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Adhesion molecules and T cell imbalance in severe eosinophilic asthma: Insights from anti-IL-5R treatment

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ABSTRACT
Rationale: Severe eosinophilic asthma (SEA) is driven by type 2 (T2) inflammation, characterised by dysregulated cytokine release and aberrant expression of adhesion molecules involved in immune cell trafficking and activation. Despite the established role of intercellular adhesion molecules (ICAMs) and L-selectin (CD62L) in these processes, their dysregulation in SEA and their potential remodulation in response to biologic therapy remain unclear.

To investigate the expression of adhesion molecules (ICAM-1, ICAM-3, CD62L) on T-cell subsets in SEA, their modulation by IL-25 and IL-33, and the immunological impact of benralizumab therapy.
Methods: Peripheral blood from SEA patients and healthy controls were analysed using flow cytometry and live-cell imaging. A subset of patients was re-evaluated after 6 months of benralizumab therapy to assess changes in T-cell phenotype, adhesion molecule expression, proliferation, and cytotoxicity.
Results: At baseline, SEA patients exhibited a marked Treg/Th2 imbalance, with increased ICAM-1 and CD62L expression on effector T cells and reduced ICAM-3 on Tregs. Alarmin stimulation enhanced ICAM-1 and CD62L expression on Th2 and Treg subsets and increased T-cell proliferation and cytotoxicity. After benralizumab therapy, Treg levels increased, and effector T-cell expression of ICAM-1, ICAM-3, and CD62L was significantly downregulated upon alarmin stimulation, suggesting a restoration of immune homoeostasis.
Conclusion: SEA is associated with alarmin-driven dysregulation of adhesion molecule expression on T cells. Treatment with benralizumab induces an at least partial immunological rebalancing by indirectly modulating T-cell responses to epithelial-derived signals.

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Severe eosinophilic asthma; adhesion molecules; IL-33; IL-25; anti-IL-5

Introduction

The heterogeneity of the inflammatory patterns underlying the pathogenesis of severe asthma has led to the identification of different disease endotypes: in particular, severe eosinophilic asthma (SEA) is characterised by dysregulated type 2 (T2) immune responses with overexpression and production of T2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 and increased numbers of activated eosinophils in the blood and bronchial tissues.[1]

The recruitment and trafficking of cells is modulated by a superfamily of intercellular cell adhesion molecules (ICAMs), some of which have been implicated in coordinating eosinophil and T2 cell adhesion and signalling in SEA.[2] ICAM-1 has shown a significant pro-inflammatory effect, boosting T cell activation, cytotoxicity and proliferation and stimulating release of pro-inflammatory cytokines.[3] Furthermore, ICAM-1 acts as a receptor for rhinoviruses, which are in turn a common cause of virus-triggered exacerbation of asthma.[4] ICAM-3 clusters specifically at the T cell surface where contact with antigen-presenting cells (APC) is initiated. It mediates strong adhesion, or immune synapses, between resting T cells and dendritic cells (DCs). L-selectin (CD62L) not only plays a pivotal role in regulating T cell trafficking in peripheral lymph nodes,[5] but is also implicated in polarisation of CD4 T cells into a T2-high phenotype.[6,7]

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Interestingly, ICAMs and CD62L expression on T2 immune cells are modulated by key cytokines, such as IL-33 and IL-25 all deeply implicated in the pathogenesis of SEA.[8] IL-33 and IL-25 have been regarded as among the main drivers of immune imbalance associated with the development of severe asthma: indeed, most innate and adaptive immune cells in the airways express receptors for IL-33 and IL-25, signalling of which triggers inflammatory pathways downstream, associated with many pathological features of SEA.[9–11] The regulation of ICAM and CD62L expression in eosinophils is also determined by IL-5, whose function as priming and survival factor enhancing eosinophil responsiveness to chemotactic signals is well-known.[12] Conversely, no solid data is still available regarding the modulation of adhesion molecules in T cells.[13] Thus, adhesion molecules are known to play a role in the pathogenesis of severe asthma and T2 inflammation, but little is known about how their expression and activity are modified under treatment, or about their implications in the evaluation of treatment response and prognosis.

Among the biological drugs licenced for severe asthma, benralizumab, a humanised monoclonal antibody against IL-5 receptor α, has proven effective in improving clinical control of SEA.[14] Besides the fast and persistent depletion of circulating and tissue eosinophils, treatment with benralizumab has also been associated with wider immunomodulatory processes, leading to rebalancing of T cell subsets, enhancing Treg activity and reducing T effector percentages.[14,15] The changes in T-cell phenotypes observed following IL-5 Rα blockade are most likely indirect, reflecting altered type 2 cytokine signalling resulting from the depletion of IL-5 Rα–expressing effector cells rather than a direct effect on T cells.

On the basis of this preliminary evidence and the link between the IL-5 pathway and ICAM expression, the aim of the present study was to explore the impact of benralizumab on T cell subset activity in SEA patients, with a view to analysing ICAM expression on the cell surface, cytokine release, modulation in response to alarmins, as well as proliferative and cytotoxic capacity.

Methods

Study design

The research project entitled 'Benralizumab effects on adhesion molecules and T regulatory cell imbalance in patients with severe eosinophilic asthma' (BE-ACTIVE) is a 3-year study designed to characterise cellular, immune and physiological features in patients with SEA and to explore any modifications during treatment with benralizumab. Given the observational nature of this translational study, there was no randomisation or formal blinding process for the investigator, as benralizumab was prescribed as normal clinical practice according to Italian national prescription criteria. Enrolment was at the Respiratory Diseases Unit of Siena University Hospital between January 2021 and December 2023. Diagnosis was made according to international European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines for the diagnosis and management of severe asthma.[16] The detailed list of inclusion and exclusion criteria is in supplementary material section A.

Written informed consent was obtained after institutional review board CEAVSE approval (BE-ACTIVE prot. n. 21 210, date: 13/12/2021). The study flow chart is depicted in supplementary Figure 1.

Study design rationale was essentially in two parts: at baseline (T0) we employed a case-control approach, enrolling a population of SEA patients and a group of healthy age- and sex-matched controls (HC), to compare adhesion molecule expression on the surface of peripheral T cell subsets and to determine their modifications after IL-33 and IL-25 stimulation. Analysis was performed in a blinded manner and unblinded if possible once the results were obtained. No predefined power analysis was performed, except when trends were observed.

In the second phase of the study, we selected a subpopulation of five SEA patients, in whom blood sampling was repeated after 6 months of treatment with benralizumab (T6), in order to investigate any immunological modifications, such as cell changes, cytokine release or functional alterations (including cell proliferative capacity or cytotoxicity) before and after stimulation with IL-33 and IL-25.

All enrolled SEA patients underwent clinical and functional follow-up, including medical examination, lung function tests, fractional exhaled nitric oxide (FeNO) analysis and validated questionnaires for the assessment of clinical control of disease (Asthma Control Test (ACT) and 7-item Asthma Control Questionnaire (ACQ-7). All these parameters, including daily dosage of oral and/or inhaled steroids, were

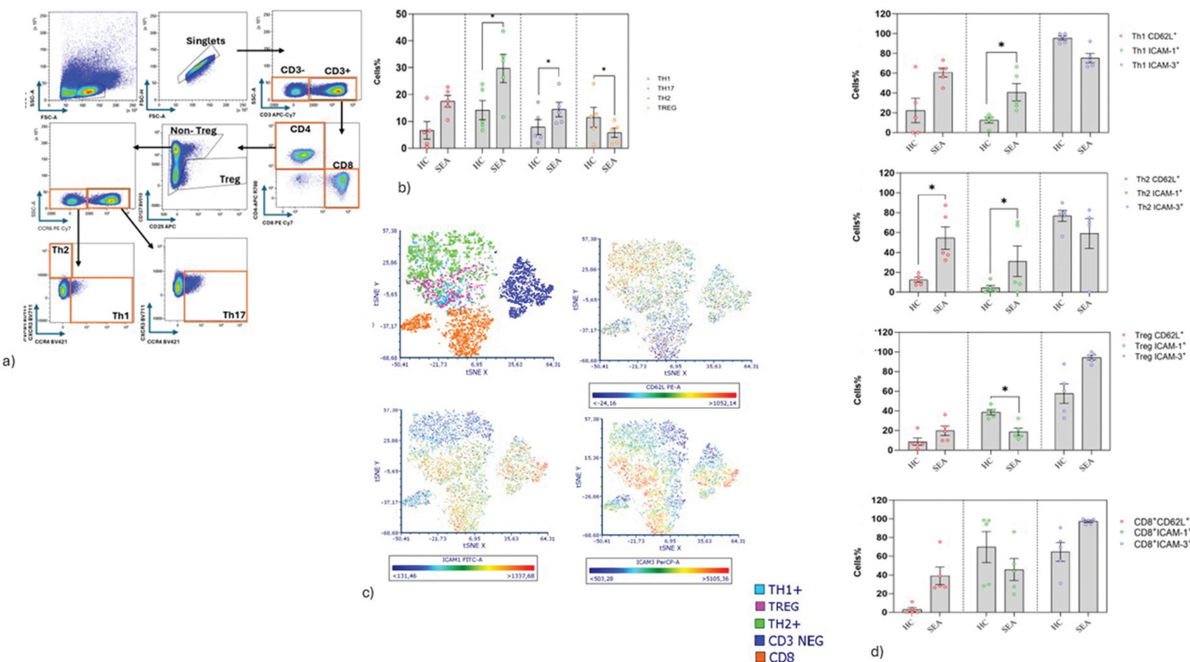

Figure 1. (a) Gating strategy used in flow cytometry assay for the detection of T cell subsets (healthy control). (b) Histograms illustrating differences in cell percentages between controls ($n = 10$) and SEA patients ($n = 10$). These percentages were derived from flow cytometric gating strategy. (c) The data was pre-gated on single, live, singlets, CD3+ and CD3- to include T cells and non-T cells. To create a t-SNE overview, data from all samples was randomly subsampled to 20 000 cells with equal contribution from SEA samples. Protein expression levels of all additional antigens are overlaid as a colour-dimension on the t-SNE map. (d) Histograms showing the percentages of ICAM-1, ICAM-3 and CD62L expressed on the surface of Th2, Th1, Treg cells and cytotoxic T cells (CD8). The data is reported as means ± SE. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Abbreviations: CD: cluster of differentiation, HC: healthy controls, SEA: severe eosinophilic asthma, Th: T helper. ICAMs: intercellular cell adhesion molecules.

collected at T0, T6 and after one year of treatment with benralizumab (T12) to investigate any correlations between clinical outcomes and cellular and immune modifications.

Detailed description of methods are reported as suppl. Mat. Section B

Statistical analysis

Data in the tables is expressed as means ± standard deviation (SD). The statistical significance of differences in parametric data was assessed by the two-tailed Student's t test or one-way analysis of variance. Differences in nonparametric data between groups were assessed by the Wilcoxon matched-pairs signed-rank test or the Kruskal-Wallis with post hoc test and correction for multiple comparisons by Dunn's test. A p-value < 0.05 was considered significant. Prism 10.2. (GraphPad) was used to analyse the data. TSNE Analysis analysis are reported as suppl. mat. Part C.

Results

Clinical features of study population

The main characteristics of our study population are reported in supplementary Table 2. No differences in the distribution of sex, age or smoking habits emerged between HC and SEA patients. In line with diagnosis and the Italian prescription criteria for benralizumab, SEA patients showed poor control of asthma at T0, as assessed by ACT and ACQ-7, associated with a high annualised exacerbation rate. At T6, we observed a significant improvement in clinical control of asthma in terms of ACT and ACQ-7, coupled with a significant increase in post-bronchodilator FEV1 and FVC values and a reduction of FeNO values.

Table 1. Clinical data of study population. Inhaled corticosteroid dosage is expressed as beclomethasone equivalent.

	T0	T1	T6	T12	p-value
FEV1%	79.48 ± 22.45	88.12 ± 20.09	97.7 ± 19.5	93.14 ± 19.38	0.003
FVC%	96 ± 19.91	103.73 ± 18.50	105.31 ± 17.56	109.58 ± 16.4	0.007
Inhaled corticosteroids dosage (mcg/day)	791.3 ± 41.7	791.3 ± 41.7	711.11 ± 176.38	626.66 ± 198.08	0.008
ACQ-7	2.18 ± 0.9	0.89 ± 0.53	0.68 ± 0.44	0.57 ± 0.56	<0.001
ACT	14.34 ± 4.86	20.43 ± 3.57	23.90 ± 1.37	23.36 ± 2.33	<0.001
Blood eosinophils count (cells/mm^3)	807.34 ± 478.17	17.36 ± 50.86	12.5 ± 35.35	25.81 ± 46.65	<0.001
FeNO (ppb)	65.61 ± 44.45	56.84 ± 35.51	30.66 ± 28.99	28.66 ± 16.56	0.016

As expected, we observed nearly complete blood eosinophilic depletion. At T12, clinical and functional improvement was confirmed, with no requirement for oral corticosteroid therapy.; accordingly, we observed a significant reduction in annualised exacerbation rate, only one patient experiencing a moderate exacerbation during the observation period (Table 1). As expected, therapy with benralizumab was not associated with any moderate-to-severe adverse events and there were no interruptions of treatment.

Treg/Th2 cell imbalance in patients with SEA

We first analysed blood lymphocyte subpopulations by flow cytometry (Figure 1(a)) in T0 samples from 10 patients with SEA and 10 HC. Patients with SEA showed a marked reduction in the number of circulating Treg cells compared to HC (Figure 1(b)) and conversely, an increase in the percentages of effector T cells, regarding both the Th2 and Th17 cell subsets (Figure 1(b)).

We therefore used t-SNE dimensionality reduction to characterise our patients' peripheral blood T cell subset subpopulations. T cells were enriched from total PBMCs by preselection based on FSC and SSC and as singlets before t-SNE (Figure 1(a)).

Our t-SNE and gating approach identified a total of six distinct cell subsets (Figure 1(c)). One was determined to be a non-CD3 cell population, another a CD8 cell population and a group of CD4 was stratified among Th1, Th2, Th17 and Treg cells. t-SNE was used only for visual support: all quantitative conclusions rely on conventional gating strategies.

Density plot was used for expression of ICAM-3, ICAM-1 and CD62L molecules (Figure 1(c)).

Concerning expression of adhesion molecules, the SEA population showed higher expression of ICAM-1 on effector Th1 and Th2 cells, whereas the opposite pattern was observed for Treg cells. Moreover, Th2 cells showed selective expansion of CD62L in SEA patients (Figure 1(d)).

Together these findings demonstrate that patients with SEA had a global reduction in circulating Treg cells associated with an increase in Th1 and Th2 cells expressing ICAM-1 and CD62L.

IL-33 selectively expands Treg cells of SEA patients

Alarmins (IL-25 and IL-33) were administered to T cells of SEA and control subgroups: their effects were evaluated by functional assay and in terms of phenotypic changes (Figure 2(a)). IL-25 administration promoted Th17, Th2 and Treg cell expansion in SEA patients, while in HC this effect was only significant for Th2 cells. On the contrary, IL-33 stimulation led to an expansion of Th1, Th2 and Treg cells in control samples and only isolated expansion of Treg cells in patients (Figure 2(b)). The combination of IL-25 and IL-33 resulted in a numerical expansion of all T cell subsets in HC, but only Treg cells were significantly increased in patients (Figure 2(b)).

Functional tests were performed by advanced live-cell imaging. The experiment was performed for 24 hours with a scan every 2 hours. Basal cytotoxicity of PBMCs increased in SEA patients with respect to HC in the first 24 hours. In the SEA subgroup, administration of IL-33 and IL-25 resulted in a significant increase in cytotoxicity after 10–12 hours compared to the control group; this effect tended to diminish after 24 hours (Figure 2(c)). Conversely, both single and combined administration of IL-25 and IL-33 induced a significant enhancement in proliferative capacity after 4 hours the effect enduring for up to 24 hours (Figure 2(d)).

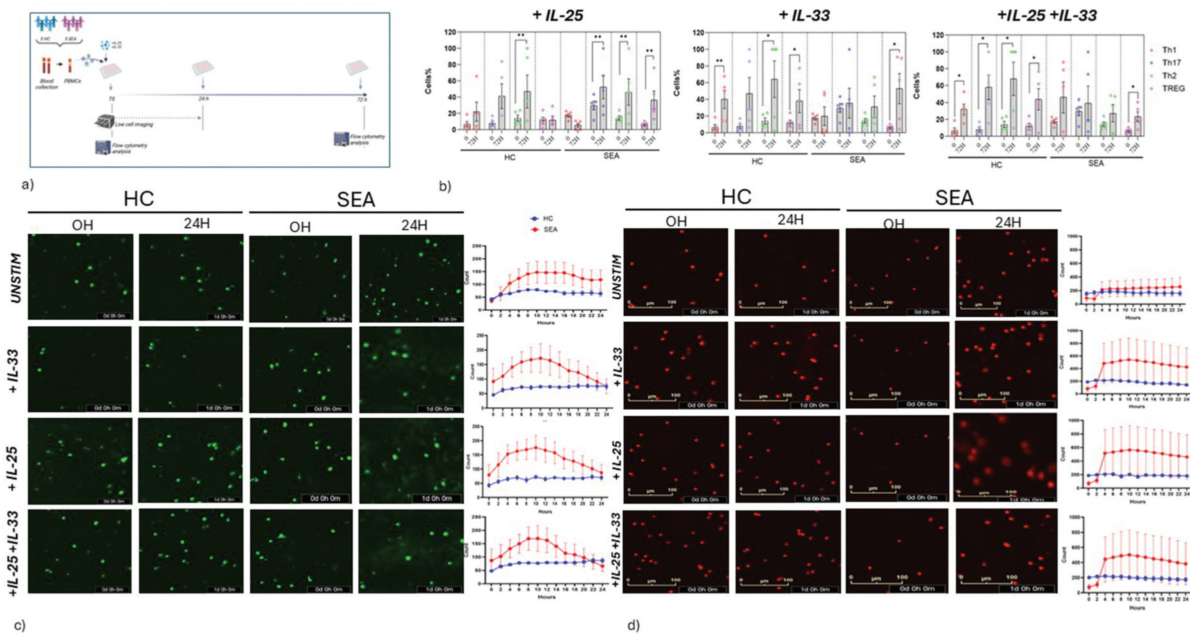

Figure 2. (a) Study design of experiments with SEA patients before treatment and HC. As illustrated, samples were collected from 5 HC and 5 SEA patients. PBMCs were used for stimulation with IL-33 and IL-25 for 72 hours. Flow cytometric analysis was performed at T0 and after 72 h of stimulation, while live cell imaging was performed for 24 hours, with a scan every 2 hours. (b) Histograms showing the percentages of Th1, Th2, Th17 and Treg in HC and SEA patients, before (T0) and after 72 hours of stimulation (72 h). (c) Live cell imaging assay to monitor cell cytotoxicity using IncuCyte® Cytotox Green Dye for 24 h. The selected images show scans at 0 and 24 hours under unstimulated conditions and after administration of IL-33 and IL-25, separately and combined. Histograms showing the mean of each scan (2 hours) for each well. The red line indicates the results for SEA at T0 and the blue line those of HC. (d) Live cell imaging assay to monitor cell proliferation using Incucyte® Nuclight rapid red Dye for 24 h. The selected images show scans at 0 and 24 hours under unstimulated conditions and after administration of IL-33 and IL-25, separately and combined. Histograms showing the mean of each scan (2 hours) for each well. The red line indicates the results for SEA at T0 and the blue line those of HC. Data is reported as means ± SE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: HC: healthy controls, SEA: severe eosinophilic asthma, IL-: Interleukin, H: Hour/s.

These findings indicate that cytotoxic activity is higher in unstimulated PBMCs from SEA patients than in those from HC, but without any concomitant increase in proliferation. Meanwhile, exposure to IL-25 and IL-33 is associated with sustained enhancement of proliferative capacity, but not cytotoxicity, in T cells.

ICAM-1, ICAM-3 and CD62L expression is mediated by alarmins

At T0 we observed strong overexpression of ICAM-1 on Treg and Th2 cells after administration of IL-25 IL-33 and IL-25 + IL-33 (Figure 3(a,b)) for 72 h in SEA patients. ICAM-3 was downregulated on Treg cells and upregulated on Th2 cells after stimulation with IL-33 or with IL-25 + IL-33 (Figure 3(a,b)) Changes of adhesion molecules expression on Th2 cells are observed in both SEA patients and HC (Figure 3(b)). CD62L expression was elevated on the surface of Th2 (Figure 3(b)) and Th17 (Figure 3(c)) cells in response to IL-25 in HC, while in SEA patients, CD62L was only significantly increased on Treg (Figure 3(a)), Th17 (Figure 3(c)) and Th1 (Figure 3(d)) cells by the combination of IL-25 and IL-33.

Effects of benralizumab on T cells: Comparison between T0 and T6

In the second phase of the project, we investigated changes in PBMCs between samples obtained at T0 and T6 from SEA patients (Figure 4(a)). T reg cells were significantly more numerous at T6 than at T0 (Figure 4(b)), but no significant changes were observed in the expression of cell surface adhesion molecules (Figure 4(c)). Regarding other cell subsets, CD62L expression decreased on effector T cells, including Th1 and Th2

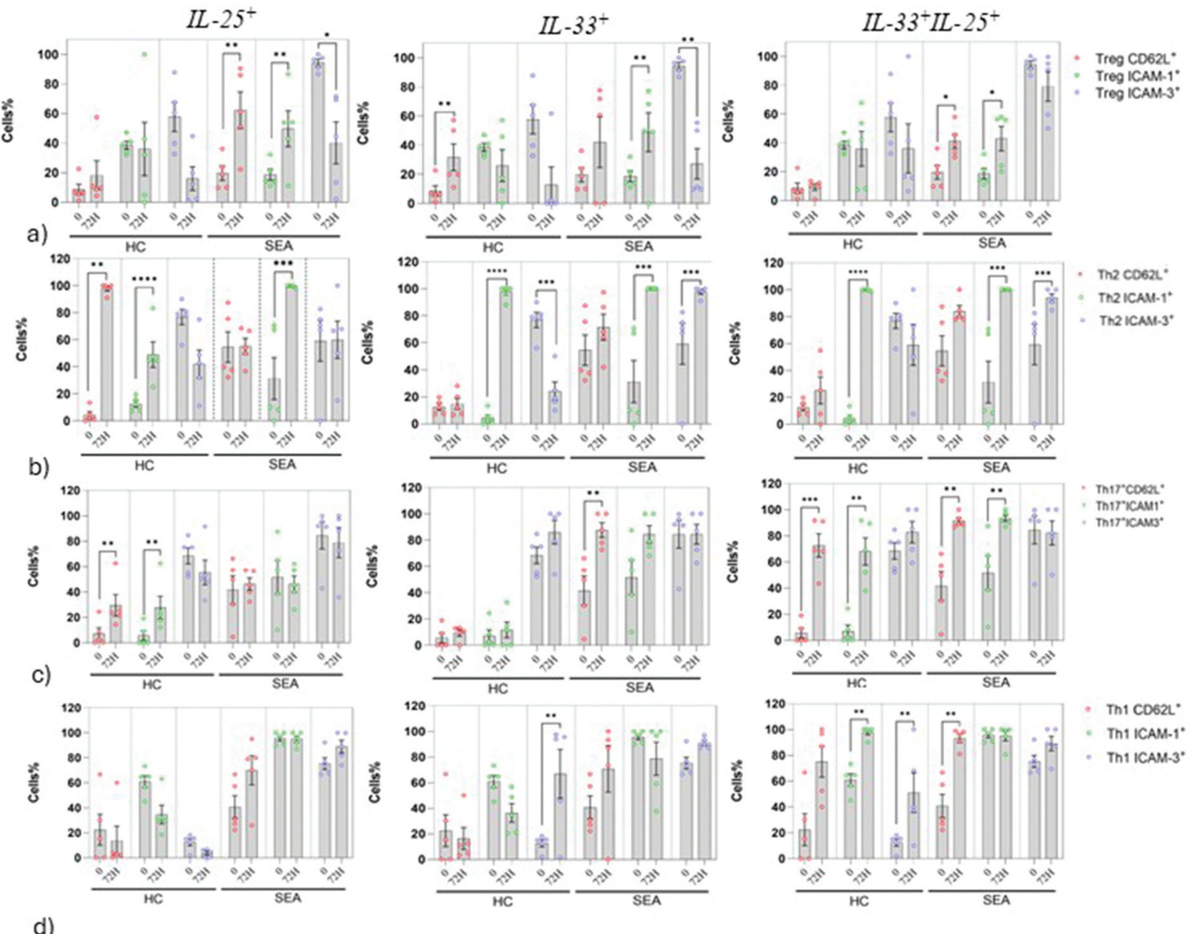

Figure 3. Histograms showing the percentages of ICAM-1, ICAM-3 and CD62L expressed on the surface of Treg (a), Th2 (b), Th17 (c) and Th1 (d). Data is reported as means ± SE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: CD: cluster of differentiation, HC: healthy controls, SEA: severe eosinophilic asthma, Th: T helper. ICAMs: intercellular cell adhesion molecules.

(Figure 4(c)), while an opposite pattern was observed for CD8 T cells (Figure 4(c)). We also observed a decrease in ICAM-3 in CD8 cells (Figure 4(c)).

Adhesion molecules were modulated by IL-33 and IL-25 stimulation at T6

PBMCs isolated from SEA patients treated with benralizumab were also stimulated with IL-25 and IL-33 (and their combination) for 72 h to assess the response of T cells in terms of expression of adhesion molecules during biological treatment (Figure 5(a)). After stimulation, we did not observe any changes in the percentages of T subsets (Figure 5(b)). The expression of adhesion molecules of Th1 and Th2 showed relevant modifications: ICAM-1 and ICAM-3 expression were significantly reduced in both cell subsets after IL-25 stimulation (Figure 5(c)). Similar results were found after stimulation with IL-33, although statistical significance was only reached in the Th1 cell subset (Figure 5(c)). IL-25 and IL-33 together downregulated expression of CD62L, ICAM-1 and ICAM-3 in Th1 and Th2 cells (Figure 5(c)). CD62L also decreased in these cell subsets, reaching statistical significance on Th1 after IL-25 induction (Figure 5(c)) and on Th2 after IL-33 induction (Figure 5(c)).

From a functional point of view, no differences in cytotoxic activity were observed in unstimulated PBMCs (Suppl. Fig. S2a). In the same unstimulated condition, proliferation rates were higher at T0 compared with T6 (Suppl. Fig. S2b).

In contrast, stimulation with IL-33 and IL-25 was associated with a significant reduction in overall PBMC proliferation at T6 (Suppl. Fig. S2b).

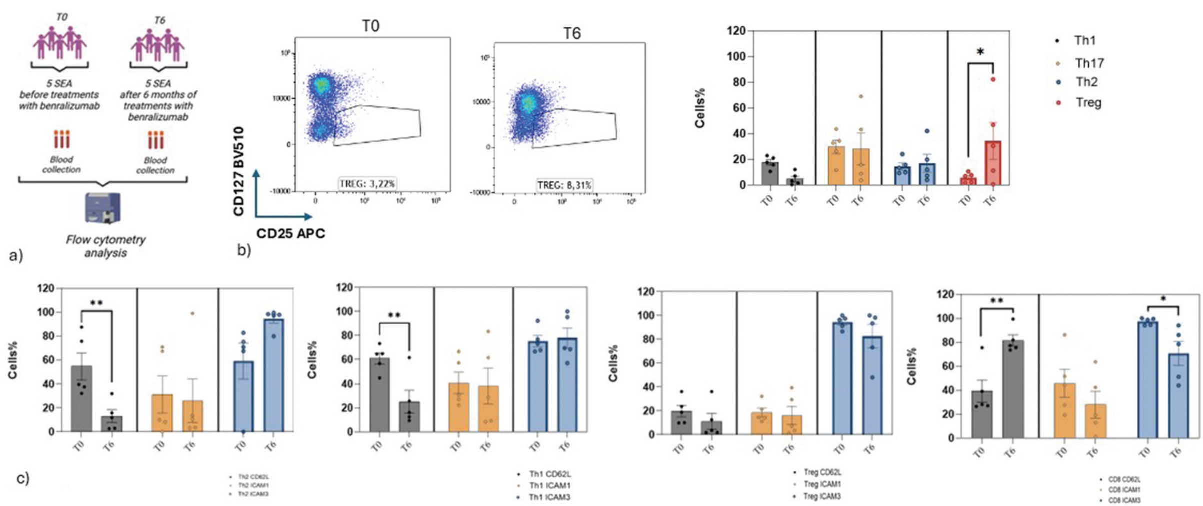

Figure 4. (a) Study design of experiments with SEA patients before treatment (T0) and after 6 months of therapy (T6). As illustrated, samples were collected from 5 SEA patients at T0 and T6. PBMCs were used for cytofluorimetric analysis. (b) Dot plot showing the different distribution of CD56 cell subsets between T0 and T6, revealing a shift from CD56br towards CD56dim, as reported in the adjacent histogram. (c) Dot plot showing the different distribution of T cell subsets between T0 and T6, revealing an increased percentage of Treg cells as reported in the adjacent histogram. (d) Histograms showing the percentages of ICAM-1, ICAM-3 and CD62L expressed on the surface of Th2, Th1, Treg cells and CD8: results at baseline (T0) and after 6 months of treatment with benralizumab (T6). Data is reported as means ± SE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: CD: cluster of differentiation, HC: healthy controls, SEA: severe eosinophilic asthma, Th: T helper. ICAMs: intercellular cell adhesion molecules.

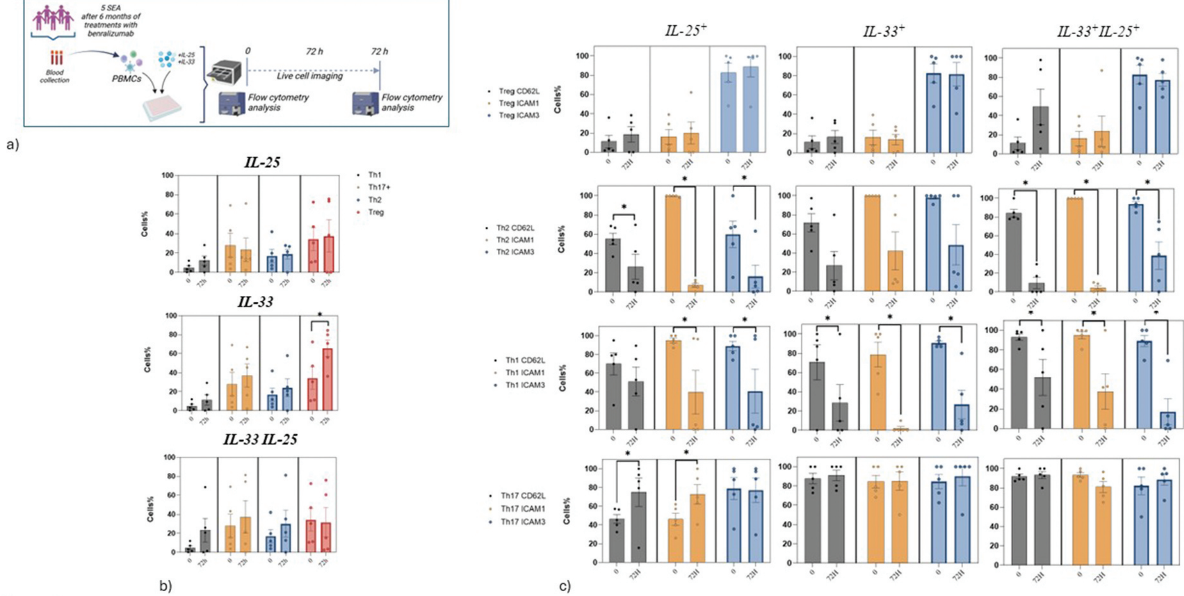

Figure 5. (a) PBMCs from patients treated with benralizumab were used for these experiments. As illustrated, samples were collected from 5 SEA patients. PBMCs were used for stimulation with IL-33 and IL-25 for 72 hours. Flow cytometric analysis was performed at T0 and after 72 h of stimulation, while live cell imaging was performed for 24 hours, with a scan every 2 hours. (b) Histograms showing the percentages of Th1, Th2, Th17 and Treg before and after 72 hours of stimulation with IL-33 and IL-25, separately and combined. (c) Histograms showing the percentages of ICAM-1, ICAM-3 and CD62L expressed on the surface of Th2, Th1, Treg cells and Th17: results at baseline (T0) and after 72 of stimulation with IL-25 and/or IL-33. Data is reported as means ± SE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: CD: cluster of differentiation, HC: healthy controls, SEA: severe eosinophilic asthma, Th: T helper. ICAMs: intercellular cell adhesion molecules.

Notably, a distinct pattern emerged at T6. Under these conditions, administration of IL-33 and IL-33 + IL-25 induced a rapid peak in proliferative activity approximately 10 hours after stimulation (Suppl. Fig. S2b). Together, these findings indicate that proliferative and cytotoxic readouts differ according to the activation state and stimulatory context and may involve distinct cellular compartments within the PBMC population. These functional changes were assessed in total PBMCs and cannot be unequivocally attributed to T-cell – intrinsic mechanisms but may instead reflect composite or indirect effects mediated by other immune subsets known to be affected by benralizumab.

Discussion

SEA is a respiratory immune disorder belonging to the family of T2 inflammatory conditions characterised by increased proliferation and activity of eosinophils. The role of adhesion molecules in asthma has been extensively investigated,[17] revealing various abnormalities in their expression on T cells.[4] ICAM-1 is expressed at very low levels on the surface of resting T cells, but is up-regulated after exposure to inflammatory mediators, including cytokines.[3,17] ICAM-1 is upregulated in asthmatics both in its circulating form and its expression on the T cell surface.[3,18,19] Notably, alarmins such as IL-33 and IL-25 May further enhance ICAM-1 expression on T cells and repetitive exposure of the lungs to inflammatory stimuli increases the expression of adhesion molecules such as ICAM-1, VCAM-1 and vWF via the IL-33 pathway.[20] Evidence that ICAM-1 serves as the primary surface receptor for rhinoviruses in respiratory epithelial cells,[21] with IL-33 also upregulating receptors involved in the entry of respiratory viruses to enhance infection,[22] combined with the fact that viral infections are among the most common causes of acute exacerbations, further underscores the critical role of these molecules in the pathobiology of asthma. IL-25 also plays a key role in enhancing survival and regulating surface expression of ICAM-1, ICAM-3 and L-selectin on human eosinophils,[11,21] but no data is available for T cells.

Our study is the first to investigate the expression of adhesion molecules on T cells in a population of SEA patients, while also conducting an extensive analysis of T cell subset percentages, proliferation rates and cytotoxic properties. Besides a significant reduction in circulating Treg cells with respect to HC, SEA patients also showed overexpression of ICAM-1 on effector Th1 and Th2 cells. Moreover, when stimulated with IL-33 and IL-25, a significant increase in ICAM-1 expression on Th2 and Treg cells was observed, supporting the concept that ICAM-1 modulation in different T cell subsets reflects responsiveness to epithelial-derived alarmins. Accordingly, these findings should be interpreted as supporting an indirect effect of epithelial damage – associated inflammatory signals on T cell phenotype. Interestingly, after 6 months of therapy with benralizumab, we did not observe any change in ICAM-1 expression in unstimulated cells, possibly reflecting that this time frame may be insufficient to detect basal phenotypic modifications; however, following IL-33 and IL-25 stimulation, ICAM-1 expression was significantly downregulated, suggesting a modification of T cell responsiveness to epithelial injury – associated stimuli.

This finding supports the hypothesis that benralizumab may modulate circulating T-cell responses to epithelial-derived alarmins, while we cannot draw any specific conclusion concerning a potential direct influence on basal ICAM-1 expression: following eosinophil depletion, changes in the inflammatory microenvironment may attenuate IL-25 and IL-33 driven activation programs, resulting in selective downregulation of ICAM-1 only under stimulated conditions.

Given the limited size of the longitudinal cohort, these findings finding do not permit firm conclusions concerning a direct immunomodulatory effect of benralizumab on T cells, but instead support, at least, an indirect effect, associated with changes in the inflammatory microenvironment. How such modulation may relate to a reduced risk of exacerbation triggered by epithelial stimuli, such as irritants or infectious agents, and thus to the well-known clinical improvement associated with benralizumab remains to be clarifiedEven if intriguing, this association remains speculative and hypothesis-generating, and future studies specifically addressing immune responses to different stimuli in SEA will be required to confirm our findings.

Regarding the other adhesion molecules investigated in our study, ICAM-3 was upregulated on Th2 cells following exposure to IL-33 or a combination of IL-25 and IL-33. This result is significant as it underscores the crosstalk between innate and adaptive immune cells in SEA, since enhanced adhesion between T cells and eosinophils is primarily regulated through binding of ICAM-3, and only minimally by ICAM-1, leading to augmented eosinophil secretion.[23] The evidence of ICAM-3

upregulation on Th2 after IL-33 and IL-25 administration in SEA suggests enhanced ligation between Th2 cells and eosinophils and their consequent hyperactivation in response to epithelial injury.[24] Thus, our experimental data supports the critical role of epithelial-derived alarmin secretion in response to environmental exposure in triggering and promoting T2 inflammatory pathways, with ICAM-3 overexpression serving as a key step in initiating the inflammatory cascade. Our findings also showed that alarmin stimulation down-regulated the expression of ICAM-3 on Treg cells: considering the critical role of ICAM-3 for many immunoregulatory activities of Tregs, as well as in facilitating cell trafficking to target tissues,[25] these results are in line with the promotion of dysregulated T2 cellular response driving eosinophil proliferation and maturation, modulated by IL-33 and IL-25.

Note that like ICAM-1, PBMCs isolated at T6 showed some significant modifications in this regard: although undetectable in unstimulated cells, ICAM-3 proved significantly downregulated in Th1 and Th2, but not Treg subsets when stimulated with IL-25 and IL-33, This observation further supports the hypothesis that benralizumab treatment may indirectly affect immune responses to epithelial injury, rather than exerting a direct effect on T cell function.

Regarding the CD62L expression pattern, our data revealed clear overexpression, particularly on Th2 cells in SEA patients, further confirming the role of this molecule in inflammatory polarisation towards a T2 profile, as previously described in the literature, where CD62L is strongly implicated in eosinophil and lymphocyte chemotaxis.[5,7] In comparison with ICAM-1 and ICAM-3, CD62L was more significantly overexpressed across all cell subsets stimulated with IL-25 and IL-33, suggesting that epithelial injury may enhance the trafficking and migration of inflammatory cells to bronchial tissue in SEA. Further studies are however needed to confirm these findings. Interestingly, CD62L expression proved completely different on PBMCs isolated from SEA patients after 6 months of benralizumab treatment. This effect should not be interpreted as immune 'rebalancing' per se, but rather as a potential downstream consequence of altered inflammatory signalling following eosinophil depletion. Furthermore, this modulation was particularly evident after stimulation with IL-33 and IL-25, where CD62L increased in Tregs and was downregulated in Th2 cells, suggesting a shift in the T2 inflammatory environment in bronchial tissue.

Finally, we investigated the profile of circulating T cells of SEA patients in terms of cytotoxicity and proliferation rate. As expected, compared to HC, unstimulated T cells from asthma patients showed higher cytotoxic potential, consistent with the higher percentage of effector Th1 and Th2 cells and the concomitant reduction of Treg cells. Interestingly, administration of alarmins induced short-term enhancement of cytotoxicity and a persistent increase in proliferative capacity, while no significant modification was observed in control samples, further confirming the dysregulated response to epithelial injury associated with SEA.

The study has some limitations to be acknowledged, including its monocentric design and the limited sample size of the cohort. In particular, the limited number of patients with longitudinal follow-up precludes any causal inference regarding direct immunomodulatory effects of benralizumab on T cells.

Furthermore, all analyses should be considered exploratory and interpreted with caution, requiring confirmation in adequately powered studies.

Moreover, even if our results suggest preventive immunomodulation of T cells in response to epithelial injury, immunological analysis of circulating cells is insufficient to draw firm conclusions on tissue modifications before and after treatment: therefore, our findings should be coupled with in vivo or in vitro bronchial samplings to also assess modifications in epithelial and resident cells. A further limitation of this study is that functional assays assessing proliferation and cytotoxicity were performed on total PBMCs rather than on purified T-cell populations; therefore, the observed functional changes cannot be unequivocally attributed to T-cell-intrinsic mechanisms and may instead reflect composite or indirect effects mediated by other immune subsets known to be affected by benralizumab.

Finally, we cannot determine whether our findings are attributable to the direct effect of benralizumab or are to be ascribed to eosinophilic inflammation switched off; in this context, a comparative analysis with other biologic drugs may be helpful.

Conclusion

Overall, our study highlighted differential expression of ICAM-1, ICAM-3 and CD62L modulated by IL-33 and IL-25 in severe eosinophilic inflammation. We also demonstrated that benralizumab treatments is associated with modifications in T cell responsiveness to alarmin stimulation in vitro, likely reflecting indirect effects mediated by changes in the inflammatory milieu. This effect may be related to modifications in the expression of adhesion molecules on T cells, which are profoundly involved in immune processes critical for the onset and progression of SEA, including susceptibility to viral infection, crosstalk between T cells and eosinophils, and migration to target tissues. Future longitudinal and mechanistic studies will be required to clarify the causal pathways underlying these associations.

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Disclosure statement

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Author contribution statement

LB and PC designed the study, PC and TP wrote the study protocol and gained ethical approval for the study. PC, TP, and EB recruited the patients and conducted the study. LB, IP, MdA performed the experiments. LB, IP and TP performed the analysis. LB, IP, TP wrote the manuscript. All authors critically revised the manuscript.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author (IP).

Ethics approval and consent to participate

All subjects gave their written informed consent to participation in the study, which was approved by our Local Ethics Committee CEAVSE (BE-ACTIVE prot. n. 21 210, date: 13/12/2021). The study complied with the Declaration of Helsinki.

References

1. Gonzalez-Uribe V, Romero-Tapia SJ, Castro-Rodriguez JA. Asthma phenotypes in the era of personalized medicine. *JCM*. 2023;12(19):6207. doi: 10.3390/jcm12196207.
2. Blight BJ, Gill AS, Sumsion JS, et al. Cell adhesion molecules are upregulated and may drive inflammation in chronic rhinosinusitis with nasal polyposis. *J Asthma Allergy*. 2021;14:585–593. doi: 10.2147/JAA.S307197.

3. Bui TM, Wiesolek HL, Sumagin R. Icam-1: a master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*. 2020;108(3):787–799. doi: 10.1002/JLB.2MR0220-549R.
4. Stanciu LA, Djukanovic R. The role of ICAM-1 on T-cells in the pathogenesis of asthma. *Euro Respir J*. 1998;11 (4):949–957. doi: 10.1183/09031936.98.11040949.
5. Yang S, Liu F, Wang QJ, Rosenberg SA, Morgan RA. The shedding of CD62L (L-selectin) regulates the acquisition of lytic activity in human tumor reactive T lymphocytes. *PLOS ONE*. 2011;6(7):e22560. doi: 10.1371/journal.pone.0022560.
6. Matsuzaki S, Shinozaki K, Kobayashi N, Agematsu K. Polarization of Th1/Th2 in human CD4+ T cells separated by CD62L: analysis by transcription factors. *Allergy*. 2005;60(6):780–787. doi: 10.1111/j.1398-9995.2005.00793.x.
7. Kanegane H, Kasahara Y, Niida Y, et al. Expression of L-selectin (CD62L) discriminates Th1- and Th2-like cytokine-producing memory CD4+ T cells. *Immunology*. 1996;87(2):186–190. doi: 10.1046/j.1365-2567.1996.446530.x.
8. Numata T, Ito T, Maeda T, Egusa C, Tsuboi R. Il-33 promotes Icam-1 expression via Nf-kb in murine mast cells. *Allergology Int*. 2016;65(2):158–165. doi: 10.1016/j.alit.2015.10.004.
9. Chow JYS, Wong CK, Cheung PFY, Lam CWK. Intracellular signaling mechanisms regulating the activation of human eosinophils by the novel Th2 cytokine IL-33: implications for allergic inflammation. *Cell Mol Immunol*. 2010;7(1):26–34. doi: 10.1038/cmi.2009.106.
10. Salter BM, Oliveria JP, Nusca G, et al. Il-25 and IL-33 induce type 2 inflammation in basophils from subjects with allergic asthma. *Respir Res*. 2016;17(1):5. doi: 10.1186/s12931-016-0321-z.
11. Cheung PFY, Wong CK, Ip WK, Lam CWK. Il-25 regulates the expression of adhesion molecules on eosinophils: mechanism of eosinophilia in allergic inflammation. *Allergy*. 2006;61(7):878–885. doi: 10.1111/j.1398-9995.2006.01102.x.
12. Luo J, Chen W, Liu W, et al. Il-5 antagonism reverses priming and activation of eosinophils in severe eosinophilic asthma. *Mucosal Immunol*. 2024;17(4):524–536. doi: 10.1016/j.mucimm.2024.03.005.
13. Terada N, Konno A, Fukuda S, et al. Interleukin-5 upregulates intercellular adhesion molecule-1 gene expression in the nasal mucosa in nasal allergy but not in nonallergic rhinitis. *Int Arch Allergy Immunol*. 1995;106(2):139–145. doi: 10.1159/000236834.
14. Bergantini L, Pianigiani T, d'Alessandro M, et al. The effect of anti-IL5 monoclonal antibodies on regulatory and effector T cells in severe eosinophilic asthma. *Biomed Pharmacother*. 2023;166:115385. doi: 10.1016/j.biopha.2023.115385.
15. Bergantini L, d'Alessandro M, Pianigiani T, Cekorja B, Bargagli E, Cameli P. Benralizumab affects NK cell maturation and proliferation in severe asthmatic patients. *Clin Immunol*. 2023;253:109680. doi: 10.1016/j.clim.2023.109680.
16. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *EUR Respir J*. 2014;43(2):343–373. doi: 10.1183/09031936.00202013.
17. Mukhopadhyay S, Malik P, Arora SK, Mukherjee TK. Intercellular adhesion molecule-1 as a drug target in asthma and rhinitis. *Respirology*. 2014;19(4):508–513. doi: 10.1111/resp.12285.
18. Lehmann JCU, Jablonski-Westrich D, Haubold U, Gutierrez-Ramos JC, Springer T, Hamann A. Overlapping and selective roles of endothelial intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 in lymphocyte trafficking 1. *J Immunol*. 2003;171(5):2588–2593. doi: 10.4049/jimmunol.171.5.2588.
19. Shiota Y, Sato T, Ono T. Serum levels of soluble ICAM-1 in asthmatic patients. *Arerugi*. 1993;42(12):1782–1787.
20. Pandher U, Kirychuk S, Schneberger D, et al. Adhesion molecules in lung inflammation from repeated glyphosate exposures. *Int J Environ Res Public Health*. 2023;20(8):5484. doi: 10.3390/ijerph20085484.
21. Yao X, Sun Y, Wang W, Sun Y. Interleukin (IL)-25: pleiotropic roles in asthma. *Respirology*. 2016;21(4):638–647. doi: 10.1111/resp.12707.
22. Akoto C, Willis A, Banas CF, et al. Il-33 induces an antiviral signature in mast cells but enhances their permissiveness for human rhinovirus infection. *Viruses*. 2022;14(11):2430. doi: 10.3390/v14112430.
23. Douglas IS, Leff AR, Sperling AI. Cd4+ T cell and eosinophil adhesion is mediated by specific ICAM-3 ligation and results in eosinophil activation. *J Immunol*. 2000;164(6):3385–3391. doi: 10.4049/jimmunol.164.6.3385.
24. Blight BJ, Gill AS, Sumsion JS, et al. Cell adhesion molecules are upregulated and may drive inflammation in chronic rhinosinusitis with nasal polyposis. *JAA*. 2021;14:585–593. doi: 10.2147/JAA.S307197/.
25. Montoya MC, Sancho D, Bonello G, et al. Role of ICAM-3 in the initial interaction of T lymphocytes and APCs. *Nat Immunol*. 2002;3(2):159–168. doi: 10.1038/ni753.