 separate HASM donors.

Th1-differentiated lymphocytes and soluble CD6 decrease expression of CD166 on HASM

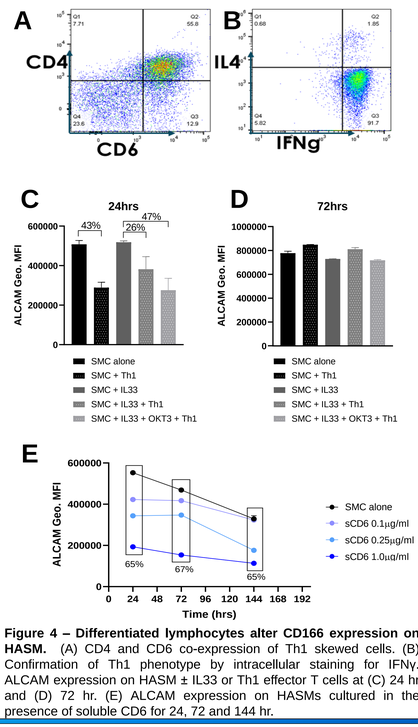

Figure 4 – Differentiated lymphocytes alter CD166 expression on HASM. (A) CD4 and CD6 co-expression of Th1 skewed cells. (B) Confirmation of Th1 phenotype by intracellular staining for IFNγ. ALCAM expression on HASM ± IL33 or Th1 effector T cells at (C) 24 hr and (D) 72 hr. (E) ALCAM expression on HASMs cultured in the presence of soluble CD6 for 24, 72 and 144 hr.

Conclusions

- CD166 is expressed on the apical membranes of epithelial cells, while CD318 is expressed basally. CD6 expression is mainly restricted to lymphocytes (CD3+). The proportion of CD3+/CD6+ cells in the tissue of severe asthma donors is significantly greater than in tissue of non-asthma donors.
- Immunophenotyping of BAL-derived cells yielded little differences in CD6 expression between non-asthma and severe asthma donors.
- Clustering of CD6 on beads elicited increased phosphorylation of actin-associated proteins, including cofilin and paxillin, but had little effect on myosin light chain.
- The combination of a Th1 signal from differentiated T cells, together with IL-33 stimulation, significantly attenuated CD166 expression on HASM.

Significance

These studies provide experimental evidence to support targeting CD6 to as an asthma therapy to potentially modulate bronchomotor tone, but may not alter HASM responses in response to virus.

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