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Short Report

Changes in Serum Immunity During Pregnancy

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ABSTRACT Pregnancy requires a host of localized immune factors that allow the mother to tolerate the fetus. Changes in the mother's serum immunity during pregnancy are less well-known. To clarify these changes, 1,351 women from the NHANES 1999–2000 were analyzed with complex survey regression to test the effect of pregnancy on adaptive and innate immune markers. Adjusting for age and BMI, pregnant women had higher C-reactive protein levels and white blood cell counts and lower measles antibody titer and lymphocyte counts than nonpregnant women. This dual pattern of immunological changes supports the hypothesis that mothers will reduce the ability of the adaptive immune system to respond to infection while increasing the activity of innate immunity during pregnancy, maintaining immune function homeostasis. The function of these homeostatic immune responses is unknown. Am. J. Hum. Biol. 21:401–403, 2009. © 2009 Wiley-Liss, Inc.

The evolutionary adaptation that allows the implantation of the embryo into the mothers womb is an immunological conundrum (Medawar, 1953). Since the fetus is not genetically identical to its mother, it should be rejected by her immune system; instead, most pregnancies are tolerated and brought fully to term. In humans, the placenta mediates tolerance and many of the immune factors are localized near this barrier.

On the maternal side of the placenta several main factors regulate immunity. There is a proliferation of macrophages and uterine natural killer cells that are thought to play a significant role in toleration of the fetus. The complement system is down-regulated to prevent inflammation. Finally, progesterone and estrogen promote local anti-inflammatory responses that help prevent rejection of the placenta. On the fetal side, major histocompatibility complex molecules are either not expressed or altered in appearance, causing most maternal T-cells to ignore the placenta. In addition, the placenta produces some anti-inflammatory effects. On both sides, there are a variety of cytokines that regulate the uterine immune environment (Poole and Claman, 2004).

To ensure the mother's survival during pregnancy the immune system patrolling the rest of the body cannot be drastically altered. However, some researchers have noted some changes in maternal immune function during pregnancy; specifically, studies indicate that the innate immune system dominates the immune system, with mixed evidence for adaptive immune system changes (Luppi, 2003).

The purpose of this report is to analyze the changes in peripheral immune function during pregnancy in a population of American women. It will examine population differences in pregnant versus nonpregnant women for four serum immune factors: measles antibody titer, lymphocyte count, white blood cell (WBC) count, and C-reactive protein (CRP) levels. I hypothesize that markers of innate (nonspecific) immunity will be higher, and adaptive (memory) immunity markers will be lower in pregnant women compared with nonpregnant women, helping maintain homeostasis during the immunological and energetic changes associated with pregnancy.

METHODS

Sample

Data were taken from the National Health and Nutrition Examination Survey 1999–2000 (National Center of Health Statistics and Center for Disease Control and Prevention, 2007). The NHANES 1999–2000 set was compiled from interviews, examinations, and laboratory tests of blood and urine. Women between the ages of 18 and 49 who had non-missing values for all dependent and independent variables were included in the study subpopulation, leaving a total n of 1,351 women out of a possible 5,082 women.

Variables

Four main dependent variables were assessed in this study: measles antibody titer, C-reactive protein (CRP) levels, lymphocyte count, and white blood cell (WBC) minus lymphocyte count.

Measles antibody level reflects circulating amounts of antibodies that are available to respond to the measles virus and reflects the ability of the adaptive humoral immunity to respond to infection. CRP is an acute phase protein that is implicated in the innate immune system and is widely used as a marker of inflammation (Marnell et al., 2005). Lymphocyte count is the total number of natural killer cells, B-cells, and T-cells circulating in the blood. The vast majority of lymphocytes are T-cells and B-cells, with natural killer cells typically making up less than 20% of the total lymphocyte count (Chng et al., 2004). Lymphocyte counts are elevated during viral infection (Moghaddam et al., 1997), making them an indicator of cell-mediated adaptive immunity. WBC count is used as a clinical marker of innate immune function. In this study,

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WBC count was calculated as the total number of white blood cells in the blood minus the lymphocyte count.

Women who had nonmissing values for CRP, WBC count, lymphocyte count, and measles antibody titer were included in the subpopulation. However, women who had an optical density index for measles of <1.0, defined by NHANES documentation as having no detectable antibodies to measles, were excluded from analysis because the data set did not contain the variables necessary to separate women who had never been exposed to measles from those that had been exposed, either through infection or vaccination, but had below-threshold values. Measles antibody and CRP levels (mg/dl) were log-transformed and lymphocyte and WBC counts were square-root transformed to normalize their distributions.

TABLE 1. Estimates for dependent variables and their predictors

	Predictors (β)		
Dependent variables	Pregnancy	Age	BMI
Measles antibody	−0.21*	0.032**	0.0028
C-reactive protein	0.80**	0.0084	0.11**
Lymphocyte count	−0.12**	−0.0033**	0.0061**
White blood cell count	0.42**	−0.0060**	0.011**

*$P < 0.05$; **$P < 0.0125$.

Pregnancy status was the main independent variable for all four dependent variables. Women were categorized as either pregnant or not pregnant based on the results from a urine pregnancy test. Approximately 20% of pregnant women could not provide their month of pregnancy, and so this variable was not included. Analyses were adjusted for age and BMI.

Statistical methods

All analyses were performed in SAS 9.1. Means and frequencies were calculated for both the total NHANES female population and the subpopulation. They were compared using *z*-tests to determine whether the subpopulation characteristics are significantly different from total population characteristics.

Following the procedures recommended by NHANES 1999–2000, the sample was weighted using appropriate 2-year strata, cluster, and weight variables from the MEC examination data set.

Sample weights and cluster variables require the entire population to be analyzed to produce unbiased estimates. Since individuals in the subpopulation may not be represented in all clusters and strata, the SAS macro %SREGSUB was used to analyze the subpopulation while

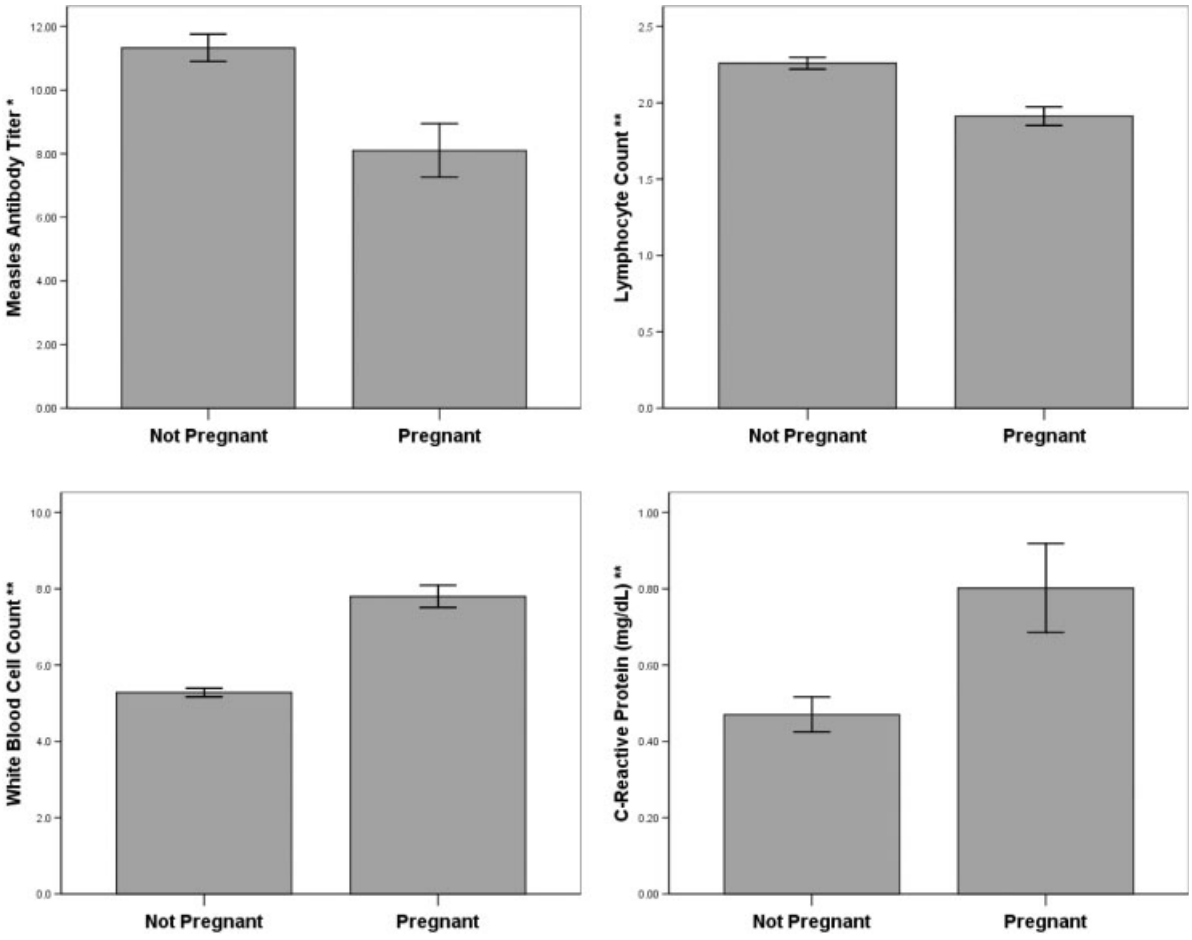

Fig. 1. Differences between pregnant and nonpregnant women in measles antibody titer, lymphocyte count, white blood cell count, and C-reactive protein.

considering observations from the entire NHANES sample (SAS Institute Inc, 2002). This macro provides extra capabilities that are not found in PROC SURVEYREG. Specifically, it restricts regression analysis to a subpopulation and incorporates missing values into variance computations in order to avoid incorrect estimates due to subgroup bias.

The α in this study is 0.05. Because four dependent variables are tested a Bonferroni correction to 0.0125 (0.05/4) is necessary to adjust the α level for each regression model so that the overall α level is maintained. The PROC REG procedure in SAS was used to determine that all variance inflation factors did not exceed 1.5, far below the recommended cutoff of 10 for determining the collinearity of variables in a model (Belsley et al., 1980). Study power was assessed using PROC POWER for multiple regression. Using one test predictor and three full predictors, a sample size of 1,351, and a small effect size (0.1), the power of each model is 95.8%.

RESULTS

Z-tests of study subpopulation characteristics versus the entire NHANES sample of women show the following comparisons. The subpopulation is not significantly different from the total population in age (31.1 vs. 30.4 years, $P = 0.14$), measles antibody level (10.8 vs. 10.6 ODI, $P = 0.17$), and lymphocyte count (2.2 vs. 2.4 SI, $P = 1$). The subpopulation is significantly higher than the general population in CRP levels (0.53 vs. 0.45 mg/dl, $P = 0.0008$), WBC counts (5.7 vs. 5.2 SI, $P < 0.0001$), BMI (28.4 vs. 25.5, $P < 0.0001$), and percentage of women pregnant (16.9% vs. 6.0%, $P = 0.005$). The subpopulation excluded nonreproductive age women, explaining the higher percentage of pregnant women.

To examine if differences in BMI, CRP, and WBC count were due to the higher proportion of pregnant women in the sample, an adjusted subpopulation ($n = 1201$) was generated containing all nonpregnant women and a random sample of 78 pregnant women from the subpopulation to approximate the 6% pregnancy rate in the general population. *Z*-tests indicate that CRP levels in the adjusted subpopulation are not significantly different than the general population ($P = 0.06$); however, WBC count and BMI remain significantly different ($P < 0.0001$ for both). Therefore, differences in CRP levels between the subpopulation and the general population can be attributed to the higher proportion of pregnant women in the sample, but differences in BMI and WBC count cannot.

A summary of regression coefficients and significance levels of all multiple regression models can be found in Table 1. There were significant differences between pregnant and nonpregnant women on three of the four dependent variables: CRP levels, lymphocyte count, and WBC count. The fourth dependent variable, measles antibody titer, was significant at $\alpha = 0.05$, but did not meet the more stringent Bonferroni-adjusted significance level. The direction of these differences can be observed in Figure 1.

DISCUSSION

These results show an increase in biomarkers related to innate immunity and a decrease in biomarkers related to adaptive immunity during pregnancy. There are two possible explanations for this dual pattern. First, it could allow the entire immune system to retain desirable levels of immune system reactivity, neither underreacting nor overreacting to threatening pathogens. It may also help prevent a systemic immune overreaction to the fetus.

There may also be a second, energetic explanation. Research indicates that the innate immune system is less energetically costly to maintain than the adaptive immune system (Lochmiller and Deerenberg, 2000). Mothers may increase the activity of the innate immune system and decrease the activity of the adaptive immune system to reserve energy for pregnancy. Repeating this study in both a nutritionally deficient population and a high inflammation population, such as women with preeclampsia, may be able to help differentiate between these two possibilities. In addition, more measures of immune system activity, such as lymphocyte subset counts, delayed hypersensitivity reactions, or response to vaccine, can provide fine-tuned insight into the nature of immune system trade-offs.

In sum, since pregnancy is accompanied by a vast array of immunological changes that allow the mother to tolerate the fetus, the present dual pattern of immunological activity supports the hypothesis that the immune system regulates itself to maintain homeostasis. Mothers accommodate increases in their innate immune system activity by lowering the ability of the adaptive immune system to respond to infection. Further research should address possible causes for this phenomenon.

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