INTERLEUKIN-2-MEDIATED STAT-5 EXPRESSION LEVELS IN $CD8^+CD25^+$ REGULATORY T CELLS: THE RELEVANCE FOR ASTHMA

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Preliminary studies demonstrated the potential role of $CD8^+CD25^+$ T regulatory cells (Tregs) in asthma. However, published data with regard to the relevance of signaling pathways that govern Tregs homeostasis are limited and conflicting. The first aim of this study was to characterize the phosphorylation of STAT-5 in $CD8^+CD25^+$ Tregs. The second aim was to investigate the ability of $CD8^+CD25^+$ Tregs from patients and controls to respond to interleukin (IL)-2 treatment *in vitro*. Twenty-five healthy subjects (NC) and 50 patients with either severe (SA) or mild-moderate (MA) asthma were enrolled in the study. STAT-5 phosphorylation was detected in purified $CD8^+CD25^+$ Tregs from healthy and asthma subjects, indicating that STAT-5 has a role in their pathobiology. At baseline, asthma cases had either significantly lower [(SA=4.5±5% *vs* NC=26±25%, $P < 0.001$) and (MA=10±7.5% *vs* NC=26±25%, $P < 0.001$)] or higher [(SA=54±58.5% *vs* 26±25%, $P < 0.01$) and (MA=71±74.5% vs 26±25%, $P < 0.01$) proportion of Tregs expressing pSTAT-5 than controls. In contrast to healthy subjects, $CD8^+CD25^+$ Tregs from asthma subjects had either increased ($pSTAT\text{-}5^{high}$) or decreased ($pSTAT\text{-}5^{low}$) phosphorylated STAT-5 levels within individual cells. These data suggest that the alteration in STAT5 phosphorylation level might be associated with asthma and is a potential molecular basis of skewed $CD8^+CD25^+$ Treg differentiation. IL-2 treatment of cells from severe asthma subjects increased the proportion of cells expressing high level of pSTAT-5 while it decreased the proportion of those expressing low level of pSTAT-5. Strikingly, IL-2 increased the proportions of both subsets from mild-moderate asthma subjects. These findings demonstrate that altered IL-2-mediated STAT-5 phosphorylation within individual circulatory $CD8^+CD25^+$ Tregs may be associated with asthma and disease severity.

Asthma is a multifactorial syndrome characterized by the activation and migration of various immune cells to the inflamed bronchial mucosa. Despite increasing awareness of asthma, its pathogenesis has not been fully established. It is known that peripheral blood $CD8^+CD25^+$ T regulatory cells (Tregs) may play a pathogenic role in asthma. Asthma death was associated with increased percentage of peripheral $CD8^+CD25^+$ T cells in the airways (1). The precise mechanisms underlying the peripheral homeostasis of this subset is unclear, nevertheless, some evidence suggests that homeostasis and function of Tregs are maintained by signaling pathways that are activated through several cytokines and related receptors. Increasing evidence suggests that a family of tyrosine kinases known as Janus kinases (JAKs) collaborates with related transcription factors, known as STATs (signal transducers and activators of transcription)

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to maintain the appropriate number of Treg cells. Likewise, other published data have reported that Interleukin (IL)-2 signaling cascade helps to maintain the appropriate quality and quantity of human Tregs (2) at the periphery. Furthermore, it has been previously reported that this cytokine signaling molecule induces the activation and phosphorylation of a great array of protein tyrosine kinases, namely JAK-1 and JAK-3. This preliminary event results in the activation of the receptor-associated JAK subunit, and serves as a precursor for the phosphorylation of the latent cytosolic STAT subunits. Phosphorylated STATs are thereafter translocated to the nucleus where they bind specific DNA sequences to regulate the transcription of target genes, which control cellular survival. The STAT pathway regulates the molecular events underlying a variety of biological processes including T-cell activation and differentiation (3), therefore, it is probable that STAT protein family inhibits or enhances autoimmune and allergic responses (4). Recent studies have demonstrated that the pathogenic implication of cytokines in immunological disorders relies on the JAK-STAT signaling pathway (5). Dysregulation of this signaling pathway partially caused by alteration of either γc or Jak3 subunits was linked to immunodeficiency (6). Signal transducers and activators of transcription 5 (STAT-5) have been reported to play a critical role in the proliferation of various cells including eosinophils (7) and mast cells (8). Furthermore, a cytokine signaling through STAT-5 has been shown to regulate the peripheral homeostasis of $CD4^+CD25^+$ Treg cells in mice (9). Some previous studies have shown that IL-2 enhances the survival of $CD4^+CD25^+$ Tregs (10). Others highlight some similarities between human $CD8^+CD25^+$ thymocytes and $CD4^+CD25^+$ Tregs (11). The significance of IL-2-mediated STAT phosphorylation for T cell survival and proliferation has prompted the investigation of this pathway in asthma. Studies in this context are extremely scarce or conflicting.

The first aim of the study was to characterize the phosphorylation of STAT-5 in $CD8^+CD25^+$ Tregs The second aim was to investigate the ability of $CD8^+CD25^+$ Tregs from patients and controls to respond to IL-2 treatment *in vitro*.

MATERIALS AND METHODS

Study subjects

Fifty patients with allergic asthma (AA) as defined by the Global Initiative for Asthma (GINA) guidelines and 25 gender- and age-matched controls (NC) were enrolled in this study. The diagnosis of asthma was confirmed by pulmonologists based on evidence of variable airway obstructions according to The Global Initiative for Asthma (GINA 2008) guidelines. Patients were divided into the severe asthma (SA) (n = 25) and mild-moderate asthma (MA) (n = 25) groups (Table I). They were treated based on European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA) guidelines. Atopy was evaluated based on skin prick tests or evidence of increase specific serum IgE. Patients with severe asthma required treatment with either high dose of inhaled steroids, long acting ß-agonists for at least 1 year. Patients with mild-moderate asthma received daily inhaled steroids in combination with symptomatic therapy. Subjects without evidence of allergic or infectious diseases served as controls.

All participants underwent a standard check-up including a questionnaire related to their general health status. In addition, all subjects with clinical symptoms of asthma filled in an Asthma Control Questionnaire. Furthermore, all participants provided written informed consent before enrolment in the study. The study was approved by the ethics committee, in accordance with the Declaration of Helsinki and good clinical practice guidelines (RNN/17/09/KE).

Assessment of asthma and its severity

Pulmonary function tests were performed using a calibrated spirometer as recommended by the American Thoracic Society/European Respiratory Society guidelines. The best of three reproducible loops was recorded for the study. The forced expiratory volume in first second (FEV_1), forced vital capacity (FVC) and the ratio of FEV_1/FVC were recorded for the differential diagnosis of asthma. Patients also underwent skin-prick tests to common allergens, and methacholine tests, and specific serum immunoglobulin E (IgE) was measured. Atopy was defined as at least one positive skin-prick test against the most common allergens and its degree was assessed based on total serum IgE levels as previously described. Allergen polysensitization was determined based on sensitization to more than 2 allergen-specific

IgEs, regardless of the allergen class. Patients with initially impaired lung function values were excluded from the challenge test. Bronchodilator and steroid drugs were discontinued 24 h before the challenge test.

Isolation of peripheral blood mononuclear cells

Peripheral blood samples were collected in EDTA vacutainer tubes, between 7–9 a.m. after 12 h of fasting and processed immediately. Briefly, peripheral blood mononuclear cells (PBMCs) were freshly isolated from peripheral buffy coats by standard Ficoll-Hypaque high-density gradient centrifugation (2500 g, 30 min).

Cell purification

After enrichment of PBMCs, $CD8^+$ T cells were isolated using biotinylated human CD8 T lymphocyte enrichment cocktail and BD™ IMag magnetic field according to the manufacturer's instructions. Cells were incubated with saturating amounts of antibodies at a ratio of 5 µl per 1 x 10^6 cells, for 15 min at 4°C. Thereafter, streptavidin particles were added at a concentration of 5 µl of particles for every 1 x 10^6 total cells and incubated for 30 min at 4°C. To maintain high purity using the Miltenyi MACS system, cells were passed through two LS columns placed on BD™ IMag magnetic field. The purity of the enriched $CD8^+$ T cells was over 90% and 93% for all samples. $CD8^+$ T cell fraction was subsequently incubated for 15 min with CD25-coated magnetic beads conjugated to anti-CD25 mAb (Miltenyi Biotec, mouse IgG1), followed by incubation with anti-human $CD25^{PE}$ mAb (Miltenyi Biotec, clone 4E3, Mouse IgG2b, k). Cells were passed through two LD columns placed on BD™ IMag magnetic field. The purity of $CD8^+CD25^+$ T cell was > 97% as assessed by flow cytometry.

In vitro *cell activation*

Next, the ability was evaluated of $CD8^+CD25^+$ Tregs from healthy and asthma subjects to respond directly to IL-2 stimulation *in vitro* in the absence of any other co-stimuli such as T Cell Receptor (TCR) activation. Specifically, the activation of the downstream signaling molecule, STAT-5, and the expression of the pSTAT-5 on the surface of $CD8^+CD25^+$ Treg cells before and after short term *in-vitro* stimulation were quantified. Firstly, the patients and healthy subjects were analyzed for the expression pattern of phosphorylated STAT-5 (pSTAT-5). For quantitative analysis, freshly isolated $CD8^+CD25^+$ T cells (1x10^6 cells/100µl) were cultured in complete RPMI medium containing 10% fetal bovine serum, 20 mM Hepes buffer, non-essential amino acids, sodium pyruvate, 2 mM L-glutamine, 100 µg/ml streptomycin and 100 units/ml penicillin (Sigma-Aldrich) supplemented with 10ng/ml recombinant human IL-2 (R & D SYSTEMS) for 15 min. The cells were incubated for 72 h in 24-well flat bottom sterile plates (Thermo Fisher Scientific Inc) at 37°C in a 5% CO_2-humidified atmosphere.

Table I. *Clinical and demographic characteristics of the study population.*

Parameters	SA	NC	MA
Number	25	25	25
Sex (Female/Male)	13/12	12/13	12/13
Age (yr±SD)	48±14	48.5±14.5	42±13
Disease duration (yr±SD)	16±8.7	NC	10±6.3
Atopy	Yes	No	Yes
Predicted FEV1(%±SD)	67±15.98	96.6±4	87.71±16.2

SA: Severe asthma; MA: Mild-to-moderate asthma; NC: Healthy controls; FEV1: forced expired volume in 1 s; NA: Not applicable

Flow cytometry analysis

Activated or non-activated $CD8^+CD25^+$ T cells were fixed with Buffer Cytofix (BD Bioscience). To increase the cell membrane permeability to anti-STAT5, 500 µl BD Perm Buffer III Phosflow (BD Bioscience) was added to the suspension which was vortexed and incubated for 30 min on ice. ($1x10^6$/100µl) cells were incubated for 30 min at 4°C with anti-human $CD8^{AmCyan}$ mAb (BD Biosciences, clone SK1, Mouse IgG1, k), and anti- human $CD25^{FITC}$ mAb (BD Biosciences, clone M-A251, Mouse IgG1, k). For intracellular staining, cells were incubated with anti-human anti-STAT5-conjugated PE (BD Biosciences, clone 236A/E7, Mouse IgG1, k) for 45 min at 4°C. Labeling of phosphorylated STAT-5 was carried out in accordance with the BD™ Phosflow Protocol. Cells were washed twice and analyzed using a BD Biosciences FACS Canto II flow cytometer and FACS DIVA software.

Statistical analyses

The Mann-Whitney U test and Kruskal-Wallis analysis were used to assess statistical differences between the study and control groups. A Spearman correlation test was performed to analyze the association between the forced expiratory volume in one second FEV_1 (% predicted) and the percentage of $pSTAT\text{-}5^+$ T cells. Data were expressed as median and interquartile range (IQR) which is the difference between the third and first quartiles. A p-value less than 0.05 was considered significant. Calculations were performed by the statistical software package Statistica (Stat Soft, Polska).

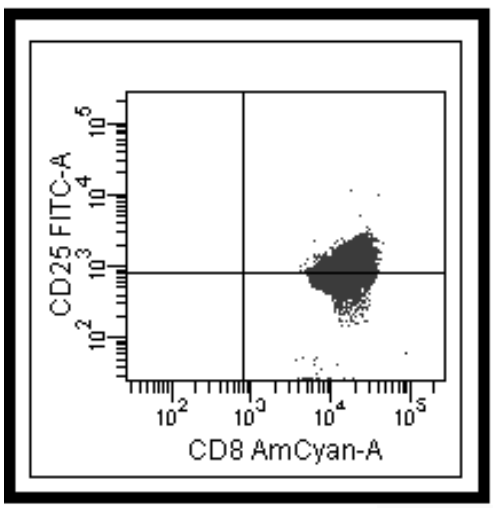

Fig. 1. *Representative flow cytometric analysis of $CD8^+CD25^+$ Tregs from fresh peripheral blood of asthma and healthy subjects. $CD8^+CD25^+$ T cells were gated from $CD8^+$ lymphocytes. An isotype control FMOs (fluorescence minus one)was used to set the positive gates. The same gates were used for data analysis of each asthma subject and healthy control. Variable pSTAT5 expression levels were detected in $CD8^+CD25^+$ Tregs from all subjects.*

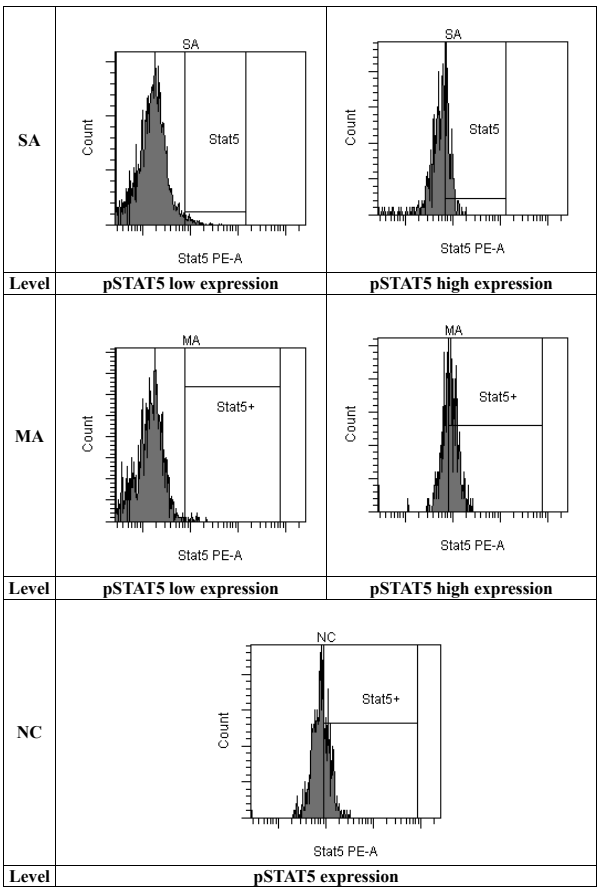

Fig. 2. *Representative histograms illustrating pSTAT-5 expression. Cells were isolated from the peripheral blood and cultured in RPMI with IL-2 for 72 hours. Cells were fixed, permeabilized, and flow cytometry performed using antibodies against CD8, CD25, and phosphorylated STAT-5 (pSTAT-5).*

RESULTS

The clinical and demographic data of the study population are presented in Table I.

Circulatory $CD8^+CD25^+$ Tregs express pSTAT-5

The existence of data suggesting the implication of STAT5 in $CD25^+CD4^+$ Treg homeostasis prompted

our attempt to characterize the phosphorylation of STAT-5 in peripheral blood $CD8^+CD25^+$ Tregs in an *ex-vivo* model and flow cytometry (Fig. 1). Variable pSTAT5 expression levels were detected in $CD8^+CD25^+$ Tregs from all subjects (Fig. 2). Asthmas had either significantly lower [(SA=4.5±5% *vs* 26±25%, $P < 0.001$) and (MA=10±7.5% *vs* 26±25%, $P < 0.001$)] (Fig. 3) or higher [(SA=54±58.5% *vs* 26±25%, $P < 0.01$) and (MA=71±74.5% *vs* 26±25%, $P < 0.01$) (Fig.3) proportion of Tregs expressing pSTAT-5 than healthy controls.

Stratification of each asthma phenotype into high and low STAT-5 profiles

Baseline analysis allowed stratification of each asthma group, based on the extent of STAT-5 expression in $CD25^+CD8^+$ Tregs. $CD8^+CD25^+$ Tregs from asthma subjects had either increased ($pSTAT\text{-}5^{high}$) (Fig. 4) or decreased (Fig. 3) ($pSTAT\text{-}5^{low}$) phosphorylated STAT-5 levels within individual cells. Patients with high-STAT-5 profile had higher levels of pSTAT-5 [(SA=54±58.5) and (MA=71±74.5)] compared with the control group (NC= 26±25) (Fig. 4) whereas those with low-pSTAT-5 profile had lower pSTAT-5 expression level [(SA=4.5±5) and (MA=10±7.5)] (Fig. 3) than the control group (NC= 26±25).

Strikingly, the proportion of cells expressing low level of pSTAT-5 was lower in the severe asthma group than in the mild-to-moderate group (SA=4.5±5% *vs* MA=10±7.5%, $P< 0.001$) (Fig. 3). Likewise, the proportion of cells expressing high level of pSTAT-5 expression was lower in the severe asthma group than in those from mild-to-moderate group (SA=54±58.5% *vs* MA=71±74.5%, $P< 0.001$) (Fig. 4).

IL-2 had pleiotropic effects on $CD8^+CD25^+$ Tregs expressing low or high pSTAT-5

IL-2 treatment of cells from severe asthma subjects increased the proportion of cells expressing high level of pSTAT-5 while it decreased the proportion of those expressing low level of pSTAT-5. Strikingly, IL-2 increased the proportions of both subsets from mild-to-moderate asthma subjects. After stimulation, statistically significant differences were noted among the asthma groups ($p<0.001$). Likewise, we observed statistically significant differences between the control and mild-to-moderate or severe asthma groups. In each case, p was lower than 0.001 (Figs. 3 and 4). IL-2

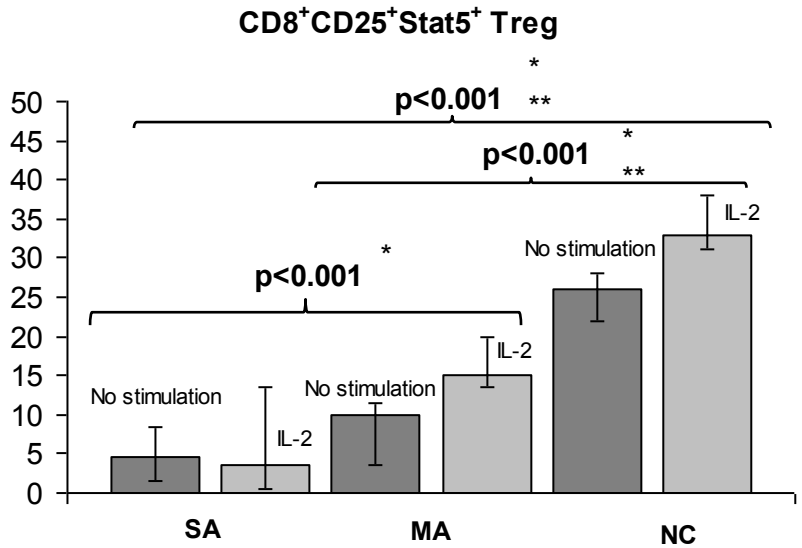

Fig. 3. *$CD8^+CD25^+$ T cells from asthma patients have decreased pSTAT-5 levels ($pSTAT\text{-}5^{high}$) as compared to healthy subjects. Cells were isolated from the peripheral blood of asthma subjects and controls. They were cultured in RPMI media supplied with IL-2 for 72 hours and analyzed as described in methods. Data are expressed as median percentages±interquartiles and are representative of three independent experiments. Significance was determined by * significant difference after stimulation and ** significant difference without stimulation; NS: no significance.*

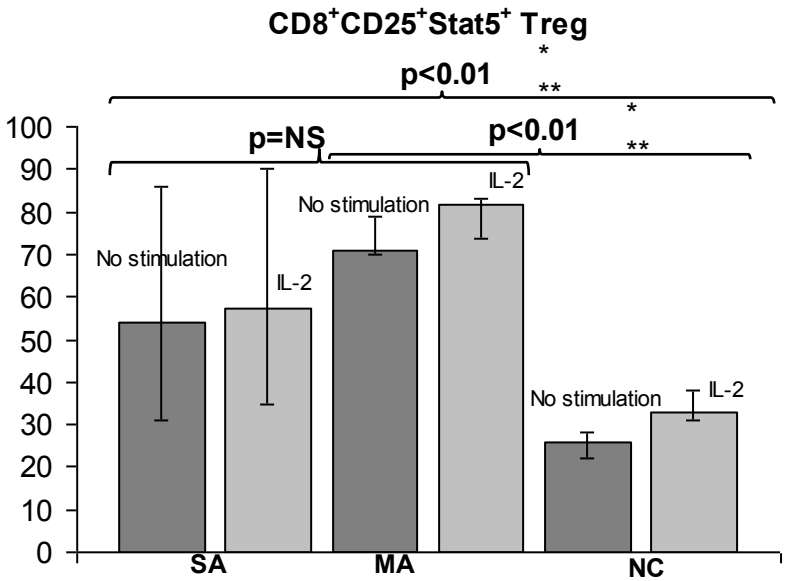

Fig. 4. *$CD8^+CD25^+$ T cells from asthma patients have increased pSTAT-5 levels ($pSTAT\text{-}5^{high}$) as compared to healthy subjects. Cells were isolated from the peripheral blood of asthma subjects and controls and were cultured in RPMI media supplied with IL-2 for 72 hours and analyzed as described in methods. Data are expressed as median percentages±interquartiles and are representative of three independent experiments. Significance was determined by * significant difference after stimulation and ** significant difference without stimulation; NS: no significance.*

stimulation increased the proportion of $CD25^+CD8^+$ Tregs expressing low or high pSTAT-5 in the control group (NC= 26±25) *vs* (NC= 33±34.5) (Figs. 3 and 4).

IL-2 increased the proportion of Tregs with high pSTAT-5 expression in the asthma groups

IL-2 increased the frequencies of $CD25^+CD8^+$ Tregs expressing high STAT-5 level in the asthma subgroups [(SA=54±58.5) *vs* (SA=57.5±62.3) and [(MA=71±74.5) *vs* (MA=81.5±78) subgroups (Fig. 3). At baselines and after stimulation, statistically significant differences were observed between controls and mild-to-moderate or severe asthma groups, In each case, *p* was lower than 0.01 (Fig. 4). IL-2 stimulation increased $STAT\text{-}5^+CD25^+CD8^+$ Treg frequencies in the control group (NC= 26±25) *vs* (NC= 33±34.5) (Figs. 3 and 4).

IL-2 decreased the proportion of Tregs with low pSTAT-5 expression in the severe asthma subgroup

IL-2 decreased the frequencies of $CD25^+CD8^+$ Tregs expressing low STAT-5 level in the severe asthma subgroup [(SA=4.5±5) *vs* (SA=3.5±7). In contrast, IL-2 stimulation increased this parameter in mild-to-moderate asthma [(MA=10±7.5) *vs* (MA=15±16.5)] subgroup. At baseline state, there were statistically significant differences in the proportion of $CD25^+CD8^+$ Tregs expressing low STAT-5 level between controls and mild-to-moderate ($p<0.001$) or severe ($p<0.001$) asthma groups.

DISCUSSION

STAT-5 phosphorylation was detected in cells purified from peripheral blood of healthy and asthma subjects. These findings indicate that the development and expansion of human $CD8^+$ $CD25^+$ Tregs is mediated by activation of STAT-5, as previously reported (12). However, they contradict previous studies suggesting that STAT-5 activation is not required for cell development (13). Aberrant STAT-5 phosphorylation has been described in chronic inflammation (14). Our study extends this preliminary data by showing that pSTAT-5 expression significantly differed between the group of healthy subjects, and those with mild-to-moderate or severe asthma. This observation corroborates with a

previous study which described the implication of the STAT-5 signaling in IgE-mediated inflammation (15) and lung diseases (16). We also argued that immune phenotyping by flow cytometry may provide a better insight of intracellular signaling pathways involved in immunological disorders. The baseline analysis of patients and controls revealed two specific patterns of pSTAT-5 expression within individual $CD8^+CD25^+$ Tregs. Some cells expressed high pSTAT-5 levels (pSTAT-5 high) while others expressed low pSTAT-5 levels (pSTAT-5 low). This observation corroborates with previous studies that demonstrated distinct STAT5 thresholds among innate lymphoid subsets (17). Collectively, our results indicate that despite being expressed by all $CD8^+CD25^+$ Tregs, different STAT5 signaling thresholds regulate these cells. Our study supports previous hypothesis of cell-specific requirements of STAT signaling in T cells (18). Our results also corroborate with previous findings suggesting that STAT-5 regulates human $CD8^+$ T-cell function in a dose-dependent manner (19). The differential intracellular pSTAT-5 expression patterns could reflect the difference in the STAT-5 intra-cellular signaling patterns in asthma. Therefore, our observations lend support to other studies suggesting the implication of different molecular pathomechanisms in asthma (20). Furthermore, our results are in agreement with a previous study suggesting that differential STAT-5 phosphorylation by various subsets can help discriminate between healthy and disease states (21). Another main finding from this study was that IL-2 plays a role in STAT-5 activation, as previously suggested (22). Specifically, flow cytometry analysis of the IL-2-mediated STAT-5 signaling demonstrated that circulatory $CD8^+CD25^+$ Treg cells from asthma subjects could phosphorylate STAT-5 at a higher or lower level than those from controls. The pleitropic effects of IL-2 on STAT-5 phosphorylation in $CD8^+CD25^+$ Tregs lend support to previous studies which highlighted the redundant role of IL-2 on STAT-5-mediated proliferation of lymphocytes (23). More importantly, our study showed that IL-2 increased the proportion of some proliferating $CD8^+CD25^+$ Tregs which express high levels of the transcription factor STAT-5. This finding is consistent with previous reports indicating that $CD8^+$ T-cell proliferation is partially driven by IL-2. Our observation corroborates the results of previous studies suggesting that IL-2 induces STAT5 phosphorylation (24). Our observations are also in agreement with previous studies suggesting that altered cytokine signaling in T cells might play a role in asthma (25). Despite remarkable progress in understanding and managing asthma, the continuous increase in the number of asthma patients with steroid-resistance or insensitivity remains a challenge. Our study may provide better insights into the regulatory mechanisms driving this phenomenon. IL-2 treatment of cells from severe asthma subjects increased the proportion of cells expressing high levels of pSTAT-5 while it decreased the proportion of those expressing low level of pSTAT-5. Moreover, IL-2 increased the proportions of both subsets from mild-to-moderate asthma subjects. This observation is in agreement with previous studies suggesting that IL-2 mediated STAT5 phosphorylation has a role in asthma and may be relevant to steroid insensitivity (26).

Taking all together, we showed that alteration in IL-2 mediated-STAT-5 phosphorylation level may be associated with asthma and is a potential molecular basis of skewed $CD8^+CD25^+$ Treg differentiation. Further studies investigating the clinical relevance of these STAT-5 phosphorylation levels may provide new approaches to identifying asthma phenotypes and endotypes.

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REFERENCES

1. O'Sullivan S, Cormican L, Faul JL, Ichinohe S, Johnston SL, Burke CM, Poulter LW. Activated, cytotoxic CD8 (+) T lymphocytes contribute to the pathology of asthma death. Am J Respir Crit Care Med 2001; 164 (4):560-64.
2. Attridge K, Walker LS. Homeostasis and function of regulatory T cells (Tregs) in vivo: lessons from TCR-transgenic Tregs. Immunol Rev 2014; 259(1):23-39.
3. Stabile H, Scarno G, Fionda C, Gismondi A, Santoni A, Gadina M, Sciumè G. JAK/STAT signaling in regulation of innate lymphoid cells: The gods before the guardians. Immunol Rev 2018; 286 (1):148-59.
4. Pfitzner E, Kliem S, Baus D, Litterst CM. The role of STATs in inflammation and inflammatory diseases.

Curr Pharm Des 2004; 10 (23):2839-50.
5. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. .JAK-stat signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs 2017; 77 (11):1261.
6. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. Immunol Rev 2009; 228(1):273-87.
7. Buitenhuis M, Baltus B, Lammers JW, Coffer PJ, Koenderman L. Signal transducer and activator of transcription 5a (STAT5a) is required for eosinophil differentiation of human cord blood-derived CD34+ cells. Blood 2003; 101(1):134-42.
8. Shelburne CP, McCoy ME, Piekorz R, et al. Stat5: an essential regulator of mast cell biology. Mol Immunol 2002; 38(16-18):1187-91.
9. Antov A, Yang L, Vig M, Baltimore D, Van Parijs L. Essential role for STAT5 signaling in CD25+CD4+ regulatory T cell homeostasis and the maintenance of self-tolerance. J Immunol 2003; 171(7):3435-41.
10. Hall BM. CD4+CD25+ T regulatory cells in transplantation tolerance: 25 years on. Transplantation 2016; 100 (12):2533-47.
11. Cosmi L, Liotta F, Lazzeri E, et al. $CD8^+CD25^+$ thymocytes share phenotypic and functional features with $CD4^+CD25^+$ regulatory thymocytes. Blood 2003; 102(12):4107-14.
12. Passerini L, Allan SE, Battaglia M, et al. STAT5-signaling cytokines regulate the expression of FOXP3 in CD41CD251 regulatory T cells and CD41CD252 effector T cells. Int Immunol 2008; 20(3):421-31.
13. Zamorano J, Wang HY, Wang R, Shi Y, Longmore GD, Keegan AD. Regulation of cell growth by IL-2: role of STAT5 in protection from apoptosis but not in cell cycle progression. J Immunol 1998; 160 (7):3502-12.
14. Franceschini D, Paroli M, Francavilla V, et al. PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. J Clin Invest 2009; 119(3):551-64.
15. Pullen NA, Barnstein BO, Falanga YT, et al. Novel mechanism for Fc {epsilon} RI-mediated signal transducer and activator of transcription 5 (STAT5) tyrosine phosphorylation and the selective influence of STAT5B over mast cell cytokine production. J Biol Chem 2012; 287(3):2045-54.
16. Kanai T, Jenks J, Nadeau KC. The STAT5b pathway defect and autoimmunity. Front Immunol 2012; 3:234.
17. Villarino AV, Sciumè G, Davis FP, et al. Subset- and tissue-defined STAT5 thresholds control homeostasis and function of innate lymphoid cells. J Exp Med 2017; 214(10):2999-3014.
18. Dabitao D, Hedrich CM, Wang F, Vacharathit V, Bream JH. Cell-Specific Requirements for STAT Proteins and Type I IFN Receptor Signaling Discretely Regulate IL-24 and IL-10 Expression in NK Cells and Macrophages. Immunol 2018; 200 (6):2154-64.
19. Nivarthi H, Prchal-Murphy M, Swoboda A, et al. Stat5 gene dosage in T cells modulates CD8+ T-cell homeostasis and attenuates contact hypersensitivity response in mice. Allergy 2015; 70(1):67-79.
20. Lötvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2011; 127(2):355-60.
21. Teofili L, Martini M, Cenci T, et al. Different STAT-3 and STAT-5 phosphorylation discriminates among Ph-negative chronic myeloproliferative diseases and is independent of the V617F JAK-2 mutation. Blood 2007; 110(1):354-59.
22. Beadling C, Ng J, Babbage JW, Cantrell DA. Interleukin-2 activation of STAT5 requires the convergent action of tyrosine kinases and a serine/threonine kinase pathway distinct from the Raf1/ERK2 MAP kinase pathway. EMBO J 1996; 15(8):1902-13.
23. Lockyer HM, Tran E, Nelson BH. STAT5 is essential for Akt/p70S6 kinase activity during IL-2-induced lymphocyte proliferation.J Immunol 2007; 179(8):5301-8.
24. Huss DJ, Mehta DS, Sharma A, et al. *In vivo* maintenance of human regulatory T cells during CD25 blockade. J Immunol 2015; 194(1):84-92.
25. Gernez Y, Tirouvanziam R, Nguyen KD, Herzenberg LA, Krensky AM, Nadeau KC. Altered phosphorylated signal transducer and activator of transcription profile of CD4+CD161+ T cells in asthma: modulation by allergic status and oral corticosteroids. J Allergy Clin Immunol 2007; 120(6):1441-48.
26. Goleva E, Kisich KO, Leung DY. A Role for STAT5 in the pathogenesis of IL-2-induced glucocorticoid resistance. J Immunol 2002; 169(10):5934-40.