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Human basophils express amphiregulin in response to T cell-derived IL-3

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Abstract

Background—Amphiregulin, a member of the Epidermal Growth Factor family, is expressed by activated mouse Th2 cells. Amphiregulin produced by mouse hematopoietic cells contributes to the elimination of a nematode infection by a Type 2 effector response.

Objective—To identify the human peripheral blood cell population expressing amphiregulin.

Methods—Amphiregulin-expressing cells were identified by flow cytometry of cell surface markers and histological staining. Histamine and amphiregulin in supernatants were measured by enzyme immunoassay. Quantitative real-time PCR was used to measure mRNA expression.

Results—Stimulation of human peripheral blood mononuclear cells by anti-CD3 + anti-CD28 antibodies induced expression of amphiregulin mRNA and protein by a non-T cell population. The amphiregulin-producing cells were basophils, as judged by morphology and expression of CD203c and CD123 (IL-3 receptor alpha chain). Activated mouse basophils also produced amphiregulin. Amphiregulin expression by basophils in response to anti-TCR stimulation required IL-3 produced by T cells, and IL-3 alone induced high levels of amphiregulin expression by purified basophils. Amphiregulin was expressed at much higher levels when human basophils were stimulated by IL-3 than by IgE cross-linking, whereas the opposite was true for IL-4 expression and histamine release. Heparin-binding Epidermal Growth Factor-like growth factor was also expressed by IL-3-stimulated human basophils. PBMC from asthmatic human subjects contained significantly higher numbers of basophils able to produce amphiregulin, compared to allergic or non-allergic controls.

Conclusion—IL-3 can induce basophils to express high levels of amphiregulin, which may contribute to tissue remodeling during type 2 immune responses such as asthma.

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Keywords

Basophils; amphiregulin; IL-3

Introduction

Amphiregulin (AR) is a member of the Epidermal Growth Factor (EGF) family, which includes EGF, AR, transforming growth factor- α , heparin-binding EGF-like growth factor (HB-EGF), betacellulin, and epiregulin ^{1, 2}. These ligands share a conserved EGF-like motif (three disulfide loop structure) and all are initially expressed as transmembrane precursor proteins that are released from the cell surface by proteolytic cleavage ^{2, 3}. EGF receptors (EGFR) also comprise a multigene family of integral membrane tyrosine kinases that are activated upon binding of the ligands. AR (and EGF) bind to the homodimer EGFR (ErbB1/ErbB1) or heterodimer ErbB1/ErbB2 ^{4, 5}.

AR is widely expressed in human tissues ⁶. EGF family members, including AR, induce proliferation and differentiation of normal and malignant epithelial cells, fibroblasts and keratinocytes ^{1, 7}. This is potentially important for embryogenesis, tissue remodeling and repair ². Although AR-deficient mice show a defect in ductal elongation during mammary gland development in puberty ⁸, these mice can still nurse young effectively. Other tissue remodeling functions appear to be normal in AR^{-/-} mice, possibly because these functions are mostly redundant with other EGF family members ^{2, 8}.

We previously reported that AR is expressed by T cell receptor (TCR)-activated mouse CD4 T cells ⁹, particularly the Th2 cells that are involved in allergic responses. AR-deficient mice ⁸ showed slower kinetics of clearance of the helminth parasite, *Trichuris muris*, that is cleared most effectively by Th2-biased responses. Lack of AR was associated with reduction of the hyperproliferation of gut villus epithelium cells ⁹ that has been implicated in the removal of intestinal worms ¹⁰. Hemopoietic cells produced the AR needed for this response, as reconstitution of irradiated AR^{-/-} mice with wild-type bone marrow cells restored normal worm elimination kinetics ⁹.

Human mast cells also produce AR upon stimulation by IgE cross-linking, or constitutively in tissue-resident mast cells in asthma patients ^{11, 12}. Human eosinophils express AR in response to granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-5 stimulation ¹³. Therefore AR is produced in the immune system in at least three cell types (mouse Th2 cells, human mast cells and human eosinophils) that are strongly involved in allergic responses.

In allergic diseases including asthma and atopic dermatitis, EGF family members such as AR have been implicated in tissue remodeling ¹⁴. AR can promote the proliferation of human lung fibroblasts ¹², increase mucin gene expression by airway epithelial primary cells ¹¹ and enhance migration of Th2 cells into the inflamed tissue by increasing TARC expression ¹⁵. AR levels in sputum were significantly higher in subjects with asthma during acute attacks and correlated with the severity of asthma symptoms and with tryptase or Eosinophil Cationic Protein (ECP) in the sputum ^{16, 17}. Thus AR might significantly contribute to human allergic diseases.

We therefore tested whether human peripheral blood mononuclear cells (PBMC) produced AR in response to T cell activation. Although we found that AR expression was indeed increased after anti-TCR-stimulation of PBMC, unexpectedly we found that very little of this AR could be attributed to T cell production. Instead, a substantial proportion of

basophils strongly upregulated AR mRNA and protein in response to TCR ligation in the overall PBMC population. The link between T cell activation and basophil production of AR was found to be IL-3, which was both necessary and sufficient to stimulate AR production by basophils.

Methods

Antibodies and Reagents

Biotinylated goat anti-human and anti-mouse AR antibodies, and recombinant human (rh) IL-3 were obtained from R&D Systems (Minneapolis, MN). Antibodies specific for human CD3 ϵ (OKT3), IL-3 (BVD8-3G11), CD69 (FN50, Pacific Blue), CD123 (6H6, PE-Cy7), and CD203c (NP4D6, PE) were purchased from BioLegend (San Diego, CA). Antibodies specific for human CD28 (CD28.2), CD4 (RPA-T4, APC-AF750 or PE-Cy7), CD19 (HIB19, PE-Cy5), and mouse Ly-6G (Gr-1, RB6-8C5, AlexaFluor700), IL-4 (BVD6-24G2, PE-Cy7), and Fc ϵ RI α (MAR-1, PE) were obtained from eBioscience (San Diego, CA). Antibodies specific for human CD8 α (3B5, PE-Texas Red), CD14 (T μ K4, PE-Cy5), mouse CD4 (RM4-5, AF405), and mouse CD19 (6D5, PE-Texas Red) were obtained from CALTAG (Carlsbad, CA). APC-conjugated anti-human CD303 was a generous gift from Dr. Ernest Wang. Polyclonal goat anti-human IgE (ϵ -chain specific) was obtained from Sigma (Saint Louis, MO). 7AAD was obtained from Calbiochem (Gibbstown, NJ). The basophil isolation kit II was obtained from Miltenyi Biotec (Auburn, CA).

Human PBMC activation and cell surface staining

Heparinized blood was obtained from healthy donors under a protocol approved by the University of Rochester Medical Center Research Subjects Review Board. PBMC were isolated by Ficoll-Hypaque (Cellgro, Herndon, VA) density gradient centrifugation. Cells were suspended in RPMI-1640 medium containing 100U penicillin/streptomycin (Invitrogen, Carlsbad, CA) supplemented with 8% heat-inactivated fetal calf serum (FCS, HyClone, Logan, UT). 10^6 PBMC per well were stimulated with medium alone or 5 μ g/ml anti-CD3 ϵ + 1 μ g/ml anti-CD28 in round-bottom 96-well plate (Costar, Corning Inc., Corning, NY) for 6 hours at 37°C. After stimulation, the cells were stained for cell surface markers AR, CD4, CD8, CD14, CD19, CD69, CD123, CD203c, CD303 and live/dead 7AAD staining, then with APC-conjugated streptavidin (BD Bioscience) at 4°C for 15min, and fixed with 2% paraformaldehyde. Samples were analyzed using an LSR II flow cytometer (BD Bioscience), and FlowJo software (Tree Star Inc., Ashland, OR).

Human basophils preparation and activation

Peripheral blood basophils were purified by cell sorting or enriched by negative selection. For studying the direct induction of AR in human basophils by IL-3, basophils were sorted from fresh PBMC by surface markers CD4–CD8–CD14–CD19–7AAD–CD123+ on a FACSARIA (BD Bioscience, San Jose, CA). Purified basophils were treated with medium alone; 5 μ g/ml anti-CD3 ϵ + 1 μ g/ml anti-CD28 (as a negative control); and 5ng/ml rhIL-3 for 16 hours. Cells were stained with 7AAD and antibodies against AR, CD123, CD203c, and CD69.

For the remaining experiments, basophils were enriched from peripheral blood by depletion of magnetically labeled cells according to the manufacturer's instructions (Basophil isolation kit II, Miltenyi Biotec, Auburn, CA). The purity of live basophils was about 20–80%. Enriched basophils were suspended in IMDM medium supplemented with 5% FCS, 1x MEM non-essential amino acids (Invitrogen), $\sim 10^5$ cells/well in 96-well plates, and treated for 0.5, 1, 4 or 16 hours with medium alone, 10ng/ml rhIL-3 or 10ng/mL anti-IgE. Supernatants (4 hrs) were collected to measure histamine release. Half of the cells were

collected for extraction of total RNA, and the remaining cells were stained with 7AAD and antibodies against AR, CD123, CD203c, FcεRIα, and CD69 to measure surface AR expression.

Quantitative real time PCR for gene expression

Total RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was prepared by reverse transcription from total RNA using SuperScript III Reverse Transcriptase (Invitrogen) and Oligo(dT)₁₂₋₁₈ (Invitrogen) as primer. Real-time PCR was performed using Applied Biosystems 7900HT Sequence Detection System. Primers and probes specific for AR, IL-3, IL-4, IL-13, HB-EGF and GAPDH were obtained from the TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA). mRNA amounts were normalized to endogenous GAPDH gene expression. Samples were run in triplicate and data were analyzed using SDS software (Applied Biosystems).

Histamine release assay

Histamine in enriched basophil supernatants was measured in duplicate by the Histamine enzyme immunoassay kit (Cayman, Ann Arbor, MI) according to the manufacturer's protocol.

Measurement of AR release

Enriched basophils were treated with medium alone, 10ng/mL rhIL-3 or 10ng/mL anti-IgE, with or without 5μg/mL anti-IL3Rα (R&D) or 50μM TAPI-1, an ADAM17 protease inhibitor¹⁸ (Calbiochem). After 24 hours, supernatant AR was measured using the human Amphiregulin DuoSet ELISA Development kit (R&D, detection limit 7.8 pg/mL). This kit may underestimate the absolute levels of AR, because the ELISA standard and the immunogen for the polyclonal antibody reagent were unglycosylated bacterial recombinant human AR, whereas natural AR is heavily glycosylated. However, studies on AR produced by eosinophils and mast cells used the same ELISA kit^{12, 13}, so our results can be directly compared with these other studies.

Intracellular staining of mouse cells

AR deficient (AR^{-/-}) mice⁸, originally obtained from David Lee were backcrossed to AR C57BL/6.PL (B6/PL) mice for ten generations and then maintained by homozygous breeding. C57BL/6.PL mice were bred in our facility.

Peripheral blood from mice was obtained by cardiac puncture. Red cells were lysed by ACK lysis buffer (0.8% NH₄Cl, 10mM KHCO₂ and 0.1mM EDTA), and the remaining cells were stimulated with medium alone, 20ng/ml recombinant mouse IL-3 (R&D Systems Minneapolis, MN) or 20ng/mL rat anti-mouse IgE (R35-92, BD Bioscience) in a 96-well plate at 10⁶ cells/well. After 16 hours stimulation (with 2μM monensin present for the last 6 hours), the cells were stained with antibodies specific for CD4, CD19, Gr-1, FcεRIα and 7AAD, then fixed and permeabilized using Fix-Perm (BD Bioscience), and stained with anti-AR and anti-IL-4 intracellularly. Cells were analyzed using an LSR II flow cytometer, and FlowJo software.

Results

Basophils from anti-CD3/CD28 stimulated human PBMC express AR

Mouse Th2 cells produced AR in response to TCR-mediated activation⁹. To determine whether human Th2 CD4 T cells produce AR we stimulated human PBMC with soluble anti-CD3 and anti-CD28 for 6 hours *ex vivo*. As IL-4- and IL-5-producing cells can be

detected in polyclonally-stimulated PBMC, this approach should have revealed Th2 production of AR, if it occurred.

As AR is initially expressed as a surface protein, stimulated and unstimulated PBMC were stained for cell-surface AR as well as other surface markers to identify CD4 T cells and other major PBMC populations. Analysis by flow cytometry (Fig 1A) showed that anti-CD3 + CD28 stimulation induced a substantial population of AR-expressing cells, but these cells did not appear to be T cells. The AR-positive cells induced by T cell activation (about 0.1% of total PBMC) were mainly CD4⁺CD8⁺CD123⁺. Within the CD4⁺CD8⁺CD123⁺ population, 26% expressed AR after anti-CD3/CD28 stimulation, whereas less than 1% of the cells expressed AR in the corresponding unstimulated population (Fig 1B). The induction of AR expression by CD123⁺ PBMC is highly reproducible, and we have observed this in more than 20 experiments (data not shown).

CD123, the ligand-specific α -chain of the heterodimeric IL-3 receptor (CD123/CD131), is expressed at high levels on blood basophils and plasmacytoid dendritic cells 19, 20. These two populations were distinguished by expression of two additional surface markers. Among hemopoietic cells, CD203c (ectonucleotide pyrophosphatase/phosphodiesterase 3 E-NPP3) is expressed on human basophils and mast cells but not on plasmacytoid dendritic cells. Expression of CD203c is increased on activated basophils 21. Conversely, CD303 (BDCA-2, a type II transmembrane C-type lectin), is expressed on blood plasmacytoid dendritic cells but not basophils 20. The major population expressing AR after anti-TCR stimulation of PBMC expressed the basophil pattern (CD123⁺CD203c⁺CD303⁺, Fig 1A). Forward and side scatter were also consistent with the properties of small granulocytes, as expected for basophils.

Final identification of these AR positive cells as basophils was made by sorting CD4⁺CD8⁺CD14⁺CD19⁺7AAD⁺AR⁺ cells from anti-CD3 + CD28-stimulated human PBMC, and analyzing these by cytospin and histological staining. More than 90 percent of these cells contained the lobulated nuclei and basophilic granules characteristic of basophils (Fig 1C).

To confirm the expression of AR protein on basophils revealed by the polyclonal goat antibodies, RNA was extracted from CD4⁺ and CD123⁺ cells sorted from non-treated and anti-CD3 + CD28 stimulated PBMC. As measured by qPCR, AR transcription in stimulated CD123⁺ cells increased greatly, whereas CD4 cells expressed much lower AR mRNA levels in either untreated or stimulated PBMC (Fig 1D). The mRNA analysis suggests that AR on basophils after stimulation represented new expression rather than release of preformed protein. This was supported by staining of permeabilized cells, showing increased intracellular AR after stimulation (data not shown).

Basophils are induced to express AR by IL-3, which is expressed by activated human T cells

Basophils do not express the T cell receptor, so why do human basophils upregulate AR expression after anti-CD3 stimulation of PBMC? We proposed that AR expression by basophils might be indirectly induced by a mediator released by activated T cells. IL-3 primes and activates basophils²², enhancing expression of CD203c and CD69^{23, 24}. The IL-3 receptor chain CD123 is highly expressed on the AR-expressing basophils (e.g. Fig 1A). By qPCR, IL-3 mRNA levels were significantly increased in CD4 T cells after PBMC cell activation by anti-CD3 + CD28 (15, 34, 41, 49, and 53-fold in five individuals), and also in human Th1 and Th2 cell lines (data not shown).

Anti-IL-3 almost completely blocked the AR expression on basophils when added during stimulation of PBMC by anti-CD3 + CD28. RhIL-3 (in the absence of anti-CD3 + CD28 stimulation) strongly induced basophil expression of AR and two other activation markers, CD203c and CD69 (Fig 2A). IL-3-induced AR expression was rapid, peaked at 12-24 hours (Figure E1 in the Online Repository), and was induced by low IL-3 concentrations (Fig 2B).

To test whether IL-3 (but not anti-CD3 + CD28) directly induced AR expression on basophils, basophils (CD4-CD8-CD14-CD19-7AAD-CD123+) were separated from PBMC by cell sorting. IL-3 induced strong AR expression on the purified basophils, whereas anti-CD3 + CD28 had no effect, as expected (Fig 2C). Thus IL-3 is necessary for AR expression on basophils in anti-TCR-activated PBMC, and sufficient to potently induce AR expression on purified basophils.

AR expression is induced more strongly by IL-3 than by IgE cross-linking

Human basophils are strongly activated by cross-linking of high affinity IgE Fc receptors (FcεRI), which leads to secretion of various mediators including histamine, leukotrienes, IL-4 and IL-13²⁵. IL-3 alone is less effective than FcεRI cross-linking, but IL-3 can prime basophils for enhanced responses to subsequent FcεRI cross-linking²². IL-3 also directly enhances expression of CD69 and CD203c on basophils^{23, 24}. We therefore tested whether FcεRI cross-linking enhanced AR expression.

IgE cross-linking was more effective than IL-3 for inducing histamine release by basophils (Fig 3A). In contrast, IL-3 was more effective than cross-linking IgE for inducing AR protein expression on the surface of basophils (Fig 3A). The ability of anti-IgE to stimulate histamine release but not AR expression was confirmed with additional anti-IgE concentrations (from 1ng/mL to 1μg/mL, Figure E2 in the Online Repository) and incubation times (data not shown). IL-3 consistently induced higher levels of AR expression than any of the anti-IgE treatments tested. Fig 3A shows Mean Fluorescent Intensity (MFI) values for AR – similar conclusions were obtained if the percentages of cells positive for AR were plotted. Although IL-3 priming synergizes with IgE cross-linking to induce IL-4 production and histamine release^{26, 27}, there was no significant difference in the induction of surface AR expression during treatment with IL-3 with or without anti-IgE (Fig 3B).

As AR can exist as an initial membrane-bound form or a soluble cleaved molecule, we tested the possibility that IgE cross-linking induced AR production, but this AR was cleaved off the basophil surface. IL-3 increased the levels of soluble AR in the supernatant more effectively than IgE cross-linking (Fig 3C), and this could be inhibited by anti-IL-3 receptor antibodies, or by the cleavage inhibitor TAPI-1. The supernatant levels of AR induced by IL-3 treatment for 24 hours were 71 ± 28 pg/million basophils in six different experiments, comparable to the AR levels produced by eosinophils (estimated 18 pg/million cells from reference 13), and mast cells (360 pg/million cell)¹². Cross-linking of IgE did not enhance soluble AR levels (Fig. 3C). However, in some experiments anti-IgE further enhanced (up to two-fold) the levels of AR released by IL-3-stimulated basophils (Figure E3 in the Online Repository).

Analysis of mRNA expression led to similar conclusions. Although anti-IgE induced rapid expression of IL-4 and IL-13 mRNA, within one hour (Fig 4), only IL-3 induced high levels of AR mRNA expression, with somewhat slower kinetics. As in previous studies²⁸, IL-13 mRNA expression was induced by IL-3 at longer times (Fig 4).

Basophils can secrete IL-3 following IgE cross-linking²⁹. Low levels of IL-3 expression by basophils were also detected by qPCR during our anti-IgE stimulation (data not shown). However, this IL-3 was not sufficient to induce substantial levels of AR expression (Figure

3 and Figure E3 in the Online Repository). The possibility that anti-IgE stimulation induced both IL-3 and an inhibitor of AR expression was ruled out by the strong AR response of anti-IgE-treated basophils to exogenous IL-3 (Fig. 3B).

Overall, AR mRNA and protein expression as well as protein shedding by basophils was induced consistently and strongly via an IL-3-dependent pathway, whereas anti-IgE stimulation, even though more effective for inducing expression of other mediators, induced lower levels of AR expression.

As IL-3 induces the synthesis of several mediators by basophils, we tested whether these conditions activated the synthesis of other EGF family members, by measuring mRNA levels using qPCR. Similar to AR, HB-EGF was expressed by basophils in response to IL-3, but at lower, more transient levels by anti-IgE. Figure 4 shows the extent of induction of HB-EGF mRNA, relative to unstimulated cells. When normalized to GAPDH mRNA levels, HB-EGF mRNA levels were lower than those of AR (data not shown). Other EGF family members were expressed at lower or undetectable levels (data not shown).

Activated mouse basophils express AR

As human basophils expressed AR, we tested whether mouse blood basophils could also express AR. After red blood cell lysis, mouse blood cells were stimulated with IL-3 or anti-IgE, and stained for expression of surface markers, intracellular IL-4 and AR. Basophils were identified as CD4⁺CD19⁺Gr-1⁺FcεRI⁺ cells³⁰. IL-3 induced substantial numbers of wild type B6/PL cells in this population to express IL-4, and about 29% of these cells also expressed AR. The specificity of AR staining in basophils was confirmed by showing that the equivalent population from AR^{-/-} mice expressed similar levels of IL-4, but undetectable AR (Fig 5). Thus IL-3 activated mouse basophils also expressed AR. Interestingly, anti-IgE stimulation was more effective on mouse than human basophils, stimulating low levels of AR production. Similar to human basophils, mouse basophils produced cytokines more rapidly in response to anti-IgE, and produced AR more slowly in response to IL-3 (results not shown).

Increased numbers of basophils from asthmatic subjects can produce AR

To investigate potential links between asthma and the ability of basophils to produce AR, we measured the levels of AR-producing basophils in PBMC from three groups of human subjects, with A) allergic asthma; B) allergy but not asthma; and C) non-allergic. Figure E4 in the Online Repository describes this study and the results. The numbers of basophils able to produce AR were increased in asthmatic subjects compared to either of the other groups. This difference was significant ($P < 0.05$) in response to stimulation with IL-3 or SEB, but did not reach significance with anti-CD3+anti-CD28. Consistent with our previous data, anti-IL-3 antibodies substantially reduced the numbers of AR⁺ cells induced by anti-CD3+anti-CD28 or SEB.

Discussion

These results clearly show that human basophils can produce the EGF ligands AR and HB-EGF, particularly in response to stimulation by IL-3. Thus basophils would be expected to produce these cytokines at inflammatory sites where T cells were activated to produce IL-3. AR is also produced upon stimulation of human mast cells by IgE cross-linking¹¹; constitutively in tissue-resident mast cells in asthma patients¹²; and on activation of eosinophils by IL-5 or GM-CSF¹³.

Immune cells known to produce AR now include Th2 cells (mouse), basophils (mouse and human), mast cells (human) and eosinophils (human)^{9, 11-13}. Mouse or human neutrophils

(data not shown) do not express significant levels of AR 13. Recent studies on mouse basophils suggest that basophils also provide a positive feed back loop enhancing type 2 responses, acting as one of the major sources of local IL-4 secretion, and directly priming T cells to induce Th2 responses 30-33. Thus the hemopoietic cell types expressing AR are all crucial components of the allergic inflammatory response 34, suggesting that the effects of immune AR are more prominent in allergic rather than Type 1 inflammation.

IL-3 also enhances mouse basophil migration into sites of inflammation ³⁵. Expression of specific chemotactic receptors, such as the prostaglandin D2 receptor CRTH2 ³⁶, on human basophils also contributes to the selective recruitment of basophils. Although IL-3 is produced by both Th1 and Th2 cells after activation ³⁷, selective migration of basophils into allergic sites due to other receptors may result in association between Type 2 responses and basophils, resulting in IL-3-dependent AR expression on basophils infiltrating the sites of allergic inflammation.

Human CD4 T cells stimulated through the TCR did not express AR in this study, which was unexpected based on mouse data showing that AR is produced by Th2 cell populations 9. However, in other studies we found that TCR stimulation and intracellular cAMP upregulation synergize to induce human CD4 T cells to express AR (manuscript in preparation). Partially-purified human CD4 T cells have also been reported to produce AR in response to cAMP agonists in another study ³⁸, but the purification method used (depletion of CD14+ and CD19+ cells) would have resulted in a population containing both basophils and T cells, thus the AR detected in that study was probably derived mainly from basophils.

AR can contribute to effector functions by enhancing parasite expulsion during worm infection ^{9, 10}, and can also cause tissue remodeling, which may be positive for repairing immunopathology, but could play a negative role in the development of asthma ¹⁴. A characteristic feature of asthma is airway remodeling, including epithelial proliferation, subepithelial fibrosis, increased airway smooth muscle mass, goblet cell hyperplasia, and mucus gland hyperplasia. AR not only promoted the proliferation of primary human lung fibroblasts ¹², but also enhanced mucin gene expression in the airway epithelium ¹¹. IL-13 can also contribute to tissue remodeling ³⁹, and both IL-13 and AR are produced by basophils in response to IL-3.

Consistent with a role for AR in asthma, mast cells expressing AR have been described in asthma patients ¹², and AR levels in sputum were associated with the severity of asthma and with tryptase and ECP levels in sputum ^{16, 17}. Although not directly addressing the role of basophils in the lung, our studies demonstrated that asthmatic subjects had increased numbers of peripheral blood basophils capable of producing AR. IL-3-dependent production of AR by human basophils could occur as part of a coordinate Type 2 response in which mast cells, eosinophils, and basophils produce AR, consistent with the correlation of AR and the mast cell and eosinophil products tryptase and ECP. The discovery that AR is produced by basophils, as well as mast cells, eosinophils and potentially Th2 cells, strongly suggests that AR is selectively produced at the site of allergic reactions and may participate in allergic reactions involving tissue remodeling, such as asthma and allergic dermatitis.

Key messages

1. Activation of T cells in human peripheral blood mononuclear cells leads to high expression of amphiregulin in basophils.
2. IL-3 induces amphiregulin expression by human basophils.

3. IL-3 is more effective than IgE cross-linking for inducing amphiregulin expression.

Capsule summary

The production of amphiregulin by basophils in response to IL-3 (produced by inflammatory T cells) may link allergic immune responses with the tissue remodeling that contributes to asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PBMC	human peripheral blood mononuclear cells
AR	amphiregulin
ECP	Eosinophil Cationic Protein
EGF	epidermal growth factor
HB-EGF	heparin-binding EGF-like growth factor
EGFR	EGF receptors
TCR	T cell receptor
GM-CSF	granulocyte macrophage colony-stimulating factor
rh	recombinant human
MFI	Mean Fluorescent Intensity
qPCR	Quantitative real-time PCR

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Figure 1. Human basophils express AR

(A) PBMC were stimulated with anti-CD3/CD28, stained, and gated as shown. (B) AR expression on unstimulated (shaded) and stimulated (line) CD4-CD8-CD123+ cells. (C) Sorted CD4-CD8-CD14-CD19-7AAD-AR+ cells were stained with Wright-Giemsa. (D) Stimulation-induced AR mRNA changes were measured in CD4+CD8-CD14-CD19-7AAD-CD123- ("CD4") and CD4-CD8-CD14-CD19-7AAD-CD123+ ("CD123") cells sorted from anti-CD3/CD28-stimulated PBMC (four subjects, 16 hours). Results are representative of at least three experiments.

Figure 2. IL-3 is necessary and sufficient for induction of AR expression on basophils by activated T cells

(A) Surface antigens on basophils (CD4–CD8–CD14–CD19–7AAD–CD203c+) were analyzed by flow cytometry in unstimulated (shaded) or stimulated (line) PBMC. (B) PBMC were treated with rhIL-3 (left, 6 hours; right, 5ng/mL). Means of triplicate cultures are shown. (C) Sorted basophils treated for 16 hours as indicated, were analyzed by flow cytometry. Results are representative of at least three experiments.

Figure 3. AR expression in human basophils is induced more strongly by IL-3 than IgE cross-linking

Enriched basophils were stimulated as indicated. (A) Histamine release and surface AR expression (four hours). Symbols represent different individuals. (B) Surface AR on basophils from five individuals (16 hours). (C) Supernatant AR after stimulation with or without anti-IL-3R α or TAPI-1 (24 hours).

Figure 4. IL-3 and IgE cross-linking induce different activation profiles in human basophils
Enriched basophils were stimulated as indicated, and mRNA measured by qPCR. Kinetics of AR, IL-4, IL-13 and HB-EGF mRNA expression in response to IL-3 or anti-IgE stimulation (expressed as the fold change relative to unstimulated cells) are shown. Similar results were obtained in at least four experiments.

Figure 5. Mouse basophils express AR in response to IL-3 activation

Mouse blood leukocytes from B6/PL or AR^{-/-} mice were treated as indicated and stained for surface markers and intracellular IL-4 and AR expression. Basophils (CD4⁺CD19⁻Gr-1⁻FcεRI⁺) were gated, and then analyzed for IL-4 versus AR. Results are representative of three experiments.