Genomics Insights

ORIGINAL RESEARCH

Transcriptional Changes of Blood Eosinophils After Methacholine Inhalation Challenge in Asthmatics

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Abstract

Background: Methacholine challenge is commonly used within the asthma diagnostic algorithm. Methacholine challenge has recently been shown to induce airway remodelling in asthma via bronchoconstriction, without additional airway inflammation. We evaluated the effect of methacholine-induced bronchoconstriction on the peripheral whole-blood transcriptome.

Methods: Fourteen males with adult-onset, occupational asthma, 26–77 years of age, underwent methacholine inhalation challenges. The concentration of methacholine eliciting a ≥20% fall in FEV_1 (PC_{20}) was determined. Blood was collected immediately prior to and two hours after challenge. Complete blood counts and leukocyte differentials were obtained. Transcriptome analysis was performed using Affymetrix GeneChip® Human Gene 1.0 ST arrays. Data were analyzed using robust LIMMA and SAM. The cell-specific Significance Analysis of Microarrays (csSAM) algorithm was used to deconvolute the gene expression data according to cell type.

Results: Microarray pathway analysis indicated that inflammatory processes were differentially affected. CsSAM identified 1,559 transcripts differentially expressed (all down-regulated) between pre- and post-methacholine in eosinophils at a false discovery cutoff of 10%. Notable changes included the *GOLGA5* and *METTL2B* genes and the protein ubiquitination and CCR3 pathways.

Conclusions: We demonstrated significant changes in the peripheral blood eosinophil-specific transcriptome of asthmatics two hours after methacholine challenge. CCR3 and protein ubiquitination pathways are both significantly down-regulated.

Keywords: asthma, CCR3, gene expression, microarray, peripheral blood, protein ubiquitination

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Introduction

Airway hyperresponsiveness (AHR) is a universal and defining feature of asthma. It has semi-independent, direct and indirect, components.[1,2] Direct AHR usually reflects persistent structural changes in the airway and is measured with direct stimuli such as methacholine (MCh) or histamine; indirect AHR reflects existing inflammation and is measured by stimuli such as exercise, cold air, hyperventilation, or AMP.[1,2] In the MCh challenge test (MCT), a subject inhales aerosolized MCh and the concentration of MCh required to elicit a 20% reduction in the forced expiratory volume in one second (FEV_1) is recorded as the PC_{20}.

MCT has been widely used in clinical practice since the PC_{20} is one of the diagnostic criteria of asthma.[3–5] It also correlates with severity and prognosis of asthma.[6,7] More importantly, MCT has been used to explore mechanisms of asthma in human and animal studies. In such scenarios, it is important to differentiate between molecular changes due to the stimulus of concern (such as viral, allergen and occupational exposure) and those due to MCh and its consequent airway narrowing. MCT may affect airway remodeling via bronchoconstriction, independent of inflammation.[8]

Gene expression profiling can identify biological molecules and pathways involved in disease pathogenesis and has been used in several asthma studies,[9] including in the analysis of whole blood and its purified lymphocyte fraction.[10,11] To our knowledge, genome-wide gene expression changes in peripheral blood due to MCh have not been reported. Given the profound importance of direct stimuli such as MCh in the current global algorithms for diagnosing asthma, an understanding of the systemic genomic response to such stimuli is of fundamental interest. Furthermore, related changes, if detected, could be useful in separating genomic response due to initial airway narrowing from genomic responses specific to other stimuli such as allergen exposure.

Recently, a new algorithm has been developed to combine whole-blood gene expression datasets and differential cell counts (CBC/diff) and successfully deconvolute genomic signatures attributable to each of the five major blood cell types measured by a standard CBC/diff.[12,13] Cell-specific Significance Analysis of Microarrays (csSAM) attributes gene expression profiling specifically to basophils, neutrophils, eosinophils, monocytes and lymphocytes. To our knowledge, the application of csSAM to whole blood transcriptomics in asthma has not been previously reported.

In the study reported in this manuscript, we hypothesized that methacholine challenge in chronic asthmatic subjects leads to significant changes in peripheral blood gene expression. Furthermore, we aimed to delineate the expression changes to specific cell types within the leukocyte fraction, using csSAM. Such analyses improve our understanding and interpretation of asthma-related research.

Material and Methods

Study subjects

This study was approved by the Institutional Review Board of the Vancouver Coastal Health Research Institute. We enrolled 14 male, non-smoking subjects amongst participants of a previous study of occupational (cedar) asthma in mill workers;[14] each provided written informed consent. A questionnaire, administered by a trained interviewer, assessed relevant demographic data, current presence and severity of asthmatic symptoms and medication usage.

Methacholine challenge test

A methacholine challenge was performed according to Cockcroft et al and PC_{20} was calculated.[15] Impairment for each subject was estimated according to ATS guidelines[16] using the sum of the scores for lung function, airway hyperresponsiveness, and asthma-related medication usage. The class of impairment was expressed as Class 0 (total score, 0), Class 1 (total score, 1 to 3), and Class 2 (total score, 4 to 6).

Blood sample collection and RNA extraction

Peripheral venous blood samples from each subject, pre-MCT and 2 hours post-MCT, were collected into PAXgene Blood RNA tubes (PreAnalytiX – Qiagen/BD, Valencia, CA, USA), stored at −80 °C until RNA extraction, and also into standard EDTA tubes. WBC counts and differentials were obtained from fresh EDTA blood using a Cell-Dyn 3700 (Abbott Diagnostics, Québec, Canada). From thawed PAXgene samples, total intracellular RNA was purified from 2.5 mL of blood according to manufacturer protocols

using the PAXgene Blood RNA Kit (PreAnalytiX – Qiagen). Quality of RNA was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Microarrays and statistical analysis

Pre- and post-MCT RNA samples from each subject were processed at the same time to avoid batch effects. Messenger RNA profiling was performed by the Centre for Translational and Applied Genomics at the BC Cancer Agency (Vancouver, BC), an Affymetrix-certified service provider. Affymetrix Human Gene 1.0 ST arrays were used (Affymetrix, Santa Clara, CA, USA), providing whole transcript coverage of well-annotated genes.[17] We used the oligo package from Bioconductor[18] to background-correct, normalize and summarize CEL files using Robust Multiarray Average (RMA) and extracted a total of 26,890 transcript clusters (genes).[19,20] Paired moderated *t*-tests were used to determine differential gene expression.[21–23] All statistical analyses were performed in R (the R Project for Statistical Computing [http://www.r-project.org/]; version 2.12.1). Microarray data were deposited in the Gene Expression Omnibus (approved GEO Series GSE32198).

Significance Analysis of Microarrays for R (SAMR), which relies on the permutation of class labels in order to estimate the false discovery rate (FDR), was used initially to determine differential gene expression in whole blood between pre- and post-MCT, with an FDR cut-off of 30% and a total of 1,000 permutations. Then, robust linear regression with the Linear Models for MicroArrays (limma) package in R was employed,[22] since it reduces the influence of outliers on the regression coefficients, with a Benjamini Hochberg FDR of 5%.[24] Cell-specific Significance Analysis of Microarrays (csSAM), a recent innovation that determines how the correlation between gene expression and relative cell-type frequencies changes between conditions, was also applied,[12] using mRNA expression data and complete blood counts obtained at pre- and post- time points. In csSAM, confidence estimates (in the form of FDRs) are generated by permuting the assignment of samples to groups, and fitting the two-group model to the data. FDR is estimated by V/R, where R is the number of genes exceeding a given threshold in the original data, and V is the average number of genes exceeding the same threshold in the permuted datasets. This yields an estimated FDR for genes for each individual cell-type comparison. Use of a positive or negative threshold yields separate FDRs for up-regulated or down-regulated genes. In our case, the number of permutations was set to 1,000 and FDR cut-offs of 10% and 30% were selected, based on inspection of plots of the number of differentially expressed transcripts called at various FDR cutoffs (essentially to identify the point of inflection). Ingenuity Pathway Analysis (IPA) was used to analyze the whole blood and cell-specific gene lists (Ingenuity® Systems, Redwood City, CA, USA).

Results

Study population characteristics

All 14 subjects are male non-smoking asthmatics previously diagnosed by positive specific inhalational challenge to plicatic acid (Table 1).[14] They were removed from cedar exposure, on average, 17 years prior to our study. The ATS asthma impairment was class 0 and 1 in 7 subjects and class 2 in 7 subjects. There was current airway hyperresponsiveness ($PC_{20} \leq 8$ mg/mL) in 10 subjects. Five subjects were using regular inhaled corticosteroid medications. Although these factors likely influence gene expression in peripheral blood leukocytes, the study design employed intra-individual self-pairing to eliminate confounding by inter-individual biological variability and thus focally detect changes due to methacholine challenge alone.

Differential gene expression in whole blood

Although no genes were differentially expressed between pre- and post-MCT (Supplementary Fig. 1) using SAMR at an FDR of 30%, robust limma revealed a total of 293 (168 up- and 125 down-regulated) differentially expressed genes (adjusted *P* value [FDR] < 0.05). Up-regulated genes included *CX3CR1*, *GATA3*, *HLA-DQA1*, *IL12RB2*, *CCRL2*, *IL10RA*, *TGFBR3* and *NCAM1* (Fig. 1). Down-regulated genes included *ALOX15*, *IFNG*, *GABRA6*, *PLA2G10*, *CLEC4E* and *DEXI* (Fig. 2). Supplementary Table 1 lists the full set of differentially expressed genes detected using robust limma.

Table 1. Demographics of study population.*

ID	Age/sex	Race†	Severity class‡	FEV_1% predicted	PC_{20} (mg/mL)	Steroid usage
059	71/male	W	2	52.22	0.28	No
503	68/male	W	2	59.06	2.14	No
504	66/male	W	1	99.66	>16	No
505	77/male	W	2	48.12	3.77	Yes
506	56/male	W	2	79.46	0.59	Yes
508	65/male	W	1	88.05	2.03	No
509	57/male	W	1	82.27	2.07	No
510	61/male	O	1	92.15	>16	No
511	26/male	O	0	113.64	>16	No
512	68/male	W	2	62.41	1.79	Yes
513	60/male	W	1	77.43	8.56	No
514	54/male	W	2	70.62	<0.5	Yes
515	62/male	W	2	78.16	0.73	Yes
516	56/male	W	1	81.62	0.77	No

Notes: *All subjects are male, non-smokers; †"W" donates non-Hispanic whites, "O" denotes other races; ‡asthma severity class: according to ATS guidelines.

Relative cell counts and cell-specific differential gene expression

Relative neutrophil cell counts significantly decreased ($P < 0.01$) whereas relative lymphocyte cell counts significantly increased ($P < 0.05$) post-MCT (Fig. 3). No other cell-types changed significantly. Deconvolution of whole blood gene expression into the five white blood cell-types detected a significant signal in eosinophils, at an FDR of 30% (Fig. 4). 1,559 and 4,919 genes were significantly down-regulated post-MCT in eosinophils at FDR cutoffs of 10% and 30%, respectively. No overlaps were found between

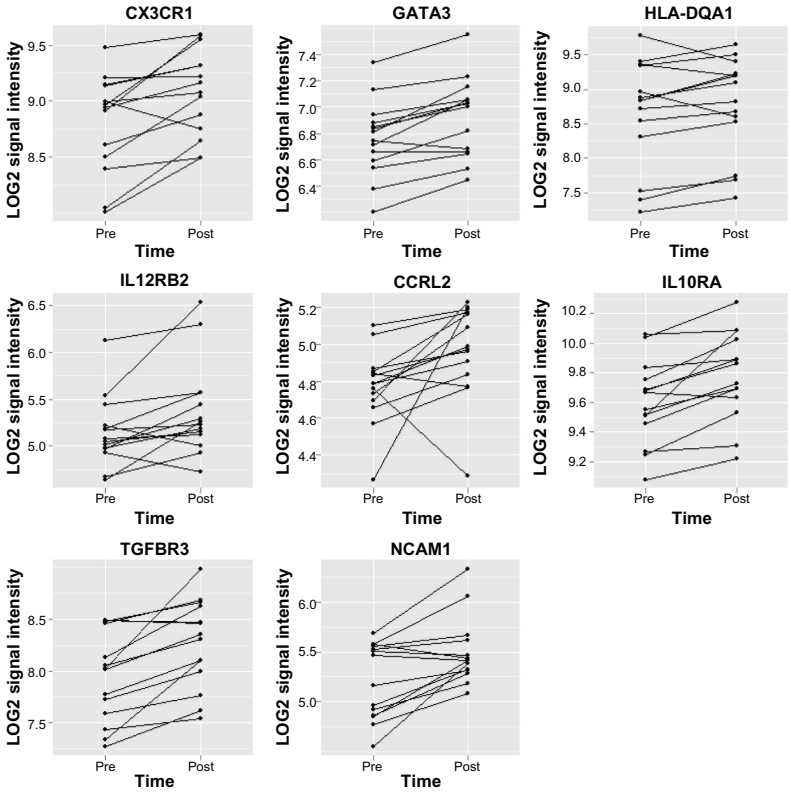

Figure 1. Selected up-regulated genes post-methacholine challenge in whole blood.
Notes: Detected by robust limma. Expression intensity given as Log2 values.

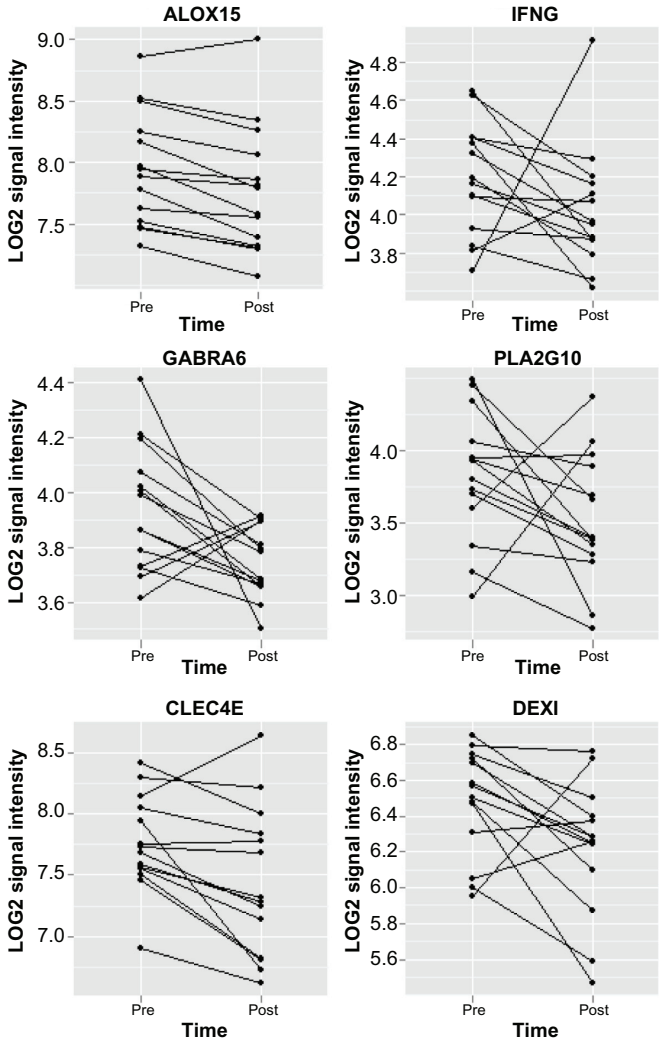

Figure 2. Selected down-regulated genes post-methacholine challenge in whole blood.
Notes: Detected by robust limma. Expression intensity given as Log2 values.

the 293 up- and down-regulated genes in whole blood and the 1,559 genes found significantly down-regulated in eosinophils. Supplementary Table 2 lists the full set of differentially expressed genes (FDR < 0.10) detected as down-regulated post-MCT in eosinophils using csSAM.

Pathway analysis

Analysis of the 293 differentially expressed genes/probe-sets identified in whole blood by robust limma revealed 223 well-annotated molecules for IPA (Supplementary Table 3). Several networks, including Cell-mediated Immune Response, Inflammatory Response, Cell Death, Cell Cycle, Cell-To-Cell Signaling and Interaction, Immune Cell Trafficking, and Cellular Growth and Proliferation, were significantly altered. Supplementary Figure 2 shows a combined network analysis of the data. Top-ranked canonical pathways are listed in Supplementary Table 4, with the most significant being the Cross-talk between Dendritic Cells and Natural Killer Cells pathway.

Following deconvolution using csSAM, analysis of the 1,559 genes/probe-sets significantly down-regulated post-MCT in eosinophils at FDR cutoff of 10% revealed 883 well annotated molecules for IPA (Supplementary Table 5). Top-ranked canonical pathways are listed in Supplementary Table 6, with the most significant being the Protein Ubiquitination Pathway (Fisher's Exact Test *P*-value = 5.49E-06; Benjamini Hochberg [B-H] Multiple Testing Correction *P*-value = 1.18E-03). Notably, the CCR3 Signaling in Eosinophils pathway was identified as significant (Fisher's Exact Test *P*-value = 4.09E-03; B-H Multiple Testing Correction *P*-value = 4.53E-02). Figure 5 shows this pathway, with the individual genes significantly down-regulated post-MCT highlighted (FDR < 0.10).

Discussion

We were interested in determining if the well-established methacholine inhalation challenge test (MCT) results in significant genome-wide transcriptional changes in peripheral blood leukocytes. Given the global reach of MCT and similar testing in the standard algorithms for diagnosing asthma, the systemic genomic response to MCT is of fundamental interest and of particular note given the recent data published by Grainge et al[8] that demonstrated an effect of methacholine-induced bronchoconstriction on airway remodeling in asthma, without eosinophilic airway inflammation.

This population of male asthmatics, whose asthma is fundamentally caused by a unique and well-characterized antigen (plicatic acid), and whose airway function has been carefully described,[25,26] provides an extremely good opportunity to study the molecular basis of methacholine challenge. However, there is no reason to believe that our results are relevant only to those with cedar asthma, as the subjects in our study were removed from cedar exposure long before the current study.

Robust limma identified a total of 293 (168 up- and 125 down-regulated) differentially expressed genes (FDR < 0.05), supporting the notion that MCT does affect the peripheral blood transcriptome.

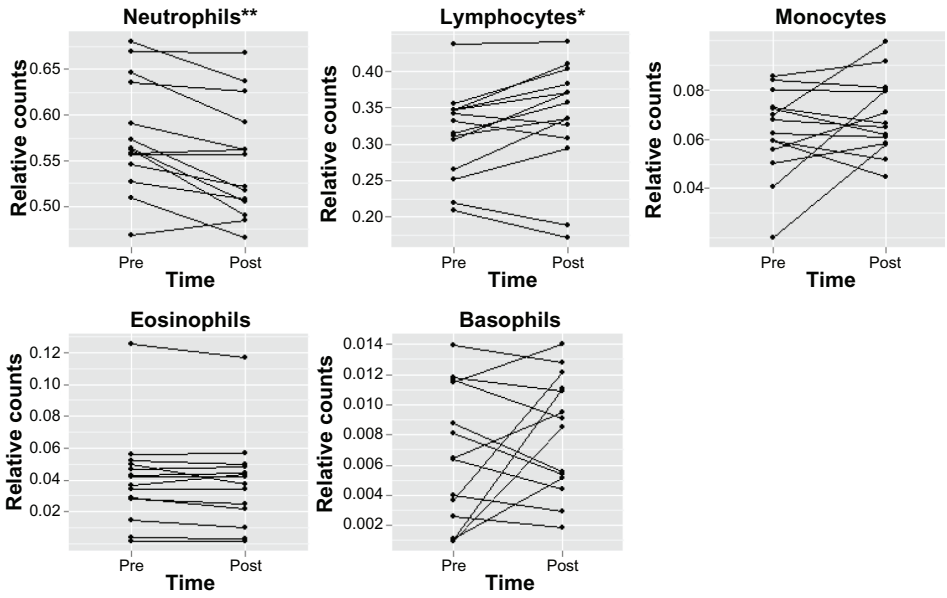

Figure 3. Changes in relative cell-type frequencies.
Notes: Neutrophils significantly (P = 0.002) decreased post-challenge whereas lymphocytes significantly (P = 0.045) increased post-challenge. Monocytes, eosinophils and basophils did not significantly change between pre- and post- methacholine challenge.

Several of the genes and related inflammatory and immune pathways reported as significantly differentially-expressed have biological plausibility. Up-regulated genes detectable generally in peripheral blood leukocytes included *CCRL2*, *CX3CR1* and *GATA3*, whilst down-regulated genes included *PLA2G10*, *CLEC4E* and *DEXI*.

Chemokines and their receptors mediate signal transduction and are critical for the recruitment of effector immune cells to site of inflammation, and several were significantly up-regulated by methacholine in our study. The *CCRL2* gene is expressed at high levels in primary neutrophils and primary monocytes, and is further up-regulated on neutrophil activation and during monocyte to macrophage differentiation.[27] CX3CR1 (chemokine [C-X3-C motif] receptor 1) is a transmembrane protein and chemokine involved in the adhesion and migration of leukocytes and implicated in cross-talk between smooth muscle cells and monocytes.[28] GATA3 (GATA binding protein 3) is a transcription factor that regulates T-cell development and plays an role in endothelial cell biology. A correlation of GATA3 expression with an increase of IgE is characteristic of long-lasting asthma, and GATA3 has been demonstrated to be over-expressed in individuals with allergic rhinitis related to an increase of ICAM-1.[29,30]

Amongst the proteins encoded by the down-regulated genes, exogenously secreted PLA2G10 (phospholipase A2, group X) has been shown to mediate cysteinyl leukotriene synthesis in eosinophils,[31] and has an important role in allergen-induced airway inflammation and remodeling in mouse asthma models.[32] CLEC4E (C-type lectin domain family 4, member E) is a member of the C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily which share a common protein fold and have diverse functions, including cell adhesion, cell-cell signaling, glycoprotein turnover, and multiple roles in inflammation and immune response.[33] DEXI (Dexi homolog; mouse) is a novel glucocorticoid-induced gene transcript that is up-regulated in emphysema.[34]

Two genes significantly down-regulated in eosinophils were *GOLGA5* and *METTL2B*. *GOLGA5* (Golgi autoantigen, Golgin subfamily A, 5) encodes a coiled-coil protein localized to the Golgi surface, and may play a role in vesicle tethering and docking.[35] The *METTL2B* gene (methyltransferase like 2B) is a member of a family of methyltransferases that share homology with, but are distinct from, the UbiE family of methyltransferases. Interestingly, several other methyltransferase genes were found to be significantly down-regulated in eosinophils using the csSAM algorithm, including *METTL10*, *METT5D1*, *METTL6* and *METTL4*. The METTL2B protein has been shown to interact with PSEN1(presenilin 1),[36] whose gene is also amongst those on our list of significantly

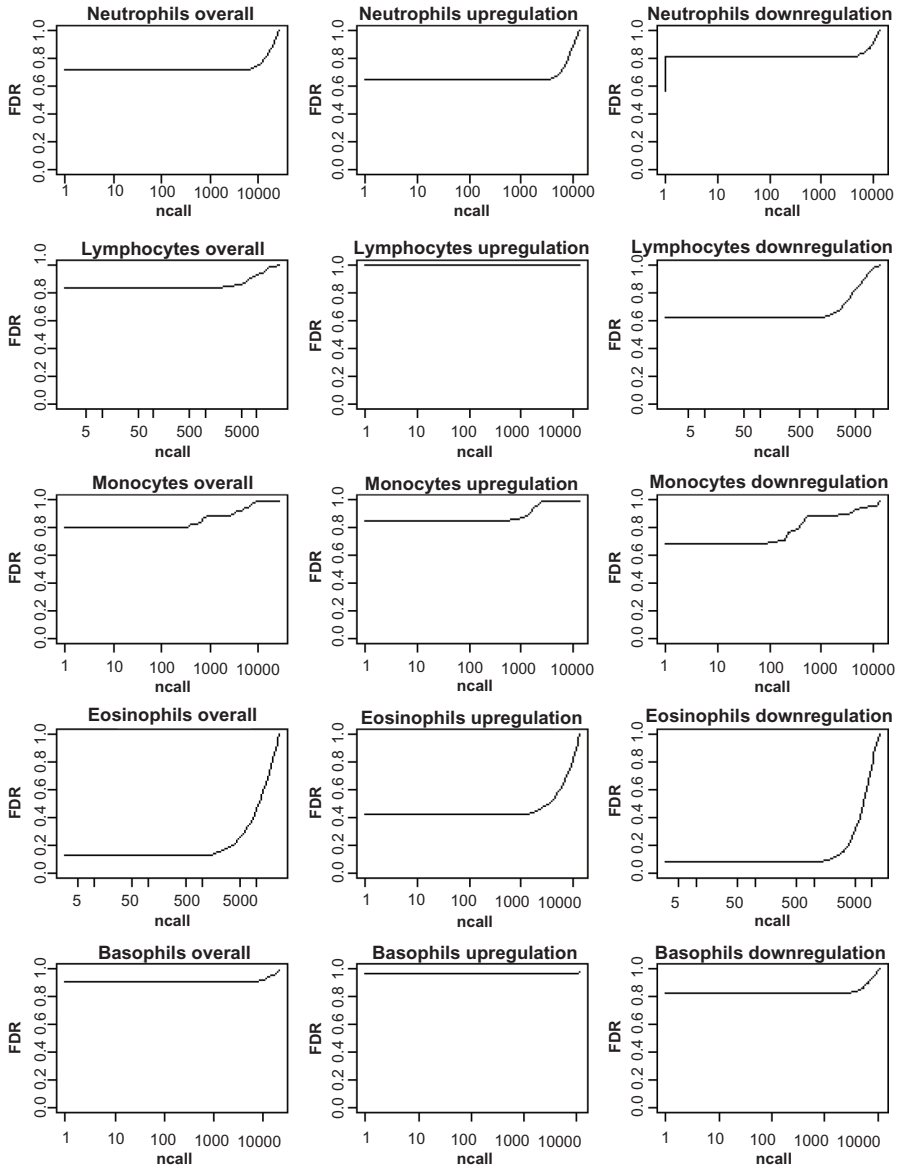

Figure 4. Deconvolution of whole blood gene expression into the 5 major cell types.
Notes: At a 30% FDR, differential genes expression is demonstrated only in eosinophils, post methacholine challenge. csSAM identified 1,559 and 4,919 genes significantly down-regulated post-challenge in eosinophils at an FDR cutoff of 10% and 30%, respectively (ncall = number of probe-sets/genes called significant at the corresponding FDR).

down-regulated transcripts in eosinophils, post-MCT. Presenilins have been implicated in both protein trafficking and proteolysis events within the cell.[37] Specifically, Guo et al have found that the subcellular distribution of Notch is affected in mutants and transgenics.[38] Of particular relevance to asthma, Notch signaling regulates the terminal differentiation and subsequent effector phenotypes of eosinophils, partly through modulation of the ERK pathway.[39,40]

Pathway analysis of the 883 well-annotated molecules down-regulated in eosinophils identified numerous significant canonical pathways. The highest ranked was the Ubiquitination pathway, which plays a major role in the degradation of damaged as well as short-lived or regulatory proteins involved in a variety of cellular processes, including cell cycle, cell proliferation, apoptosis, DNA repair, transcriptional regulation, regulation of cell surface receptors and ion channels, and antigen presentation. Degradation of proteins via this highly organized and complex pathway involves two successive steps: (1) conjugation of multiple ubiquitin moieties (Ub) to the target protein; (2) degradation of the polyubiquitinated protein by the 26S proteasome complex.[41] Whilst mild or transient

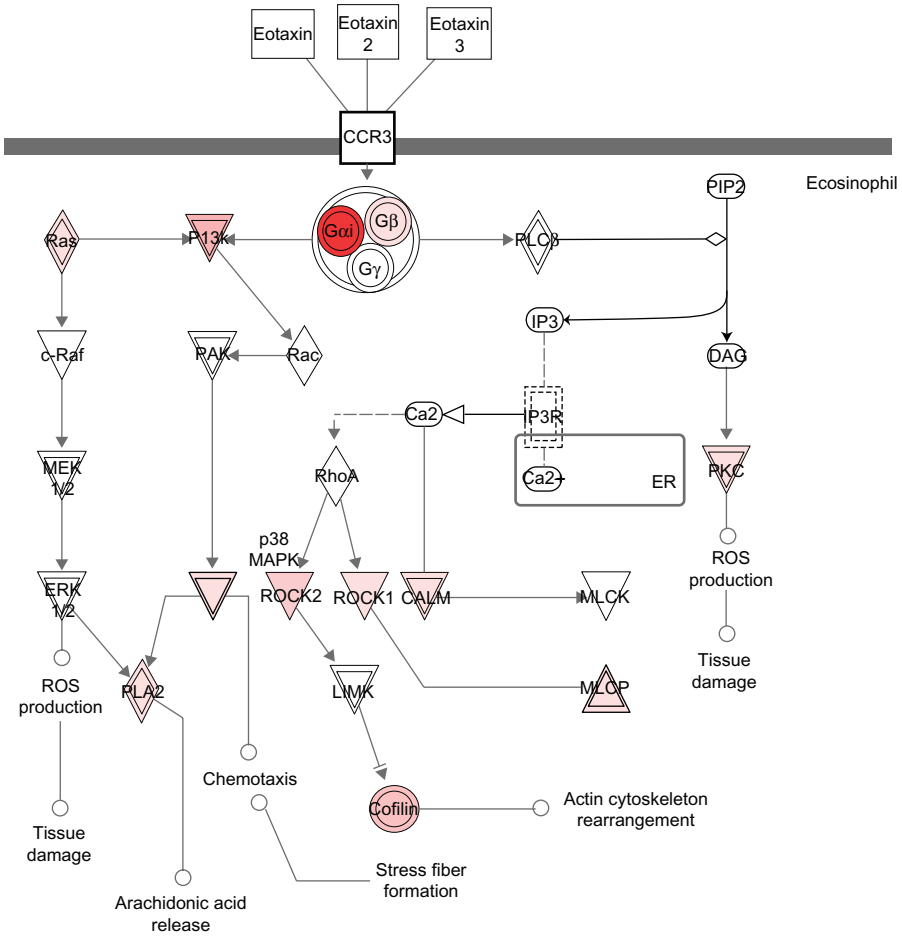

Figure 5. CCR3 Signaling in Eosinophils pathway.
Notes: The individual genes significantly down-regulated (FDR < 0.10) post-MCT are displayed in red (darker colour indicates greater fold change).

oxidative stress can up-regulate the ubiquitination system and proteasome activity in cells and tissues, severe or sustained oxidative stress impairs the function of the system and decreases intracellular proteolysis. Indeed, both the ubiquitin-conjugating enzymes and the proteasome can be inactivated by sustained oxidative stress, especially the 26S proteasome.[41]

A particularly compelling canonical pathway identified was CCR3 Signaling in Eosinophils.[42,43] The chemotactic response of eosinophils is mostly mediated by CC Chemokine Receptor-3 (CCR3), linked to G-Proteins. Chemokines such as Eotaxin, Eotaxin 2, and Eotaxin 3 signal exclusively via CCR3 to recruit eosinophils to the site of inflammation and activate them. Eosinophils express at least two additional chemokine receptors, CCR1 and CXCR2, though CCR3 achieves by far the highest expression levels. At sites of inflammation, eosinophils are responsible for tissue damage by the release of reactive oxygen species and toxic granule proteins. Moreover, CCR3 recruitment by eotaxins stimulates a set of downstream signaling pathways, which are responsible for eosinophil chemotaxis, degranulation, and propagation of the inflammatory response through the secretion of cytokines and chemokines.[44] CCR3 recruitment by eotaxin activates MAPKs ERK1/2 and p38 in eosinophils, which are indispensable for eosinophil chemotaxis and degranulation. ERKs are regulated through the PI3 K-γ-Ras-Raf1-MEK-ERK pathway. Although the upstream signal of p38 in the CCR3 pathway is unclear, Rac and PAKs have an active participation. On the signaling level, activation of MAPK pathway (ERK2 and p38) mediates arachidonic acid release catalyzed by cytosolic PLA2, leading to inflammatory responses, prolonged bronchoconstriction and increased bronchial mucus production. Sehmi et al have suggested that allergen-induced fluctuations in CCR3 expression on bone marrow CD34+ cells

obtained from asthmatic subjects are of significance for the mobilization of haemopoietic progenitor cells in allergic inflammation.[45] Antisense RNA therapy against CCR3 has been demonstrated to attenuate allergen-induced eosinophilic responses.[46]

One limitation of our study is that, while our intention was to document the systemic genomic signal inherent to the 'pure' bronchoconstriction of a direct agonist such as methacholine, we are unable to rule out an effect of methacholine itself directly on blood cells, due to leaking or spill-over of methacholine into the blood-stream from the airway. However, we have no reason to believe that methacholine so translocates into the blood stream.

It is reasonable to speculate upon the biological plausibility for a general down-regulation of eosinophil-related gene expression in circulating blood, including a marked quiescence in the protein ubiquitination pathway and CCR3 signaling pathway. Ubiquitination is part of the unfolded protein response, a general stress response believed to allow cells to deal with rapid increases in protein production and turnover.[47] That ubiquitination appears down-regulated in eosinophils might indicate a skewing towards relatively immature eosinophils, less capable of ubiquitination, as more mature blood eosinophils migrate into the airways. This would be consistent with stable overall eosinophil counts in blood; we are limited by not having data on cell surface markers which would reflect cell maturity. Similarly, CCR3 signaling could be enhanced primarily in those eosinophils routed to the airways.

A previous report has shown increased numbers of sputum eosinophils, four hours post-MCT, in at least some asthmatic individuals.[48] Other studies have evaluated methacholine challenge on the airway by measuring bronchoalveolar lavage (BAL) fluid cell count and function, showing no effects within 30 minutes of MCT,[49] but increased BAL fluid leukotrienes and prostaglandins 5 hours post-MCT.[50] We obtained neither sputum nor BAL samples from our research participants and thus were unable to validate these finding, though our data derived from peripheral blood supports mild inflammatory changes at least two hours post-challenge.

In conclusion, in this exploratory analysis we have demonstrated that methacholine challenge in chronic asthmatic subjects leads to changes in peripheral blood gene expression, most significantly correlated to eosinophils. CCR3 and protein ubiquitination pathways are both significantly down-regulated, two hours post-challenge compared to pre-challenge. These findings increase our understanding of changes associated with direct stimulation of bronchoconstriction, by looking beyond the airways and into the peripheral circulation. Independent replication of our findings in additional subjects is important, and will be part of future work.

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Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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Supplementary Figures

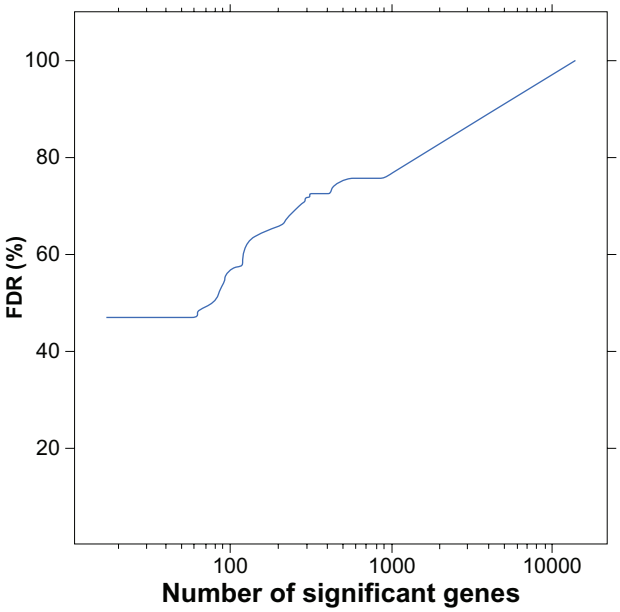

Figure S1. Differentially expressed genes in whole blood.
Notes: Using a false discovery rate (FDR) of 30%, no genes were identified by SAMR to be differentially expressed in whole blood between pre- and post-methacholine challenge test.

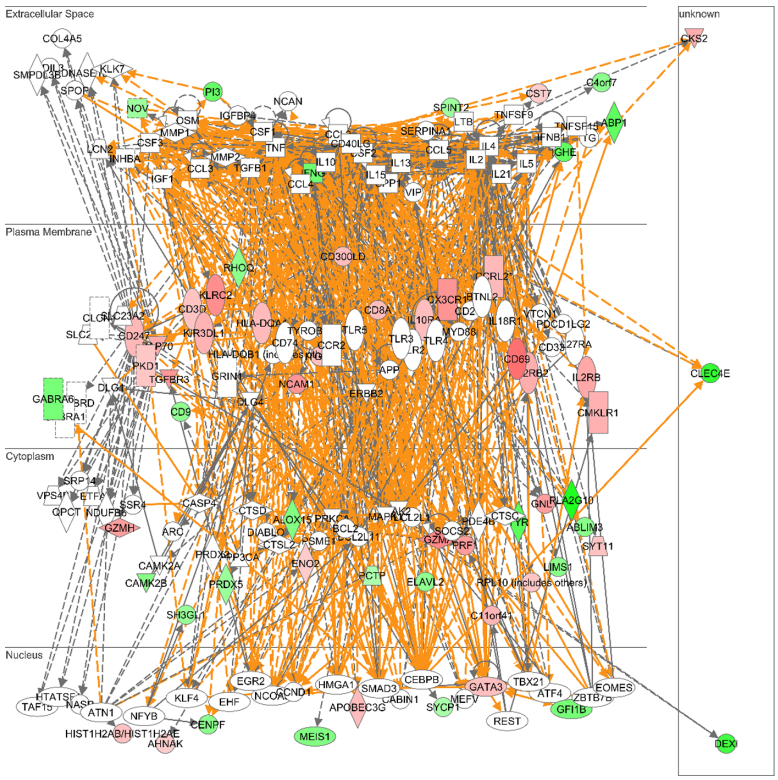

Figure S2. Combined network analysis (Ingenuity) of differentially expressed genes identified in whole blood by robust limma (FDR < 0.05).
Notes: Up-regulated and down-regulated genes displayed in red and green, respectively (darker colour indicates greater fold change).

Supplementary Tables

Supplementary Table 1. Differentially expressed genes (FDR $<$ 0.05) in peripheral whole blood, post- versus pre-methacholine challenge, detected using robust limma.

Supplementary Table 2. Differentially expressed genes (FDR $<$ 0.10) detected as down-regulated post-MCT in eosinophils using csSAM.

Supplementary Table 3. 223 well annotated molecules for Ingenuity pathway analysis, mapping to differentially expressed genes/probe-sets identified in whole blood by robust limma (FDR $<$ 0.05).

Supplementary Table 4. Top-ranked canonical pathways derived from the 223 well annotated molecules for Ingenuity pathway analysis, mapping to differentially expressed genes/probe-sets identified in whole blood by robust limma (FDR $<$ 0.05).

Supplementary Table 5. 883 well annotated molecules for Ingenuity pathway analysis, mapping to differentially expressed genes/probe-sets identified as down-regulated in eosinophils by csSAM (FDR $<$ 0.10).

Supplementary Table 6. Top-ranked canonical pathways derived from the 883 well annotated molecules for Ingenuity pathway analysis, mapping to differentially expressed genes/probe-sets identified as down-regulated in eosinophils by csSAM (FDR $<$ 0.10).

Supplementary Tables 1-6 are available from 9125Supplementarytables.zip