Published in final edited form as:

J Matern Fetal Neonatal Med. 2005 April ; 17(4): 247–252.

INCREASED CONCENTRATION OF THE COMPLEMENT SPLIT PRODUCT C5a IN ACUTE PYELONEPHRITIS DURING PREGNANCY

ELEAZAR SOTO^{1,2}, KARINA RICHANI^{1,2}, ROBERTO ROMERO¹, JIMMY ESPINOZA^{1,2}, TINNAKORN CHAIWORAPONGSA^{1,2}, JYH KAE NIEN¹, SAMUEL EDWIN¹, YEON MEE KIM^{1,3}, JOON-SEOK HONG¹, LUIS F. GONCALVES^{1,2}, and MOSHE MAZOR⁴

¹ Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, and Detroit, MI, USA;

² Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI USA;

³ Department of Pathology, Wayne State University School of Medicine, Detroit, MI USA

⁴ Department of Obstetrics and Gynecology, Soroka Medical Center, Ben Gurion University of the Negev, Beer Sheva, Israel

Abstract

OBJECTIVE—Pregnant women with acute pyelonephritis develop acute respiratory distress syndrome (ARDS) more frequently than non-pregnant women. The reasons for this remain unknown. The complement system is a complex set of self-assembling proteins that have been implicated in the pathophysiology of ARDS and sepsis. The purpose of this study was to determine if activation of the complement system occurs in pregnant women with acute pyelonephritis.

METHODS—A cross-sectional study was conducted to determine the plasma concentrations of C3a, C4a and C5a (i.e., complement split products) in pregnant patients with acute pyelonephritis (n=38) and normal pregnant women (n=38). The complement split products C3a, C4a and C5a were measured using ELISA. Data were analyzed using non-parametric statistics.

RESULTS—1) The median plasma concentration of C5a in pregnant patients with acute pyelonephritis was significantly higher than that in normal pregnant women ($p < 0.001$); 2) there was no statistical difference in the median plasma concentration of C3a and C4a between the two groups ($p > 0.05$); and 3) concentrations of C3a, C4a and C5a were not different among patients with acute pyelonephritis with and without bacteremia.

CONCLUSIONS—1) Pyelonephritis in pregnant women is associated with increased plasma concentration of C5a, but not C3a and C4a; 2) an excess of C5a can predispose pregnant women to develop ARDS and multi-organ failure in pyelonephritis. This finding may have clinical implications since blocking C5a improves ARDS in experimental sepsis.

Keywords

Pregnancy; complement system; anaphylatoxins; acute pyelonephritis; innate immunity; C3a; C4a; C5a

INTRODUCTION

The complement system, a major component of the innate immune response, plays an important role in host defense against invading pathogens. The complement cascade is formed by several plasma proteins with catalytic properties that react in a sequential manner, yielding active biological mediators and lytic components to clear foreign cells. In addition, the complement system is involved in the regulation of the adaptive immune response.[1]

Infection can trigger activation of the complement system directly by microbial surfaces or products, or indirectly by antigen-antibody complexes. Anaphylatoxins C3a, C4a and C5a are pro-inflammatory peptides released from the cleavage of their native components following activation of the complement cascade. These molecules induce vascular permeability,[2–4] smooth muscle contraction[3–5], the release of pro-inflammatory cytokines[6–8] and white blood cell chemotaxis.[9–11] Among anaphylatoxins, C5a is the most potent.[4] Its interaction with phagocytic cells increases oxidative burst,[12,13] induces expression of adhesion molecules[14,15] and inhibits polymorphonuclear (PMN) apoptosis.[16] C3a effects are predominantly on mast cells and eosinophils, and include chemotaxis,[10,11] granule release,[11,17] expression of adhesion molecules (β_2 integrin) and L-selectin shedding.[14] C4a, the weakest anaphylatoxins,[4] can only be generated through the classical pathway. Little is known about C4a receptor interactions and its biological functions in humans.

Even though “complement split products” participate in microbial clearance, excessive production may be harmful to the host.[18] Indeed, plasma and serum concentrations of anaphylatoxins are higher in septic patients with a poor outcome than in those with a favorable outcome.[19–21] Moreover, C5a has been implicated in the generation of acute respiratory distress syndrome (ARDS).[22,23]

Pregnancy is a unique immune state characterized by activation of the innate immune response and suppression of adaptive immunity.[24] This is thought to provide protection to the mother against infection[24] and promote tolerance of the fetal semi-allograft. Nonetheless, pregnant women are more susceptible to the effects of microbial products.[25] For example, urinary tract infections are frequently encountered during pregnancy,[26,27] and acute pyelonephritis affects 1 to 2% of pregnant women, particularly during the last two trimesters.[28] Pregnant women with acute pyelonephritis are at risk of developing sepsis and ARDS.[29–33] complications that are rare in non-pregnant women with urinary tract infections. The reasons for this remain unknown.

Previous studies have demonstrated complement activation during normal pregnancy,[34] as determined by higher plasma concentrations of anaphylatoxins (C3a, C4a and C5a) in pregnant women than in non-pregnant women.[35] However, the plasma concentrations of these complement split products in acute pyelonephritis during pregnancy has not been reported. The purpose of this study was to determine the plasma concentration of anaphylatoxins in pregnant patients with and without acute pyelonephritis.

PATIENTS AND METHODS

Study Design

A cross-sectional study was conducted by searching our clinical database and bank of biological samples. This study was designed to compare the plasma concentration of complement split products C3a, C4a and C5a among normal pregnant women (n=38) and pregnant patients with acute pyelonephritis (n=38). The control group consisted of pregnant women from 20 to 42 weeks, not in labor, and without medical or obstetrical complications. Acute pyelonephritis was diagnosed in the presence of fever (temperature $\geq 38^\circ\text{C}$), clinical

signs or symptoms of upper urinary tract infection (e.g., flank pain, costovertebral angle tenderness), pyuria and a positive urine culture. Blood cultures were also performed. Both groups were matched for gestational age at venipuncture within two weeks. Eligible patients were approached at the Detroit Medical Center/Hutzel Women's Hospital in Detroit, Michigan. All women provided written informed consent prior to the collection of samples. The collection of samples was approved by the IRBs of both the National Institute of Child Health and Human Development and Wayne State University. Many of these samples have been previously used in other studies.

Blood Collection

Samples of peripheral blood for complement determinations were collected in tubes containing EDTA (ethylene diamine tetraacetic acid). The samples were centrifuged at 1068g and stored at -70°C . Specific and sensitive enzyme-linked immunoassays (ELISA) were used to determine concentrations of complement C3a, C4a and C5a. Immunoassay systems for C3a and C4a were obtained from Assay Designs, Inc. (Ann Arbor, MI). C5a immunoassays were obtained from American Laboratory Products Company (Windham, NH). Complement C3a, C4a and C5a assays were performed according to the manufacturer's instructions. Briefly, maternal plasma samples were incubated in duplicate wells of microtiter plates, which had been pre-coated with antigen specific (C3a, C4a or C5a) antibodies. Complement C3a, C4a or C5a present in the standards or maternal plasma samples were immobilized by their specific pre-coated antibodies (forming antigen antibody complexes) during this incubation. Repeated washing and aspiration was conducted to remove unbound materials from the assay plates. This step was followed by incubation with a specific antibody-enzyme reagent. Following a wash step to remove excess unbound materials, a substrate solution was added to the wells of the microtiter plates and color developed in proportion to the amount of antigen bound in the initial step of the individual assay. The color development was stopped with the addition of an acid solution and the intensity of color was read using a programmable microtiter plate spectrophotometer (Ceres 900 Microplate Workstation, Bio-Tek Instruments, Winooski, VT). The concentrations of complement C3a, C4a or C5a in maternal plasma samples were determined by interpolation from individual standard curves composed of purified human C3a, C4a or C5a. The calculated inter-assay coefficients of variation (CV) for C3a, C4a and C5a immunoassays in our laboratory were 5.4%, 6.1%, and 4.0%, respectively. Calculated intra-assay CV for C3a, C4a and C5a were 6.6%, 6.9%, and 2.3%, respectively. The detection limit (sensitivity) was 0.13 ng/ml for C3a, 0.32 ng/ml for C4a, and 0.06 ng/ml for C5a.

Statistical Analysis

The Shapiro-Wilk test was used to test for normal distribution of the data. Mann-Whitney U tests were utilized to compare the medians of normal pregnant women and pregnant patients with acute pyelonephritis. A chi-square test was used for comparison of proportions. The statistical package used was SPSS 12 (SPSS Inc., Chicago, IL). A probability value < 0.05 was considered statistically significant.

RESULTS

Clinical and obstetrical characteristics of women in each group are displayed in Table I. Women with acute pyelonephritis delivered neonates with lower birthweight than those in the control group (pyelonephritis median: 3175 g, range: 1080–4090g vs. normal pregnancy median: 3372 g, range 2610–4080g; $p = 0.04$). The other characteristics were similar between the two groups.

Among women with acute pyelonephritis, blood cultures were positive in 11 (28.9%) patients. Microorganisms isolated from urine cultures included: *Escherichia coli* (31), *Klebsiella* (1), *Proteus mirabilis* (1), *Pseudomonas* species (1), *Staphylococcus aureus* (1), *Lactobacilli* (2),

Gardnerella vaginalis (2), *Citrobacter koseri* (1), *Streptococcus* species (1) and mixed flora (1). Blood cultures were positive for *Escherichia coli* (8), *Enterobacter aerogenes* (1), and *Staphylococcus aureus* (2).

The median plasma concentration of C5a in pregnant patients with acute pyelonephritis was significantly higher than in normal pregnant women (acute pyelonephritis median 20.1 ng/ml, range 9.7 – 61.8 ng/ml vs. normal pregnancy median 12.3 ng/ml, range: 1.5 – 87.1 ng/ml; $p < 0.001$; Figure 1, C). There was no significant difference in the median plasma C3a and C4a concentrations between the two groups ($p = 0.4$ and $p = 0.1$, respectively; Figures 1a and 1b). Among patients with acute pyelonephritis, no significant difference in the median plasma concentration of C5a, C4a and C3a was observed between those who had both positive urine and blood cultures and those who had only positive urine cultures (C5a, $p = 0.76$; C4a, $p = 0.34$; and C3a, $p = 0.22$).

DISCUSSION

This study demonstrated that pregnant women with acute pyelonephritis had a higher median plasma concentration of C5a than normal pregnant women (Figure 1, C). To our knowledge, this is the first study that has determined complement anaphylatoxins in pyelonephritis in pregnancy.

C5a exerts its activities by coupling with its seven loop transmembrane receptor C5aR/CD88. [36] C5aR has been identified in a variety of cells including myeloid, lymphoid, endothelial, and cells from different tissues such as liver, lung, kidney and brain.[37–41] Recently, it has been shown that C5a-C5aR interaction participates in the production of antiviral CD8+ T cell response.[42] C5a activities on white blood cells include the release of granules containing elastase,[43] glucuronidase,[11] histamine[44] and eosinophil cationic protein (ECP),[17] stimulation of granulocyte and monocyte oxidative burst with the release of reactive oxygen species, [12,13,45,46] an increased expression of β_2 -integrin adhesion molecules and shedding of L-selectin,[14] an increase in vascular permeability[2,3] and delayed neutrophil apoptosis. [16] These mechanisms of C5a have been implicated in the induction of a potent inflammatory response and prolongation of the life span of neutrophils in the presence of foreign antigens. [16]

A solid body of evidence indicates that C5a stimulates the white blood cell release of pro-inflammatory cytokines. In vitro studies have shown that C5a induces the expression of IL-1 [8,47,48] and IL-8[6] by monocytes. In animal and in vitro studies, C5a augmented LPS-induced release of pro-inflammatory cytokines such as IL-6[7] from neutrophils and TNF α [8,49] from mononuclear cells. On the other hand, these pro-inflammatory cytokines induced the expression of anaphylatoxins in white blood cells. Indeed, IL-1 induces the expression of C3a and C5a receptors in monocytes.[50]

During normal pregnancy, maternal immunity is modified presumably to adapt to the presence of the fetus. Changes in the innate immunity involving leukocytes include increased leukocyte concentration,[51] enhanced oxidative burst and baseline intracellular reactive oxygen species, [52] increased expression of granulocyte/monocyte adhesion molecules,[52] reduced neutrophil apoptosis[53] and increased concentration of plasma granulocyte elastase.[54] Patients with pyelonephritis have marked phenotypical and metabolic changes in granulocytes and monocytes than those detected in normal pregnancy.[52] Neutrophil elastase and myeloperoxidase, as well as macrophage serine proteases, can cleave C5 directly and generate C5a.[55–58] Thus, it is possible that leukocyte activation may account for the high concentration of C5a found in the plasma of pregnant patients with acute pyelonephritis. It is unclear why C3a and C4a were not different between the groups.

There is growing evidence that excessive C5a can be harmful to the host. Indeed, patients with sepsis who had a higher concentration of C5a had lower survival rates than those who had lower C5a serum levels.[19–21] High concentrations of C5a during sepsis are associated with the loss of expression of C5aR due to granulocyte internalization of the C5a-C5aR complex [59–61] with subsequent reduction of the oxidative burst and generation of reactive oxygen species, chemotaxis and impaired bacterial killing.[60–62] In addition, excessive C5a has been linked to the pathophysiology of ARDS.[22,23] Indeed, C5a is increased in bronchoalveolar lavage[63] and plasma[22] of patients with this condition. Furthermore, the administration of anti-C5a improved the outcome of sepsis and ARDS in animal models. [23,62,64,65]

In summary, pregnancy complicated with acute pyelonephritis is characterized by increased maternal plasma C5a concentration. C5a may act on leukocytes and other host cells to potentiate the mechanisms necessary to effectively remove pathogens. On occasion, persistent or exaggerated activation of C5a could lead to detrimental effects to the mother such as ARDS.

References

1. Carroll MC. The complement system in regulation of adaptive immunity. *Nat Immunol* 2004;981–986. [PubMed: 15454921]
2. Schumacher WA, Fantone JC, Kunkel SE, Webb RC, Lucchesi BR. The anaphylatoxins C3a and C5a are vasodilators in the canine coronary vasculature in vitro and in vivo. *Agents Actions* 1991;345–349. [PubMed: 1810146]
3. Cochrane CG, Muller-Eberhard HJ. The derivation of two distinct anaphylatoxin activities from the third and fifth components of human complement. *J Exp Med* 1968;371–386. [PubMed: 4383923]
4. Gorski JP, Hugli TE, Muller-Eberhard HJ. C4a: the third anaphylatoxin of the human complement system. *Proc Natl Acad Sci U S A* 1979;5299–5302. [PubMed: 291947]
5. Dias DS, Lepow IH. Complement as a mediator of inflammation. II. Biological properties of anaphylatoxin prepared with purified components of human complement. *J Exp Med* 1967;921–946. [PubMed: 4960742]
6. Ember JA, Sanderson SD, Hugli TE, Morgan EL. Induction of interleukin-8 synthesis from monocytes by human C5a anaphylatoxin. *Am J Pathol* 1994;393–403. [PubMed: 7508686]
7. Riedemann NC, Guo RF, Hollmann TJ, Gao H, Neff TA, Reuben JS, Speyer CL, et al. Regulatory role of C5a in LPS-induced IL-6 production by neutrophils during sepsis. *FASEB J* 2004;370–372. [PubMed: 14688199]
8. Okusawa S, Yancey KB, van der Meer JW, Endres S, Lonnemann G, Hefter K, Frank MM, et al. C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro. Comparison with secretion of interleukin 1 beta and interleukin 1 alpha. *J Exp Med* 1988;443–448. [PubMed: 3260938]
9. Shin HS, Snyderman R, Friedman E, Mellors A, Mayer MM. Chemotactic and anaphylatoxic fragment cleaved from the fifth component of guinea pig complement. *Science* 1968;361–363. [PubMed: 4175690]
10. Hartmann K, Henz BM, Kruger-Krasagakes S, Kohl J, Burger R, Guhl S, Haase I, et al. C3a and C5a stimulate chemotaxis of human mast cells. *Blood* 1997;2863–2870. [PubMed: 9108406]
11. Daffern PJ, Pfeifer PH, Ember JA, Hugli TE. C3a is a chemotaxin for human eosinophils but not for neutrophils. I. C3a stimulation of neutrophils is secondary to eosinophil activation. *J Exp Med* 1995;2119–2127. [PubMed: 7760001]
12. Mollnes TE, Brekke OL, Fung M, Fure H, Christiansen D, Bergseth G, Videm V, et al. Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 2002;1869–1877. [PubMed: 12176911]
13. Ehrenguber MU, Geiser T, Deranleau DA. Activation of human neutrophils by C3a and C5A. Comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst. *FEBS Lett* 1994;181–184. [PubMed: 8013630]
14. Jagels MA, Daffern PJ, Hugli TE. C3a and C5a enhance granulocyte adhesion to endothelial and epithelial cell monolayers: epithelial and endothelial priming is required for C3a-induced eosinophil adhesion. *Immunopharmacology* 2000;209–222. [PubMed: 10741901]

15. Tonnesen MG, Anderson DC, Springer TA, Knedler A, Avdi N, Henson PM. Adherence of neutrophils to cultured human microvascular endothelial cells. Stimulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. *J Clin Invest* 1989;637–646. [PubMed: 2521491]
16. Perianayagam MC, Balakrishnan VS, King AJ, Pereira BJ, Jaber BL. C5a delays apoptosis of human neutrophils by a phosphatidylinositol 3-kinase-signaling pathway. *Kidney Int* 2002;456–463. [PubMed: 11849385]
17. Takafuji S, Tadokoro K, Ito K, Dahinden CA. Degranulation from human eosinophils stimulated with C3a and C5a. *Int Arch Allergy Immunol* 1994;27–29. [PubMed: 8156000]
18. Ward PA. The dark side of C5a in sepsis. *Nat Rev Immunol* 2004;133–142. [PubMed: 15040586]
19. Nakae H, Endo S, Inada K, Yoshida M. Chronological changes in the complement system in sepsis. *Surg Today* 1996;225–229. [PubMed: 8727941]
20. Nakae H, Endo S, Inada K, Takakuwa T, Kasai T, Yoshida M. Serum complement levels and severity of sepsis. *Res Commun Chem Pathol Pharmacol* 1994;189–195. [PubMed: 8091004]
21. Bengtson A, Heideman M. Anaphylatoxin formation in sepsis. *Arch Surg* 1988;645–649. [PubMed: 3282496]
22. Hammerschmidt DE, Weaver LJ, Hudson LD, Craddock PR, Jacob HS. Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome. Pathophysiological relevance and possible prognostic value. *Lancet* 1980;947–949. [PubMed: 6103300]
23. Stevens JH, O'Hanley P, Shapiro JM, Mihm FG, Satoh PS, Collins JA, Raffin TA. Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. *J Clin Invest* 1986;1812–1816. [PubMed: 3711336]
24. Sacks G, Sargent I, Redman C. An innate view of human pregnancy. *Immunol Today* 1999;114–118. [PubMed: 10203701]
25. Mori W. The Shwartzman reaction: a review including clinical manifestations and proposal for a univisceral or single organ third type. *Histopathology* 1981;113–126. [PubMed: 7194306]
26. Dixon HG, Brant HA. The significance of bacteriuria in pregnancy. *Lancet* 1967;19–20. [PubMed: 4163738]
27. Little PJ. The incidence of urinary infection in 5000 pregnant women. *Lancet* 1966;925–928. [PubMed: 4162367]
28. Gilstrap LC III, Cunningham FG, Whalley PJ. Acute pyelonephritis in pregnancy: an anterospective study. *Obstet Gynecol* 1981;409–413. [PubMed: 7243084]
29. Cunningham FG, Morris GB, Mickal A. Acute pyelonephritis of pregnancy: A clinical review. *Obstet Gynecol* 1973;112–117. [PubMed: 4720190]
30. Cunningham FG, Lucas MJ. Urinary tract infections complicating pregnancy. *Baillieres Clin Obstet Gynaecol* 1994;353–373. [PubMed: 7924012]
31. Catanzarite VA, Willms D. Adult respiratory distress syndrome in pregnancy: report of three cases and review of the literature. *Obstet Gynecol Surv* 1997;381–392. [PubMed: 9178312]
32. Cunningham FG, Lucas MJ, Hankins GD. Pulmonary injury complicating antepartum pyelonephritis. *Am J Obstet Gynecol* 1987;797–807. [PubMed: 3578394]
33. Pruett K, Faro S. Pyelonephritis associated with respiratory distress. *Obstet Gynecol* 1987;444–446. [PubMed: 3543771]
34. Hopkinson ND, Powell RJ. Classical complement activation induced by pregnancy: implications for management of connective tissue diseases. *J Clin Pathol* 1992;66–67. [PubMed: 1740520]
35. Richani K, Soto E, Romero R, Espinoza J, Chaiworapongsa T, Nien J, Edwin S, et al. Normal pregnancy is characterized by systemic activation of the complement system.
36. Gerard NP, Gerard C. The chemotactic receptor for human C5a anaphylatoxin. *Nature* 1991;614–617. [PubMed: 1847994]
37. Chenoweth DE, Hugli TE. Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. *Proc Natl Acad Sci U S A* 1978;3943–3947. [PubMed: 279010]
38. Gasque P, Chan P, Fontaine M, Ischenko A, Lamacz M, Gotze O, Morgan BP. Identification and characterization of the complement C5a anaphylatoxin receptor on human astrocytes. *J Immunol* 1995;4882–4889. [PubMed: 7594492]

39. Haviland DL, McCoy RL, Whitehead WT, Akama H, Molmenti EP, Brown A, Haviland JC, et al. Cellular expression of the C5a anaphylatoxin receptor (C5aR): demonstration of C5aR on nonmyeloid cells of the liver and lung. *J Immunol* 1995:1861–1869. [PubMed: 7836770]
40. Nataf S, Davoust N, Ames RS, Barnum SR. Human T cells express the C5a receptor and are chemoattracted to C5a. *J Immunol* 1999:4018–4023. [PubMed: 10201923]
41. Zahedi R, Braun M, Wetsel RA, Ault BH, Khan A, Welch TR, Frenzke M, et al. The C5a receptor is expressed by human renal proximal tubular epithelial cells. *Clin Exp Immunol* 2000:226–233. [PubMed: 10931135]
42. Kim AH, Dimitriou ID, Holland MC, Mastellos D, Mueller YM, Altman JD, Lambris JD, et al. Complement C5a receptor is essential for the optimal generation of antiviral CD8+ T cell responses. *J Immunol* 2004:2524–2529. [PubMed: 15294968]
43. Haeger M, Unander M, Norder-Hansson B, Tylman M, Bengtsson A. Complement, neutrophil, and macrophage activation in women with severe preeclampsia and the syndrome of hemolysis, elevated liver enzymes, and low platelet count. *Obstet Gynecol* 1992:19–26. [PubMed: 1727579]
44. Siraganian RP, Hook WA. Complement-induced histamine release from human basophils. II. Mechanism of the histamine release reaction. *J Immunol* 1976:639–646. [PubMed: 56398]
45. Goldstein IM, Roos D, Kaplan HB, Weissmann G. Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis. *J Clin Invest* 1975:1155–1163. [PubMed: 171281]
46. Wymann MP, Kernen P, Deranleau DA, Baggiolini M. Respiratory burst oscillations in human neutrophils and their correlation with fluctuations in apparent cell shape. *J Biol Chem* 1989:15829–15834. [PubMed: 2777766]
47. Okusawa S, Dinarello CA, Yancey KB, Endres S, Lawley TJ, Frank MM, Burke JF, et al. C5a induction of human interleukin 1. Synergistic effect with endotoxin or interferon-gamma. *J Immunol* 1987:2635–2640. [PubMed: 3116090]
48. Schindler R, Gelfand JA, Dinarello CA. Recombinant C5a stimulates transcription rather than translation of interleukin-1 (IL-1) and tumor necrosis factor: translational signal provided by lipopolysaccharide or IL-1 itself. *Blood* 1990:1631–1638. [PubMed: 2207333]
49. Riedemann NC, Guo RF, Sarma VJ, Laudes IJ, Huber-Lang M, Warner RL, Albrecht EA, et al. Expression and function of the C5a receptor in rat alveolar epithelial cells. *J Immunol* 2002:1919–1925. [PubMed: 11823527]
50. Takabayashi T, Shimizu S, Clark BD, Beinborn M, Burke JF, Gelfand JA. Interleukin-1 upregulates anaphylatoxin receptors on mononuclear cells. *Surgery* 2004:544–554. [PubMed: 15118592]
51. Efrati P, Presentey B, Margalith M, Rozenszajn L. Leukocytes of normal pregnant women. *Obstet Gynecol* 1964:429–432. [PubMed: 14128474]
52. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. *Am J Obstet Gynecol* 2001:1118–1123. [PubMed: 11717644]
53. von Dadelszen P, Watson RW, Noorwali F, Marshall JC, Parodo J, Farine D, Lye SJ, et al. Maternal neutrophil apoptosis in normal pregnancy, preeclampsia, and normotensive intrauterine growth restriction. *Am J Obstet Gynecol* 1999:408–414. [PubMed: 10454692]
54. Greer IA, Haddad NG, Dawes J, Johnston TA, Johnstone FD, Steel JM. Increased neutrophil activation in diabetic pregnancy and in nonpregnant diabetic women. *Obstet Gynecol* 1989:878–881. [PubMed: 2586951]
55. Vogt W. Complement activation by myeloperoxidase products released from stimulated human polymorphonuclear leukocytes. *Immunobiology* 1996:334–346. [PubMed: 8877407]
56. Ward PA, Hill JH. C5 chemotactic fragments produced by an enzyme in lysosomal granules of neutrophils. *J Immunol* 1970:535–543. [PubMed: 4985169]
57. Huber-Lang M, Younkin EM, Sarma JV, Riedemann N, McGuire SR, Lu KT, Kunkel R, et al. Generation of C5a by phagocytic cells. *Am J Pathol* 2002:1849–1859. [PubMed: 12414531]
58. Vogt W. Cleavage of the fifth component of complement and generation of a functionally active C5b6-like complex by human leukocyte elastase. *Immunobiology* 2000:470–477. [PubMed: 10776801]

59. Furebring M, Hakansson LD, Venge P, Nilsson B, Sjölin J. Expression of the C5a receptor (CD88) on granulocytes and monocytes in patients with severe sepsis. *Crit Care* 2002;363–370. [PubMed: 12225614]
60. Guo RF, Riedemann NC, Bernacki KD, Sarma VJ, Laudes IJ, Reuben JS, Younkin EM, et al. Neutrophil C5a receptor and the outcome in a rat model of sepsis. *FASEB J* 2003;1889–1891. [PubMed: 12897064]
61. Solomkin JS, Jenkins MK, Nelson RD, Chenoweth D, Simmons RL. Neutrophil dysfunction in sepsis. II. Evidence for the role of complement activation products in cellular deactivation. *Surgery* 1981;319–327. [PubMed: 7256544]
62. Czermak BJ, Sarma V, Pierson CL, Warner RL, Huber-Lang M, Bless NM, Schmal H, et al. Protective effects of C5a blockade in sepsis. *Nat Med* 1999;788–792. [PubMed: 10395324]
63. Robbins RA, Russ WD, Rasmussen JK, Clayton MM. Activation of the complement system in the adult respiratory distress syndrome. *Am Rev Respir Dis* 1987;651–658. [PubMed: 3826891]
64. Huber-Lang MS, Sarma JV, McGuire SR, Lu KT, Guo RF, Padgaonkar VA, Younkin EM, et al. Protective effects of anti-C5a peptide antibodies in experimental sepsis. *FASEB J* 2001;568–570. [PubMed: 11259369]
65. Hangen DH, Stevens JH, Satoh PS, Hall EW, O'Hanley PT, Raffin TA. Complement levels in septic primates treated with anti-C5a antibodies. *J Surg Res* 1989;195–199. [PubMed: 2493531]

Figure 1.

Plasma anaphylatoxin concentration in normal pregnancy and pregnancy complicated with acute pyelonephritis. **a**, The median plasma C3a concentration was not significantly different between pregnant patients with acute pyelonephritis and normal pregnant women (median 2414.5 ng/ml, range 769.3 – 19150 ng/ml vs. median 2393.4 ng/ml, range 557.9 – 6642.7 ng/ml; $p=0.4$). **b**, the median plasma C4a concentration was not significantly different between pregnant women with and without acute pyelonephritis (median 5894.6 ng/ml, range 619.9 – 33400 ng/ml vs. median 7827 ng/ml, range 850.7 – 27380 ng/ml; $p=0.1$). **c**, In contrast, pregnant patients with acute pyelonephritis had a median plasma C5a concentration higher than normal pregnant women (median 20.1 ng/ml, range 9.7 – 61.8 ng/ml vs. median 12.3 ng/ml, range 1.5 – 87.1 ng/ml; $p<0.001$).

Table I
Clinical characteristics of the study population and comparison of anaphylatoxin plasma concentrations

	Normal Pregnancy n = 38	Pyelonephritis n = 38	P
Maternal age (y)	24 (17–34)	22 (16–41)	0.4
Nulliparity	13 (34.2)	13 (34.2)	1
Smoking	4 (10.5)	5 (13.2)	0.7
Gestational age at venipuncture (wks)	31.6 (20.5–41.7)	31.6 (20.7–42.2)	0.9
Gestational Age at delivery (wks) [†]	39.0 (37–41.8)	39.2 (28.5–42.7)	0.8
Birthweight (g) [‡]	3372 (2610–4080)	3175 (1080–4090)	0.04*
C3a (ng/ml)	2393.4 (557.9 – 6642.7)	2414.5 (769.3 – 19150)	0.4
Quartiles			
25	1765.2	1913.9	
75	3136.1	3328.8	
C4a (ng/ml)	7827 (850.7 – 27380)	5894.6 (619.9 – 33400)	0.1
Quartiles			
25	3755.4	3500	
75	12925	8464.7	
C5a (ng/ml)	12.3 (1.5 – 87.1)	20.1 (9.7 – 61.8)	<0.001*
Quartiles			
25	7.5	16.2	
75	16.2	27.9	

Values expressed as median (range) or number (percent)

* Statistically significant, $P < .05$

[†] Pyelonephritis n= 37

[‡] Pyelonephritis n= 36