

Relationship of Circulating C5a and Complement Factor H Levels With Disease Control in Pregnant Women With Asthma

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BACKGROUND: Asthma often complicates pregnancy and represents a risk of serious pregnancy complications. The complement system contributes to asthma pathogenesis and is up-regulated in healthy gestation as well. The anaphylatoxin C5a has a major pro-inflammatory role, and the complement factor H is a main soluble regulator protein both in asthma and during pregnancy; however, peripheral levels of these complement factors and their relationship to disease control have not yet been evaluated in pregnant subjects with asthma. **METHODS:** The present study aimed to investigate circulating C5a and complement factor H levels in asthma (non-pregnant subjects with asthma; $n = 19$) and in pregnancy with asthma (pregnant subjects with asthma; $n = 22$), compared with healthy non-pregnant ($n = 21$) and healthy pregnant women ($n = 13$) and to test their relationship to clinical parameters of asthma (lung function, airway inflammation, and symptoms). **RESULTS:** Circulating C5a levels were higher in the pregnant asthma subject group compared with the healthy non-pregnant, healthy pregnant, and non-pregnant asthma groups: median 2.629 (interquartile range [IQR] 2.257–3.052) ng/mL versus 1.84 (IQR 1.576–2.563), 1.783 (IQR 0.6064–2.786), and 2.024 (IQR 1.232–2.615) ng/mL, respectively ($P = .02$ in all cases). C5a correlated negatively with FEV₁ ($r = -0.44$, $P = .039$) and FVC values ($r = -0.64$, $P = .001$) in the pregnant asthma group and positively with fraction of exhaled nitric oxide levels in the non-pregnant asthma group ($n = 12$, $r = 0.78$, $P = .004$). Complement factor H levels were elevated in both the healthy pregnant and pregnant asthma subject groups compared with the healthy non-pregnant group (median 1,082 [IQR 734.9–1,224] and 910.7 [IQR 614.5–1076] $\mu\text{g/mL}$ vs 559.7 [IQR 388.7–783.1] $\mu\text{g/mL}$, $P = .002$ and $P = .004$, respectively) but not in the pregnant asthma group compared with the non-pregnant asthma group (median 687.4 [IQR 441.6–947.6] $\mu\text{g/mL}$, $P = .10$). **CONCLUSIONS:** Asthma during pregnancy increases the circulating level of pro-inflammatory C5a, which is accompanied by impaired lung function and partly counteracted by the gestation-specific elevation of regulatory complement factor H level (detected in pregnancy both in healthy and subjects with asthma). *Key words:* asthma; pregnancy; complement; C5a; complement factor H; biomarker. [Respir Care 2016;61(4):502–509. © 2016 Daedalus Enterprises]

Introduction

Asthma is a chronic inflammatory disease of the airways characterized by variable and recurring symptoms,

heightened local inflammation, and reversible air flow obstruction. It imposes a high burden of morbidity, especially if not controlled, which occurs in up to 50% of cases worldwide.¹ At the same time, asthma is one of the most common chronic diseases complicating pregnancy, occurring in 8–12% of all gestations.² It represents a risk of

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potentially serious maternal and fetal morbidities, including preterm delivery, gestational hypertension, preeclampsia, low birthweight, and neonatal mortality.^{3,4} In addition, pregnancy may also influence asthma control with the deterioration of symptoms in one third of all pregnant women with asthma.⁵ Notably, if the disease is well controlled, the risks of poor pregnancy outcomes decrease⁶; therefore, maintaining optimal control in this period is crucial. Hence, it would be especially important to understand the immune interactions between asthma and gestation and to have a more detailed view into inflammatory processes of asthma during pregnancy. Furthermore, because pregnancy itself can influence spirometry results,⁷ and because most of the available techniques used to determine asthma airway inflammation (eg, induced sputum) are semi-invasive and therefore cannot be used in pregnancy, circulating, non-invasively obtainable biomarkers related to asthma control could help in identifying pregnant women with asthma with elevated risk. However, to date, objective circulating markers reflecting asthma control are not known. Previously, we investigated 2 promising blood markers, but both of them proved to be helpful in asthma alone rather than in asthma during pregnancy.^{8,9}

The complement system is an ancient danger-sensing component of innate immunity. Its functions include recognizing and eliminating microorganisms, immune complexes, and apoptotic cells, and it has a regulatory role influencing both innate and adaptive immunity.¹⁰ It can be activated through the so-called classical, alternative, and lectin pathways. The complement system participates in the pathogenesis of asthma on several levels.^{11,12} An interesting element of this complex system is the fragment C5a, because it has a dual role in allergic inflammation. On one hand, it protects against Th2-mediated airway inflammation in the initial phase of sensitization by modulating dendritic cells, but on the other hand, it enhances airway inflammation and hyper-reactivity in already established disease.^{11,12} In the latter case, it behaves as a major pro-inflammatory mediator by promoting chemotaxis and activation of many cell types, which results in release of cytokines, increased vascular permeability, and smooth muscle contraction. Elevated C5a levels were measured not only in bronchoalveolar lavage fluid¹³ and induced sputum of subjects with asthma,¹⁴ but also in their plasma¹⁵; moreover, plasma levels correlated with asthma severity.¹⁵

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QUICK LOOK

Current knowledge

Asthma management during pregnancy can be difficult, and asthma that is not well controlled represents a risk of potentially serious maternal and fetal morbidities. The complement system participates in the pathogenesis of asthma and is involved in healthy pregnancy as well, but excess activation leads to pregnancy complications.

What this paper contributes to our knowledge

Plasma C5a levels were increased in pregnant women with asthma, and higher levels implied worse lung function. Plasma C5a levels were also associated with airway inflammation in non-pregnant subjects with asthma. A pregnancy-specific elevation of plasma complement factor H levels were observed in pregnant subjects regardless of concomitant asthma.

Complement factor H is one of the soluble inhibitor molecules that regulate complement activation, and it is a major alternative pathway regulator. Imbalances in complement factor H and its regulatory role contribute to tissue injury and can result in autoimmune diseases.¹⁶ In our earlier study,¹⁷ we found increased sputum (but not circulating) complement factor H concentration in subjects with asthma, which correlated with asthma control but not with plasma complement factor H.

Normal human pregnancy is characterized by an up-regulated complement system but at the same time by an immune tolerance. Thus, excess complement activation leads to pregnancy complications. Both C5a¹⁸ and complement factor H¹⁹ levels were found to be higher in healthy pregnancy than in non-pregnant controls, but plasma C5a levels were further elevated in spontaneous abortion²⁰ and preeclampsia.^{21,22} However, to date, data on circulating C5a and complement factor H levels and their possible relationship to disease control are scarce in asthma and lacking for pregnant women with asthma.

Therefore, the present study aimed to investigate circulating C5a and complement factor H levels in non-pregnant and pregnant subjects with asthma. Furthermore, to determine the utility of C5a and complement factor H as a help in the evaluation of asthma control in pregnant subjects with asthma, we also investigated their relationship to major asthma control determinants (lung function parameters, airway inflammation, and symptoms).

Methods

Ethics Statement

Written informed consent was obtained from the subjects, and our study was reviewed and approved by an independent ethics committee of the institution (Institutional and Regional Research Ethics Committee of Semmelweis Medical University). The study adhered to the tenets of the most recent revision of the Declaration of Helsinki.

Study Participants

The study had a cross-sectional design. Nineteen non-pregnant subjects with asthma, 22 pregnant subjects with asthma, 21 healthy non-pregnant subjects, and 13 healthy pregnant subjects were enrolled. Subjects with asthma were assessed at their regular visit at the out-patient clinic of the Department of Pulmonology, Semmelweis University. They had persistent disease, and asthma had been diagnosed according to the current guidelines¹ at least 6 months before enrollment. Exclusion criteria were current smoking or >5 pack-years of smoking history, any other chronic disease (except for allergic rhinitis), acute infection within 4 weeks of measurement, fetal infection, and multi-fetal gestation. There were no complications in the 2 pregnant groups. Subjects were asked not to use their medication 12 h before visits. Healthy pregnant subjects were recruited when attending their scheduled visit at the first Department of Obstetrics and Gynecology, Semmelweis University. Healthy non-pregnant controls were volunteers and had a negative history and negative asthma status upon detailed physical and routine laboratory examination.

Measurement of Serum C5a and Complement Factor H Levels

Venous blood samples were collected in EDTA tubes and were processed within 2 h. Plasma was separated and stored at -80°C until analyses. The plasma levels of C5a (Quidel, San Diego, CA) and complement factor H (Hy-cult, Uden, Netherlands) were determined with ELISA kits in the same samples.

Lung Function, Fraction of Exhaled Nitric Oxide Measurements, and Asthma Control Evaluation

Lung function was measured by means of an electronic spirometer (PDD-301/s, Piston, Budapest, Hungary) according to the American Thoracic Society guidelines.²³ Three technically acceptable maneuvers were performed, and the best was used. FEV₁, forced FVC, peak expiratory flow, and airway resistance were recorded in subjects with

asthma (non-pregnant subjects with asthma, $n = 17$; pregnant subjects with asthma, $n = 20$).

Airway inflammation was assessed with the fraction of exhaled nitric oxide (F_{ENO}), which was measured with a NIOX MINO airway inflammation monitor (Aerocrine AB, Solna, Sweden) according to the European Respiratory Society/American Thoracic Society recommendations²⁴ in 12 non-pregnant subjects with asthma and 18 pregnant subjects with asthma.

Asthma control was assessed using the Asthma Control Test (non-pregnant subjects with asthma, $n = 16$; pregnant subjects with asthma, $n = 20$) recommended by the current asthma guideline.¹

Statistics

Data distribution was analyzed by the D'Agostino-Pearson normality test. Comparisons between the study groups were made with the Mann-Whitney, Kruskal-Wallis, and Dunn post hoc multiple comparison tests. Correlation analyses were performed using the Spearman test due to non-normal distribution of data. Area under the curve values of receiver operating characteristic curves were calculated using standard methods, and data are presented as area under the curve receiver operating characteristics (95% CI). *P* values of $<.05$ were considered significant. The tests used were 2-tailed. Statistics were calculated using GraphPad Prism 5 (GraphPad Software, La Jolla, California). Data are expressed as median (interquartile range [IQR]).

Results

Clinical Characteristics

Clinical data and inflammatory parameters of the 4 study groups are summarized in Table 1. The median age of participants in the 4 groups was comparable. Gestational age at blood sampling and at delivery or fetal birthweight did not differ between the pregnant subjects with asthma and healthy pregnant subject groups. The median (IQR) gestational age was 26.5 (17.75–33.25) and 24 (20.25–25) weeks in the pregnant subjects with asthma and healthy pregnant subject group, respectively. Every healthy pregnant woman was in the second trimester, whereas 41% of the pregnant women with asthma were in the third trimester. None of the subjects who were pregnant showed any obstetric complications. No difference was detected in the severity or control of asthma or in the F_{ENO} levels between the non-pregnant and pregnant asthma groups (Table 1). Daily dose of inhaled corticosteroids (ICS) showed a trend to be higher in the non-pregnant asthma subject group than in the pregnant asthma subject group (median 400 [IQR 400–800] μg vs 300 [IQR 0–500] μg of

Table 1. Clinical Data in the 4 Study Groups

| Parameters | HNP (<i>n</i> = 21), Median (IQR) | HP (<i>n</i> = 13) | | ANP (<i>n</i> = 19) | | AP (<i>n</i> = 22) | |
|---|---------------------------------------|---------------------|----------|----------------------|----------|---------------------|----------|
| | | Median (IQR) | <i>n</i> | Median (IQR) | <i>n</i> | Median (IQR) | <i>n</i> |
| Age, y | 29 (26.25–33.25) | 33 (28.5–36) | | 35 (26–37) | | 31.5 (26.5–35) | |
| Gestational age at sampling, wks | | 24 (20.25–25) | | | | 26.5 (17.75–33.25) | |
| 2nd/3rd trimester, <i>n</i> | | | 13/0 | | | | 13/9 |
| Gestational age at delivery, wks | | 39 (39–40) | 11 | | | 40 (38–41) | 16 |
| Fetal birth weight, g | | 3,600 (3,228–3,715) | 12 | | | 3,415 (3,029–4,030) | 20 |
| FEV ₁ , L | | | | 3.01 (2.57–3.37) | 17 | 2.87 (2.76–3.18) | 20 |
| FEV ₁ , % predicted | | | | 98 (85–107) | 17 | 92 (85–99.25) | 20 |
| FVC, L | | | | 3.94 (3.43–4.205) | 17 | 3.82 (3.3–4.165) | 20 |
| FVC, % predicted | | | | 108 (96.5–116) | 17 | 102.5 (96.25–111.5) | 20 |
| PEF, L/s | | | | 5.92 (5.25–7.185) | 17 | 6.31 (5.56–6.815) | 20 |
| PEF, % predicted | | | | 81 (74.5–101.5) | 17 | 84 (76.75–93) | 20 |
| R _{aw} , % predicted | | | | 123 (105.5–154) | 17 | 115 (82–136) | 20 |
| ACT total score | | | | 20 (15.5–24) | 16 | 20.5 (13–23.5) | 20 |
| F _{ENO} , ppb | | | | 19 (10.5–22) | 12 | 19 (14.25–39) | 18 |
| Daily dose of ICS, µg of beclomethasone equivalent | | | | 400 (400–800) | 17 | 300 (0–500) | |
| Steroid-naïve/steroid-treated subjects, <i>n</i> | | | | | 2/15 | | 10/12 |

HNP = healthy non-pregnant
 HP = healthy pregnant
 ANP = asthma, non-pregnant
 AP = asthma, pregnant
 IQR = interquartile range
 PEF = peak expiratory flow
 R_{aw} = airway resistance
 ACT = Asthma Control Test
 F_{ENO} = fraction of exhaled nitric oxide
 ICS = inhaled corticosteroid

beclomethasone equivalent, respectively, *P* = .067; Table 1). Fifteen non-pregnant and 12 pregnant subjects with asthma received ICS treatment. The median (IQR) Asthma Control Test total scores of 20 (15.5–24) in the non-pregnant asthma subject group and 20.5 (13–23.5) in the pregnant asthma subject group showed similar levels of disease control.

Comparison of Circulating C5a and Complement Factor H Levels Among the 4 Groups

Asthma itself did not influence peripheral C5a and complement factor H levels, because they were similar in the healthy non-pregnant subject and non-pregnant asthma subject groups (median 1.84 [IQR 1.576–2.563] ng/mL vs 2.024 [IQR 1.232–2.615] ng/mL C5a, respectively, *P* = .98, and 559.7 [IQR 388.7–783.1] µg/mL vs 687.4 [IQR 441.6–947.6] µg/mL complement factor H, respectively, *P* = .30; Fig. 1, A and B). Median (IQR) C5a levels were markedly higher in the pregnant asthma subject group (2.629 [2.257–3.052] ng/mL) compared with the healthy non-pregnant subject (1.84 [1.576–2.563] ng/mL, *P* = .02), healthy pregnant subject (1.783 [0.6064–2.786] ng/mL, *P* = .02), and

non-pregnant asthma subject groups (2.024 [1.232–2.615] ng/mL, *P* = .02; Fig. 1A). On the other hand, both healthy pregnancy and pregnancy in subjects with asthma were associated with elevation in complement factor H levels compared with the healthy non-pregnant subject group (1,082 [IQR 734.9–1,224] and 910.7 [IQR 614.5–1,076] µg/mL vs 559.7 [IQR 388.7–783.1] µg/mL, *P* = .002 and *P* = .004, respectively), whereas complement factor H levels were elevated only in the healthy pregnant group (*P* = .03) and not in the pregnant asthma subject group (*P* = .10) when compared with the non-pregnant asthma subject group (687.4 [IQR 441.6–947.6] µg/mL; Fig. 1B). The complement factor H levels did not differ between the healthy pregnant subject and pregnant asthma subject groups (*P* = .29; Fig. 1B). The 2 complement factors correlated with each other only in the healthy non-pregnant subject group (*r* = 0.5, *P* = .02).

Relationship of C5a and Complement Factor H to Asthma Control Determinants

Pointing to the possible relationship between complement activation and airway inflammation, there was a sig-

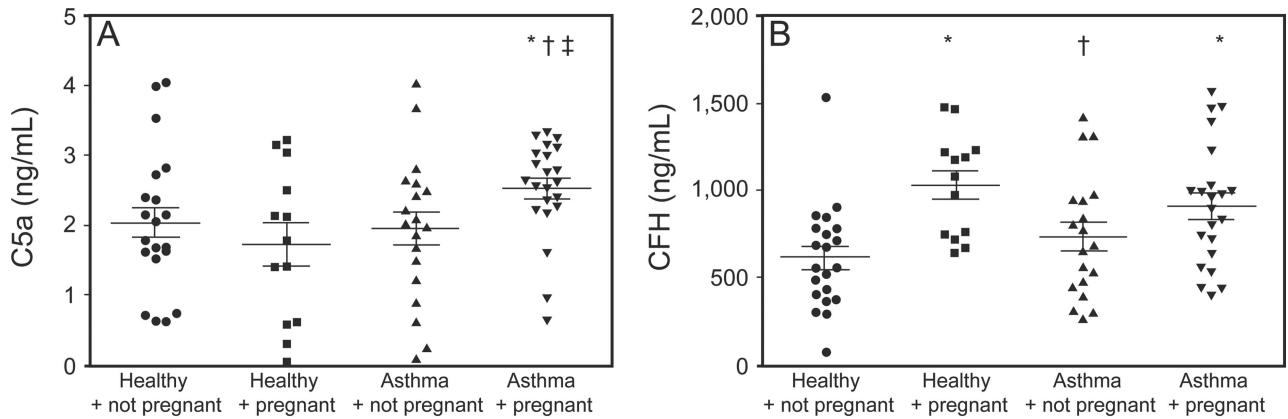


Fig. 1. Median (interquartile range) circulating C5a (A) and complement factor H (CFH) (B) levels measured in non-pregnant and pregnant healthy subjects and in non-pregnant and pregnant subjects with asthma; C5a, 1.84 (1.576–2.563), 1.783 (0.6064–2.786), 2.024 (1.232–2.615), and 2.629 (2.257–3.052) ng/mL, respectively; complement factor H, 559.7 (388.7–783.1), 1,082 (734.9–1,224), 687.4 (441.6–947.6), and 910.7 (614.5–1,076) μ g/mL, respectively. * $P < .05$ versus healthy + non-pregnant subjects. † $P < .05$ versus healthy pregnant subjects. ‡ $P < .05$ versus asthma + non-pregnant subjects.

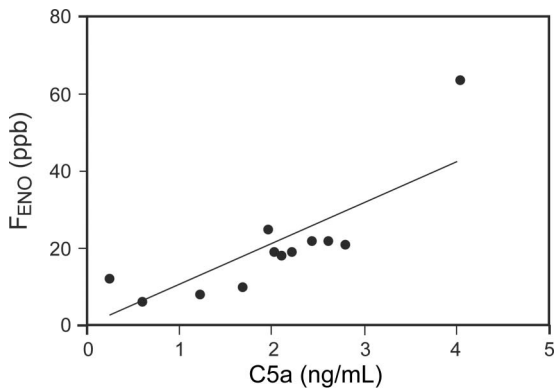


Fig. 2. Positive correlation between circulating C5a and fraction of exhaled nitric oxide (F_{ENO}) levels in non-pregnant subjects with asthma ($n = 12$).

nificant positive correlation between C5a and F_{ENO} levels ($n = 12$, $r = 0.78$, $P = .004$; Fig. 2) in the non-pregnant asthma subject group. Furthermore, in the pregnant asthma subject group, C5a levels correlated negatively with FEV_1 ($r = -0.44$, $P = .039$; Fig. 3A) and FVC values ($r = -0.64$, $P = .001$; Fig. 3B).

To obtain a more detailed view on the interactions of asthma air flow obstruction and inflammation, the relationship of complement factor H levels with lung function was also examined. However, a trend toward a correlation between complement factor H levels and peak expiratory flow values ($n = 11$; $r = 0.61$, $P = .052$) could be observed only in the subgroup of pregnant subjects with asthma in the second trimester and not in the whole pregnant asthma subject cohort ($P > .05$). We did not find further correlations between the complement factors and any of the remaining lung function parameters or Asthma Control Test total scores.

Age of the participants did not influence the level of the 2 complement factors in any of the groups. The prescribed dose of the ICS was not related to either complement level.

ROC Analysis of C5a and Complement Factor H Levels in Controlled and Uncontrolled Asthma

In order to evaluate the possible utility of C5a and complement factor H measurements in the detection of uncontrolled asthma, ROC analyses of C5a and complement factor H levels were performed in subgroups of pregnant and non-pregnant subjects with asthma with peak expiratory flow above and below 80% and Asthma Control Test total score above and below 20, because the current GINA guideline suggests these cut-off values to differentiate between optimal and suboptimal asthma control.¹ We did not find any significant effect of the 2 proteins in the detection of suboptimal asthma control.

Relationship of Complement Factors and Functional Parameters to Obstetric Data

C5a and complement factor H concentrations were not related to gestational age at sampling in the pregnant asthma subject nor in the healthy pregnant subject group. There was no difference between C5a or complement factor H values of pregnant women with asthma in the second versus the third trimester.

We could not detect any association between C5a or complement factor H levels and obstetric data, such as gestational age at delivery and fetal birthweight. In the healthy pregnant group, women carrying female fetuses had slightly higher complement factor H levels compared

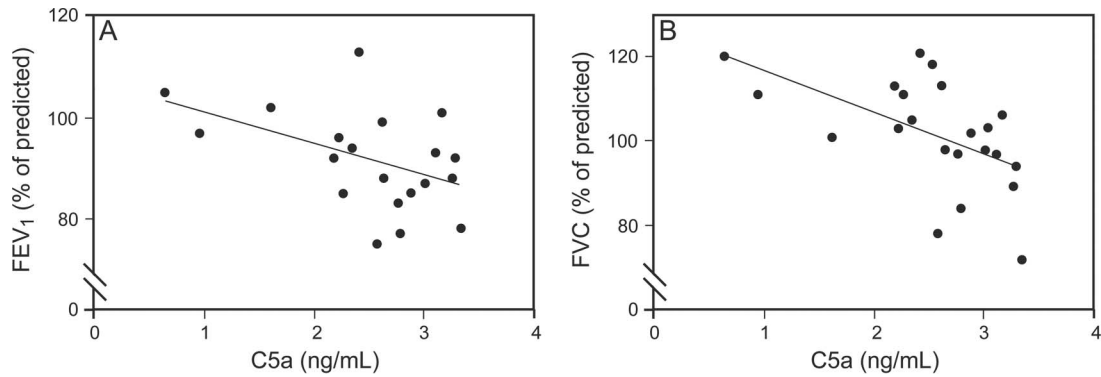


Fig. 3. Negative correlation between circulating C5a levels and FEV₁ (A) and FVC (B) values in pregnant women with asthma ($n = 20$).

with those carrying male fetuses, a difference that reached only the level of a trend ($P = .07$).

Discussion

The complement system plays an important role in the development of asthma^{11,12}; therefore, evaluation of this complex system could potentially help to better understand the disease and may even serve as a biomarker pool for asthma control. This latter possibility is especially important during pregnancy in order to maintain optimal asthma control and thus prevent unwanted effects of the disease on pregnancy outcomes.⁶ For the first time, in the present study, we investigated 2 major proteins of the complement cascade, C5a and complement factor H, in pregnant subjects with asthma compared with non-pregnant subjects with asthma and with healthy pregnant and non-pregnant subjects. C5a levels were increased solely in pregnant women with asthma, and higher C5a levels implied worse lung function in these subjects with regard to both FEV₁ and FVC. C5a levels were found to be associated also with airway inflammation because they correlated with F_{ENO} levels in non-pregnant subjects. On the other hand, a pregnancy-specific elevation of regulatory complement factor H levels was demonstrated in both pregnant groups regardless of concomitant asthma.

The anaphylatoxin C5a, a cleaved fragment of C5, is one of the highly inflammatory key effector molecules of the complement system.²⁵ In contrast, complement factor H is a major soluble regulator primarily of the alternative and classical pathways.²⁶ Having homeostatic roles, a certain degree of spontaneous activation of the complement system can always be found in healthy people, indicating a controlled working of this ancient danger-sensing system in normal conditions; however, a delicate balance of activation and regulation is important to preclude normal self-tissue damage.^{10,27} We found a correlation between C5a and complement factor H in the healthy control population, presumably suggesting a balanced state between activation and regulation.

C5a has a dual role in asthma: During the phase of sensitization, it protects against the initiation of Th2-mediated airway inflammation, but in already established disease, it becomes highly pro-inflammatory and further enhances airway hyperreactivity.^{11,12,28} C5a may contribute to airway inflammation in asthma,^{11,12,28} which was reflected in the correlation between C5a and F_{ENO} levels in non-pregnant subjects with asthma in our study. Furthermore, inhibition of C5a improved lung function, airway hyperreactivity, and airway inflammation in animal models and in mild allergic asthma subjects.^{29,30} F_{ENO} levels were not evaluated in the healthy pregnant group, since, according to our previous data, pregnancy itself does not have an influence on normal F_{ENO} values.³¹

As a novel finding of our study, we demonstrated an asthma-induced increase in circulating C5a levels in pregnant subjects with asthma, which was not detected either in healthy pregnant women or in any other group and which was associated with impaired lung function with regard to both FEV₁ and FVC in pregnant subjects with asthma. A limitation of our study was that lung function was not measured in healthy control groups, and therefore our results confirm a negative correlation between C5a levels and FEV₁ and FVC values only in the pregnant asthma subject group; no conclusion can be drawn regarding this issue in healthy pregnancy. Higher concentrations of complement anaphylatoxins (eg, C5a) may cause recurrent spontaneous abortion,^{20,32} preterm birth,^{33,34} and preeclampsia,^{21,22} whereas treatment with the C5 inhibitor eculizumab may have therapeutic relevance in these conditions.³⁵ Importantly, eculizumab seems to be administrable safely in pregnancy in the long term.^{36,37} In our study, we found similar C5a levels in healthy pregnant and non-pregnant women and could not detect any association between C5a levels and obstetric parameters, such as fetal birthweight or gestational week at delivery; of note, these pregnancies were without complications.

Elevation of complement factor H in healthy pregnancy was already known earlier,¹⁹ and we confirmed this again, but here we also tested whether this pregnancy-specific

elevation is present in pregnant women with asthma as well. Indeed, asthma did not suppress the elevation of complement factor H in our pregnant subjects compared with healthy ones, so that complement factor H was elevated in both pregnant groups regardless of the presence of asthma; however, it must be noted that the majority of pregnant women with asthma were ICS-treated and well-controlled. In addition, hormonal and physiological changes of pregnancy may differ across trimesters, which might influence our results. Nevertheless, if regulators (such as complement factor H) are dysfunctional during pregnancy, excessive complement activation may lead to placental damage and preeclampsia.^{38–40} In our study, none of the women showed any obstetric complications in any pregnant group, which may be, at least partly, a result of well balanced complement activation.

In our previous study, only induced sputum levels of complement factor H were increased in asthma and were related to worse lung function, whereas plasma levels were not elevated and did not correlate with any clinical parameter.¹⁷ This latter finding was supported by the current study, because in non-pregnant subjects, we could not demonstrate any association between plasma complement factor H levels and asthma control determinants. However, in the subgroup of second-trimester pregnant women with asthma, complement factor H levels showed a trend toward a direct correlation with peak expiratory flow. This phenomenon might imply that more rigorous control of complement activation could be beneficial during the pregnancy of asthma patients; notably, a significantly large sample size evaluation would be warranted to confirm our observation. A possible reason why pregnant subjects received a somewhat lower dose of ICS is that pregnant women are less adherent in taking the prescribed ICS regularly.^{2,5} This may theoretically also explain why the association between complement factor H and peak expiratory flow was detectable only in pregnant and not in non-pregnant subjects. However, because ICS dose did not influence plasma complement factor H levels significantly in any of the groups, this explanation remains hypothetical.

Conclusions

According to our data, the circulating level of pro-inflammatory C5a is elevated in pregnant women with asthma and shows a relationship to impaired lung function in this patient group. On the other hand, pregnancies of both healthy and subjects with asthma increase the level of regulatory complement factor H, which seems to be a pregnancy-specific change independent of concomitant asthma.

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