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# T regulatory cells from asthmatic individuals show a Th2-like phenotype

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**T regulatory cells from people with asthma show a Th2-like phenotype**

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experimental infection study was supported by a Medical Research Council Clinical Research Fellowship (to S.D.M.), a British Medical Association H.C. Roscoe Fellowship (to S.D.M.), British Lung Foundation/Severin Wunderman Family Foundation Lung Research Program Grant P00/2, Asthma UK Grants 02/027 and 05/067, Welcome Trust Grants 063717 and 083567/Z/07/Z for the Centre for Respiratory Infection, Imperial College, and the National Institute for Health Research (NIHR) Biomedical Research Center funding scheme. S. L. Johnston is the Asthma UK Clinical Professor (grant CH11SJ), is an NIHR Emeritus Senior Investigator and was supported by MRC Centre Grant G1000758, Asthma UK Centre Grant AUK-BC-2015-01 and European Research Council Advanced Grant 788575. K.C. Nadeau is supported by NIH grant U19 AI104209 (Asthma and Allergic Diseases Cooperative Research Center), U01 AI140498 and R01 AI140134 and the Naddisy Foundation.

To the editor,

Asthma is the most common chronic inflammatory disease of the lung, characterised by wheezing, shortness of breath and variable airflow obstruction. It is a heterogeneous disease that can be classified into different endotypes of which T2-high - allergic asthma is one of the most common forms, especially in children. Allergic asthma is characterised by increased IgE and type-2 cytokines, including IL-5, IL-4 and IL-13[1].Thus far, it is not completely understood why these type-2 responses are poorly controlled in asthma. T regulatory cells (Treg cells) are key mediators in controlling type 2 responses. However, under certain conditions, Treg cells can display a pathogenic and proinflammatory phenotype and contribute to disease pathogenesis[2]. Treg cells of food allergic children showed a T helper 2 (Th2)-like phenotype. Whether this Th2-like phenotype of Treg cells is also present in asthmatic individuals is unknown.

Therefore, in this exploratory study, we compared the gene-expression profile of Tregs from people with stable allergic-asthma to non-allergic controls without asthma. We isolated PBMCs from 5 people with asthma and 4 controls (Table S1) and sorted Treg cells with flow cytometry ($CD3^{+}CD4^{+}D25^{hi}CD127^{low}$). Then, we isolated RNA from the sorted Treg cells and performed RNA-seq (See Supplemental information for detailed methods). In total, 369 genes were differentially expressed between Treg cells from asthmatic individuals and controls ($P<0.01$) (Supplemental Figure 1). We clustered the genes into different groups: Treg cell markers, cytokine receptors, virus related, transcription factors, cytokines and others (Figure 1A). Interestingly, we found that the expression of *FOXP3*was reduced in Treg cells from asthmatic individuals (Figure 1B). This is in line with a previous study that observed a lower expression of*FOXP3* in Treg cells from individuals with asthma[3]. Interestingly *FOXP3* expression inversely correlated with the IgE levels found in the serum (Figure 2A), supporting the finding that Treg cells can suppress IgE production[4].

In addition, we found a significant upregulation of *IL13* mRNA expression and a trend to increased expression of *IL4* and*IL5* mRNAs in Tregs in asthma, indicating a Th2-like phenotype as was reported in Tregs from children with food allergies[2]. Furthermore, we found an upregulation of the prostaglandin D2 receptor (*PTGDR2* ) or CRTH2, in line with a previous study that reported an increased amount of $CRTH2^{+}$ Tregs in asthma[5].

Interestingly, several cytokine receptors were differentially expressed between Tregs from asthmatic individuals compared to controls. The IL-4 receptor alpha transcript *IL4RA* was significantly reduced in asthma. The expression of *IL4RA* also strongly correlated with the levels of IgE in the serum (Figure 2A). Previously, it was shown in mice that IL-4 receptor signalling is essential in controlling Th2 responses and airway inflammation[6]. Our data suggest a similar role of *IL4RA* in humans. Likewise, we observed a downregulation of TNF receptor superfamily member 25 (*TNFRSF25* ), which was shown to contribute to preventing allergic lung inflammation[7] and downregulation of OX40 (*TNFRSF4* ).

Additionally, we observed a difference in virus/type-I interferon(IFN)-related genes in asthma, which was also observed in single-cell transcriptomic data of allergen-specific Tregs from individuals with asthma[8]. Curiously, the expression of the type 1 IFN receptors *IFNAR1/2* were lower expressed in asthma, which could indicate a deficiency against respiratory viruses and chronicity.

Lastly, we performed an enrichment analysis to see up or downregulation of pathway maps, process networks

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and go processes with MetaCore (Table 1). The pathway maps and process networks included upregulation of pathways related to immune functions already described. However, the affected GO processes were mostly related to epigenetic mechanisms including nucleosome organisation, nucleosome assembly and chromatin organisation. With the tool STRING, we performed a pathway analysis that showed a cluster of histone genes (Figure 2B). So far, there is no data reporting the function of histone genes in Tregs or related to asthma, but perhaps this finding could be related to changes in epigenetics. It was reported that in asthma Tregs have increased CpG methylation of the*FOPX3* locus compared to individuals without asathma[3].

In conclusion, Tregs from individuals with asthma show reduced expression of several molecules related to Treg suppressive functionality, while having increased expression of Th2-like characteristics that could lead to their reduced control of allergic airway inflammation. Further studies are needed to confirm these findings in a larger population and investigate their contribution to disease pathology.

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**Figure 1: Tregs from asthmatic individuals show a distinct phenotype compared to controls.** (A) Genes that are significantly changed in Tregs cells from asthmatic individuals compared to controls (log 2 ratio)– clustered in the groups: Treg markers, cytokine receptors, virus related, transcription factors, cytokines and others. (B) Fragments per kilo base per million mapped reads (FPKM) values of genes of

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interest (FOXP3, IL13, IL5, IL4, IL4R, PTGDR2, TNFRSF25, TNFRSF4, IFNAR1, IFNAR2) of all donors. N = 4 (healthy), 5 (asthma). *** p<0.001 , ** p<0.01, * p<0.05

**Figure 2: Phenotype of Tregs might be associated to Treg function** . (A) Correlation between expression of FOXP3 (left) and IL4RA (right) with IgE serum levels. (B) Satellite plot showing a cluster of known interactions related to nucleosome assembly. Genes higher expressed in asthmatic individuals are shown in red, and lower expression in blue.

**Table 1: Differentially expressed pathways maps, process networks and Go processes in Tregs from asthmatic and healthy subjects.**

| **Pathway maps** | Genes |
|---|---|
| Immune response IFN alpha/beta signalling via PI3K and NF-kB pathways | CCND3, MAPK3, IFI17, IFITM1, IFN |
| Immune response OX40L/ OX40 signalling pathway | IL13, MALT1, NFKB2, FOXP3, TNFI |
| Th2 cytokine- and TNF-alpha-induced inflammatory response in asthmatic airway | CXCL8, MAPK3, IL13, ITGA5, NFKI |
| **Process networks** | **Process networks** |
| Inflammation_IL-10 anti-inflammatory response | CCND3, CCNG1, CXCL8, IRAK2, NI |
| Inflammation_IL-4 signaling | MAPK3, HLADQA1, IL13, CXCL8, I |
| Inflammation_Interferon signaling | MAPK3, IFITM1, IFITM2, KLF4, IFI |
| **Go processes** | **Go processes** |
| Nucleosome Assembly | HIST1H1C, HIST1H1D, HIST1H1E, H |
| Nucleosome Assembly | |
| Chromatin Assembly | |

Red colour indicates a higher expression in asthmatic individuals, while blue indicates a higher expression in healthy individuals.

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