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NORMAL PREGNANCY IS CHARACTERIZED BY SYSTEMIC ACTIVATION OF THE COMPLEMENT SYSTEM

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Abstract

BACKGROUND: The complement system, a major component of innate immunity, has recently been implicated in the mechanisms of fetal loss and placental inflammation in the anti-phospholipid antibody syndrome. Inhibition of complement has been proposed as an absolute requirement for normal pregnancy. Yet, pregnancy is characterized by a generalized activation of the innate immune system. This study was conducted to determine whether normal pregnancy is associated with complement activation in the maternal circulation.

METHODS: Anaphylatoxins (C3a, C4a and C5a) were determined in the plasma of normal pregnant (20-42 weeks; n=134) and non-pregnant women (n=40). These complement split products (C3a, C4a and C5a) were measured using specific immunoassays. Non-parametric statistics were used for analysis.

RESULTS: 1) The median plasma concentration of C3a, C4a and C5a was significantly higher in normal pregnant women than in non-pregnant women (all $p<0.001$); 2) The concentration of C3a, C4a and C5a did not change with gestational age ($p>0.05$); and 3) The plasma concentration of C3a had a positive correlation with the plasma C4a and C5a concentrations (r 0.36, $p<0.001$ and r 0.35, $p<0.001$, respectively).

CONCLUSION: 1) Normal human pregnancy is associated with evidence of complement activation, as determined by increased concentrations of the anaphylatoxins C3a, C4a and C5a in the maternal circulation; and 2) We propose that physiologic activation of the complement system during pregnancy is a compensatory mechanism aimed to protect the host against infection.

Keywords

Complement system; anaphylatoxins; innate immunity; pregnancy; C3a; C4a; C5a

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INTRODUCTION

The complement system, a major component of the innate immune system, is comprised of different plasma proteins reacting with one another in a proteolytic cascade, generating active and powerful products that provide early defense mechanisms against pathogens. Opsonization, chemotaxis and lysis of microorganisms may result from activation of the complement cascade. Furthermore, the complement system has been attributed to modulate adaptive immunity through regulation of B and T cell responses[1] and its involvement in antiviral response has recently been suggested.[2]

Complement can be activated by three different mechanisms: 1) the classical pathway, triggered by the binding of C1q to antigen-antibody complexes or directly to the surface of a microorganism; 2) the alternative pathway, triggered by deposition of spontaneously activated complement components directly on microbial surfaces; or 3) the mannan binding lectin (MBL) pathway, initiated by the binding of MBL to mannose-containing carbohydrates on microorganisms. All these pathways converge in C3 protein; the subsequent cleavage of C5 initiates the assembling of the membrane attack complex (MAC), which is essential for complement function. C3a, C4a and C5a are a group of peptides released from the cleavage of their native proteins during the activation of the complement system. These “complement split products” are also known as anaphylatoxins, due to their ability to increase vascular permeability[3-5] and stimulate smooth muscle contractions.[4,5] Serum carboxypeptidases modulate the activity of all three anaphylatoxins by the removal of a C-terminal arginine residue, generating inactive (C3a desArg and C4a desArg) or less active products (C5a desArg).[6] It has been suggested that detection of complement split products in plasma results from increased complement activation and saturation of the binding capacity of their receptors,[7] or is the consequence of an uncontrollable inflammatory response.[8] Since anaphylatoxins are inactivated rapidly (approximately 2 min),[6] determination of their metabolites (C3a desArg, C4a desArg, and C5a desArg) accurately represents the degree of complement activation.[9] Aside from its role in host defense, the complement system has been implicated in the pathophysiology of sepsis,[10,11] asthma,[12] ischemic/hypoxic injury,[13] rheumatoid arthritis,[14,15] systematic lupus erythematous,[16] and delayed type hypersensitivity.[17]

Complement activation could potentially harm the developing fetus. Recent evidence in murine models have implicated Crry deficiency, a C3 convertase inhibitor, and C3 products in the mechanisms of pregnancy loss.[18] Additionally, fetal injury and pregnancy loss in experimental antiphospholipid-antibody syndrome has been attributed to the effects of complement, mainly C5a.[19] Therefore, it has been proposed that inhibition of the complement system is an absolute requirement for normal pregnancy.[20] Yet, the presence of immune complexes and products of the complement cascade have been identified in trophoblast tissue in normal gestation.[21-23] Moreover, pregnancy is characterized by enhancement of the innate immune system and suppression of the adaptive immune response.[24] Accumulating evidence in support of this view includes: 1) an increased number of granulocytes in maternal blood;[25] 2) phenotypic and metabolic changes in granulocytes and monocytes (increased expression of adhesion molecules, baseline intracellular reactive oxygen, and oxidative burst);[26,27] 3) an increased concentration of acute phase proteins (fibrinogen, clotting factors, globulin);[28,29] and 4) a shift of the T helper-1 to T helper-2 cell cytokine profile.[30,31]

Studies conducted on complement system activation during normal pregnancy have demonstrated increased complement protein concentrations and total complement hemolytic activity (CH50).[32-35] Complement regulatory proteins including “decay accelerating factor” (DAF), membrane cofactor protein (MCP), and CD59 have been reported to be highly expressed in trophoblast[23,36,37] and may play a role in protecting the fetus from damage

generated from complement activation. However, there is a paucity of data on maternal plasma C3a, C4a and C5a concentration in normal pregnancy. This study was designed to determine if the plasma concentrations of C3a, C4a and C5a change during normal pregnancy.

PATIENTS AND METHODS:

Study design: A cross-sectional study was conducted by searching our clinical database and bank of biological samples. This study included 174 women in the following two groups: 1) non-pregnant women (n=40); and 2) normal pregnant women (n=134). The non-pregnant group consisted of healthy women ranging in the luteal phase of the menstrual cycle from 18 to 40 years of age. Normal pregnant women were enrolled from either a labor-delivery unit (in cases of scheduled cesarean section that were not in labor) or from our antenatal clinic. Normal pregnant women were defined as those without medical, obstetrical or surgical complications. Their gestational ages ranged from 20 weeks to term gestation. Eligible patients were approached at the Detroit Medical Center/Hutzel Women's Hospital in Detroit, Michigan. All women provided informed consent prior to the collection of the plasma samples. The collection of samples was approved by the Human Investigation Committees and their utilization for research purposes by the IRBs of both the National Institute of Child Health and Human Development and Wayne State University. Many of these samples have been used in prior studies.

Blood Collection and anaphylatoxin immunoassays: Samples of peripheral blood were collected in tubes containing EDTA. The samples were centrifuged and stored at −70° C. Specific and sensitive enzyme-linked immunoassays 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 the American Laboratory Products Company (Windham, NH). Complement C3a, C4a and C5a assays were performed according to the manufacturers' instructions. Briefly, maternal plasma samples were incubated in duplicate wells of the 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 removed all other 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 and 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 assays. 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 Micro plate 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: Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for normal distribution of the data. Mann-Whitney U tests were utilized for comparison of results among non-pregnant and normal pregnant women. A chi square test was used for comparison of the proportions and Spearman's rank test was used for correlations. The statistical package employed 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. Normal pregnant women had a significantly higher median plasma concentration of C3a, C4a and C5a than non-pregnant women (all $p < 0.001$; Figure 1). The concentration of C3a, C4a and C5a did not change with gestational age (Spearman's correlation coefficient $r = -0.16$, $p = 0.05$; $r = -0.08$, $p=0.35$; and $r = 0.15$, $p= 0.08$, respectively; Figure 2). While a positive correlation was found between plasma concentrations of C3a, C4a and C5a ($r = 0.36$, $p<0.001$ and $r = 0.35$, $p<0.001$, respectively), no correlation was noted between plasma concentrations of C4a and C5a.

DISCUSSION

This study demonstrated that normal pregnancy is associated with activation of the plasma complement system as determined by increased maternal plasma C3a, C4a and C5a concentrations (Figure 1). In addition, the concentrations of these anaphylatoxins did not change with advancing gestational age (from 20 weeks to term gestation) (Figure 2).

The determination of plasma anaphylatoxin concentration was used, since these molecules are more sensitive markers of complement activation in vivo than total hemolytic complement activity (CH50) or native complement proteins.[9] Previous studies have demonstrated that the concentration of complement proteins or CH50 in maternal blood during normal pregnancy is increased.[32,34,38-40] Moreover, Johnson et al.[41] reported that the concentrations of some complement proteins (C2, C4, C3, C5, C6, and Factors B and H) were increased during pregnancy, while others remained unchanged (C1q, C1r and P). However no study has compared the plasma anaphylatoxin concentrations of non-pregnant and normal pregnant women. Although Haeger et al.[42] reported higher plasma C3a and C5a concentrations in preeclamptic patients than in normal pregnant women, no comparisons were performed between normal pregnant and non-pregnant women.

The hormonal regulation of complement tissue expression has been assessed in the endometrium of humans[43] and mice.[44] Hasty et al.[43] determined that human endometrial C3 expression and synthesis was up-regulated during the luteal phase of the menstrual cycle. In contrast, in animal models, the up-regulation of C3 appeared to be influenced by estrogens.[44] The increment in systemic anaphylatoxins during gestation could be attributed, in part, to a stimulation of their precursors by high levels of steroid hormones. Futures studies are required to evaluate the relationship between systemic complement activation and endocrine factors.

The plasma concentration of C1 esterase inhibitor (C1INH), which regulates the activation of the first component of the classical pathway, has been reported to be either low[45-47] or unchanged[40] during normal pregnancy. Its reduction may lead to the activation of the complement cascade and subsequent elevation of C3a, C4a and C5a in maternal plasma. Furthermore, a decrease in the expression of complement receptor 1 (CR1) and DAF on red blood cells has been observed during normal pregnancy.[48] These findings could also explain high levels of complement split products in maternal plasma.

C5a, the most potent anaphylatoxin, can induce oxidative burst in neutrophils and stimulate oxygen radical species production,[49-51] enhance phagocytosis,[49] chemoattract granulocytes,[7,52] and reduce neutrophil apoptosis.[53] Interestingly, these findings have been described in normal pregnant women.[26,27,54] Activated phagocytic cells have the ability to cleave C5 into C5a and C5b fragments.[55-57] Vogt et al. showed that oxidants generated by PMN myeloperoxidase and kallikrein activity resulted in the cleavage of C5 and subsequent production of C5a.[56,58] Additionally, the enzymatic activity of neutrophil elastase[57,59] and macrophage serine protease[55] can also lead to the generation of this

anaphylatoxin. Considering that pregnancy is associated with an increase in the white blood cell count,[25] as well as "activation" of granulocytes and monocytes,[26,27] these may, at least in part, explain the increased C5a concentrations.

It is unknown when complement split products C3a, C4a and C5a begin to rise during normal gestation. However, our results indicate that their plasma concentrations remain unchanged from 20 weeks of gestation to term. Previous studies by Baines et al.[32] and Kitzmiller et al.[34] reported no difference in plasma CH50 concentration in the first trimester when compared to those of non-pregnant women. Stabile et al.[60] did not observe a correlation between maternal complement protein concentrations (C3, C4, factor B) and gestational age during the second trimester, but the interval period of the study was short. Kovar et al.[61] reported similar results during the third trimester. The weak positive correlation between plasma concentration of C3a and the other two anaphylatoxins studied (C4a and C5a) support the view that not all C3a peptides are generated after C4, and that other routes of complement activation may be involved (i.e., alternative pathway). Similarly, other factors besides C3 activation may be involved in the production of C5a, as previously described.[55-57,59]

In conclusion, our study demonstrated that normal pregnancy is associated with increased maternal plasma C3a, C4a and C5a concentration. The activation of the complement system constitutes further evidence of activation of the innate immune system during normal pregnancy. We propose that complement activation may compensate for the decreased adaptive immunity observed in normal pregnancy, and is aimed to protect the host (mother and/or fetus) from microorganisms and other potential antigens.

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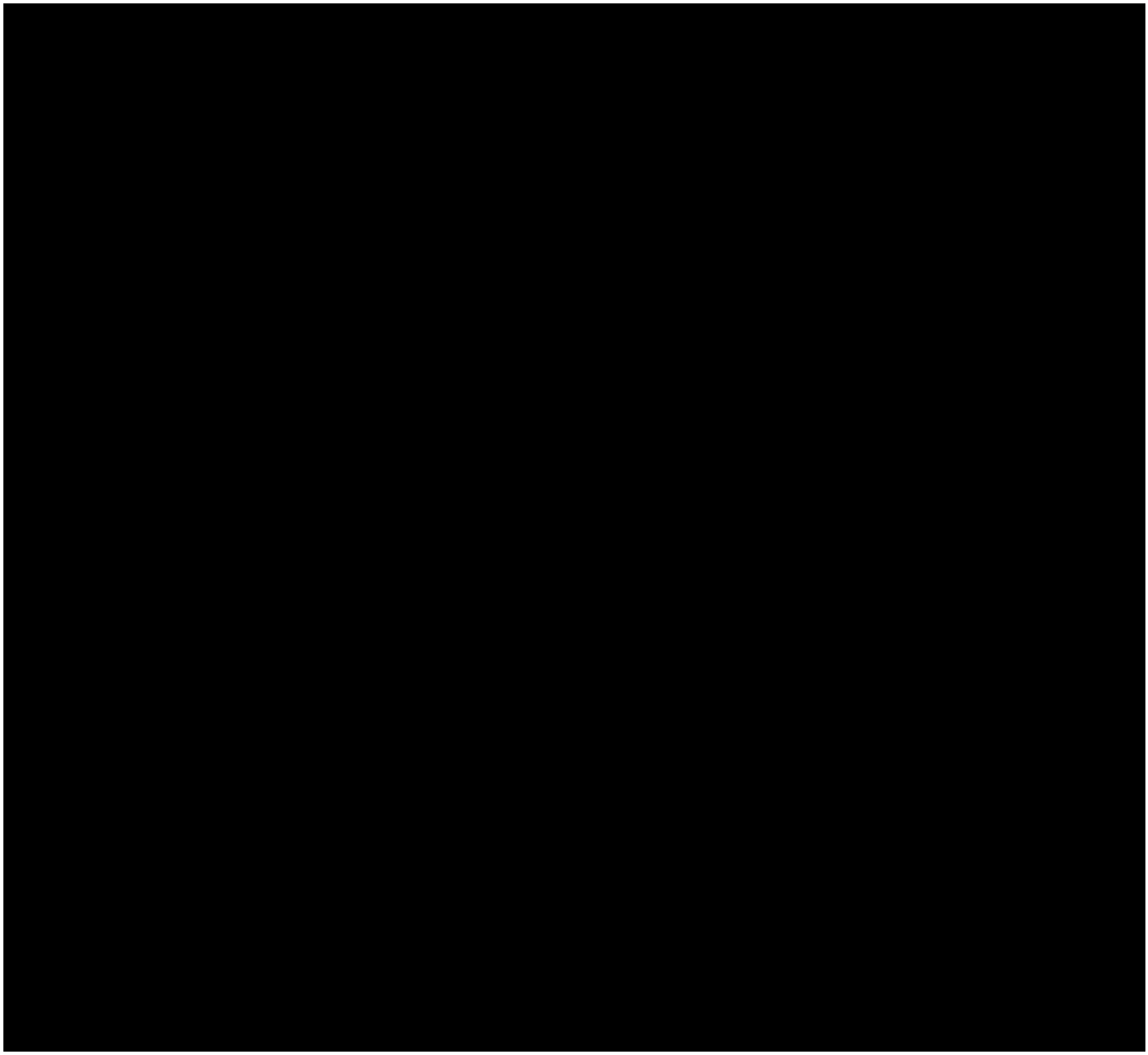

Figure 1.

Plasma anaphylatoxin concentrations in non-pregnant and pregnant women**.** Please note the difference of the scales on the *y*-axis between **A**, **B** and **C. A,** The median plasma concentration of C3a was higher in normal pregnant women than nonpregnant women (median 2364.7 ng/ml, range 557.9 – 6642.7 ng/ml vs. median 1340.4 ng/ml, range 367.4 – 6722.4 ng/ml; $p<0.001$). **B,** Normal pregnant women had a median plasma C4a concentration higher than nonpregnant women (median 10125.4 ng/ml, range 850.7 – 32640 ng/ml vs. median 2625.4 ng/ml, range 304 – 21380 ng/ml; $p<0.001$). **C,** Normal pregnant women had a median plasma C5a concentration higher than nonpregnant women (median 12.4 ng/ml, range 1.2 - 87.1 ng/ml vs. median 4.1 ng/ml, range 0.9 - 13.1 ng/ml; $p<0.001$).

Figure 2.
Relationship between plasma anaphylatoxin concentrations and gestational age. In normal pregnant women, the plasma C3a, C4a and C5a concentrations did not show a correlation with advancing gestational age from 20 weeks to term (C3a $r = -0.16$, $P=0.05$; C4a $r = -0.08$, $P=0.35$ and C5a $r = 0.15$, $P=0.08$).

Table I

Clinical characteristics of the study groups and plasma anaphylatoxin concentrations

	No pregnancy n = 40	Normal pregnancy n = 134	P
Age (y)	26 (18-40)	25 (17-40)	0.33
Gestational age at venipuncture (wks)	--	35.5 (20-41.7)	
Gestational age at delivery (wks)	--	39.2 (37-42.4)	
Birthweight (g)	--	3345 (2610-4080)	
C3a (ng/ml)	1340.4 (367.4 - 6722.4)	2364.7 (557.9 - 6642.7)	<0.001*
Quartiles			
25	1007.3	1723.5	
75	2440.1	3120.3	
C4a (ng/ml)	2625.4 (304 - 21380)	10125.4 (850.7- 32640)	<0.001*
Quartiles			
25	1609.9	5481.2	
75	4567.9	15872.5	
C5a (ng/ml)	4.1 (0.9 - 13.1)	12.4 (1.2 - 87.1)	<0.001*
Quartiles			
25	2.54	8.6	
75	6.9	17.3	

Values are expressed as median (range)

*Statistically significant, $P < 0.05$