Received: 23 February 2022 | Revised: 4 April 2022 | Accepted: 26 April 2022

DOI: 10.1111/cea.14153

ORIGINAL ARTICLE

WILEY

High proportion of inflammatory CD62L^{low} eosinophils in blood and nasal polyps of severe asthma patients

Andrea Matucci[1] | Francesca Nencini[1] | Giandomenico Maggiore[2] | Fabio Chiccoli[3] | Matteo Accinno[3] | Emanuele Vivarelli[1] | Chiara Bruno[2] | Luca Giovanni Locatello[2] | Annarita Palomba[4] | Elena Nucci[4] | Valentina Mecheri[3] | Margherita Perlato[3] | Oliviero Rossi[1] | Paola Parronchi[3] | Enrico Maggi[5] | Oreste Gallo[2] | Alessandra Vultaggio[1]

[1]Immunoallergology Unit, Careggi University Hospital, Florence, Italy

[2]Otolaryngology Clinic, Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy

[3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

[4]Department of Pathology, Careggi University Hospital, Florence, Italy

[5]Translational Immunology Unit, Immunology Area, Bambino Gesù Children's Hospital, I.R.C.C.S, Rome, Italy

Correspondence
Andrea Matucci, Immunoallergology Unit, Careggi University Hospital, Florence, Italy.
Email: andrea.matucci@unifi.it

Enrico Maggi, Translational Immunology Unit, Immunology Area, Bambino Gesù Children's Hospital, I.R.C.C.S., Rome, Italy.
Email: enrico.maggi@opbg.net

Abstract

Background: In mice models, eosinophils have been divided into different subpopulations with distinct phenotypes and functions, based on CD62L and CD101 patterns of membrane expression. Limited data are available in humans.

Objective: To investigate eosinophils subpopulations in peripheral blood (PB) and nasal polyp tissue (NP) from severe eosinophilic asthma (SEA) patients plus concomitant chronic rhinosinusitis with nasal polyps (CRSwNP).

Methods: We recruited 23 SEA patients (14 with CRSwNP); as controls, we enrolled 15 non-severe asthma patients, 15 allergic rhinitis patients without asthma and 15 healthy donors. Eosinophils were isolated from PB and NP and analysed by FACS. Eotaxin-3 and eotaxin-1 mRNA expression in NP tissue was also evaluated.

Results: A significantly higher percentage of circulating CD62L^{low} cells was observed in SEA, as compared with controls, expressing higher levels of CCR3, CD69 and lower levels of CD125 (IL-5R), CRTH2, CD86 and CD28 in comparison with CD62L^{bright} cells. In NP, eosinophils showed a high proportion of CD62L^{low} phenotype, significantly greater than that observed in PB. Surface expression of IL-3R, IL-5R, CD69 and CD86 was significantly higher in CD62L^{low} eosinophils from NP than in those from blood. Moreover, eotaxin-3 mRNA expression positively correlated with the percentage of CD62L^{low} cells in NP.

Conclusion: Two different eosinophil subphenotypes can be identified in blood and NP of SEA patients, with a preferential accumulation of CD62L^{low} inflammatory cells in NP.

KEYWORDS
inflammatory eosinophil, nasal polyps, resident eosinophil, severe asthma

1 | INTRODUCTION

Eosinophils are both circulating and tissue-resident leukocytes, traditionally regarded as damaging effector cells involved in asthma, chronic rhinosinusitis with nasal polyps (CRSwNP), gastrointestinal disorders and eosinophilic vasculitis; their presence is a hallmark of type 2 inflammatory response.[1–5] Eosinophils primed by interleukin (IL)-5, IL-3, granulocyte macrophage colony-stimulating factor (GM-CSF), eotaxins and arachidonic acid derivatives can express membrane receptors and adhesion molecules involved in activation and tissue migration.[5,6] Eosinophils have been subdivided into different subpopulations with distinct phenotypes and functions.[7–9] A role for resident eosinophils (rEos) in healthy tissue and metabolic homeostasis has been described,[7] including the maintenance of tissue microenvironment during physiological organ development, along with the modulation of host innate and adaptive immune responses.[10] More recently, rEos have also been described in lung tissue. In mice, specifically, Siglec8$^+$ rEos and inflammatory eosinophils (iEos) have been distinguished, based on CD62L and CD101 patterns of membrane expression and lung compartmentalization.[11] Resident eosinophils are thought to contribute to tissue homeostasis in the lung, adipose tissue and gastrointestinal tract, whereas the inflammatory subtypes are associated with inflammatory responses typically of both interstitial and epithelial compartments.[12] Limited data are available in humans; lung iEos have been characterized as CD62L^{low}IL-3R$^+$ cells, even though a similar cell subset has been also identified in peripheral blood (PB).[11,13]

The aim of this study was to investigate the presence of rEos and iEos cell subpopulations in both PB and nasal polyp tissue from severe eosinophilic asthma (SEA) patients with concomitant CRSwNP.

Key messages

- The existence of two sub-phenotypes of circulating and tissue eosinophils in SEA patients with CRSwNP has been confirmed
- CD62L^{low} eosinophils are enriched in nasal polyp tissue and display an increase of activation markers
- Circulating iEos could identify the tissue inflammatory process and become a new biomarker of type 2 inflammation

2 | MATERIALS AND METHODS

2.1 | Study subjects

Twenty-three SEA patients, 14 of whom with concomitant CRSwNP were enrolled; polyp tissue was not available in 2 patients from the latter group, the analysis of polyp tissue being performed on the remaining 12 patients. Fifteen non-severe asthma (NSA) patients, 15 allergic rhinitis (AR) patients without asthma and 15 non-allergic healthy donors (HD) were also enrolled as control groups. The following exclusion criteria were applied: airway infection and/or use of oral corticosteroids during the previous month before enrolment, smoking, and ongoing or previous biological therapy for asthma patients. Demographic and clinical characteristics are summarized in Table 1. All study participants were informed about the study protocol and gave written consent. The study protocol was approved by the Ethics Committee of University Hospital of Careggi, Florence (19295_bio).

2.2 | Reagents

Dulbecco's phosphate buffered saline (PBS) was purchased from Euroclone (Milan, Italy). Low-endotoxin RPMI 1640 medium (VLE-RPMI 1640, Biochrom AG, Germany) was supplemented with 2 mM L-glutamine, 2 mM 2-mercaptoethanol, 100 U/mL penicillin and 100 μg/mL streptomycin, 1% non-essential amino acids and 1% sodium pyruvate (all from Sigma Chemical Co, Milan, Italy) (complete medium). Fetal bovine serum (FBS) was purchased from GE Healthcare (Helsinki, Finland). Antibodies and isotype controls for cytometric analysis were purchased from Miltenyi Biotec (Bologna, Italia). Additional reagents are described in the respective sections below.

2.3 | Human eosinophils isolation from peripheral blood, staining and cytometric analysis

Peripheral blood (20 mL) was collected into sterile ethylenediaminetetraacetic acid-containing vacutainer tubes (BD Bioscience, San Jose, CA, USA). Density-gradient centrifugation was performed using Lymphoprep™ (STEMCELL Technologies): 20 mL of whole blood were carefully layered over 20 mL of reagent in a 50 mL conical tube and centrifuged at 600× g force for 30 min at room temperature. The supernatant was discarded, and the bottom layer with granulocytes and erythrocytes was collected. To remove the erythrocytes from the cell suspension, they were lysed by adding one volume of cell suspension with 10 volumes of 1× Red Blood Cell Lysis Solution (Miltenyi Biotec) according to the manufacturer's instructions. Isolated granulocytes were counted, and their viability and quantity in peripheral blood were assessed. 10^6 cells were stained with the panel of monoclonal antibodies (mAbs) towards surface markers (recommended antibody dilution is 1:50 in a final volume of 100 μL) reported in Table S1. The cells were then analysed with "BD FACSCanto II" flow cytometer (Becton-Dickinson, Franklin Lakes, NJ USA) and BD FACSDiva software.

2.4 | Analysis on nasal polyps

Samples from patients suffering from CRSwNP were obtained during functional endoscopic sinus surgery (FESS) procedures.

Three polyp tissue samples were taken from each patient: (i) one was embedded in paraffin and then processed for histology, (ii) one was frozen and subsequently processed for quantitative mRNA

TABLE 1 Demographic and clinical characteristics of patients at enrolment

	HD	AR	NSA	SEA
Number, *n*	15	15	15	23
Gender, M/F	5/10	5/10	2/13	12/11
Age, years	41.9 ± 3.8	41.1 ± 5.1	39.5 ± 3.4	53.7 ± 2.3[a]
Atopy, *n* (%)	0 (0%)	15 (100%)	12 (80%)	15 (65%)
CRSwNP, *n* (%)	-	-	0 (0%)	14 (61%)
ACT	-	-	22.5 ± 0.5	19.3 ± 1[b]
SNOT22	-	11.8 ± 1.9	14 ± 2.8	40.1 ± 3.9[c]
Blood eosinophils count, *n*/μL	109 ± 22	144 ± 24	205 ± 3	604 ± 79[d]
ECP, μg/L	18.5 ± 2.6	18.5 ± 3.8	18.8 ± 2.8	76 ± 14.1[e]
Total IgE, kU/L	53.7 ± 20.5	175 ± 49.3	183 ± 34.5	235 ± 59.8

Note: All values are reported as mean (±standard error of the mean).

Abbreviations: ACT, asthma control test; AR, allergic rhinitis; CRSwNP, chronic rhinosinusitis with nasal polyps; ECP, eosinophilic cationic protein; HD, healthy donors; NSA, non-severe asthma; SEA, severe eosinophilc asthma; SNOT22, 22-item SinoNasal Outcome Test.

a$p < .05$ SEA vs all groups.

b$p < .05$ SEA vs NSA.

c$p < .001$ SEA vs all groups.

d$p < .05$ SEA vs all groups.

e$p < .005$ SEA vs all groups.

analysis, (iii) one was transferred to a GentleMACs Tube (Miltenyi Biotec) with 10 mL of complete medium and 2% FBS and then processed for cell isolation.

2.5 | Nasal polyp histology

Nasal polyp tissue was fixed in 4% formalin, paraffin-embedded and cut in 5 μm thick sections. H&E stained polyp slices were subjected to histopathological examination for the presence of eosinophils. Cells were counted in 10 random fields by an observer blinded to the experimental conditions and each polyp tissue sample was scored according to the absolute count of infiltrating eosinophils per high power field (hpf) (a, <10 eosinophils (eos)/hpf; b, 10–25 eos/hpf; c, 26–50 eos/hpf and d, >50 eos/hpf).

2.6 | RNA extraction and quantitative real-time RT-PCR

Total RNA from snap-frozen polyp was extracted using TRIzol reagent (RNAwiz; Invitrogen, Milan, Italy). cDNA from each sample was synthesized with TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, Calif), according to the manufacturer's protocol.

Real-time PCR was performed with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems), according to the manufacturer's instructions. All PCR amplifications were performed on MicroAmp optical 96-well reaction plate with Taqman Gene Expression Master Mix and with Predesigned TaqMan Gene Expression Assays (Applied Biosystems). Each assay was carried out in duplicate and included a no-template sample as a negative control. Beta-actin was used as a housekeeping gene for normalization.

2.7 | Human eosinophils isolation from nasal polyps, staining and cytometric analysis

Nasal polyp specimens were processed in the Gentle MACs (Miltenyi Biotec) by a mixing step. The gentle MACs tube with tissue was centrifuged for 5 min at 300 g; the supernatant was discarded and replaced by complete medium containing 2 mg/mL collagenase (Worthington, USA) and 0.04 mg/mL DNAse I (Roche Diagnostics, Belgium). After the incubation of 45 min at 37°C, the second mixing step in the GentleMACs was performed. Cell suspension was dispersed and passed through a 70 μm cell strainer (Miltenyi Biotec). Red blood cells were lysed by adding one volume of cell suspension with 10 volumes of 1x Red Blood Cell Lysis Solution (Miltenyi Biotec) according to the manufacturer's instructions. Cells were counted and stained with the panel of mAbs towards the surface markers reported in Table S1. Cells were gated as described in Figure S1 (panel A).

The cells were then analysed with "BD FACSCanto II" flow cytometer (Becton-Dickinson, Franklin Lakes, NJ USA) and BD FACSDiva software.

2.8 | Serum cytokines and chemokines dosage

Serum obtained from the centrifugation of peripheral blood were assayed for IL-3, IL-5, IL-13, GM-CSF, eotaxin-1 and -3 by using

commercially available ELISA kits (R&D Systems). The limits of detection for IL-3, IL-5, IL-13, GM-CSF and Eotaxins 1–3 were 31.3, 3.9, 125, 7.8, 15.6 and 3.1 pg/mL, respectively.

2.9 | Statistical analysis

The results are presented as mean values ± standard error of the mean (SEM). Statistical analysis was performed using Mann-Whitney *U* test or ANOVA associated with post-hoc Tukey test, according to the data type. Correlations were analysed by the Spearman correlation test. A p-value < .05 was considered statistically significant.

3 | RESULTS

3.1 | Increase of circulating CD62L^{low} eosinophils in SEA patients

Severe eosinophilic asthma patients were characterized by a higher number of eosinophils at the differential blood count as compared with NSA, AR and HD groups (Figure 1A). Accordingly, a significant increase in CD45^{+}Siglec8^{+}CD16^{-} circulating cells was found in SEA patients (Figure 1B). Flow cytometry analysis (FACS) of Siglec8^{+} cells revealed the presence of two sub-phenotypes based on the expression of CD62L. Both populations (CD62L^{bright} and CD62L^{low}) were also detectable in control groups, including HD. However, a significantly higher percentage of the CD62L^{low} sub-phenotype was found in SEA patients as compared with control groups (Figure 1C–E).

3.2 | Surface profile expression of CD62L^{low} and CD62L^{bright} blood eosinophils in SEA patients

To gain deeper insight into the characteristics of both Siglec8^{+}CD62L^{low} and Siglec8^{+}CD62L^{bright} cells, we evaluated the expression of several surface markers with FACS analysis in SEA patients. In Figure S2 (panel B) a representative FACS analysis on CD62L^{low} cells is illustrated. A lower percentage of CD62L^{low} cells expressed CD125 (IL-5R), CRTH2, CD86 and CD28, when compared with CD62L^{bright} cells. On the other hand, a significantly higher percentage of CD62L^{low} cells expressed CCR3 and CD69 (Figure 2A). Regarding CRTH2, CD28 and CD86 we also observed a significant reduction of mean fluorescence intensity (MFI) in CD62L^{low} cells (Figure 2B). Similarly, CD62L^{low} cells expressed lower levels of CD101 and the adhesion molecule very late antigen (VLA)-4. Of note, we did not observe any difference of CD123 (IL-3R), CD101 and VLA-4 expression between CD62L^{bright} and CD62L^{low} in SEA patients.

Finally, no differences were observed in the percentage and the surface profile expression of both CD62L^{low} and CD62L^{bright} in SEA patients with and without NP (Figure S2).

3.3 | Enrichment of CD62L^{low} IL-3R^{+} eosinophils in polyp tissue

Eosinophils are primarily tissue-dwelling leucocytes and polyp tissue represents an ideal setting for evaluating their subphenotypes, taking into account the typical eosinophilic nature of CRSwNP. Based on this premise, we further analysed the SEA patients with CRSwNP (n = 12) included in our study population. All of them, but one, displayed an eosinophilic phenotype of NP at the histological analysis (>10 eos/hpf); the quantification of the eosinophilic infiltration showed that a high proportion of patients' samples (5/11, 45.4%) had an eosinophil count >50 cells/hpf. We found a significant positive correlation between peripheral and polyp tissue Siglec8^{+} cells at the FACS analysis (Figure 3A). More importantly, with respect to the characterization of eosinophil subphenotypes, a high percentage of eosinophils in polyp tissue displayed a Siglec8^{+}CD62L^{low} phenotype and this proportion was significantly higher than that observed in corresponding PB samples (42.3 ± 6.1% vs. 12.3 ± 3.5%, p < .001) (Figure 3B). Of note, we observed that the greater the percentage of tissue Siglec8^{+}CD62L^{low} cells, the higher the grading of eosinophil tissue infiltration though with borderline statistical significance probably due to the small sample size (Figure 3C).

We then compared the expression of other surface markers on CD62L^{low} eosinophils obtained from NP with the corresponding cell population of PB. We found a significant increase of MFI expression of CD125, CD101, CD69 and CD86 and a decrease in CCR3 and VLA-4 in NP in comparison to PB (Figure 3D).

3.4 | Eotaxin-3 mRNA expression correlates with CD62L^{low} cells in nasal polyp tissue

To explore the mechanisms underlying the enrichment of CD62L^{low} tissue eosinophils, we analysed serum levels and NP tissue mRNA expression of IL-3, GM-CSF, IL-5, IL-13 and eotaxin-1 and −3. The analysis of sera did not allow to detect any cytokines in any group of patients, while both eotaxins were clearly detectable: notably, a significant positive correlation was found between their serum levels (eotaxin-3: 42.8 ± 2.4 pg/mL; eotaxin-1: 389 ± 21.4 pg/mL - r = 0.59; p < .05). However, serum eotaxin-3 and eotaxin-1 did not correlate neither with total blood eosinophil count nor with circulating CD62L^{low} eosinophils subset. Similarly, no correlation was found between serum levels of eotaxins and eosinophils (total and CD62L^{low}) in NP (Figure S3A–D). In agreement with what was observed in serum, IL-3, IL-5, GM-CSF and IL-13 mRNA expression was not detectable in NP tissue, whereas all samples tested positive for both eotaxin-3 and eotaxin-1 mRNA (Figure S3 panel E). A positive correlation (r = 0.75, p < .005) between eotaxin-1 and eotaxin-3 mRNA expression was found also at the polyp level (Figure 4A). Noteworthy, a significant correlation between the degree of eotaxin-3 mRNA expression in tissue and the percentage of CD62L^{low} cells in NP was found, but not with total

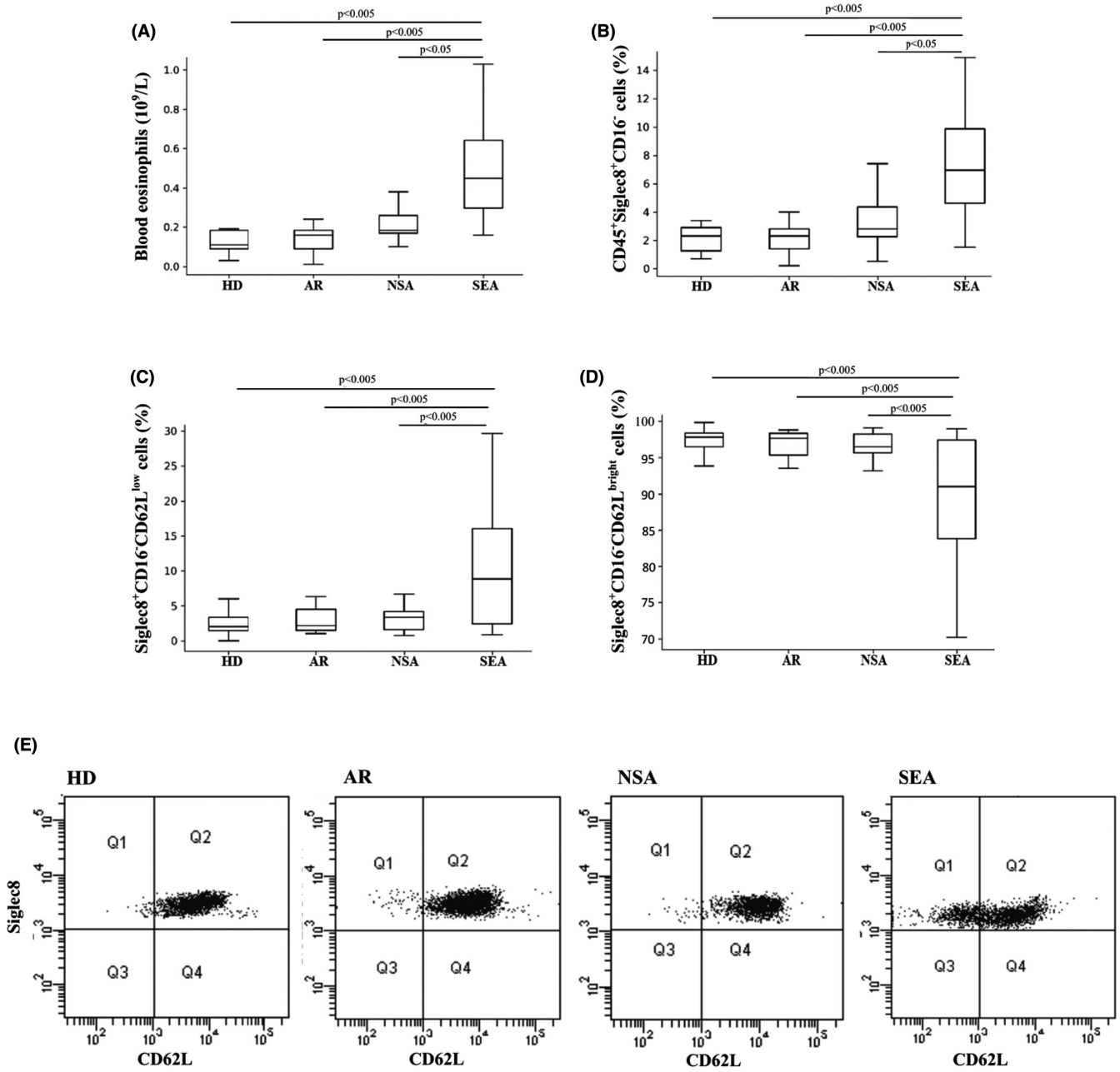

FIGURE 1 Blood eosinophil sub-phenotypes (A) Blood eosinophil count in severe asthma patients (SEA) and control groups (healthy donors, HD; allergic rhinitis, AR; non-severe asthma, NSA); horizontal bars represent median values, $p < .0005$. (B) Percentage of $CD45^{+}Siglec8^{+}CD16^{-}$ cells in study populations, $p < .00001$. (C) Percentage of $CD62L^{low}$ cells among Siglec8+ cells in study populations, $p < .0005$. (D) Percentage of $CD62L^{bright}$ cells among Siglec8+ cells in study populations, $p < .0005$. (E) Representative dot plot for $CD62L^{low}$ and $CD62L^{bright}$ cells among $Siglec8^{+}$ cells in each study group. *p* values are referred to preliminary analysis performed with ANOVA test; *p* values shown on figures are referred to Tukey test

tissue eosinophils ($CD45^{+}Siglec8^{+}$ cells) (Figure 4B). We also found a similar trend for eotaxin-1 mRNA, even though it did not reach statistical significance (Figure 4C).

4 | DISCUSSION

This study was aimed at investigating the presence of different Eos sub-phenotypes in PB and NP tissue from SEA patients with or without CRSwNP. Similar to rEos ($SiglecF^{int}CD62L^{+}CD101^{low}$) and iEos ($SiglecF^{high}CD62L^{-}CD101^{high}$) observed in mice, our FACS analysis confirms the presence of two circulating eosinophil subsets distinguishable on the basis of CD62L expression in humans, including HD. In all studied groups, most circulating eosinophils express the $CD62L^{bright}$ phenotype, but a marked increase in $CD62L^{low}$ cells was observed in SEA, as compared with the three control groups (NSA, RA and HD) where few circulating $CD62L^{low}$ cells were found. More importantly, an enrichment of

CD62L^{low} cells occurs in the nasal tissue of SEA patients with CRSwNP. In agreement with already published data, we'll refer to CD62L^{low} cells as iEos and to CD62L^{bright} as rEos.[11,13]

Our data obtained from blood are at odds with those previously reported by other authors who described a lower proportion of circulating iEos in SEA patients compared with mild allergic asthmatics and HD, and hypothesized that such finding could be a consequence of a major recruitment of these inflammatory cells at the tissue level.[13] This discrepancy might be due to the different methods used in our study as well as by the heterogeneity of baseline clinical characteristics and ongoing drug regimens of enrolled patients.

CD62L^{low} cells were characterized by a reduced expression of CRTH2, of costimulatory molecules (CD28 and CD86) and of IL-5R (CD125), and conversely by an increased expression of the activation marker CD69 and of the receptor for eotaxin-3 (CCR3). The down-regulation of CRTH2 expression by iEos might be explained by its internalization and degradation due to the ongoing prostaglandin D2 stimulation which occurs during type-2 inflammation.[14,15] Similarly, the reduced expression of IL-5R that we observed is in agreement with the previous data showing that type 2 cytokines induce the downregulation of their receptors on Eos.[16] The decreased expression of costimulatory molecules may be the consequence of an ongoing inflammatory stimulation of Eos cells, too. Overall, the modulation of many surface expression molecules and receptors on eosinophils depends on microenvironmental conditions.[16]

One of the most important aspects of our study is the analysis of eosinophils populations at the NP tissue level in patients with SEA, and the comparison between circulating and tissue Eos in the same patients. Results confirm the presence of two phenotypically distinct Eos populations also in NP tissue. Of note, not only iEos, as expected, but also rEos have been found in tissue even if to date their role in NP tissue remains to be defined. Our data underscore that, when type 2 inflammation is active as in eosinophilic CRSwNP

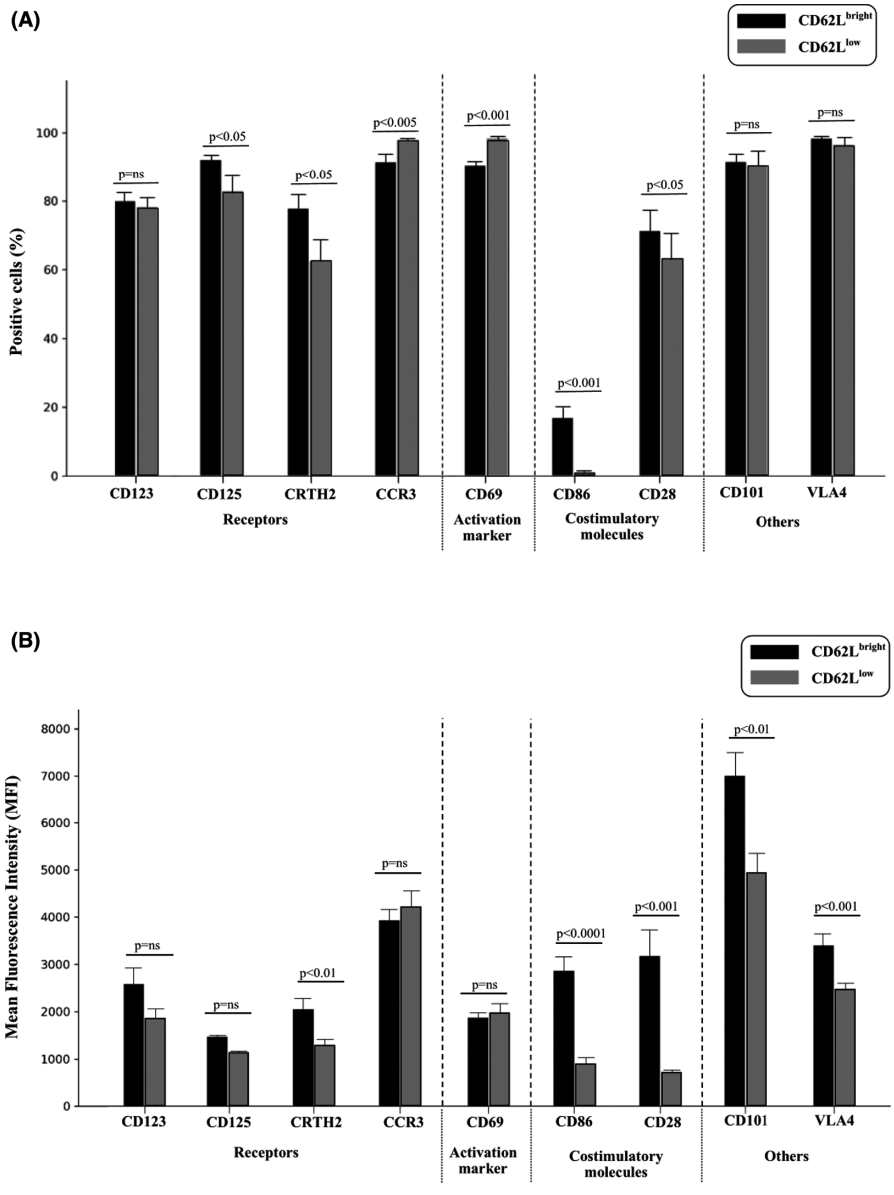

FIGURE 2 Expression of surface markers on blood eosinophil subphenotypes (A) Percentage of positive cells (±mean standard error) among Siglec8^{+}CD62L^{low} and Siglec8^{+}CD62L^{bright} cells. (B) Surface markers expression (expressed as MFI ± mean standard error) on blood Siglec8^{+}CD62L^{low} and Siglec8^{+}CD62L^{bright} cells

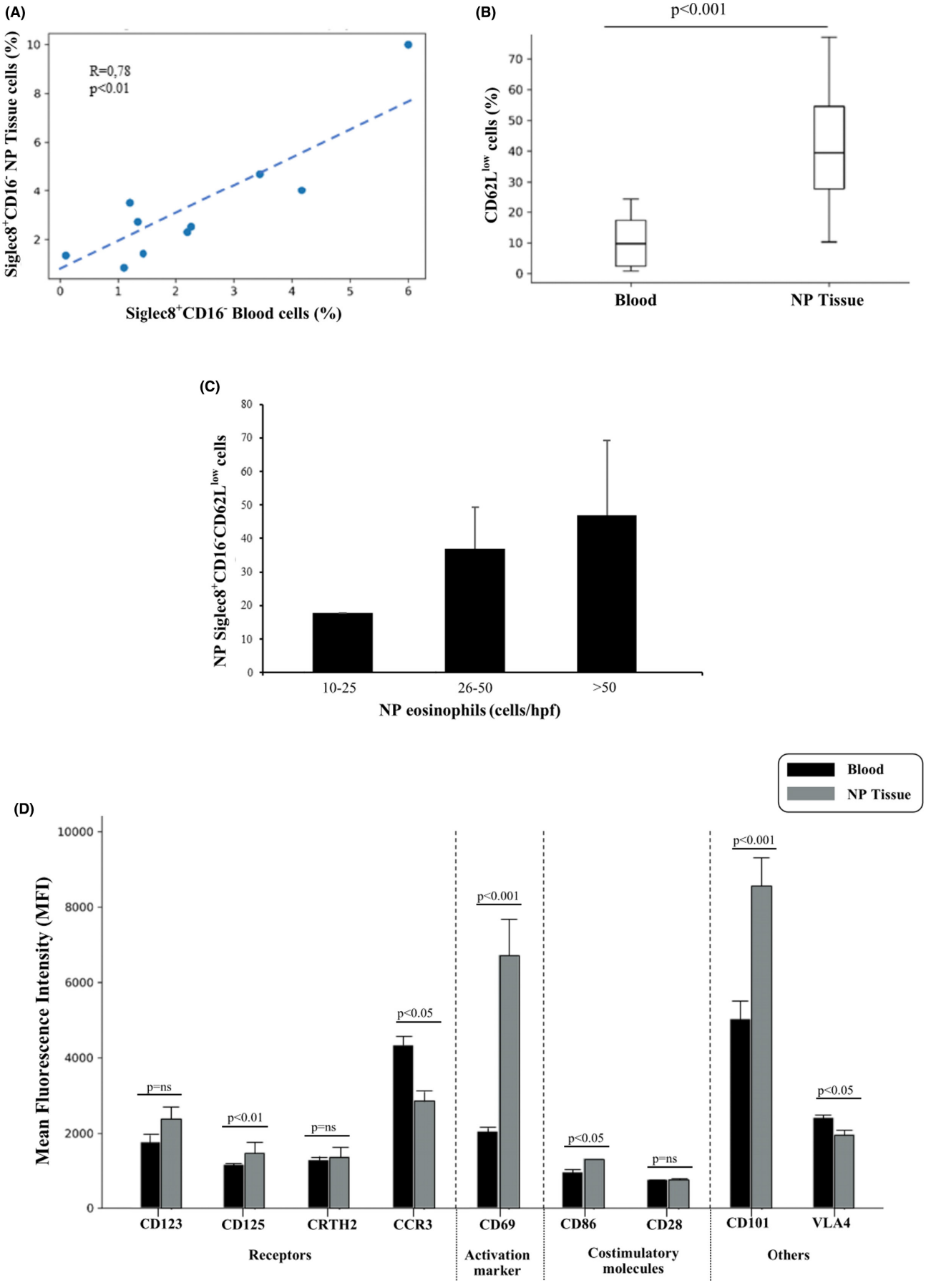

FIGURE 3 Eosinophil sub-phenotypes in nasal polyp tissue from patients with SEA and CRSwNP. (A) Correlation between the percentage of Siglec8$^+$CD16$^-$ cells in peripheral blood and the percentage of Siglec8$^+$CD16$^-$ cells in nasal polyp tissue as quantified by flow cytometry. (B) CD62L^{low} eosinophils in blood and nasal polyp tissue among CD45$^+$Siglec8$^+$ cells. (C) Surface markers expression (expressed as MFI) on Siglec8$^+$ CD62L^{low} cells obtained from nasal polyps

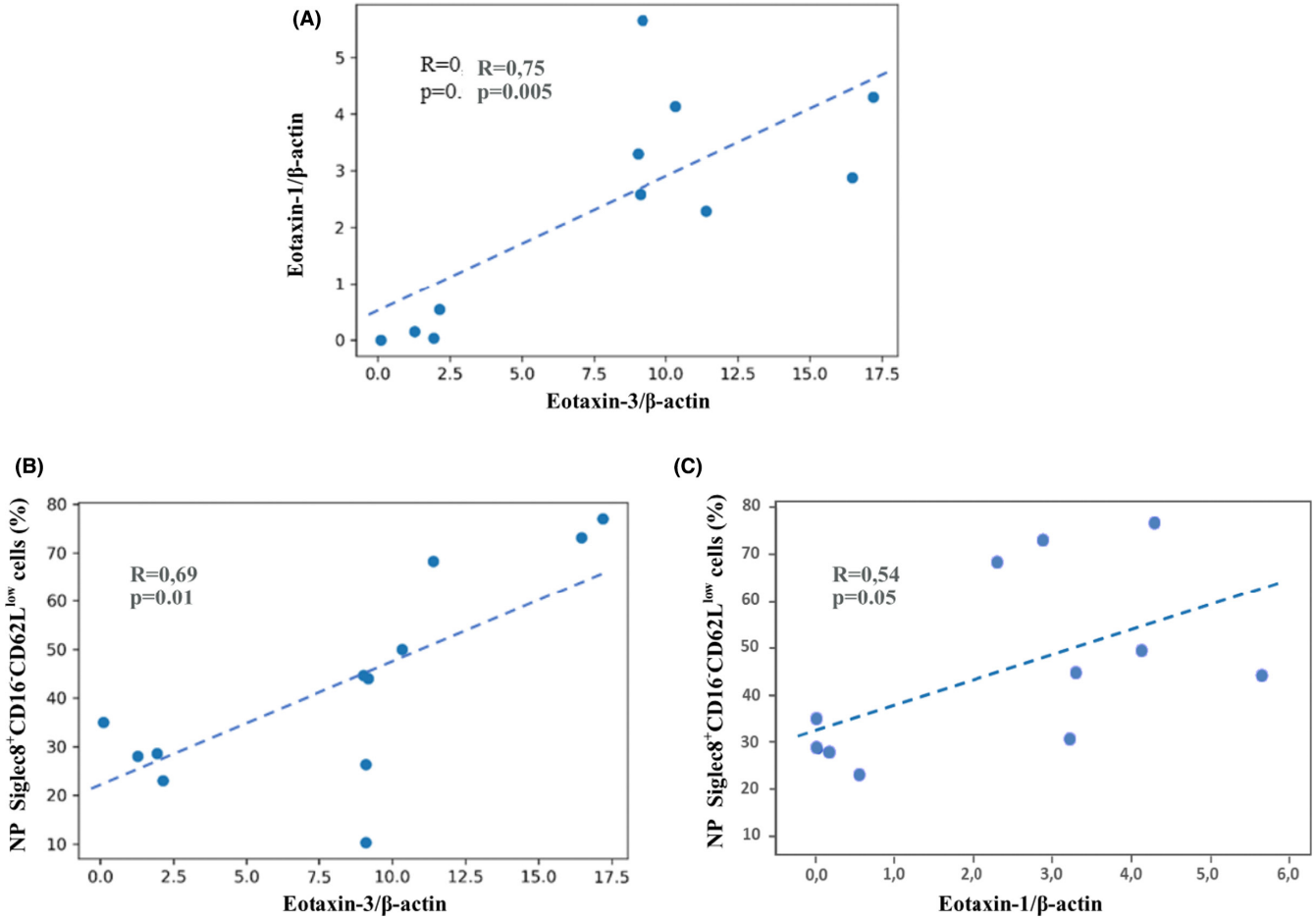

FIGURE 4 CD62L^{low} cells correlate with Eotaxin-3 mRNA expression in polyp tissue. (A) Correlation between eotaxin-1 and eotaxin-3 mRNA expression in nasal polyp tissue. (B) Correlation between eotaxin-3 mRNA expression and the percentage of tissue Siglec8$^+$CD62L^{low} cells. (C) Correlation between eotaxin-1 mRNA expression and the percentage of tissue Siglec8$^+$CD62L^{low} cells. The amounts of eotaxins mRNA expression were normalized with endogenous control β-actin

phenotype, a marked enrichment of iEos cells, and a consensual decrease of rEos cells, occurs in tissue, in comparison with the peripheral blood compartment. Interestingly, the grading of eosinophils infiltration evaluated at the histological analysis tends to reflect the amount of tissue iEos accumulation. Although to be confirmed in a wider sample of patients, our results suggest that the traditional histological evaluation may represent an inflammation surrogate sustained by iEos.

Tissue iEos are characterized by a more pronounced activation state, as shown by the expression of high levels of CD69, a marker of eosinophil activation.[17,18] Our data are in agreement with the results of Yun et al. that found a 30-fold increase in CD69 expression on tissue eosinophils.[19] Of note, it has been shown that IL-3 and IL-5 and GM-CSF induce CD69 expression on peripheral blood eosinophils.[19] In addition, our data suggest that upon tissue recruitment, the expression of the costimulatory molecule CD86 increases on iEos. The exact biological role of CD86 expression by iEos is not fully understood, but it is important to note that co-stimulation provided by CD80/CD86 molecules is involved in the successful presentation of peptides and protein antigens to T lymphocytes by eosinophils.[20] It has been shown that CRSwNP is characterized by a predominant type 2 inflammatory microenvironment with increased levels of mediators including IL-4, IL-5, IL-13 and eotaxins.[19,21,22] The high expression of IL-5R and IL-3R on tissue iEos in our cases suggests a role for both cytokines in the activation and prolonged survival of these cells at this level. To our knowledge, no previous studies have analysed the expression of IL-5R and IL-3R on eosinophils collected from NP tissue, so additional studies must focus on this aspect, including the comparison between NP and bronchial tissue. In our analysis the lack of mRNA

expression in nasal tissue of all the cytokines studied seems to be in contrast with an eosinophilic type 2 inflammatory process; the treatment with inhaled cortico-steroids that all patients were receiving may have influenced these findings.

The eotaxins/CCR3 receptor axis plays an important role in the recruitment of eosinophils in nasal polyps[23]; indeed, a positive correlation between the eosinophilic inflammation and eotaxin levels in nasal polyp extracts was previously found.[24] In our study, eotaxins expression directly correlated with iEos infiltration thus supporting the role of eotaxins/CCR3 axis in the nasal recruitment of iEos from PB compartment. Although eotaxin-1 and eotaxin-3 may have differential rules on eosinophils recruitment because eotaxin-3 seems to be more effective to induce eosinophils' tissue migration,[25] our data are not sufficient to demonstrate a differential role in the NP context. Trend for eotaxin-1 is similar to that obtained for eotaxin-3, probably limited by the power of our casisistics due to the low number of cases.

The reduced tissue expression of CCR3 detected in our cases is in agreement with previously published data obtained in both BAL and NP tissue[26] and could be explained by a downregulation of the receptor due to internalization induced by exposure to its agonist (eotaxin). We suggest that CRSwNP in SEA patients could benefit from novel therapeutic strategy targeting CCR3/eotaxins axis on inflammatory eosinophils. However, we cannot rule out the involvement of other chemotactic factors that we have not investigated, which represents a limit of our study. An additional limit is that all patients were receiving inhaled corticosteroids that may have influenced our results. At present, it is unknown whether the phenotypic profile of iEos from the lung and from NP tissue overlap, thus representing a blind spot. Anyway, if this overlap is proven, NP histological analysis might represent a simple tool to define the endotype of lung inflammatory process in patients with SEA and concomitant CRSwNP.

One of the major concerns about eosinophils' subpopulations is represented by the definition of their origin.[27] Specifically, iEos and rEos found in tissue, as well as in blood, could be the same eosinophilic population with just different expression levels of the various molecules in a continuum of activation states, or otherwise different cellular endotypes originated from different bone marrow precursors. A more detailed morphological and functional characterization of these subpopulations would help to provide insights into these aspects.

5 | CONCLUSION

Overall, our findings confirm the existence, also in humans, of two phenotypic subsets of circulating and tissue eosinophils with differential surface expression of CD62L, and suggest that the PB constitutes the likely source of tissue iEos with pathogenic activity. This implicates that the count of circulating iEos could provide an insight into the inflammatory state of both pulmonary and nasal tissues and eventually become a new biomarker of type 2 inflammation, as IL-5 has been shown to cause CD62L downregulation or shedding.[28] Furthermore, circulating levels of iEos might represent a biomarker predictive for the clinical response to an anti-IL-5 therapeutic strategy. Additional confirmatory studies on more expanded case series are needed, aimed at investigating in more depth the circulating and tissue iEos features and their pathophysiological role.

AUTHOR CONTRIBUTIONS

Acquisition of data: A.M., F.N., G.M., F.C. M.A., C.B., L.G.L., V.M., M.P., O.R., P.P., O.G. **Conception and design**: A.V., A.M., E.V. **Analysis and interpretation:** A.V., A.M., E.V., F.N., M.A., F.C., A.P., E.N. **Wrote the manuscript**: A.V., A.M., E.V., F.C. **Approved and edited the manuscript**: A.M., F.N., E.V., G.M., F.C. M.A., C.B., L.G.L., A.P., E.N, V.M., O.R., P.P., O.G., E.M., A.V.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Chiara Bruno https://orcid.org/0000-0002-2506-4452

REFERENCES

1. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 2019;50:975-991.
2. Chaigne B, Terrier B, Thieblemont N, Witko-Sarsat V, Mouthon L. Dividing the Janus vasculitis? Pathophysiology of eosinophilic granulomatosis with polyangitis. *Autoimmun Rev*. 2016;15:139-145.
3. Fujieda S, Imoto Y, Kato Y, et al. Eosinophilic chronic rhinosinusitis. *Allergol Int*. 2019;68:403-412.
4. Gonsalves N. Eosinophilic gastrointestinal disorders. *Clin Rev Allergy Immunol*. 2019;572:272-285.
5. McBrien CN, Menzies-Gow A. The biology of eosinophils and their role in asthma. *Front Med (Lausanne)*. 2017;4:93-106.
6. Luna-Gomes T, Bozza PT, Bandeira-Melo C. Eosinophil recruitment and activation: the role of lipid mediators. *Front Pharmacol*. 2013;4:27-347.
7. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: characteristics and functions. *Front Med (Lausanne)*. 2017;4:101-106.
8. Berek C. Eosinophils can more than kill. *J Exp Med*. 2018;215:1967-1969.
9. Abdala-Valencia H, Coden ME, Chiarella SE, et al. Shaping eosinophil identity in the tissue contexts of development, homeostasis, and disease. *J Leukoc Biol*. 2018;104:95-108.
10. Klion AD, Ackerman SJ, Bochner BS. Contributions of eosinophils to human health and disease. *Annu Rev Pathol*. 2020;15:179-209.
11. Mesnil C, Raulier S, Paulissen G, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest*. 2016;126:3279-3295.
12. Wechsler ME, Munitz A, Ackerman SJ, et al. Eosinophils in health and disease: a state-of-the-art review. *Mayo Clin Proc*. 2021;96:2694-2707.
13. Januskevicius A, Jurkeviciute E, Janulaityte I, Kalinauskaite-Zukauske V, Miliauskas S, Malakauskas K. Blood eosinophils subtypes and their survivability in asthma patients. *Cells*. 2020;9:1248-1265.
14. Hamada K, Yamada Y, Kamada Y, et al. Prostaglandin D2 and interleuckin-5 reduce CRTH2 surface expression on human eosinophils. *Allergol Int*. 2004;53:179-184.

15. Gregory B, Kirchem A, Phipps S, et al. Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor alpha-chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor alpha expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor alpha expression. *J Immunol*. 2003;170:5359-5366.
16. Kanda A, Yun Y, Bui DV, et al. The multiple functions and subpopulations of eosinophils in tissues under steady-state and pathological conditions. *Allergol Int*. 2021;70(1):9-18.
17. Yun Y, Kanda A, Kobayashi Y, et al. Increased CD69 expression on activated eosinophils in eosinophilic chronic rhinosinusitis correlates with clinical findings. *Allergol Int*. 2020;69:232-238.
18. Miyata J, Fukunaga K, Kawashima Y, et al. Dysregulated fatty acid metabolism in nasal polyp-derived eosinophils from patients with chronic rhinosinusitis. *Allergy*. 2019;74:1113-1124.
19. Walsh GM, Williamson ML, Symon FA, Willars GB, Wardlaw AJ. Ligation of CD69 induces apoptosis and cell death in human eosinophils cultured with granulocyte-macrophage colony-stimulating factor. *Blood*. 1996;87:2815-2821.
20. Kambayashi T, Laufer TM. Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? *Nat Rev Immunol*. 2014;14:719-730.
21. Stevens WW, Ocampo CJ, Berdnikovs S, et al. Cytokines in chronic rhinosinusitis. role in eosinophilia and aspirin-exacerbated respiratory disease. *Am J Respir Crit Care Med*. 2015;192:682-694.
22. Van Crombruggen K, Zhang N, Gevaert P, Tomassen P, Bachert C. Pathogenesis of chronic rhinosinusitis: inflammation. *J Allergy Clin Immunol*. 2011;128:728-732.
23. Sabroe I, Artnell A, Jopling LA, et al. Differential regulation of eosinophil chemokine signaling via CCR3 and non-CCR3 pathways. *J Immunol*. 1999;162:2946-2955.
24. Felton JM, Bouffi C, Schwartz JT, et al. Aiolos regulates eosinophil migration into tissues. *Mucosal Immunol*. 2021;14(6):1271-1281. doi:10.1038/s41385-021-00416-4
25. Provost V, Larose M-C, Langlois A, Rola-Pleszczynski M, Flamand N, Laviolette M. CCL26/eotasin-3 is more effective to induce migration of eosinophils of asthmatics than CCL11/eotaxin-1 and CCL24/eotaxin-2. *J Leukoc Biol*. 2013;94:213-222.
26. Liu LY, Jarjour NN, Busse WW, Kelly EA. Chemokine receptor expression on human eosinophils from peripheral blood and bronchoalveolar lavage fluid after segmental antigen challenge. *J Allergy Clin Immunol*. 2003;112:556-562.
27. Van Hulst G, Bureau F, Desmet CJ. Eosinophils as drivers of severe eosinophilic asthma: endotypes or plasticity? *Int J Mol Sci*. 2021;22:10150-10167.
28. Johansson MW. Activation states of blood eosinophils in asthma. *Clin Exp Allergy*. 2014;44:482-498.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Matucci A, Nencini F, Maggiore G, et al. High proportion of inflammatory CD62L^{low} eosinophils in blood and nasal polyps of severe asthma patients. *Clin Exp Allergy*. 2023;53:78–87. doi:10.1111/cea.14153