Peripheral blood intermediate monocyte protease-activated receptor-2 expression increases during asthma exacerbations and after inhalation allergen challenge

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ABSTRACT

Background: Myeloid cells, especially dendritic cells and macrophages, play important roles in asthma pathophysiology. Monocytes (Mo) and macrophages express protease-activated receptor-2 (PAR-2), a proinflammatory serine protease receptor implicated in the pathophysiology of allergic airway inflammation. We have revealed that patients with severe asthma and those with a history of frequent asthma exacerbations exhibit increased PAR-2 expression on peripheral blood monocytes.

Objective: To determine PAR-2 expression on peripheral blood intermediate monocytes (IMMo) in subjects with increased PAR-2 expression on peripheral blood monocytes.

Methods: A total of 16 adults who presented to the emergency department with asthma exacerbations were recruited and underwent inhalation allergen challenge after providing an informed consent. Immune cell profiling was performed by whole blood flow cytometry in both groups of patients.

Results: PAR-2 expression in peripheral blood IMM0 increased in patients with an asthma exacerbation compared with those with stable disease, but this expression decreased after treatment of the asthma exacerbation. Subjects with mild asthma had an increase in percentages of IMM0 expressing PAR-2 after an allergen challenge.

Conclusion: Increased PAR-2 expression on Mo during periods of increased airway inflammation may initiate a positive feedback loop leading to systemic inflammatory changes.

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Introduction

Asthma is a chronic inflammatory disease of the airways that affects children and adults across the globe. 1 It is an important cause of impaired quality of life for patients and contributes to health care costs through clinic, emergency department (ED) hospital, and inpatient encounters. 2 In particular, ED visits for asthma exacerbations are an important contributor to high health care costs, 3 especially in children. Understanding the pathogenesis of asthma exacerbations and the role of airway inflammation during asthma exacerbations may allow the development of novel therapeutic interventions.

Among myeloid cells, macrophages and dendritic cells (DCs), particularly their role in asthma, have been extensively studied and
reviewed; however, the role of monocytes (Mo) is still poorly understood. Monocytes circulate in the blood with a short half-life followed by migration into tissues, especially inflamed tissues, where they can maintain their monocyctic characteristics and functions, differentiate into macrophages, or give rise to DC. Depletion of circulating Mo in mice suggests that these cells have a proinflammatory role in asthma, in contrast to alveolar macrophages that, in certain models, are anti-inflammatory. In particular, depletion of circulating Mo decreases the number of eosinophils infiltrating into the lungs, the levels of Th1, Th2, and Th17 cytokines, and airway hyperresponsiveness. This effect of Mo could be mediated through either direct interleukin (IL)-5 secretion or their ability to increase the capacity of T lymphocytes to produce IL-5. Alternatively, another important eosinophil survival factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), can also be released by Mo. The above-mentioned in vivo observations, however, have not been validated with human Mo and/or in human asthma.

Protease-activated receptor-2 (PAR-2), a proinflammatory receptor for serine proteases, is expressed on multiple cells, and its activation has been implicated in the pathophysiology of asthma. Monocytes are the main cell expressing PAR-2 in peripheral blood. Human peripheral blood Mo are heterogeneous. Mo are classified into CD14++CD16− (classical—Cmo), CD14++CD16+ (intermediate—IMMo), and CD14−CD16+ (nonclassical—NCMo), subsets, which can be identified and characterized separately by flow cytometry. Very little is known on the presence, functions, and activation status of Mo subsets in human asthma. The information presented previously suggests that Mo may play important roles in asthma through various mechanisms, including release of proinflammatory mediators and facilitation of recruitment, differentiation, and transformation into DC and macrophages of lymphocytes and eosinophils in the airways.

We have revealed that IMMo of patients with severe asthma exhibit higher surface PAR-2 expression compared with those from subjects with mild and moderate disease, whereas we found no difference in PAR-2 expression on CMo between patients with asthma of varying severities. That study raised the question whether increased PAR-2 expression was dependent on increased inflammation in patients with severe asthma or was associated with other determinant of disease severity.

To answer this question, here we evaluated PAR-2 expression on peripheral blood Mo subsets in patients experiencing asthma exacerbations (increased inflammation and loss of asthma control independent of severity of the disease) or owing to an inhalation allergen challenge (increased airway inflammation in a background of mild asthma).

**Methods**

**Subjects**

Patients aged more than 18 years who presented to the ED of University of Alberta Hospital with an asthma exacerbation, according to ED attending physician diagnosis, were recruited by trained research nurses. Recruitment took place between March 2016 and December 2018. The study was approved by the University of Alberta institutional review board (Pro00052055), and all patients provided written informed consent.

After providing consent, patients were interviewed, charts were reviewed, and a blood sample was obtained, treated as per ED protocol (including at least a 5-day course of oral corticosteroids) without any study-specific intervention, and patients were asked to return to the clinic 14 days after the index ED visit. In addition to the clinical data collected in ED visit, spirometry was obtained, and patients completed the asthma control questionnaire (ACQ6) and the asthma quality-of-life questionnaire (AQLQ) during both the exacerbation (on presentation to the ED) and treatment (14 days after) visits.

on clinical characteristics of these patients have been presented elsewhere. Data were compared between the 2 visits. Data from the asthma exacerbation visit were also compared with data obtained from a cohort of patients with stable asthma we reported previously. Briefly, these patients with stable asthma attended 4 clinic visits in 3-month intervals and had no substantial worsening of their symptoms (exacerbation) in between the visits.

To study the effect of inhalation allergen challenge on peripheral blood myeloid cells, patients with mild asthma (ie, treated only with as-needed bronchodilators) were recruited between March 2016 and January 2019 and inhalation allergen challenges were performed in accordance with the University of Alberta protocol that is based on the description by O’Byrne et al. This part of the study had been approved separately by the University of Alberta health review ethics board (Pro00069879). All recruited individuals provided written informed consent. Briefly, standard allergen extracts were prepared by study personnel and administered to patients by inhalation. A filter was positioned on the exhalation side of the valve to prevent the allergen from being nebulized or exhaled into the room. The starting concentration of allergen extract for inhalation during screening was up to 4 doubling concentrations below the concentrations that are predicted to cause a 20% decline in forced expiratory volume in 1 second (FEV1), estimated from the methacholine and allergen skin test results as described. FEV1 was measured 10, 20, 30, 45, 60, 90, and 120 minutes after allergen inhalation and then each hour until 7 hours after allergen inhalation.

The early asthmatic response (EAR) was defined as the largest decline in FEV1 within 3 hours after allergen inhalation, and the late asthmatic response (LAR) as the largest decline in FEV1 between 3 and 7 hours after allergen inhalation. For each subject, both EAR and LAR were recorded and the trapezoidal area under the curve was determined for the early (0-3 hours) and late (3-7 hours) responses by calculating the area of the percent decline in FEV1 vs time response.

**Profiling of Peripheral Blood Mo and DC**

Blood was collected in sodium heparin tubes (BD Bioscience, Mississauga, Ontario, Canada), and whole blood staining for flow cytometry was performed at room temperature. The following antibodies were used for cell identification: anti-CD14 (PerCP-Cy5.5, clone 61D3; eBioscience), anti-CD16 (Phycoerythrin, clone 3G8; BD Bioscience), biotinylated anti-CD19 (allophycocyanin, SAM-11, Santa Cruz), Lin-1 (consisting of anti-CD3, anti-CD14, anti-CD16, anti-CD19, anti-CD20, and anti-CD56) (FITC, BD Bioscience), anti-HLA-DR (PerCP-Cy5.5, clone G46-6; BD Bioscience), anti-CD11c (BV510, clone B-ly6; BD Bioscience), anti-CD123 (BV421, clone 6H6; eBioscience), and anti-Fc epsilon receptor I (FcRn1) alpha (phycoerythrin, clone AER-37 [CRA1]; eBioscience).

Flow cytometry data were collected on a BD LSR Fortessa-II (BD Bioscience, California) using FACS Diva software (BD Bioscience, California) and gates set in accordance with the profiles of the isotype control and negative control beads. Results were analyzed using FlowJo (TreeStar, Ashland, Oregon). Gating strategies to identify Mo and DC subsets are found in eFigure 1 and eFigure 2, respectively. Details of the flow cytometry protocol have been published previously.

**Statistical Analysis**

Values of continuous parameters are reported using median and interquartile range (IQR). Correlations between immune parameters were analyzed using Spearman’s rank correlation coefficient. Values between patients having asthma exacerbation and those with stable asthma (Table 1) were compared using 2-way analysis of variance and were adjusted for current smokers. Change in immune
parameters between the exacerbation and treatment visits was analyzed by nonparametric Wilcoxon rank test (Table 2).

For the Allergen Challenge Study, the overall difference between data from 0-hour, 6-hour, and 24-hour time points was calculated using Friedman test. Pairwise comparisons were then performed using Nemenyi multiple comparisons between interindividual groups (Table 4).

Statistical analyses were performed using Statistical Product and Service Solutions (version 21.1; IBM, Chicago, Illinois) and R (R Core Team, Vienna, Austria).

Results
PAR-2 Expression on Peripheral Blood IMMo During Asthma Exacerbations
To test whether loss of asthma control is associated with changes in PAR-2 expression on peripheral blood Mo, we recruited patients who presented to the ED with an asthma exacerbation and evaluated PAR-2 expression on Mo by flow cytometry. The demographics of this population have been previously reported in detail.19 Briefly, the recruited subjects had a median age of 30 years (IQR, 25-40), body mass index (BMI) of 29.4 (IQR, 23.8-32.2), and 50% were of female sex. Only 12.5% had a history of smoking greater than 10 pack years, whereas 37.5% were current smokers. The median FEV1 (percentages predicted) and the ratio of FEV1 and forced vital capacity (FVC) were 67.2 (IQR, 42.7-77.7) and 63.3 (IQR, 52.6-71.6), respectively. The median ACQ6 score was 2.9 (IQR, 2.2-4.4), whereas the median AQLQ was 4.0 (IQR, 3.3-5.1).

We compared data from these patients who presented with an asthma exacerbation with data from a cohort of patients with stable asthma we recruited and reported previously.19 The demographics of this stable asthma population have been previously reported in detail.19 Briefly, the median age between the asthma exacerbation and stable asthma was significantly different although no difference was observed in biological sex and median BMI. There was significantly a greater number of current smokers in asthma exacerbation compared with stable asthma. Furthermore, patients with asthma exacerbation were more likely to have poor asthma control compared with those with stable asthma and had a lower FEV1 (percentages predicted) and FEV1/FVC ratio.

There was no difference in the percentages of Mo, percentages of IMMo, or percentages of CMo in the peripheral blood between the 2 populations after adjusting for current smoking (Table 1). We adjusted for smoking because the 2 populations had a significant difference in the numbers of current smokers included (previously published article19). Nevertheless, the percentages of IMMo and CMo expressing PAR-2 were higher in patients who presented to the ED

### Table 1
Characteristics of Mo and DC Subsets Between Patients Presenting With an Asthma Exacerbation and Stable Asthma

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exacerbation (N = 16)</th>
<th>Stable (N = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo subsets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Mo in PB</td>
<td>4.68 (4.05-11.04)</td>
<td>7.4 (5.25-10.5)</td>
<td>.73</td>
</tr>
<tr>
<td>Percentage of IMMo in PB</td>
<td>1.13 (0.47-1.39)</td>
<td>1.11 (0.48-2.21)</td>
<td>.54</td>
</tr>
<tr>
<td>Percentage of IMMo expressing PAR-2</td>
<td>15.45 (10.09-26.82)</td>
<td>10.35 (4.25-16.4)</td>
<td>.02</td>
</tr>
<tr>
<td>Percentage of CMo in PB</td>
<td>0.81 (0.63-1.34)</td>
<td>0.85 (0.33-1.37)</td>
<td>.32</td>
</tr>
<tr>
<td>Percentage of CMo expressing PAR-2</td>
<td>6.36 (3.50-12.5)</td>
<td>3.07 (1.48-4.67)</td>
<td>.02</td>
</tr>
<tr>
<td>DC subsets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of DC in PB</td>
<td>0.16 (0.10-0.32)</td>
<td>0.47 (0.26-0.87)</td>
<td>.09</td>
</tr>
<tr>
<td>Percentage of mDC in PB</td>
<td>0.08 (0.03-0.21)</td>
<td>0.24 (0.12-0.63)</td>
<td>.02</td>
</tr>
<tr>
<td>Percentage of pDC in PB</td>
<td>0.05 (0.01-0.17)</td>
<td>0.08 (0.05-0.12)</td>
<td>.25</td>
</tr>
<tr>
<td>Percentage of mDC expressing FcεRI</td>
<td>65.1 (50.0-75.0)</td>
<td>84.2 (37.7-90.9)</td>
<td>.16</td>
</tr>
<tr>
<td>Percentage of pDC expressing FcεRI</td>
<td>63.3 (36.7-80.5)</td>
<td>78.7 (74.3-89.8)</td>
<td>.009</td>
</tr>
<tr>
<td>MFI of FcεRI on mDC</td>
<td>39.5 (9.0-243.2)</td>
<td>48.6 (3.7-115.8)</td>
<td>.30</td>
</tr>
<tr>
<td>MFI of FcεRI on pDC</td>
<td>203.9 (12.7-57.3)</td>
<td>17.6 (8.6-55.3)</td>
<td>.31</td>
</tr>
</tbody>
</table>

### Table 2
Characteristics of Mo and DC Subsets Between the Exacerbation and Treatment Visits for Patients Recruited During an Asthma Exacerbation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exacerbation (N = 16)</th>
<th>Treatment (N = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo subsets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Mo in PB</td>
<td>4.61 (4.10-7.47)</td>
<td>8.79 (6.11-10.31)</td>
<td>.03</td>
</tr>
<tr>
<td>Percentage of IMMo in PB</td>
<td>0.88 (0.40-1.33)</td>
<td>1.12 (0.40-2.52)</td>
<td>.14</td>
</tr>
<tr>
<td>Percentage of IMMo expressing PAR-2</td>
<td>15.45 (10.43-35.35)</td>
<td>8.52 (5.03-13.22)</td>
<td>.01</td>
</tr>
<tr>
<td>Percentage of CMo in PB</td>
<td>0.77 (0.58-1.17)</td>
<td>1.43 (0.86-2.24)</td>
<td>.11</td>
</tr>
<tr>
<td>Percentage of CMo expressing PAR-2</td>
<td>7.29 (2.84-222.95)</td>
<td>3.0 (1.87-6.98)</td>
<td>.07</td>
</tr>
<tr>
<td>DC subsets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of DC in PB</td>
<td>0.16 (0.10-0.31)</td>
<td>0.35 (0.19-0.46)</td>
<td>.01</td>
</tr>
<tr>
<td>Percentage of mDC in PB</td>
<td>0.07 (0.03-0.17)</td>
<td>0.25 (0.07-0.62)</td>
<td>.01</td>
</tr>
<tr>
<td>Percentage of pDC in PB</td>
<td>0.04 (0.01-0.08)</td>
<td>0.10 (0.02-0.19)</td>
<td>.04</td>
</tr>
<tr>
<td>Percentage of mDC expressing FcεRI</td>
<td>57.1 (38.8-72.1)</td>
<td>90.1 (41.4-92.7)</td>
<td>.31</td>
</tr>
<tr>
<td>Percentage of pDC expressing FcεRI</td>
<td>69.1 (43.6-86.8)</td>
<td>81.6 (70.2-100.0)</td>
<td>.12</td>
</tr>
<tr>
<td>MFI of FcεRI on mDC</td>
<td>12.2 (6.3-115.8)</td>
<td>53.4 (8.8-380.6)</td>
<td>.03</td>
</tr>
<tr>
<td>MFI of FcεRI on pDC</td>
<td>19.2 (10.3-66.1)</td>
<td>51.0 (19.2-100.0)</td>
<td>.33</td>
</tr>
</tbody>
</table>

Abbreviations: CMo, classical monocyte; DC, dendritic cell; FcεRI, Fc epsilon receptor I; IMMo, intermediate monocyte; PB, peripheral blood; mDC, myeloid dendritic cell; Mo, monocyte; pDC, plasmacytoid dendritic cell.

NOTE. Data are presented as median (interquartile range). Change in immune parameters between the exacerbation and treatment visits was analyzed by non-parametric Wilcoxon rank test. Bold indicates a statistically significant difference with a P-value less than 0.05.
compared with those with stable asthma (Fig 1A). Patients who presented to the ED were treated by the ED physician according to a standard protocol without any study-specific intervention. Of these subjects, 10 returned 14 days after for repeat evaluation (treatment visit). We have published\textsuperscript{20} that FEV\textsubscript{1} (percentages predicted) increased and ACQ\textsubscript{6} decreased in this population between the exacerbation and treatment visits, indicating treatment of the exacerbation improved both lung function and asthma symptoms. Comparisons of the numbers of Mo subsets and percentage of Mo subsets expressing PAR-2 between the 2 visits are found in Table 2. The percentages of Mo in peripheral blood increased at the treatment visit compared with the exacerbation visit, whereas percentages of IMMo expressing PAR-2 decreased (Fig 1B) and percentages of CMo expressing PAR-2 revealed a trend for decrease (Table 2). Changes in percentages of IMMo and CMo expressing PAR-2 between the 2 visits did not correlate with changes found in FEV\textsubscript{1} or ACQ\textsubscript{6} (data not revealed).

Peripheral Blood DC During Asthma Exacerbations

We then evaluated DC subsets, myeloid DC (mDC, also referred as conventional DC), and plasmacytoid DC (pDC) in peripheral blood and their expression of HLA-DR and high-affinity IgE receptor, also known as Fc\textsubscript{e}RI. Our data indicate that peripheral blood DC of patients with asthma do not express PAR-2 (unpublished observation), and therefore, we did not analyze PAR-2 expression on these cells. The percentages of mDC in peripheral blood, but not pDC or total DC in peripheral blood, were lower in subjects who presented with an asthma exacerbation compared with those with stable asthma (Table 1). Fc\textsubscript{e}RI expression on mDC (percentages of cells expressing Fc\textsubscript{e}RI and median fluorescence intensity [MFI] of Fc\textsubscript{e}RI on mDC) revealed no difference between the 2 groups (Table 1). In contrast, the percentages of pDC expressing Fc\textsubscript{e}RI were lower in patients with an asthma exacerbation, but MFI was not different (Table 1). In patients who presented to the ED, all DC populations increased during the treatment visit compared with the exacerbation visit (Table 2). There was also a trend toward increased Fc\textsubscript{e}RI expression on pDC after treatment, although this difference did not reach statistical significance (Table 2). The MFI of HLA-DR expression on these cells did not change between exacerbation and treatment visits (data not found).

PAR-2 Expression on IMMo After Inhalation Allergen Challenge

To further understand whether the changes in PAR-2 expression by Mo during an asthma exacerbation are dependent on increased airway inflammation, we used a human model characterized by acute induction of allergic airway inflammation. A total of 11 patients with mild asthma were recruited and underwent inhalation allergen challenge. Blood was obtained for analysis before the challenge (0 hour) and then again at 6 and 24 hours after the challenge. Demographics, clinical characteristics, and response to the allergen challenge for the 11 subjects included in our study are found in Table 3. The recruited population had a median age of 23 (IQR, 19-39) years and a median BMI of 23.6 (IQR, 21.8-27.9) and included 45% female subjects. Of the subjects, 7 exhibited isolated EAR whereas 4 had dual response (DR).
The percentage of total Mo, IMMo, or CMo in peripheral blood did not change after the challenge, but there was statistically significant increase in percentages of IMMo expressing PAR-2 after the allergen challenge (Table 4). Post hoc analysis using Nemenyi multiple comparison test revealed statistically significant increase in IMMo expressing PAR-2 from 0 hour to 6 hours and 0 hour to 24 hours, but not from 6 hours to 24 hours (Fig 2). In contrast, the percentages of CMo expressing PAR-2 did not change after allergen challenge.

Peripheral Blood DC After Inhalation Allergen Challenge

We also characterized mDC and pDC in the peripheral blood of subjects who underwent an inhalation allergen challenge. There were no differences in the percentages of total DC and mDC in the peripheral blood after the allergen challenge, and only a trend for decreased percentages of pDC at 6 hours after the allergen challenge returning to baseline by 24 hours was found (Table 4). At baseline, almost 88% of mDC expressed FcεRI and there was no change in this parameter or in MFI of FcεRI expression on mDC after the challenge. Approximately 85% of pDC expressed FcεRI at baseline. Furthermore, there were overall difference in both pDC expressing FcεRI and MFI of FcεRI on pDC between the 3 time points using Friedman test and decreased MFI of FcεRI on pDC at 6 hours compared with 0 hour. In addition, there was no change in the MFI of HLA-DR expression on any of the DC subsets after the challenge (data not revealed).

Correlation of Immune Parameters With Allergen Challenge-Induced Changes in FEV1

To understand whether any immune parameters we studied are associated with the development of early bronchoconstriction after allergen challenge, we studied whether there was any correlation between the baseline immune parameters and the degree of the early drop in FEV1 after allergen challenge. Baseline percentages of Mo, IMMo, or CMo in peripheral blood and percentages of IMMo and CMo expressing PAR-2 did not correlate with the peak drop in FEV1 during the early response or with AUC0-3 (data not revealed). Similarly, percentages of mDC and the expression of FcεRI by DC subsets did not correlate with these parameters (eTable 1). In contrast, percentages of DC and pDC in peripheral blood (eTable 1) and (Fig 3A and Fig 3B) at baseline positively correlated with the peak drop in FEV1 during the early response, but not with AUC0-3. Because only 4 subjects had a late response, we did not analyze the correlation of immune parameters with drop in FEV1 during the late response.

Discussion

Our previous results suggested that PAR-2 expression on Mo subsets may be a novel biomarker of asthma severity and asthma control18,19 but provided no further insight. The studies presented here address the relation between airway inflammation and PAR-2.
expression by peripheral blood Mo subsets using the following 2 human models: airway inflammation associated with an asthma exacerbation or inflammation after an inhalation allergen challenge. The results suggest that airway inflammation is associated with changes in numbers and function of peripheral blood myeloid cells. We first revealed that the percentages of IMMo and CMo expressing PAR-2 on their cell surface were higher in subjects who experienced an asthma exacerbation compared with patients with stable asthma. After treatment of the exacerbation, the percentages of IMMo expressing PAR-2 decreased significantly, whereas the percentages of CMo expressing PAR-2 revealed only a trend for decrease. We also found that the percentages of IMMo expressing PAR-2 increase after inhalation allergen challenge.

We know little on the regulation of PAR-2 expression on Mo. A single publication suggests that freshly isolated Mo have low levels of PAR-2 surface expression that increases from intracellular stores after mechanical stress and with differentiation toward DC or macrophages. Nevertheless, there is no other information on the regulation of PAR-2 expression to explain our observation of increased PAR-2 expression on Mo subsets during asthma exacerbations or at times of increased airway inflammation. We hypothesize that a number of inflammatory mediators generated during allergic inflammation may be the cause of the up-regulation. This is supported by the observation that PAR-2 expression on Mo also increases in various other inflammatory conditions. The exact mediators, however, are not known at this point. It is interesting that IMMo from patients with chronic obstructive pulmonary disease have higher CCR5 expression compared with cells from healthy controls and this increase is dependent on IL-6. We do not know whether the same CCR5 changes are present in patients with asthma, but IL-6 levels are increased in the serum of patients with asthma and correlate with frequency of exacerbations. IMMo also exhibit selective surface expression of certain cytokine and chemokine receptors and one or more of those may render IMMo more sensitive to allergic inflammatory signals. In particular, CCR5, a receptor for CCL5, is preferentially on IMMo and may mediate increased PAR-2 expression. In our 2 cohorts, we did not find any correlation between CCL5 levels and PAR-2 expression on IMMo (data not found). Nevertheless, in a population of patients with asthma of varying severities (n = 36) (we have reported other results from that population), we saw that serum CCL5 levels correlated with IMMo expressing PAR-2 (unpublished data). The lack of correlation in our current study may be because of low sample size of both cohorts.

Our observation that PAR-2 expression was increased on IMMo and CMo in patients with an asthma exacerbation compared with those with an independent cohort of stable asthma, but increased only on IMMo after allergen challenge, indicates differential regulation of PAR-2 expression by these 2 cell subsets. It is possible that the systemic component of allergic inflammation is able to up-regulate PAR-2 expression on IMMo, but that different signals or a combination of signals present only during an asthma exacerbation are required to increase PAR-2 expression on CMo.

Our understanding of the physiological or pathophysiologic role of PAR-2 surface expression on Mo or Mo subsets is also limited. PAR-2—mediated activation of peripheral blood Mo leads to release of inflammatory mediators, such as IL-6, IL-8, and IL-1β. PAR-2—mediated Mo activation may also have protective effects against bacterial and viral infections by increasing phagocytosis and enhancing IFN-activated pathways. Whether the changes in PAR-2 expression in peripheral blood Mo in subjects with increased airway inflammation have significant effects on Mo function is not understood. In mice, PAR-2 expression is required for DC development in vitro and in vivo. If the same is true in humans, Mo differentiation to DC after recruitment to peripheral tissues, such as the lung, may be PAR-2 dependent. This may suggest that the increased PAR-2 expression we observed during asthma exacerbations or after an inhalation allergen challenge could support increased DC development in peripheral tissues. More detailed, in vitro studies with isolated Mo will allow us to determine the role of PAR-2 expression on Mo in asthma, but also in other inflammatory diseases.

DCs are pivotal in regulating allergic airway inflammation. For these reasons we also studied the presence and activation status of mDC and pDC subsets. We revealed that mDC numbers, but not pDC numbers, in peripheral blood are lower in subjects who experienced an asthma exacerbation compared with patients with stable asthma and that these mDC numbers increase after treatment of the exacerbation. In contrast, there was a trend of decrease of pDC, but not mDC, 6 hours after an inhalation allergen challenge and the numbers returned to baseline by 24 hours.

There is little information on changes in DC numbers and activation status in patients with asthma and comes almost exclusively from allergen challenge studies. Numbers of pDC and mDC increase in the sputum of subjects with mild asthma after an inhalation allergen challenge, but the situation for peripheral blood DC is not clear; one study revealed no changes, whereas 2 other studies...
revealed decreased numbers. The lack of a significant effect in our challenge study may be the result of having studied a slightly different population, because in our study, most subjects exhibited isolated early responses, whereas other studies included only patients with dual responses. Decreased pDC numbers after allergen challenge may be the result of cells migrating to the airways, because pDC are increased in induced sputum during asthma exacerbations, and in mice they drive the development of airway inflammation in allergic models. Although we do not have direct data, decreased pDC numbers in subjects with an asthma exacerbation may indicate a viral cause for the exacerbations, as has also been found in children during RSV-induced asthma exacerbations. Nevertheless, we have published that our subjects with an asthma exacerbation also had lower peripheral blood lymphocyte numbers, something that can be found during viral infections. DC also express FcεRI in humans. FcεRI expression increases in patients with asthma and fccRI-positive DC increase within the airway epithelium of subjects with Th2-high asthma. We observed high level of FcεRI expression on the surface of DCs in all patients in both models. We also saw a decrease of FcεRI surface expression on pDC during the exacerbation visit and an early decrease of FcεRI surface expression on these cells after inhalation challenge. The reason for this decrease is not clear. Toll-like receptor ligation can decrease FcεRI expression on human pDC, but we do not know if pattern recognition receptor ligation is the cause in our case. It is also possible that pDC expressing high levels of FcεRI are preferentially recruited to the lungs during episodes of increased airway inflammation leading to decreased presence of these cells in the periphery. Further studies will be needed to clear this question. It is interesting that in our study the percentages of DC and pDC in peripheral blood at baseline correlated with the degree of early bronchoconstriction after allergen challenge. The role of pDC in asthma is not very well understood. Studies in mouse models indicate that they may have a dual role, because they may prevent the development of allergic sensitization but in the case of established disease are recruited to the lung and drive Th2 inflammation. The presence of higher numbers of pDC in the blood followed by recruitment of pDC to the lungs and airways after allergen challenge and the subsequent pDC-induced airway inflammation may be the link between the abundance of pDC in peripheral blood and the degree of bronchoconstriction after allergen challenge, as we revealed in our study. Larger studies to validate this observation would be required to better understand the role of pDC in asthma.

Our study has several limitations that need to be considered. First, both components of the current study have small sample sizes, which may limit our ability to identify small differences in cell numbers and cell surface markers. We did not measure monocyte-specific cytokines, such as tumor necrosis factor, IL-10, and IL-6, partly because our sample size is not big enough to achieve adequate power. As for our Allergen Challenge Study, we included both patients with isolated EAR and patients with dual responses. This again may have limited our ability to identify changes that would be restricted to 1 of these 2 groups. Lastly, because we conducted a number of statistical comparisons, there is a possibility that certain significant differences we identified may be a chance occurrence owing to the multiple tests we conducted. Notwithstanding these concerns, this study provides first inhuman evidence indicating allergic airway inflammation and possibly also other systemic changes during asthma exacerbations affect the characteristics of peripheral blood Mo. In vitro studies to elucidate the mechanisms of PAR-2 regulation on Mo and individual Mo subsets and to describe the effects of PAR-2-mediated Mo activation will allow us to understand the biological significance of increased PAR-2 expression on Mo during allergic inflammation. Furthermore, a comparison between changes in monocyte populations in the blood and induced sputum after an allergen challenge will provide greater insight into the processes we described here.

Acknowledgments

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References


**eTable 1**

Correlation Between Baseline Immune Parameters and EAR After Inhalation Allergen Challenge

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Percentage peak drop in FEV&lt;sub&gt;1&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;0-3 h&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of DC in PB</td>
<td>R = 0.736</td>
<td>R = 0.282</td>
</tr>
<tr>
<td></td>
<td>P = .01</td>
<td>P = .40</td>
</tr>
<tr>
<td>Percentage of mDC in PB</td>
<td>R = 0.082</td>
<td>R = −0.191</td>
</tr>
<tr>
<td></td>
<td>P = .81</td>
<td>P = .57</td>
</tr>
<tr>
<td>Percentage of pDC in PB</td>
<td>R = 0.682</td>
<td>R = 0.191</td>
</tr>
<tr>
<td></td>
<td>P = .02</td>
<td>P = .57</td>
</tr>
<tr>
<td>Percentage of mDC expressing FcεRI</td>
<td>R = 0.545</td>
<td>R = −0.400</td>
</tr>
<tr>
<td></td>
<td>P = .08</td>
<td>P = .22</td>
</tr>
<tr>
<td>Percentage of pDC expressing FcεRI</td>
<td>R = 0.000</td>
<td>R = 0.041</td>
</tr>
<tr>
<td></td>
<td>P &gt; .99</td>
<td>P = .90</td>
</tr>
<tr>
<td>MFI of FcεRI on mDC</td>
<td>R = 0.118</td>
<td>R = 0.200</td>
</tr>
<tr>
<td></td>
<td>P = .72</td>
<td>P = .55</td>
</tr>
<tr>
<td>MFI of FcεRI on pDC</td>
<td>R = 0.018</td>
<td>R = 0.009</td>
</tr>
<tr>
<td></td>
<td>P = .96</td>
<td>P = .98</td>
</tr>
</tbody>
</table>

Abbreviations: DC, dendritic cells; EAR, early asthmatic response; FcεRI, Fc epsilon receptor I; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell.

NOTE. Correlations between parameters were analyzed using Spearman's rank correlation coefficient. Bold indicates a statistically significant difference with a P-value less than 0.05.
**eFigure 2.** Gating strategy for flow cytometry analysis of dendritic cell subsets.