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Human Airway Eosinophils Exhibit Preferential Reduction in STAT Signaling Capacity and Increased CISH Expression

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Allergic asthma, a chronic respiratory disorder marked by inflammation and recurrent airflow obstruction, is associated with elevated levels of IL-5 family cytokines and elevated numbers of eosinophils (EOS). IL-5 family cytokines elicit peripheral blood EOS (EOSPB) viability, recruit EOSPB to the airways, and, at higher concentrations, induce degranulation and reactive oxygen species generation. Although airway eosinophils (EOSA) remain signal ready in that GM-CSF treatment induces degranulation, treatment of EOSA with IL-5 family cytokines no longer confers a survival advantage. Because the IL-5 family receptors have common signaling capacity, but are uncoupled from EOSA survival, whereas other IL-5 family induced endpoints remain functional, we tested the hypothesis that EOSA possess a JAK/STAT-specific regulatory mechanism (because JAK/STAT signaling is critical to EOS survival). We found that IL-5 family–induced STAT3 and STAT5 phosphorylation is attenuated in EOSA relative to blood EOS. Additionally, following allergen challenge, EOSA express significantly more CISH and SOCS1 mRNA and CISH protein than EOSPB counterparts. In EOSPB, long-term pretreatment with IL-5 family cytokines, to varying degrees, attenuates IL-5 family–induced STAT5 phosphorylation. These data support a model in which IL-5 family cytokines trigger a selective downregulation mechanism in EOSA for JAK/STAT pathways. The Journal of Immunology, 2013, 191: 2900–2906.

Eosinophils (EOS) contribute to the pathophysiology of allergic diseases, including asthma, through activation and subsequent release of inflammatory mediators and profibrotic factors (1–5). Our laboratory and others (2, 6–9) have observed that allergic airway inflammation leads to an elevated number of airway EOS (EOSA) and that this increased number of EOSA is associated with higher concentrations of proinflammatory IL-5 family cytokines (i.e., IL-5, IL-3, and GM-CSF) found in asthmatic bronchoalveolar lavage (BAL) fluid 48 h after segmental bronchoprovocation with allergen (SBP-Ag) (6, 8, 10–12). Incidentally, these IL-5 family cytokines are critical for normal EOS biology and function and signal through heterodimeric receptors comprised of a shared common β-chain (IL-5Rb) and a ligand-specific α-chain (i.e., IL-5Rα) (13, 14). EOS activation via these cytokines leads to a number of signaling pathways (including the JAK/STAT and Ras–Raf–ERK/MEK pathway) but that EOSA survival is no longer enhanced by IL-5 family cytokines (12, 20, 30), we found that IL-5 family–induced STAT3 and STAT5 phosphorylation is attenuated in EOSA relative to blood eos from airway allergen–challenged donors. However, IL-5 family–induced ERK1/2 phosphorylation is not altered between EOSA and EOS from airway allergen–challenged donors. These observations suggest EOSA possess a regulatory mechanism for suppressing STAT signaling distinct from ERK1/2 activation. Furthermore, we found, in EOSPB, IL-5 family cytokines induce members of the suppressors of cytokine signaling (SOCS) genes, CISH and SOCS1. Additionally, following allergen challenge, EOSA express significantly more CISH and SOCS1 mRNA and CISH protein than EOSPB counterparts. In EOSPB, long-term pretreatment with IL-5 family cytokines, to varying degrees, attenuates IL-5 family–induced STAT5 phosphorylation. These data support a model in which IL-5 family cytokines trigger a selective downregulation mechanism in EOSA for JAK/STAT pathways.
IL-5 family receptor expression, there is/are (a) pathway-specific mechanism(s) regulating EOS A responsiveness to IL-5 family cytokines.

One possible selective attenuation pathway involves the suppressor of cytokine signaling (SOCS) family proteins. JAK/STAT signaling upregulates SOCS family proteins, which serve as negative-feedback regulators in several immune systems (31, 32). SOCS family proteins block phosphorylation of membrane receptors and JAK family members and, subsequently, inhibit STAT binding and phosphorylation (31, 33, 34), all necessary to induce downstream JAK/STAT signaling, which has been linked to IL-5 family–induced survival in EOS (22, 23). Interestingly, in a murine model of allergic inflammation, Lee and colleagues (35) found two members of the SOCS family of genes, SOCSI and CHS, also referred to as CIS1, were, respectively, modestly and highly expressed in hematopoietic cells trafficking to the lungs after OVA challenge. These findings, combined with the observation that CHS is inducible in human EOSPB (17), led us to CHS and SOCSI upregulation as a possible IL-5 family signaling regulatory mechanism in human EOS.

The data presented in this study represent a unique opportunity to test these questions with highly purified EOSPB and EOSA from patients with asthma undergoing an allergic airway challenge, of which the literature is extremely sparse, as well as with control EOSPB from unchallenged participants. We found EOSA exhibit decreased IL-5 family–stimulated p-STAT5 and p-STAT3 compared to IL-5 family stimulation of same-day isolation of blood EOS from the SBP-Ag–challenged individuals (EOSCPB), whereas IL-5 family–stimulated p-ERK1/2 remained unaltered between EOSA and EOSCPB. We also found that EOSA express significantly more CHS and SOCSI mRNA and CISH protein than EOSCPB, whereas EOSCPB in turn express more CISH and SOCSI mRNA and CISH protein than EOSPB from independent, nonchallenged donors. Additionally, we observed 24-h pretreatment of EOSPB with IL-5 family cytokines, to varying degrees, attenuates the ability of subsequent IL-5 family stimulation to induce p-STAT5. Taken together, these data suggest distinct regulation mechanisms by which STAT3 and STAT5 signal transduction may be altered in EOSA, as compared with regulation of other pathways stimulated by IL-5 family cytokines.

Materials and Methods

Subjects

These studies were approved by the University of Wisconsin-Madison Center for Health Sciences Human Subjects Committee. Each subject provided informed, written consent prior to participation. We recruited atopic and nonatopic volunteer donors who had EOS comprising 2–10% of their peripheral blood leukocytes.

Isolation and treatment of peripheral EOSBP and EOSA

Human EOSPB were purified from heparinized peripheral blood, as described previously (23). Briefly, a granulocyte pellet was obtained by centrifugation of the blood through a Percoll monolayer (1.090 g/ml) and subsequent hypotonic lysis of erythrocytes. The resulting granulocytes were resuspended in HBSS supplemented with 2% newborn calf serum and incubated with anti-CD16–conjugated paramagnetic microbeads (MACS system; Miltenyi Biotec, San Jose, CA) to deplete contaminating neutrophilic cells. As above, purification continued with the interface between the two layers. As above, eosinophils were resuspended in HBSS supplemented with 2% newborn calf serum, and incubation with anti-CD16–conjugated paramagnetic microbeads. Eosinophilic populations were at least 98% pure and 97% viable.

Fluid from BAL was recovered, and EOSA were isolated 48 h after SBP-Ag, in which Ag dose for SBP defined and BAL performed as described (27, 36). In brief, EOSA were obtained after centrifuging BAL fluid through a Percoll bilayer (1.085/1.100 g/ml), with the recovered EOS population at the interface between the two layers. As above, purification continued with hypotonic lysis of erythrocytes, resuspension in HBSS plus 2% newborn calf serum, and incubation with anti-CD16–conjugated paramagnetic microbeads. Eosinophilic populations were at least 98% pure and 97% viable.

EOSA were isolated and purified from the same donors who underwent SBP-Ag (challenged peripheral blood, EOSCPB), obtained from phlebotomy that occurred immediately after recovery from the bronchoscopy procedure. Control experiments were performed in all cases on the same day with peripheral blood from a different, unchallenged donor to validate that the purification process did not result in EOS activation.

For all experiments, freshly isolated EOS (2–4 million per recovery source [i.e., EOSA, EOSCPB, or EOSPB]) were divided evenly between treatments and incubated at 37°C in 25 mM HEPES-buffered RPMI 1640 containing 0.1% human serum albumin for 30 min and then stimulated with IL-5, IL-3, or GM-CSF at the concentrations and times indicated in the figure legends. Loading controls were used to appropriately compare differences in cell number between groups and donors.

Immunoblotting

Primary human EOS cultures were diluted with ice-cold STOP buffer (20 mM Tris [pH 7.4], 137 mM NaCl, 1 mM EDTA, 1 mM Na2VO3, 20 mM β-glycerophosphate, 10 mM NaF, and 1% mannunal protease inhibitor mixture), pelleted by centrifugation, and lysed in RIPA buffer (STOP buffer containing 0.1% v/v glycerol, 0.01% Triton X-100, 0.1% SDS, and 0.25% deoxycholic acid). Lysates were sonicated and centrifuged at 15,800 × g for 10 min to remove the insoluble fraction. Supernatants were assayed for total protein content using the Pierce Micro BCA protein assay (Pierce, Rockford, IL), electrophoresed on SDS-PAGE gels, and immunoblotted as described in the figure legends using Abs against the following: phospho-Tyr1056 STAT5 and phospho-Tyr1047 STAT3 (Cell Signaling Technology, Danvers, MA), p-Thr185,p- Tyr187 ERK1/2 (Invitrogen, Carlsbad, CA), CISH (BD Biosystems, Minneapolis, MN), STAT5 and STAT3 (Santa Cruz Biotechnology, Santa Cruz, CA), and actin Ab-5 (BD Biosciences, San Jose, CA). Membranes were visualized using the Echigen II darkroom (UVP, Upland, CA) equipped with a 12-bit cooled CCD camera. Relative immunoblot band densities were obtained via analysis with National Institutes of Health ImageJ software version 1.38X. Alterations in expression or phosphorylation of protein were normalized to appropriate loading control levels.

Isolation of mRNA and subsequent quantitative PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) from EOSPB, media-treated or activated in vitro with GM-CSF, IL-3, or IL-5, and from EOSPB (n = 3), EOSA (n = 4), and EOSCPB (n = 6) isolated 48 h after an in vivo allergen challenge. The reverse-transcription reaction was performed using the Superscript III system (Invitrogen/Life Technologies, Grand Island, NY). Expression of mRNA was determined by quantitative PCR using SYBR Green Master Mix (SABiosciences, Frederick, MD). Human CISH– (forward 5’-GTCAGCCGCCGATCCGGAAGC-3’ and reverse 5’-CTGCCACCGTGACAGCAGAG-3’), and SOCSI-specific (forward 5’-GTGCGCCTCCCTTGATAGT-3’ and reverse 5’-TCGTTGGGACGACTGTTGCT-3’) primers were designed using Primer Express 3.0 (Applied Biosystems, Carlsbad, CA) and blasted against the human genome to determine specificity using http://www.ncbi.nlm.nih.gov/tools/primer-blast. The reference gene, β-glucuronidase (GUSB) (forward 5’-CAGGGATCTCGGACACAGAG-3’ and reverse 5’-CTCGCA-CAGCATGGGTAAG-3’), was used to normalize the standards. Curve parameters were performed, and efficiencies were determined for each set of primers. Efficiencies ranged between 94 and 96%. Data are expressed as fold change relative to the control blood cycle through the ΔΔCT method as described previously (37). The values presented in Fig. 4A and 4B are fold change (2–ΔΔCT) compared with the level in untreated EOSPB that was fixed at 1 (n = 5). The values presented in Fig. 5A and 5B are fold change (2–ΔΔCT) compared with the level in EOSPB that average level was fixed at 1 (± 0.168 for CHS and ± 0.287 for SOCSI; n = 3).

Statistical analyses

Phosphorylated signaling markers and mRNA expression measures were compared among cell sources (EOSA, EOSCPB, and EOSPB) and stimulants (media, IL-3, IL-5, and GM-CSF) using either one-way ANOVA or linear mixed-effect ANOVA models with fixed-effect terms for cell source and stimulant and random-effect terms for donor and experiment to account for within-donor and within-experiment correlation. Measures were log-transformed as appropriate to achieve normal distributions and homogeneity of variance. A two-sided p value <0.05 was regarded as statistically significant.

Results

IL-5 family cytokine–induced ERK1/2 phosphorylation is unaltered between EOSA and EOSCPB, whereas STAT5 and STAT3 phosphorylation is reduced in EOSA

Compared to EOSCPB, EOSA exhibit phenotypic differences, including enhanced inflammatory capacity and prolonged survival uncoupled from IL-5 family cytokines (20, 30), while remaining
responsive to IL-5 family cytokines regarding certain physiological end points, such as degranulation (27). Accordingly, we tested the idea that downregulation in specific intracellular signaling pathways is associated with these phenotypic differences observed between EOSA and EOSCPB. In this regard, IL-5 family cytokines have been shown by us and others (4, 15–18, 21) to act via signaling cascades JAK/STAT (critical for IL-5 family–induced survival) and MAPK (crucial in IL-5 family–induced degranulation), thus we chose to examine these signaling cascades (at respective peak-activation time points) as potential markers of differences in EOSA and EOSCPB/EOSCPB activation. As shown in Fig. 1, we found IL-5, IL-3, and GM-CSF elevates p-ERK1/2 in EOSA compared with media control (p < 0.05, p < 0.05, and p < 0.001 respectively) (Fig. 1A, 1B) to statistically equivalent levels relative to the respective cytokine stimulation of p-ERK1/2 in EOSCPB (Fig. 1B). Control experiments using EOSCPB from unchallenged donors purified on the same day showed that the purification process did not alter baseline or IL-5 family cytokine–stimulated p-ERK1/2 levels (Supplemental Fig. 1A). These data are consistent with the previously observed reduction of IL-5Rα and elevation of IL-3Rα and GM-CSFRα on EOSA cell surface relative to circulating EOS (27, 28). Collectively, EOSA retain the capacity to activate ERK1/2 after stimulation ex vivo with IL-5 family cytokines.

In contrast to the IL-5 family activation of p-ERK1/2 in EOSA, little to no p-STAT5 was detected in EOSA despite the fact that IL-5, IL-3, and GM-CSF elevated p-STAT5 in EOSCPB, whereas immunoblotting for total STAT indicates no change in total STAT expression (Fig. 2A). IL-5–, IL-3–, and GM-CSF–stimulated p-STAT5 is significantly decreased in EOSA compared with the EOSCPB from that same challenged donor (p < 0.0001, p = 0.02, and p = 0.02, respectively) (Fig. 2B). The purification process did not alter basal or IL-5 family cytokine–stimulated p-STAT5 levels in EOSCPB from unchallenged donors (Supplemental Fig. 1B).

As shown in Fig. 3, the p-STAT3 observations are similar to p-STAT5 following stimulation with IL-5, IL-3, and GM-CSF. These treatments failed to induce p-STAT3 in EOSA (p = 0.001, p = 0.009, and p = 0.001, respectively) relative to EOSCPB but did not alter total STAT3 levels (Fig. 3A, 3B). Again, the purification process did not alter basal or IL-5 family cytokine–stimulated p-STAT3 levels in EOSPB from unchallenged donors (Supplemental Fig. 1C). Combined with observations that IL-3Rα and GM-CSFRα are present and even elevated on EOSA surfaces relative to EOSCPB (27, 28), these data suggest there is potentially a JAK/STAT-specific inhibition of IL-5 family cytokine signaling in EOSA.

**FIGURE 1.** IL-5 family cytokine–induced ERK1/2 activation is unaltered in EOSA. (A) Representative blot of EOSA (lanes 1–4) and EOSCPB (lanes 5–8) stimulated for 7 min with media ± 1 nM GM-CSF, 1 nM IL-3, or 1 nM IL-5. (B) Pooled data, n = 7 unless indicated on bar, corrected with loading control and log-transformed. Error bars indicate SEM. p values from one-way ANOVA.

**FIGURE 2.** IL-5 family cytokine–induced STAT5 activation is attenuated in EOSA. (A) Representative blot of EOSA (lanes 1–4) and EOSCPB (lanes 5–8) stimulated for 30 min with media ± 1 nM GM-CSF, 1 nM IL-3, or 1 nM IL-5. (B) Pooled data, n = 6 unless indicated, corrected with loading control and log-transformed. Error bars indicate SEM. p values from mixed-effect ANOVA.
each IL-5 family cytokine upregulates CISH protein expression at statistically significant levels \((n = 9; \text{IL-5, } p < 0.05; \text{IL-3, } p < 0.001; \text{and GM-CSF, } p < 0.05)\) (Fig. 4C, 4D).

**Fig. 3.** IL-5 family cytokine–induced STAT3 activation is attenuated in EOSA. (A) Representative blot of EOSA \((\text{lanes 1–4})\) and EOSCPB \((\text{lanes 5–8})\) stimulated for 15 min with media ± 1 nM GM-CSF, 1 nM IL-3, or 1 nM IL-5. (B) Pooled data, \(n = 5\), corrected with loading control. Error bars indicate SEM. \(p\) values from mixed-effect ANOVA.

**Fig. 4.** IL-5 family cytokines induce CISH and SOCS1 gene mRNA and protein in EOSPB. EOSPB were purified and treated for 2 h with 1 nM GM-CSF, IL-3, or IL-5, then mRNA was extracted and purified, or lysates were prepared for immunoblots. Real-time PCR was used to quantify CISH \((A)\) and SOCS1 \((B)\) mRNA levels \((\text{relative to housekeeping gene GUSB})\) in five sample sets. Whiskers indicate range, box indicates the inner quartiles, and central bar indicates the mean; \(p\) values from mixed-effect ANOVA. (C) Lysates were immunoblotted for CISH with pooled data, \(n = 9\) and normalized to actin. (D) Data displayed fold change from IL-5 treated. Error bars indicate SEM. \(p\) values from one-way ANOVA.

**EOS isolated after SBP-Ag express more CISH/SOCS1 compared with unchallenged EOS**

Given our observations that IL-5 family members induce SOCS family gene expression and that EOSA are refractory to IL-5 family cytokine–mediated STAT3/5 phosphorylation, we examined basal mRNA expression of CISH and SOCS1 and protein levels of CISH from untreated EOSA compared with untreated EOSCPB and EOSPB. At baseline, EOSA exhibit statistically elevated CISH and SOCS1 mRNA compared with EOSCPB isolated from the same individual \((p = 0.005\) and \(p = 0.03\), respectively) (Fig. 5A, 5B). Interestingly, mRNA transcripts from EOSCPB are also statistically elevated compared with expression levels from EOSPB at baseline \((\text{CISH, } p = 0.004; \text{SOCS1, } p = 0.001)\) (Fig. 5A, 5B). This could suggest that the modest systemic elevation of these cytokines in acute inflammation causes a low-level activation of EOS. Immunoblotting for CISH protein confirmed the mRNA expression results, showing elevated CISH protein in EOSA compared with undetectable-to-low protein levels in both EOSCPB and EOSPB \((n = 3; p < 0.05, p < 0.001\), respectively) (Fig. 5C).

**EOSPB exposed to GM-CSF recapitulate characteristic insensitivity of EOSA to IL-5 family cytokine–induced STAT5 phosphorylation**

In vitro priming, achieved by exposing purified EOSPB to low concentrations of IL-5 family cytokines, recapitulates many behaviors/properties observed in EOSA, which are considered to be primed in vivo \((1, 5, 8, 30, 38)\). Among other changes to EOS biology, as mentioned previously, priming EOSPB imparts decreased responsiveness to IL-5 family cytokines \((24)\) through a relatively poorly understood mechanism that warrants additional research. To further our understanding of this decreased IL-5 family sensitivity \((\text{and therefore the process by which EOSPB progress to an EOSA state})\), EOSPB were pretreated for 24 h with media, IL-5, GM-CSF, or IL-3, then restimulated for 30 min with media, IL-5, GM-CSF, or IL-3, and p-STAT5 was assessed. As hypothesized, 24-h media pretreatment permits each IL-5 family cytokine to induce significant levels of p-STAT5 after 30-min treatment relative to media-alone restimulation \((p < 0.0001\) for each cytokine; Fig. 6). Interestingly, 24 h GM-CSF pretreatment blocks any significant elevation of p-STAT5 relative to media-stimulated, regardless of which IL-5 family cytokine was used to restimulate. Furthermore, 24-h pretreatment with IL-5, IL-3, or...
GM-CSF inhibits IL-5 restimulation from significantly elevating p-STAT5 significantly above the respective media-alone stimulation in agreement with previously discussed decreases in IL-5Rα surface expression. In either IL-5 or IL-3 pretreatment conditions, both IL-3 and GM-CSF restimulation significantly elevates p-STAT5 (IL-5 priming: \( p < 0.001 \) both GM-CSF and IL-3; IL-3 priming: \( p < 0.001 \) both GM-CSF and IL-3) (Fig. 6).

Discussion

IL-5 family cytokines modulate many EOS functions, including enhancing the inflammatory capacity and potentiating survival of the cell. To better manage certain inflammatory diseases, including allergic asthma, it is critical to better understand the phenotypic differences between EOSA and EOSPB. Elucidating mechanisms underlying the phenotypic differences would potentially provide insight into the efficacy of a range of therapeutics currently used as well as provide molecular targets for manipulation to alleviate EOS-associated symptoms present in allergic disorders like asthma. The goal of the current study was to determine the effect of IL-5 family cytokines on modulation of intracellular signaling in both EOSA and EOSCPB/EOSPB in terms of p-ERK1/2, p-STAT5, and p-STAT3 and expression of SOCS family members CISH and SOCS1.

Accordingly, we find that EOSA are refractory to IL-5 family-induced p-STAT3 and p-STAT5 while inducing p-ERK1/2 at levels with no significant difference to that induced by IL-5 family cytokines in EOSCPB. Furthermore, expression levels of SOCS family members CISH and SOCS1 and CISH protein are elevated by IL-5 family cytokine stimulation. Interestingly, we observed an elevation of both CISH and SOCS1 mRNA and CISH protein in EOSA relative to both EOSCPB/EOSPB, with EOSPB expressing significantly more CISH and SOCS1 mRNA compared with EOSPB. Also, 24-h IL-5 family cytokine pretreatment of EOSPB attenuated the ability of IL-5 restimulation to induce p-STAT5. Furthermore, GM-CSF pretreatment additionally inhibited p-STAT5 induction by both IL-3 and GM-CSF. It is intriguing that only GM-CSF pretreatment attenuated IL-5 family restimulation of STAT5, regardless of the fact that all three IL-5 family cytokines induced CIS1 and SOCS1 genes. These collective data, gathered entirely from human donor samples from blood draws and/or BAL post-SBP-Ag, collectively point to a complex and specific system/mechanism of regulation that may influence EOS physiology and therefore inflammatory capacity and enhanced survival.

Given the differences our laboratory and others (23, 27, 28) have observed between EOSA and EOSCPB/EOSPB, alterations in receptor subunit expression for the IL-5, GM-CSF, and IL-3Rα-chains and the common β-chain following SBP-Ag cannot completely explain the general refractory nature of EOSA toward IL-5 family cytokines. For example, although the expression levels of the common β-chain are decreased on EOSA, GM-CSF still induces release of eosinophil-derived neurotoxin, whereas IL-5 cannot (27). If attenuation of downstream signaling was largely based on receptor common β-chain expression levels, we would hypothesize that phosphorylation of ERK1/2, STAT3, and STAT5 would...
all be decreased in response to all IL-5 family cytokines in EOS\(_A\). If the downstream signaling attenuation was due to alterations in the ligand-specific \(\alpha\)-chains, it would follow that only IL-5–induced signaling only would be attenuated, with both IL-3– and GM-CSF–induced signaling potentially increasing due to the elevated surface expression. However, neither of these models fit with the observations. Another plausible explanation would be that the decreased signaling may be a function of a threshold response needed for activation of one pathway versus another or potentially represent a specific signaling pathway regulatory event. We know that IL-3 and GM-CSF have functional receptors on EOS\(_A\) (27), which led us to test the hypothesis of altered signaling instead of pursuing the idea of an activation threshold. Also, the concentration of IL-5, IL-3, and GM-CSF used in the STAT3/5 and ERK1/2 phosphorylation experiments is in excess of the \(K_D\) values of each receptor/ligand pairing (13, 22, 39–41). Our data indicate that phosphorylation of STAT5 and STAT3 is selectively attenuated in EOS\(_A\) relative to ERK1/2 phosphorylation. These observations are in line with previous findings that indicate even after long-term exposure to IL-5, additional stimulation with IL-5 can phosphorylate MEK (24), indicating MEK/ERK signaling remains intact. We therefore hypothesized there is/are selective mechanism(s) that differentially affect(s) responses to these cytokines in the signaling pathways under consideration.

Our laboratory and others (1, 5, 20, 30, 38, 42, 43) have found that in vitro stimulation of EOS\(_{PB}\) with IL-5 family cytokines recapitulates certain signaling and physiological behaviors of EOS\(_A\) regarding the enhanced responsiveness to proinflammatory chemokines, which EOS\(_{PB}\) are normally minimally responsive to. In this study, our data indicate that in vitro IL-5 family pretreatment of EOS\(_{PB}\) is able to also recapitulate to different degrees the decreased responsiveness to IL-5–family–induced p-STAT5 observed in EOS\(_A\), proving a valuable model for EOS\(_A\), which are far less readily available than EOS\(_{PB}\). Although the data in this study regarding the IL-5 family pretreatment indeed only represent a small subset of EOS\(_A\) physiology, given the extremely limited literature surrounding the refractory nature of EOS\(_A\) relative to IL-5 family stimulation (24, 27), this in vitro EOS\(_{PB}\) system provides an exciting opportunity to research the EOS behavior upon IL-5 family exposure.

Reports from other immune cell systems have revealed that the SOCS family genes are upregulated following STAT-induced transcriptional activation and selectively downregulate signaling through JAK/STAT pathways (31, 34). Our data indicate that relative to EOS\(_{CPP}\)/EOS\(_{PB}\), EOS\(_A\) express elevated levels of CISH and SOCS1 mRNA and CISH protein. These data are in line with previous studies that indicate IL-5 and GM-CSF can induce CISH mRNA in human EOS\(_{PB}\) (18) as well as systems in which SOCS family members downregulate JAK/STAT signaling (31, 33, 34). For example, CISH has been found to block STAT5b activity by binding to activating/docking sites on signaling receptors (44, 45), whereas other members of the SOCS family proteins (i.e., SOCS1, SOCS2, and SOCS3) additionally block STAT5b activity (44). Interestingly, SOCS1 is capable of binding directly to the activating loop of JAK2 (46), thereby inhibiting several JAK/STAT-regulated signaling systems (reviewed in Ref. 47). These previous data, combined with our findings, therefore present a possible mechanism for the JAK/STAT selective attenuation of signaling in response to IL-5 family cytokines in human EOS. Furthermore, based on our findings that all three IL-5 family cytokines induce CISH and SOCS1 mRNA products as well as CISH protein, we theorize that the systemic elevation of IL-5 family cytokines in disorders like allergic asthma may alter the responsiveness and inflammatory capacity of EOS\(_A\) in distinct ways, which is further supported by the results that EOS\(_{CPP}\) expresses significantly more CISH and SOCS1 mRNA than the non-challenged EOS\(_{PB}\). Additional support for the idea that expression of SOCS family members modulates EOS activation comes from a murine model of allergic airway inflammation. Deletion of Soesl in an Ifng knockout mouse showed an overall intensification of asthma-like symptoms, including elevated EOS recruitment to the lungs, enhanced IgE serum levels, and increased cytokine and gene transcription product compared with the Ifng knockout alone (35), suggesting that in humans, SOCS1 could play a crucial role in normal regulation of airway inflammation and EOS\(_{PB}\) recruitment.

In terms of MEK/ERK signaling, IL-5 family cytokines are known to enhance EOS responsiveness to secondary stimuli that signal via G-protein–coupled receptors (GPCRs) (4, 38, 43, 48). Because GPCRs often exhibit extensive cross-talk with MEK/ERK signaling pathways (49), including chemokine-induced GPCR signaling in EOS\(_A\) (30), it would be crucial that many MEK/ERK signaling molecules remain signal-ready, which our observations support.

Given these data, future studies are necessary to further characterize the functional alterations that are observed between EOS\(_A\) and EOS\(_{PB}\), including investigation of physiological endpoints and expression of any additional SOCS family members. One promising SOCS family member is SOCS3, which López and colleagues (32) found is inducible at the mRNA level in EOS\(_{PB}\) and elevated in EOS from asthmatics and patients with eosinophilic bronchitis when compared with EOS from healthy control donors. Furthermore, SOCS3 has been observed to perform similar functions to both CISH and SOCS1, blocking JAK2 tyrosine kinase activity, and has been linked to suppression of STAT3 activity (reviewed in Ref. 47). The overlapping nature of these SOCS family members and these SOCS3 data further support the idea that there is a very finely tuned regulation system in these cells in which the smallest variations may lead to disease states and that further research is warranted.

In summary, this study supports the idea that phenotypic differences observed between EOS\(_{PB}\) and EOS\(_A\) may be explained as functions of alterations in the signaling pathways that are modulated by IL-5 family cytokines. These cytokines serve to both recruit and activate EOS\(_{PB}\) while providing negative-feedback mechanisms to regulate the inflammatory response of recruited EOS. This negative-feedback mechanism perhaps protects against inappropriate activation once the EOS\(_{PB}\) have migrated to the site of allergic inflammation and are thus inundated with proinflammatory cytokines, including those of the IL-5 family. Manipulation of these regulatory pathways may provide new and novel therapeutics to alleviate symptoms in eosinophilic-driven disorders such as allergic asthma.

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Disclosures

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