

ORIGINAL ARTICLE

Allergen challenge of peripheral blood mononuclear cells from patients with seasonal allergic rhinitis increases IL-17RB, which regulates basophil apoptosis and degranulation

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Summary

Background Previously, expression profiling has been used to analyse allergen-challenged T-helper type 2 cells, nasal biopsies and nasal fluid cells from patients with seasonal allergic rhinitis (SAR). Allergen-challenged peripheral blood mononuclear cells (PBMCs) provide a human *in vitro* model of how antigen-presenting cells, CD4⁺ T cells and effector cells such as basophils interact in allergic inflammation.

Objective To identify novel genes and pathways in allergen-challenged PBMCs from patients with SAR using gene expression profiling and functional studies.

Methods PBMCs from 11 patients with SAR and 23 healthy controls were analysed with gene expression profiling. mRNA expression of *IL17RB* in basophils was evaluated using quantitative real-time PCR. Membrane protein expression and apoptosis of basophils were examined by flow cytometry. Degranulation of basophils was assessed by measuring β -hexosaminidase release. Cytokine release was measured using ELISA.

Results Gene expression microarray analysis of allergen-challenged PBMCs showed that 209 out of 44 000 genes were differentially expressed in patients compared with controls. *IL17RB* was the gene whose expression increased most in patients ($P < 0.0001$). FACS analysis of PBMCs showed, for the first time, that basophils express IL-17RB. Following allergen challenge, IL-17RB protein increased significantly on basophils from patients compared with controls ($P < 0.05$). IL-3 significantly increased both mRNA and protein expressions of *IL17RB*. Activation of IL-17RB by its ligand, IL-25, inhibited apoptosis of basophils. Moreover, IgE-mediated degranulation was enhanced by IL-25.

Conclusion Increased expression of IL-17RB on allergen-challenged basophil is regulated by IL-3, inhibits apoptosis and promotes IgE-mediated degranulation of basophils.

Keywords apoptosis, basophils, degranulation, gene expression microarray, IL-17RB, seasonal allergic rhinitis

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Introduction

IgE-mediated reactions against specific allergens play a key role in the pathogenesis of allergic disease. Decades-long research efforts have implicated an increasing number of genes and their products in the regulation of IgE-mediated reactions [1–4]. New technologies, such as gene expression microarrays, can be used to examine the

mRNA expression of the majority of known genes and variants. During the last decade, gene expression microarray studies of cells and tissues from allergic patients and mouse models of asthma have contributed to the identification and validation of novel genes and pathways. These studies have included nasal fluid cells, nasal mucosa, lungs, eczematous skin, as well as a variety of cell populations, such as peripheral blood mononuclear cells

(PBMcs), T-helper type 1 (Th1), Th2 and Th17 cells, allergen-challenged CD4⁺ cells, all major leucocytes and keratinocytes [5–22].

Thus, these studies span from individual cell types to whole tissues. The advantage of analysing tissues or groups of cells compared with individual cell types is that this may contribute to the understanding of how different cell types interact *in vivo*. However, because of variable cellular composition, it may be difficult to link the observations to specific cell types. On the other hand, such analyses can be used to generate hypotheses that can be validated in individual cell types.

Here, we analysed allergen-challenged PBMcs from patients with seasonal allergic rhinitis (SAR) and healthy controls. This provides an *in vitro* model of how antigen-presenting cells (APCs), CD4⁺ T cells and effector cells such as basophils interact in allergic inflammation [20].

Using gene expression profiling, we found that *IL17RB* was the most differentially expressed gene in allergen-challenged PBMcs, and reported for the first time that human basophils express IL-17RB. Basophils play a key role in allergic inflammation, in that IgE-mediated degranulation of these cells results in the release of inflammatory mediators including histamine, LTC₄ as well as IL-4 and IL-13 [23–25]. IL-3 enhances the release of these mediators from basophils [26–30]. Using cell sorting, we found that allergen challenge of PBMcs resulted in increased IL-17RB on basophils from patients with SAR compared with healthy controls. Further analyses showed that IL-17RB was up-regulated by IL-3, inhibited basophil apoptosis and promoted IgE-mediated degranulation. IL-17RB signalling has previously been shown to play a key role in murine models of allergic inflammation. This has been attributed to the activation of Th2 cells and non-B/non-T cells [31–36]. Our results show that IL-17RB also is involved in regulating basophil survival and activation.

Methods

Reagents and antibodies

The basophil isolation kit was purchased from Miltenyi Biotec (Bergisch-Gladbach, Germany). Mouse anti-human CD4-FITC (SK3), rat anti-human CD294-Alexa Fluor 647 (CRTH2) (BM16), mouse anti-CD123-APC (7G3), mouse anti-HLA-DR- PerCP (L243), purified NA/LE mouse anti-human CD123 (7G3), Annexin V-FITC and all matched isotype controls were purchased from BD Pharmingen (San Diego, CA, USA). Recombinant human IL-17E (IL-25), monoclonal mouse anti-human IL-17RB-PE (170 220), goat anti-human IL-17RB and all matched isotype controls were purchased from R&D Systems Inc. (Minneapolis, MN, USA). Recombinant human IL-3, mouse anti-human CD14-APC (MEM-15), mouse anti-human CD54-PE (1H4), mouse anti-human CD18-FITC

(MEM-48), mouse anti-human CD49d-FITC (BU49), mouse anti-human CD62L-FITC (LT-TD180) and all matched isotype controls were purchased from ImmunoTools GmbH (Friesoythe, Germany). Human IgG and goat anti-human IgE were purchased from Sigma-Aldrich (St Louis, MO, USA). miRneasy Mini Kit, High-Capacity cDNA Reverse Transcription Kit, Taqman PCR master mix and FAM/MGB probe for human *IL17RB* (Hs00914532_m1) and *GUSB* (β -glucuronidase) (Hs99999908_m1) were purchased from Applied Biosystems (Foster City, CA, USA). Grass and birch extract were purchased from ALK Abell  (H rsholm, Denmark). ELISA kits for IL-4, IL-8 and IL-13 were purchased from eBiosciences (San Diego, CA, USA).

Subjects

PBMcs were purified from eleven patients with SAR induced by either birch or grass pollen and 23 healthy controls, which were analysed using gene expression microarrays. Another seven patients and three controls were recruited for basophil functional analysis. In addition, CD4⁺ cells from 19 patients with SAR were analysed using gene expression microarrays after challenge with allergen or diluent as described previously [18]. The diagnosis of birch or grass pollen-induced SAR was based on a positive history of SAR for at least 2 years and positive skin prick test (SPT) responses. All patients were studied outside the pollen season and were asymptomatic at the time of inclusion in the study. Exclusion criteria included a history of perennial symptoms and treatment with local or systemic corticosteroids or antihistamines for the last 2 months. The control subjects were all symptom-free, had no history of allergic rhinitis or any other atopic diseases and had negative SPTs. Informed consent was obtained from all subjects involved in the study. The study was approved by the Ethics Committee of the Medical Faculty of the University of Gothenburg.

Cell culture

Human PBMcs from patients with SAR or healthy controls were stimulated with relevant allergen extract (Grass or birch, 100 μ g/mL) or diluent as described previously [37]. For the CD123 blocking assay, PBMcs from patients with SAR were stimulated with or without allergen extract (Grass or birch, 100 μ g/mL) for 4 days. Purified NA/LE mouse anti-human CD123 (1 μ g/mL) was added for CD123 blocking, while mouse anti-human IgG (1 μ g/mL) was utilized as an isotype control.

Basophils were purified from buffy coat using a basophil purification kit from Miltenyi Biotec. The purity of basophils was higher than 97%. For the apoptosis assay, basophils at a density of 1×10^5 cells/well were stimulated with or without IL-25 (100 ng/mL) for 48 h. Goat anti-human IL-17RB (100 ng/mL) was used for IL-17RB

blocking, while goat anti-human IgG (100 ng/mL) was used as an isotype control.

Gene expression microarray analysis

The expression of 44 000 genes and variants were measured using gene microarrays (Illumina's Sentrix[®] Human-6 Expression BeadChips, Illumina Inc., San Diego, CA, USA). The hybridization and analysis were performed according to the manufacturer's instructions and the Minimum Information About a Microarray Experiment guideline. Signal values and arrays were background corrected and normalized using the robust multiarray average. Differentially expressed genes were identified using the Bioconductor package LIMMA for R. A complete list of differentially expressed genes is provided in the online supplement. The Ingenuity Pathway Analysis (IPA) software (<http://www.ingenuity.com>, Ingenuity Systems Inc., Redwood City, CA, USA) was used to identify immunological pathways enriched for differentially expressed genes as described previously [22].

Flow cytometry

Membrane expression of IL-17RB was examined by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA). Before staining, cells were blocked with human IgG. Th2 central memory cells (CD4⁺CRTH2⁺), Monocytes (CD14⁺) and basophils (CD123⁺HLA-DR⁻ or CD4⁺CRTH2^{high} [29, 38]) were identified using the aforementioned antibodies. Monoclonal mouse anti-human IL-17RB was used to detect IL-17RB. A goat anti-human IL17RB polyclonal antibody was also used for validation. Matched isotype controls were used in all flow cytometry analyses. The data were expressed as mean fluorescence intensity (MFI). To determine the level of apoptosis, basophils were stained with Annexin V-FITC, mouse anti-CD123 and mouse anti-HLA-DR. Annexin V-positive basophils were considered to be apoptotic. The adhesion molecules ICAM-1 (CD54), L-selectin (CD62L), leucocyte function-associated antigen (LFA-1) (CD11a/CD18) and very late antigen-4 (VLA-4, CD49d/CD29) on basophils were examined by flow cytometry after treatment with IL-25 (100 ng/mL) for 1 and 18 h, respectively.

RNA preparation and cDNA synthesis

Basophils at a density of 5×10^5 cells/well were stimulated with or without IL-3 (10 ng/mL). After 18 h, cells were harvested for RNA extraction using a miRneasy Mini Kit. cDNA synthesis was performed using a High-Capacity cDNA Reverse Transcription Kit.

Real-time polymerase chain reaction

Real-time PCR was performed on an ABI 7900HT Real-Time PCR cycler (Applied Biosystems) with the Taqman Gene

expression Master Mix. *GUSB* was used as an internal control. The mRNA expression of *IL17RB* was calculated relative to *GUSB* by using a delta-delta CT method.

Degranulation studies of basophils

To determine basophil degranulation, β -hexosaminidase analysis was carried out as described previously [39, 40]. Essentially, purified basophils from buffy coat at a density of 5×10^5 cells/well were treated with or without IL-25 (100 ng/mL) in the presence of IL-3 (10 ng/mL) for 18 h. After washing, cells were challenged with anti-IgE (100 ng/mL) for 20 min and the supernatant was collected for β -hexosaminidase analysis by a colorimetric method. Four samples and three replicates of each sample were analysed for β -hexosaminidase release, which was expressed as a percentage of total cellular β -hexosaminidase.

Cytokine assays

To investigate cytokines expression of basophils, purified basophils from buffy coat were stimulated with or without IL-25 (100 ng/mL) alone, or in combination with IL-3 (10 ng/mL), or with anti-IgE (100 ng/mL), or with both IL-3 (10 ng/mL) and anti-IgE (100 ng/mL) for 12 h. IL-4, IL-8 and IL-13 were measured using the ELISA kit from eBiosciences.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, Version 17.0. All data were expressed as the mean $\pm$ SEM. Pearson's correlation coefficient was used for the correlation analysis. Differences between two paired experimental groups were analysed using a paired Student's *t*-test. A *P*-value < 0.05 was considered to be significant.

Results

Differentially expressed pathways and genes in allergen-challenged peripheral blood mononuclear cells from patients with seasonal allergic rhinitis

Gene expression microarray analysis of allergen-challenged PBMCs from patients with SAR and healthy controls showed that 209 genes differed in expression. Pathway analysis using the IPA software revealed that three immunological pathways were significantly enriched for differentially expressed genes, namely *IL-4 signalling*, *B cell receptor signalling* and *Fc Epsilon R1 signalling* (supporting information Fig. S1). The differentially expressed genes included several of known relevance to allergy, such as *IL5*, *FCER2*, *GATA3*, *NFAT* and *MAF* (Table S1). However, the most differentially expressed gene was *IL17RB*, 2046 ± 369 in patients vs. 332 ± 117 in controls

($P < 0.00001$). IL-17RB has previously been described to be expressed on some of the cell types that are part of PBMCs, namely monocytes and activated Th2 central memory cells, and to play an important role in allergy [33–36, 38, 41, 42]. Consistent with previous studies, we also found a significant increase of *IL17RB* in gene expression microarray data from allergen-challenged $CD4^+$ cells from patients with SAR, compared with diluent-challenged cells, 1500 ± 250 vs. 42 ± 34 ($P < 0.0001$). The analyses also showed that the expression levels of *IL17RB* were significantly correlated with the Th2 cytokines *IL4*, *IL5* and *IL13*, $r = 0.75$, 0.78 and 0.7 , respectively (All $P < 0.0001$). However, before our work, it was not known whether basophils express IL-17RB.

Human basophils express IL-17RB, which increases after allergen challenge in patients with seasonal allergic rhinitis

In order to determine whether basophils express IL-17RB, freshly isolated PBMCs from buffy coat were examined by flow cytometry. We found that IL-17RB was expressed on basophils (Fig. S2) and to a limited extent on monocytes, but not on freshly isolated Th2 central memory cells (Fig. 1a). To further examine whether its expression increased on allergen-challenged basophils from patients with SAR, PBMCs from patients and healthy controls were challenged with diluent or allergen extract for 1 week. Expression of IL-17RB on allergen-challenged basophils from patients increased significantly after 4 days of culture (Fig. 1b, $P < 0.05$).

Interleukin-3 up-regulates the expression of IL-17RB on basophils

IL-3 is reported to regulate the expression of several membrane receptors on basophils [29, 30]. To clarify whether IL-3 is involved in regulating IL-17RB expression, purified basophils from buffy coat were cultured with or without IL-3. IL-3 dramatically enhanced the membrane expression of IL-17RB (Fig. 2a). Further studies, in which basophils were cultured with different concentrations of IL-3, confirmed this result (Fig. 2b). To demonstrate this mechanism on the transcriptional level, quantitative real-time PCR was used to examine *IL17RB* in basophils after IL-3 stimulation. We found that IL-3 significantly increased *IL17RB* expression (Fig. 2c, $P < 0.01$). To investigate whether IL-3 was involved in the up-regulation of IL-17RB on allergen-challenged basophils from patients with SAR, PBMCs from patients with SAR were blocked with anti-CD123 (IL-3 receptor α chain) or isotype control in the presence of allergen extract for 4 days. IL-17RB on basophils increased after allergen challenge ($P < 0.01$), but decreased after IL-3 receptor blockade ($P < 0.05$), indicating that IL-3 up-regulates IL-17RB on allergen-challenged basophils from patients with SAR (Fig. 2d).

Activation of IL-17RB inhibits apoptosis of basophils

Activation of IL-17RB has been reported to inhibit apoptosis of eosinophils [43]. To investigate whether IL-17RB also contributes to apoptosis of terminally differentiated basophils in peripheral leucocytes, purified basophils

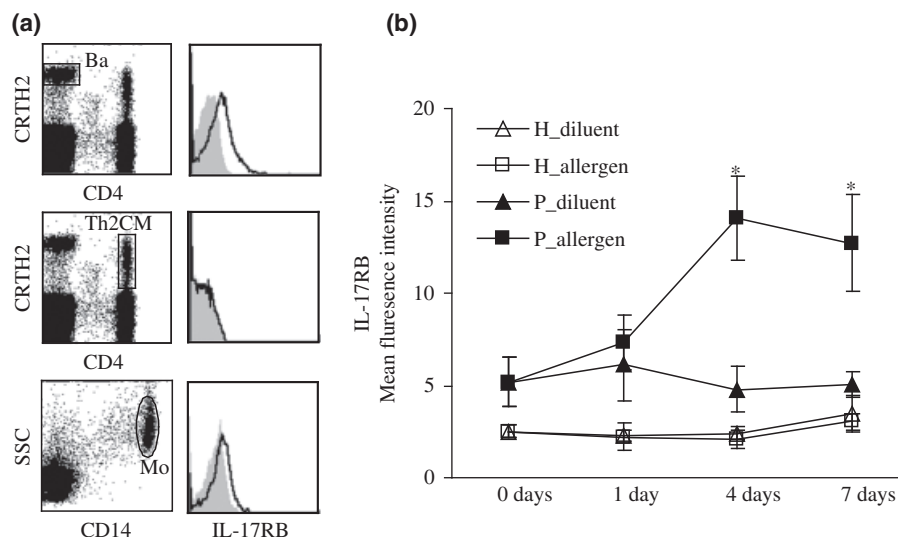


Fig. 1. Human basophils express IL-17RB. (a) Expression of IL-17RB on freshly isolated PBMCs from buffy coat was examined by flow cytometry. Representative histograms from one of ten donors show IL-17RB expression on basophils (Ba), Th2 central memory cells (Th2CM) and monocytes (Mo). The shaded histogram represents isotype control; the open histogram represents IL-17RB; SSC, Side Scatter. (b) PBMCs from patients ($n = 3$) or controls ($n = 3$) were challenged with or without allergen. IL-17RB on basophils was determined by flow cytometry. H, healthy controls; P, patients, * $P < 0.05$.

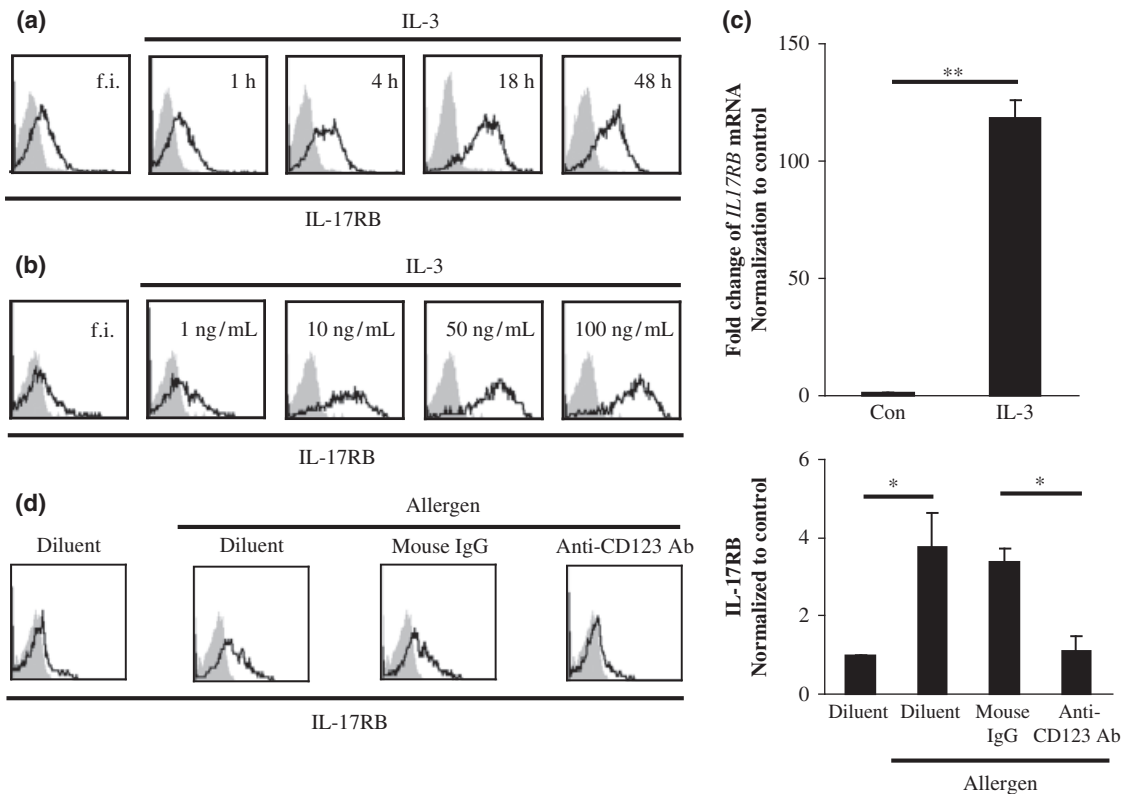


Fig. 2. IL-3 up-regulates the basophil expression of IL-17RB. (a) Purified basophils from buffy coat stimulated with or without IL-3 (10 ng/mL) or (b) with different concentrations of IL-3 for 12 h. Representative histograms are from one of three individuals. f.i., freshly isolated. (c) Purified basophils ($n = 3$) were stimulated with or without IL-3 (10 ng/mL) for 12 h. *IL17RB* mRNA was analysed by real-time PCR and the expression levels were calculated using the delta-delta CT method. (d) PBMCs from patients with SAR were blocked with mouse anti-CD123 in the presence of allergen challenge for 4 days. Representative histograms on the left are from one of four individuals. The shaded histograms represent isotype control; the open histogram represents IL-17RB. The histogram on the right shows the mean $\pm$ SEM of all the four samples * $P < 0.05$, ** $P < 0.01$.

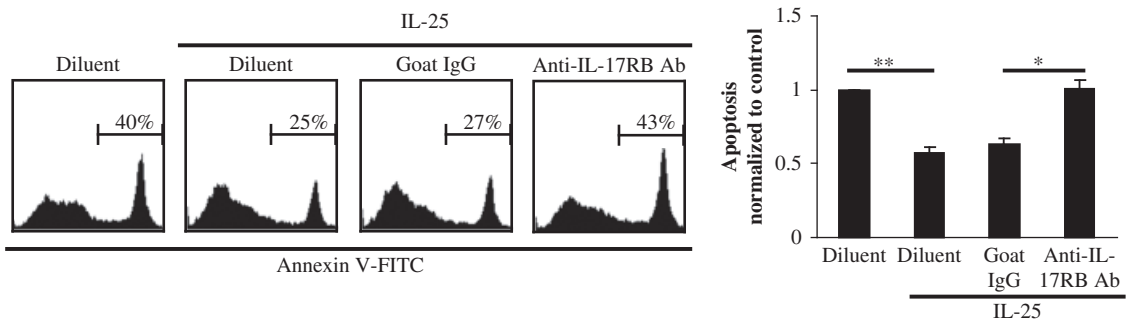


Fig. 3. Activation of IL-17RB inhibits apoptosis of basophils. Purified basophils (1×10^5 cells/well) from buffy coat were blocked with goat anti-human IL-17RB (100 ng/mL) in the presence of IL-25 (100 ng/mL) for 48 h. Representative histograms (left) are from one of four independent experiments. The histogram on the right shows mean $\pm$ SEM of all four samples * $P < 0.05$, ** $P < 0.01$.

from buffy coat were treated with or without IL-25 for 48 h. Goat anti-human IL-17RB was used to block IL-17RB. IL-25 significantly decreased basophil apoptosis ($P < 0.01$), while goat anti-human IL-17RB neutralized this effect ($P < 0.05$), indicating that IL-25 performs its anti-apoptotic function via IL-17RB (Fig. 3).

Activation of IL-17RB enhances immunoglobulin E-mediated degranulation of basophils

To investigate whether IL-17RB regulates the degranulation of basophils, basophils were purified from buffy coat and β -hexosaminidase measurement was used as a read-

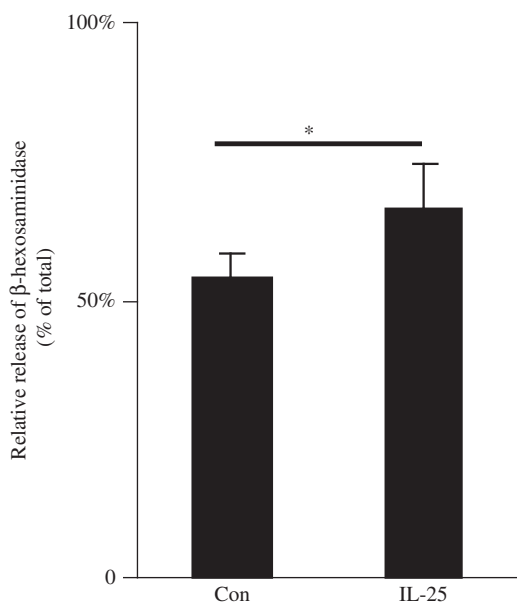


Fig. 4. Activation of IL-17RB enhances the degranulation of basophils. Purified basophils from buffy coat were treated with or without IL-25 (100 ng/mL) in the presence of IL-3 (10 ng/mL) for 18 h and then stimulated with anti-IgE (100 ng/mL) for 20 min. * $P < 0.05$.

out [39]. Pre-treatment with IL-25 in the presence of IL-3 significantly enhanced the degranulation of basophils following stimulation with anti-IgE (Fig. 4, $P < 0.05$).

Activation of IL-17RB does not affect basophil cytokine release and expression of adhesion molecules

To examine whether IL-25 regulates the release of cytokines from basophils, purified basophils from buffy coat were stimulated with or without IL-25 alone, or in combination with IL-3, or with anti-IgE, or with both IL-3 and anti-IgE for 12 h. As expected, IgE and/or IL-3 stimulation resulted in significantly increased release of IL-4, IL-8 and IL-13. However, even though the concentrations of IL-3 and anti-IgE were optimized, IL-25, alone or in combination with IL-3 and/or anti-IgE, did not exert any effect on the release of those cytokines (data not shown).

Activation of IL-17RB has been shown to regulate adhesion molecule expression on eosinophils [43]. To assess whether IL-17RB on basophils also regulates the expression of adhesion molecules, PBMCs from buffy coat were treated with or without IL-25 for 1 and 18 h, respectively. However, this treatment did not affect the expression of the adhesion molecules that were examined, namely ICAM-1 (CD54), L-selectin (CD62L), LFA-1 (CD11a/CD18) and VLA-4 (CD49d/CD29) (Fig. S3).

Discussion

Allergen-challenged PBMCs from patients with SAR provide an *in vitro* model of how APCs, CD4⁺ T cells and

effector cells such as basophils interact in allergic inflammation. Using gene expression microarrays, we found that *IL17RB* was the most differentially expressed gene in allergen-challenged PBMCs from patients with SAR. Further cell sorting studies revealed, for the first time, that IL-17RB was expressed on basophils and increased after allergen challenge in patients with SAR, but not in healthy controls. Functional analysis showed that the up-regulation of IL-17RB on allergen-challenged basophils was dependent on IL-3. We also found that the activation of IL-17RB inhibited apoptosis of basophils and enhanced the IgE-mediated degranulation of basophils. Previously, IL-17RB had been reported to be expressed on undefined non-T/non-B cells (C-kit+Fc ϵ RI-) and activated Th2 central memory cells. Activation by its ligand, IL-25, results in the release of Th2 cytokines such as IL-4, IL-5 and IL-13, eosinophil infiltration, goblet cell hyperplasia and increased serum IgE [32–36, 38, 44]. Studies of a murine asthma model show that IL-25 enhances Th2-dependent airway inflammation [44]. In agreement with previous studies, our gene expression microarray analyses of CD4⁺ cells from patients with SAR confirmed the increased expression of *IL17RB* following allergen challenge [5]. We also found highly significant correlations between the expression of *IL17RB* and the Th2 cytokines *IL4*, *IL5* and *IL13*. Moreover, we recently analysed gene expression microarray data from allergen-challenged CD4⁺ cells that had been treated with corticosteroids [22], and found that *IL17RB* was one of the genes that decreased most in expression. This indicated that IL-17RB may, in itself, be a therapeutic target. Taken together, these findings indicate that IL-17RB-dependent activation of Th2 cells plays an important role in allergic inflammation.

However, it is not known whether IL-17RB is expressed on or regulates basophils. These cells are increasingly recognized both as regulators and as effector cells in allergic inflammation. Basophils act as regulators by the release of Th2-type cytokines, in quantities that may be larger than those released from Th2 cells. From an effector perspective, IgE-mediated release of histamine and other pro-inflammatory mediators plays a key role in allergy [23–25, 45]. Despite this, there is relatively limited information about basophils in allergic inflammation. One important reason is their low cell number, which is <1% of the total peripheral leucocytes. On the other hand, cell-sorting studies of allergen-challenged PBMCs allow the analysis of basophils in the context of the cells that they naturally interact with. Our finding that IL-17RB increased on allergen-challenged basophils from patients with SAR but not from controls indicates a novel and potentially important role of IL-17RB in the pathogenesis of allergic inflammation.

IL-3 is a key regulator of basophils, and therefore, we hypothesized that IL-3 would increase the expression of IL-17RB. This hypothesis was confirmed, which suggests

that IL-17RB is an integrated part of the activation of basophils. Because basophils have been reported to release IL-3 in response to anti-IgE [46], we also challenged purified basophils from buffy coat with anti-IgE. It turned out that no increased IL-17RB after anti-IgE challenge was observed (data not shown). Therefore, we suspected that increased IL-17RB on basophils from allergen-challenged PBMCs from patients with SAR might be regulated by IL-3, which might come from activated T cells [47]. However, we only observed increased IL-17RB on basophils from allergen-challenged PBMCs at the late time points (Fig. 1b), indicating that IL-17RB might be up-regulated indirectly by IL-3 released from activated T cells.

Basophils have a short life span and do not proliferate after maturation. Many factors, such as FAS ligation [48] and corticosteroids [49], may induce apoptosis of basophils. Therefore, basophils are subject to various death induction signals and they normally survive for only a few days. However, with their short life span and the fact that they account for only 1% of the infiltrates, basophils are responsible for the induction of the IgE-mediated and late-phase chronic allergic inflammation in the absence of mast cells [50]. Therefore, altered regulation of basophil apoptosis may play a crucial role. In a previous report, activation of IL-17RB on eosinophils was shown to inhibit apoptosis [43]. Therefore, we hypothesized that IL-17RB would also inhibit apoptosis in basophils. Of note, basophils have been reported to release IL-25 after activation [38], which might in turn inhibit the apoptosis of basophils by binding IL-17RB. Even though IL-3 has been reported to play a dominant role in sustaining the survival of basophils [27, 49], we believe that any factor that contributes to prolonging the survival of basophils, such as IL-25, may result in potentially significant pathophysiological consequences.

Because degranulation of basophils plays a key role in allergy, we also wanted to examine whether IL-17RB was involved in regulating this function. We found that IL-25 promoted IgE-mediated degranulation of basophils in the presence of IL-3. However, in this study, we were not able to block the function of IL-25 with goat anti-human IL-17RB antibody as we did in our apoptosis assay. A possible explanation is that basophils express Fc receptors that may be activated by the blocking antibodies. This agrees with our observation that even isotype goat IgG induced degranulation. However, because IL-25-induced signalling is dependent on IL-17RB [42, 51], the observed increase of basophil degranulation in our study indicates that IL-17RB contributes to the IgE-mediated degranulation.

Because IL-17RB induces the release of Th2 cytokines from activated Th2 cells [38], we hypothesized that this would also be the case for basophils. However, we could not demonstrate that IL-17RB activation induced an increased release of Th2 cytokines from these cells. Basophils and eosinophils share many characteristics,

including regulation by common growth factors and similar patterns of mediator release [52]. Previously, activation of IL-17RB on eosinophils was reported to regulate the expression of adhesion molecules. However, in this study, we could not demonstrate any effect of IL-17RB on basophil adhesion molecule expression.

Two previous studies have made diverging observations about IL-17RB expression on monocytes [38, 41]. In agreement with one of those studies [38], we observed that only a fraction of monocytes expressed detectable IL-17RB. Our findings were based on two different antibodies, one monoclonal and one polyclonal, both of which yielded similar results. This supports the finding that the majority of monocytes do not express IL-17RB.

Taken together with observations made by others, our study suggests a model in which IL-17RB synchronizes the activation of basophils and Th2 central memory cells. Because both cell types play important and interdependent roles in allergy, IL-17RB could have potential as a therapeutic target. Further studies are warranted to elucidate in detail the effects of IL-17RB signalling on basophils and Th2 central memory cells, and how these interact. In conclusion, our study shows for the first time that basophils are an IL-17RB-expressing cell type in human peripheral leucocytes. We also demonstrate that the activation of IL-17RB inhibits apoptosis and promotes IgE-mediated degranulation of basophils. This indicates that IL-17RB on basophils acts in synergy with IL-17RB on Th2 cells and plays an important role in allergic inflammation.

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Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1. Pathway analysis of the differentially expressed genes in allergen-challenged PBMCs. Differentially expressed genes in patients vs. healthy controls were

submitted to Ingenuity Pathway Analysis (IPA). Pathways above the threshold have a $P < 0.05$. Yellow square represents the ratio of differentially expressed genes in each pathway.

Fig. S2. The percentage of basophils in CD4-CRTH2^{high} cells from PBMCs. Freshly isolated PBMCs from buffy coat were stained with mouse anti-CD4-FITC, rat anti-CRTH2-PE, mouse anti-HLA-DR-PerCP and mouse anti-CD123-APC. The percentage of CD4-CRTH2^{high} cells in gated CD123+HLA-DR- cells (down) and CD123+HLA-DR- cells in gated CD4-CRTH2^{high} cells (upper) were counted by Cellquest analysis.

Fig. S3. Activation of IL-17RB does not affect the expression of adhesion molecules on basophils. PBMCs from buffy coat were treated with or without IL-25 for one hour (a) and 18 h (b), respectively. Representative histograms are from one of four individuals. Shaded histogram represents isotype control; broken line represents no treatment; solid line represents treatment with IL-25.

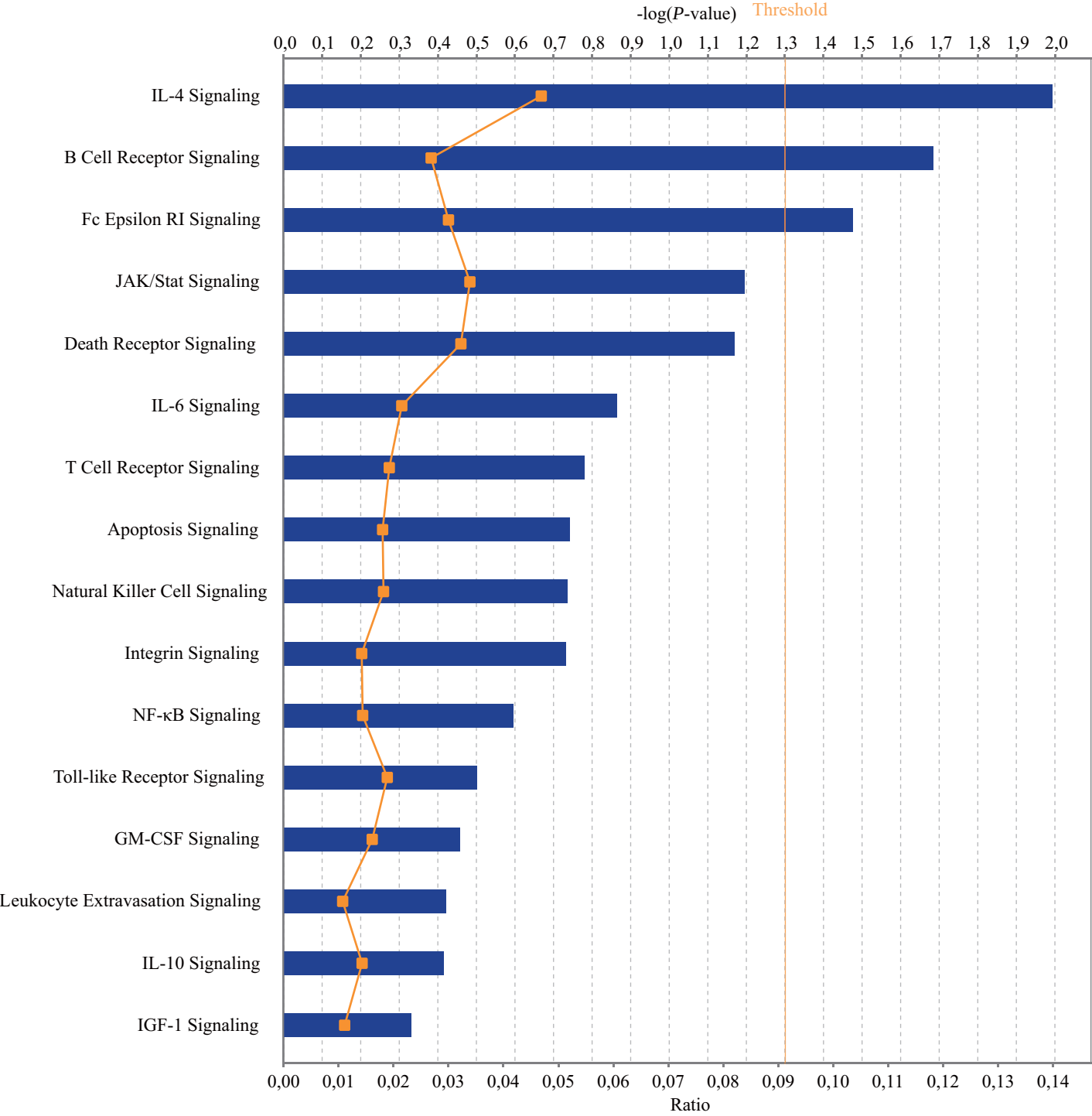
Table S1. The fold change of differentially expressed genes in allergen-challenged PBMCs from Patients with SAR vs. healthy controls

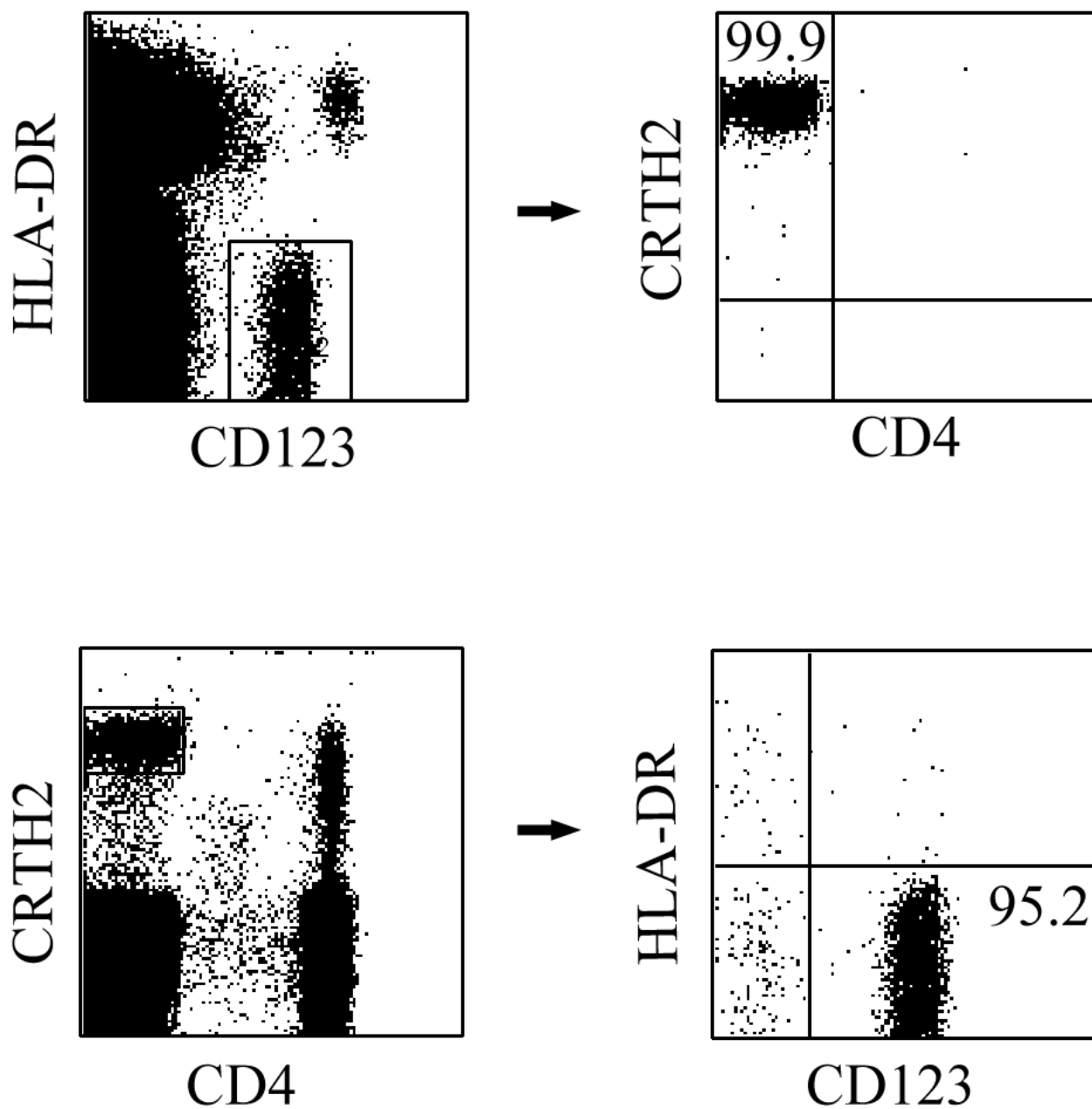
Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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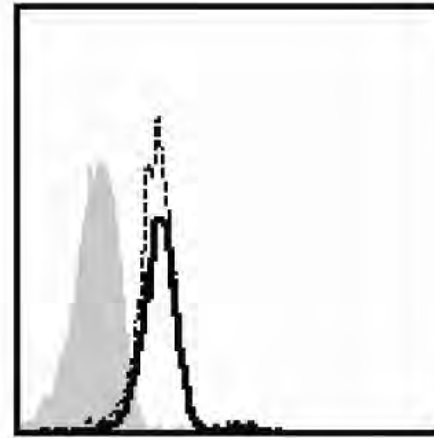
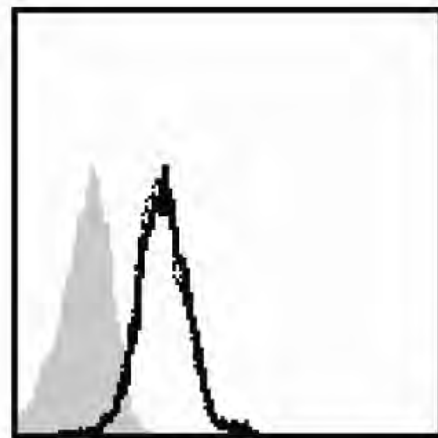
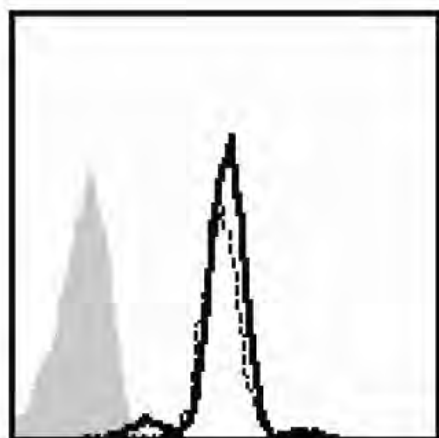
CD18

CD49d

ICAM-1

L-selectin

A



B

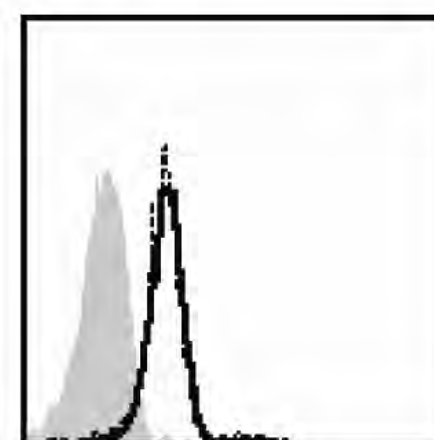
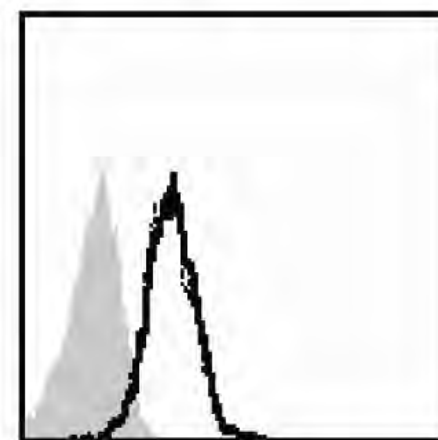
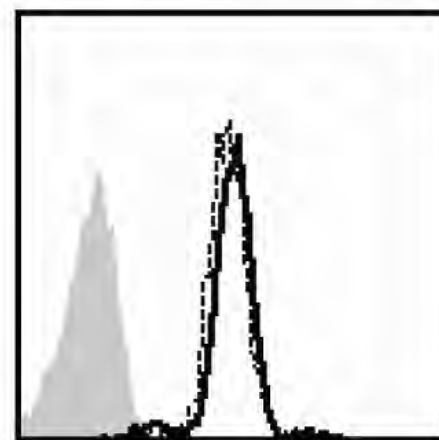


Table S1. The fold change of differentially expressed genes in allergen-challenged PBMCs from Patients with SAR versus healthy controls

Probe	Symbol	Log ₂ FC	P Value
ILMN_6687	IL17RB	2.70947866	0.00009328
ILMN_16243	PMCH	1.99651429	0.00015964
ILMN_12474	PGDS	1.94282639	0.00041295
ILMN_12030	FCER2	1.48341891	0.00008746
ILMN_8746	IL5	1.38324681	0.00361943
ILMN_8145	IL9R	1.28323438	0.00009779
ILMN_25774	KLRB1	1.13636203	0.00067854
ILMN_23533	CD209	1.10262143	0.00070871
ILMN_14583	CXCR6	1.05095370	0.00472478
ILMN_9279	CTNNAL1	1.00774041	0.00060114
ILMN_26722	ENPP2	0.93752810	0.00229451
ILMN_17651	SPTA1	0.87483346	0.00362418
ILMN_17008	TSPAN13	0.85713368	0.00200423
ILMN_729	PYHIN1	0.83214380	0.00261554
ILMN_15631	HRASLS3	0.82643881	0.00610498
ILMN_18336	FANK1	0.81661048	0.00031456
ILMN_22620	TGFBR3	0.80317758	0.00070309
ILMN_6510	QPCT	0.75259516	0.00893256
ILMN_4835	SUCNR1	0.74180572	0.00386706
ILMN_6387	GATA3	0.73478738	0.00670162
ILMN_137280	PTPLA	0.73339172	0.00053359

ILMN_12129	PYHIN1	0.70786262	0.00325134
ILMN_15540	SLC37A3	0.68414669	0.00423421
ILMN_10583	SH2D1B	0.67934780	0.00233234
ILMN_6603	LIMA1	0.66471438	0.00384231
ILMN_19059	KIAA1913	0.63378828	0.00453176
ILMN_28506	DKFZP564O0823	0.61837148	0.00947412
ILMN_12613	KLRG1	0.61823448	0.00379483
ILMN_19651	MCEMP1	0.58249609	0.00972041
ILMN_13979	C8orf70	0.57937024	0.00122285
ILMN_28039	SPINT2	0.57749381	0.00069122
ILMN_15072	MAF	0.57157834	0.00319332
ILMN_139125	CD79B	0.56027506	0.00540785
ILMN_30018	TGFA	0.54615872	0.00781023
ILMN_7155	FLJ22104	0.54569839	0.00134575
ILMN_3518	ELOVL5	0.54445313	0.00765395
ILMN_15656	MAF	0.54145494	0.00283838
ILMN_9068	FAS	0.53668541	0.00423300
ILMN_587	RNF138	0.53268441	0.00994809
ILMN_11103	TMEM2	0.52898147	0.00324918
ILMN_12662	ITGA2	0.52845872	0.00730690
ILMN_2369	PLA2G4A	0.52708035	0.00832985
ILMN_18056	RABEP1	0.51616323	0.00922963
ILMN_5164	MIB1	0.50989887	0.00697617
ILMN_28094	EMB	0.50845260	0.00579748

ILMN_10462	CISH	0.50505471	0.00572333
ILMN_1448	DNAJC18	0.49093850	0.00455993
ILMN_14733	DTX3L	0.48556465	0.00135515
ILMN_25398	FLJ14213	0.48541339	0.00922924
ILMN_26832	EIF2AK3	0.48415018	0.00244521
ILMN_10323	RSBN1L	0.47870733	0.00095657
ILMN_137297	CYB5R4	0.47535637	0.00327051
ILMN_2733	C14orf104	0.47258486	0.00295706
ILMN_8807	B3GNT3	0.46619517	0.00738023
ILMN_138841	RABGAP1L	0.46021388	0.00335124
ILMN_4996	SLA	0.45428284	0.00315315
ILMN_7556	DGKE	0.45411522	0.00886255
ILMN_14620	SLC41A2	0.44990782	0.00969238
ILMN_16203	TP53INP1	0.43437264	0.00373551
ILMN_18558	GSTA4	0.43216866	0.00530127
ILMN_10396	OSRF	0.43127911	0.00172740
ILMN_8647	C1orf24	0.43012694	0.00730123
ILMN_25419	FAM102B	0.42971772	0.00438242
ILMN_137329	ITR	0.42635928	0.00930780
ILMN_16224	ANKRA2	0.42221404	0.00598136
ILMN_29538	BBS7	0.42219534	0.00469683
ILMN_26768	PLEKHF2	0.41990890	0.00392880
ILMN_9113	BIVM	0.41381920	0.00491149
ILMN_27043	SUB1	0.41277801	0.00555220

ILMN_24639	ST7L	0.41253562	0.00287201
ILMN_20422	RWDD3	0.41006167	0.00183630
ILMN_19868	BTBD1	0.40277863	0.00774148
ILMN_15905	ZNFN1A5	0.40182955	0.00642745
ILMN_478	ARID4B	0.39862668	0.00844511
ILMN_5888	SNX4	0.39202780	0.00123590
ILMN_15187	ATXN1	0.38780756	0.00720465
ILMN_1157	HSPH1	0.37960863	0.00954795
ILMN_19680	MRPL50	0.37715902	0.00782564
ILMN_17373	PCGF6	0.37540189	0.00971201
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ILMN_7760	PPM1D	0.37516705	0.00151969
ILMN_10118	TBL1XR1	0.37417384	0.00959140
ILMN_28747	GCNT1	0.37313107	0.00061519
ILMN_25907	MED4	0.37252327	0.00776905
ILMN_9202	GOLGA4	0.37248077	0.00488123
ILMN_4851	SOCS2	0.36970449	0.00887471
ILMN_26831	NEK1	0.36939668	0.00064375
ILMN_15461	SPAST	0.36315164	0.00979827
ILMN_21364	LOC339745	0.36157573	0.00694426
ILMN_9076	SMG1	0.36122974	0.00968405
ILMN_8800	C1orf124	0.36120199	0.00991949
ILMN_12452	CMPK	0.35992633	0.00635738
ILMN_17714	ARID4A	0.35973683	0.00219590

ILMN_9182	TRNT1	0.35023245	0.00985910
ILMN_2331	FLJ33167	0.34851714	0.00746460
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ILMN_8556	SNX25	0.34378054	0.00099645
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ILMN_25241	MLKL	0.33286573	0.00292536
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ILMN_18559	SLC33A1	0.32947062	0.00836223
ILMN_13827	RAB18	0.32855416	0.00396758
ILMN_8142	NFATC3	0.32722139	0.00677862
ILMN_29637	KTN1	0.32550818	0.00917873
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ILMN_4382	GNA13	0.32220136	0.00987377
ILMN_25169	NMD3	0.32081256	0.00548042
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ILMN_15425	USP8	0.31559120	0.00919268
ILMN_19790	FAM35A	0.30924962	0.00638530
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ILMN_3380	FBXO30	0.30600497	0.00812835
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ILMN_13548	ACTR8	0.29672066	0.00256039
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ILMN_11053	RRM2B	0.28798020	0.00769819
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ILMN_137185	ARL5	0.27735406	0.00878026
ILMN_8298	LOC57149	0.27456539	0.00331458
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ILMN_21228	SMEK2	0.23182001	0.00842363
ILMN_1444	C6orf55	0.21989109	0.00322436
ILMN_6315	C1orf25	0.21196633	0.00969475
ILMN_1801	GATAD2A	-0.19406565	0.00866455
ILMN_24445	LOC126208	-0.24376980	0.00585836
ILMN_12885	FLJ43093	-0.24587489	0.00782040
ILMN_13593	UBE2J2	-0.24730258	0.00500361
ILMN_29872	CECR1	-0.24937063	0.00959723
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ILMN_25041	RPS6KB2	-0.31083994	0.00196540
ILMN_18393	PTGES2	-0.32566423	0.00734365
ILMN_20923	REXO1	-0.32736606	0.00623078
ILMN_24784	THRAP5	-0.33409539	0.00195833
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ILMN_1073	MSTO1	-0.34691414	0.00819082
ILMN_26304	C1orf198	-0.36257082	0.00458585
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ILMN_1491	FLJ22531	-0.41083906	0.00924542
ILMN_12751	OAF	-0.41971067	0.00266040
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ILMN_714	FBXW5	-0.80174801	0.00513490
ILMN_13180	K6IRS3	-0.84005186	0.00470971
ILMN_1546	MARCKS	-0.84441120	0.00237742
ILMN_2558	C1orf178	-0.86484347	0.00737282
ILMN_28417	STAC2	-0.86611832	0.00574849
ILMN_2477	TPST1	-0.89062890	0.00075025
ILMN_3550	KAL1	-0.91167304	0.00563769
ILMN_2045	KCTD7	-0.91533305	0.00106746
ILMN_4623	MYEOV	-1.10975250	0.00561006
ILMN_20186	ALS2CR16	-1.11227668	0.00598181
ILMN_16568	CDH23	-1.13719368	0.00759128
ILMN_12328	PDPN	-1.31214050	0.00911516
ILMN_11442	ITGA3	-1.54159737	0.00471247
ILMN_10981	HTRA1	-1.86259010	0.00009337
ILMN_1879	LOC153561	-1.91112373	0.00186887
ILMN_137475	CNTNAP2	-2.04193729	0.00256050

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