Immunity During Pregnancy:
Lymphocyte Subpopulations and
Mitogen Responsiveness

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ABSTRACT

Many hypotheses have been proposed to explain the alteration of maternal immune status that allows the fetus to escape rejection. Published data using monoclonal antibodies have stated that there are small variable reductions in circulating T-lymphocytes and little or no change in helper-to-suppressor ratios. Specific decreased levels of helper T-cells have been claimed by other workers. Our laboratory has previously reported alterations in tritiated thymidine uptake ($^3$H-TdR) and HLA antibodies during pregnancy. The present study evaluates total T-cells, lymphocyte T-cell subsets, helper-to-suppressor ratios of T-cells, B-cells, and lymphocyte blast transformation (LBT) throughout pregnancy. These lymphocyte measurements were compared to hormonal changes occurring during pregnancy to determine whether or not hormonal levels have a significant correlation on the maternal immune response during gestation.

Data from 100 women revealed no significant alteration of total T-cells or T-cell subsets during pregnancy or after parturition, as measured by monoclonal antibodies. Helper-to-suppressor ratios were within normal limits. B-cells showed a significant decrease ($P < 0.001$) during the postpartum period. There was decreased lymphocyte responsiveness to mitogenic stimulation by phytohemagglutinin-P (PHA-P), concanavalin-A (CON-A), and pokeweed mitogen (PWM) in the first, second and third trimesters ($P < 0.01$). The mechanisms of fetal protection from maternal immune recognition remain obscure.

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Introduction

Many hypotheses have been proposed to explain maternal toleration of an otherwise histo-incompatible fetus. These theories include that: (1) the fetus may be antigenically immature and these “antigens” are such that the mother does not recognize the fetus; (2) there may be an immunologic barrier which otherwise separates the mother and the fetus so that they are not recognized; (3) the mother may be immunologically suppressed during pregnancy; (4) there may be hormonal alterations which are associated with maternal immune suppression; and (5) there may be a decrease in circulating T-cells or alteration of helper-to-suppressor ratio which regulates cell-mediated immunity during pregnancy.

Previous work suggests that there is a decrease in total T-lymphocytes during the first two trimesters of pregnancy. There is decreased lymphocyte blast transformation (LBT) when maternal lymphocytes were exposed to phytohemagglutinin-P (PHA-P), concanavalin-A (CON-A), and pokeweed mitogen (PWM). Whether or not this decrease in LBT is associated with changes in helper-to-suppressor ratio is still unsettled. There are conflicting data in the literature as to whether or not there are changes in helper-to-suppressor ratios at various times of gestation.

The purpose of this study was to examine possible alterations in lymphocyte populations during pregnancy. Total T-cells, lymphocyte subsets, helper-to-suppressor ratios, B cells, and lymphocyte blast transformation were studied in non-pregnant women. The presence or absence of circulating antibodies to HLA antigens during pregnancy was evaluated. Serum levels of estradiol, testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), were measured in the pregnant population.

Materials and Methods

One hundred pregnant women with a mean age of 23.9 ± 5.6 years (range 15 to 41) made up the study group. These women consisted of 35 white and 65 black females in various trimesters. Twenty-one women were in their first trimester, 47 in the second, and 32 in the third trimester. One hundred healthy non-pregnant females served as the control group. Their mean age was 27.1 ± 9.3 years with a range from 21 to 43. This group consisted of 45 white and 55 black women. One hundred healthy men were used as a control group. Their mean age was 31.3 ± 8.8 years with a range from 23 to 41. There were 63 white and 37 black males.

Anticoagulated peripheral blood (EDTA and heparin) was drawn for determinations of complete blood count, T and B cell analyses, LBT, and a serum sample for radioimmunoassay (RIA) hormonal analyses. The time of gestation for this study was determined from the history by using the first day of the last menstrual period or by ultrasound technique.

Pan T-cells (OKT3), T-cell subsets (OKT4-helper and OKT8-suppressor), E-rosette receptor (OKT11), and B-cells (Leu-12) were measured by the use of commercially available monoclonal antibodies using an automated flow-cell cytometer. Helper-to-suppressor ratios and absolute numbers of lymphocytes were evaluated for each group from this data. Lymphocyte blast transformation on lymphocytes from whole blood was performed using PHA-P, CON-A and PWM at optimum doses. Serum estradiol, testosterone, FSH and LH levels were measured by RIA during each of three trimesters. T and B lymphocytes, subsets of T-cells, and LBT's were measured in the pregnant population.

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LYMPHOCYTE SUBPOPULATIONS IMMUNITY AND PREGNANCY

### TABLE I

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>PHA-A</th>
<th>CON-A</th>
<th>PWM</th>
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<tr>
<td>I Trimester</td>
<td></td>
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</tr>
<tr>
<td>NP</td>
<td>297 ± 62</td>
<td>29343 ± 3642</td>
<td>24799 ± 1240</td>
<td>14295 ± 4152</td>
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<td>P</td>
<td>212 ± 68</td>
<td>3752 ± 1169</td>
<td>3095 ± 1063</td>
<td>4026 ± 1128</td>
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<tr>
<td>II Trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NP</td>
<td>225 ± 76</td>
<td>3674 ± 4695</td>
<td>3595 ± 1095</td>
<td>3205 ± 2590</td>
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<tr>
<td>P</td>
<td>297 ± 87</td>
<td>638 ± 628</td>
<td>534 ± 1041</td>
<td>445 ± 1022</td>
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<tr>
<td>III Trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>287 ± 71</td>
<td>3406 ± 6190</td>
<td>2830 ± 2794</td>
<td>1294 ± 2886</td>
</tr>
<tr>
<td>P</td>
<td>306 ± 44</td>
<td>777 ± 2635</td>
<td>474 ± 1503</td>
<td>507 ± 2010</td>
</tr>
</tbody>
</table>

PHA-P = Phytohemagglutinin-P  
CON-A = Concanavalin-A  
PWM = Pokeweed mitogen  
\(^3H\)-Tdr = Tritiated thymidine  
n = Number of patients in population, \(p < 0.01\)  
NP = Non-pregnant  
P = Pregnant  
* = Laboratory data are expressed as counts per minute (cpm) of \(^3H\)-Tdr cells with and without mitogens (mean ± 1 SD).

PHAs were assayed during each of the three trimesters. Sera of mothers were screened for the presence of HLA antibodies.

Statistical data analyses were done by using student’s unpaired t-test.

### Results

The lymphocytes of the pregnant women showed statistically significant decreased LBT responses to each of the three mitogens tested during all three trimesters of pregnancy (table I).

In Table II are shown the results of lymphocyte percentages of women in the first, second, and third trimesters and post-partum period versus the non-pregnant controls. The difference in total T-cells and subsets of T-cells was not significant for any group \((P = n.s.)\). B-cell results remained within normal ranges throughout pregnancy but decreased during the period following delivery as compared to all the other groups, and the differences were statistically significant \((P < 0.001)\).

The results of the cytotoxicity screen-

### TABLE II

| Patient Population | Absolute Lymphs\(|\times 10^9|\) | (PAN-T) | (TH\(_A\)) | (T\(_{\text{R}}\)) | (B-ROS.) | (4/8 Ratio) | B-Cell Percent |
|--------------------|---------------------------------|---------|------------|-------------|---------|-------------|---------------|
| NP                 | 65                              | 2099 ± 695 | 73.2 ± 6.3 | 45.1 ± 8.6 | 26.7 ± 5.6 | 79.8 ± 5.8 | 1.7 ± 1.0 |
| 1st Trimester      | 10                              | 2373 ± 521 | 74.0 ± 9.0 | 45.9 ± 9.0 | 26.5 ± 8.1 | 81.4 ± 4.9 | 1.7 ± 1.1 |
| 2nd Trimester      | 20                              | 2010 ± 480 | 71.7 ± 9.0 | 43.9 ± 7.4 | 27.1 ± 6.7 | 79.7 ± 6.5 | 1.6 ± 1.1 |
| 3rd Trimester      | 20                              | 2195 ± 552 | 74.3 ± 5.5 | 45.3 ± 8.7 | 27.0 ± 4.6 | 80.7 ± 4.2 | 1.8 ± 1.2 |
| Post-Partum        | 20                              | 2032 ± 418 | 75.6 ± 6.5 | 41.4 ± 7.3 | 31.4 ± 8.8 | 83.2 ± 6.6 | 1.3 ± 0.8 |

The values are expressed as the mean ± 1 SD with the range in parentheses.  
n = Number of patients in population  
NP = Non-pregnant women  
* = Results are expressed as absolute number of lymphocytes per \(\text{mm}^3\) whole blood  
\# = Number of patients in Leu-12 population = 47  
\(\uparrow\) = \(p < 0.001\)  
TH\(_A\) = Helper T lymphocytes  
T\(_{\text{R}}\) = Suppressor lymphocytes  
E-ROS. = E-Rosette Receptor
ing indicated that approximately 35 percent of the pregnant women tested had antibodies to HLA antigens. Seven of the 11 women who had spontaneous abortions were positive for circulating HLA antibodies while 30 of 37 women with circulating HLA antibodies had normal term deliveries.

Data for estradiols, testosterone, FSH, and LH showed expected changes consistent with pregnancy. Lowest estradiol was seen in the first trimester rising to a peak in the third trimester. Testosterone was lowest in the first trimester and highest in the third trimester. Follicle stimulating hormone remained constantly low throughout pregnancy, and LH was elevated in all trimesters (figure 1).

**Discussion**

Pregnancy is not associated with a significant decrease in total T-lymphocytes. There seems to be minimal alteration in the helper-to-suppressor ratio. The decreased LBT in response to mitogens is dramatic in the first two trimesters. It is possible that there are yet unrecog-

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**Figure 1.** Sera levels in pregnant women.

<table>
<thead>
<tr>
<th></th>
<th>I (n=21)</th>
<th>II (n=47)</th>
<th>III (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>33.3</td>
<td>73.8</td>
<td>134.4</td>
</tr>
<tr>
<td>Testosterone</td>
<td>49.2</td>
<td>57.7</td>
<td>95.0</td>
</tr>
<tr>
<td>FSH</td>
<td>1.30</td>
<td>1.50</td>
<td>1.34</td>
</tr>
<tr>
<td>LH</td>
<td>1687</td>
<td>1316</td>
<td>1162</td>
</tr>
</tbody>
</table>
nized circulating factors which result in suppression of the lymphocyte blast transformation. The possibility does exist that there may be low molecular weight compounds related to the mother which could have a suppressive effect on the lymphocytes themselves. Low molecular weight compounds have been described in a variety of other diseases and are known to cause decreased lymphocyte blast transformation. The possibility exists that these low molecular weight factors or compounds may come from either fetal antigenic substances or metabolic products.

Other workers have studied the effects of a variety of hormones on T- and B-cells and their immune responses. Other investigators have studied the effects of a variety of hormones on T- and B-cells and their immune responses. 14 It does not appear that the hormones themselves cause any of the alterations which have been noted.

In this study, HLA antibodies were present in a higher percentage (64 percent) of the patients with spontaneous abortions. These antibodies were also found in 81 percent of women who had normal term deliveries. Similar observations have been made previously. 4

The reason for the successful growth and development of the fetus as a histoincompatible graft tolerated by the mother remains a mystery. The most consistent and dramatic aspect of immunoregulation which is altered during pregnancy is decreased response to mitogens as measured by lymphocyte blast transformation. Humoral responsiveness is not impaired during pregnancy. Helper-to-suppressor ratios do not seem to be markedly altered. Whether or not these differences in cell subsets, decreased 3H-Tdr uptake responses in pregnant women to mitogens and some variations in antibodies to HLA antigens are significant in protecting the fetus is not fully apparent. In order to understand more precisely the immune response during pregnancy, continued study using new and innovative experimental approaches will be necessary.

Acknowledgments

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