Maternal Adjustments in the Immune System in Normal Pregnancy

Stanley A. Gall, MD
Duke University Medical Center
Durham, North Carolina

The concept of normal pregnancy implies the adjustment of the maternal organism to an antigenically foreign fetus and placenta. The observed fact that pregnancies are successful is proof that the mother's immune system is able to adjust to this foreign insult and maintain the pregnancy in an undamaged state. Therefore, we are left with the task of attempting to explain the immunologic changes that occur in the mother during normal gestation.

The immunobiologic relationship of the fetal-maternal unit has been reviewed from a number of viewpoints. Immunology in general and the immunology of pregnancy specifically is not well understood by the practicing obstetrician. In fact, the rapid accumulation of new information has made understanding more difficult by those working in the field every day. There has been a rapid expansion of knowledge, and those publishing are combining the various aspects of the immunology of pregnancy rather than attempting to better define the particular aspect of the immune system being affected. The immunologic relationship may be described under the following areas:

1. The immunology of the maternal-fetal interaction
2. The immunologic alterations in the mother
3. The immunologic response of the fetus
4. The immunology of preeclampsia and other pregnancy-associated conditions
5. Autoimmune disease in pregnancy

In this article I will attempt to explain the immunologic alterations occurring in the mother with regard to the immunology of the maternal-fetal interaction. A number of mechanisms have been proposed to explain the nonrejection of the fetus and placenta, recently summarized by Beer et al. Some of these include:

1. Complete separation of maternal and fetal blood circulation
2. Immunologic buffer zone at the maternal-fetal level interface
3. Masking of the surface alloantigens on the trophoblastic cells
   a. Sialomucin coating on trophoblastic cells that decreases or prevents contact by maternal lymphocytes
   b. Acquisition of agents that results in "masking" or "blocking"
   1. Transferrin that binds to specific receptors on trophoblastic cells

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2. Antibodies or antibody-antigen complexes that bind via Fc receptors to the trophoblastic cell surface
4. Synthesis by syncytiotrophoblast and maintenance in local high concentrations of hormones and other agents that play an immunosuppressive role, e.g., progesterone, estrogens, chorionic gonadotropin, cortisol-binding globulin
5. Production by the fetus of immunosuppressive agents or cells that enter the maternal circulation, e.g., fetal suppressor T cells, α-fetoprotein, lymphokines from stimulated fetal lymphocytes
6. Production of immunoregulatory agents by the mother that alter the immune attack on the fetoplacental unit
   a. Increased synthesis of adrenal corticosteroids, pregnancy-associated plasma proteins (PAPP), early pregnancy factor, α2-globulins
   b. Synthesis of "blocking" antibodies
   c. Presence of suppressor cells
   d. Inversion of T- and B-cell numbers in peripheral blood

Although these mechanisms, as well as others, have been proposed as explanations for the maintenance of the fetoplacental unit as an allograft, the significance of each mechanism as part of the entire process has not been elucidated. Obviously, some mechanisms are of greater importance and must be considered primary, whereas others are secondary. Because of the rapid expansion of basic immunologic information in the area of human reproduction, it is not possible to be definitive about which proposed mechanisms are primary. Therefore, as new information becomes available, there will be an expansion in some areas and elimination of some hypotheses or mechanisms.

**Physiologic Alterations During Pregnancy Affecting the Immune System**

**Blood Volume**

The maternal blood volume increases dramatically during pregnancy. Pritchard calculated blood volume in normal pregnant women using $Cr^{51}$-labeled red blood cells (RBCs). He showed a 48% increase in singleton pregnancies and a 51% increase in twin gestations (Table 1), with RBC volumes increasing 32% and 31%, respectively. Individual variation in patients is the rule, with increases of 20-100% of nonpregnant volume found in normal pregnancies.

Other methods to measure plasma volume have been used and include Evans blue dye (T-1824) and radioactive iodinated serum albumin (RISA). The results of many investigators have demonstrated an increase of 30-60% (mean 42%). Chesley and Duffus found that posture in the third trimester affected plasma volume and probably accounts for wide discrepancies in the values that have been reported. In addition, there are different findings when the plasma volume is reported as total milliliters or in relation to body weight (milliliters per kilogram). Pirani et al. showed that plasma volume in total milliliters increases until 30-34 weeks, then remains the same until delivery. Lund and Donovan measured plasma volume in relation to weight (milliliters per kilogram) and found a progressive rise until 24 weeks, followed by a minimal increase until term.

The volume changes affect formed elements, including lymphocytes. As a result of the rapid rise in plasma volume in early pregnancy (10% increase at 10 weeks' gestation, 20% increase at 20 weeks' gestation) and the later rise in RBC volume, the hematocrit drops in the first trimester by as much as 10% of the original volume. This has been referred to as physiologic anemia of pregnancy, but there is in fact an actual increase in total RBC volume.

No known single physiologic mechanism is solely responsible for the increase in blood volume during pregnancy. Aldosterone, estrogen, and progesterone, either synergistically or antagonistically have been implicated as causative factors in the expansion of blood volume. The fetus is not a requisite for the development of hypervolemia, because significant increases in blood volume have been observed in cases of hydatidiform mole.

Therefore, it is seen that blood volume
TABLE 1. Blood and Red Cell Volumes in Normal Women in Late Pregnancy and When Not Pregnant

<table>
<thead>
<tr>
<th></th>
<th>Late Pregnant</th>
<th>Nonpregnant</th>
<th>Increase (ml)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single fetus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood volume</td>
<td>4820</td>
<td>3250</td>
<td>1570</td>
<td>48</td>
</tr>
<tr>
<td>RBC volume</td>
<td>1790</td>
<td>1355</td>
<td>430</td>
<td>32</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>37.0</td>
<td>41.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood volume</td>
<td>5820</td>
<td>3865</td>
<td>1960</td>
<td>51</td>
</tr>
<tr>
<td>RBC volume</td>
<td>2065</td>
<td>1580</td>
<td>485</td>
<td>31</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>35.5</td>
<td>41.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


expands by 20–100%, starting in early gestation, continuously until 34 weeks, when a plateau occurs. The amount of expansion is 1500–2500 ml, with plasma volume expansion averaging 1500 ml and red blood cell volume 110–500 ml. The extent of plasma and RBC expansion during pregnancy must be kept in mind when one addresses the concentration and changes during pregnancy of other formed elements and hormones.

**Blood Constituents**

The hemodilution that occurs during pregnancy has an effect on other plasma components. Serum osmolality falls in the first trimester from a nonpregnant level of 290 mOsm/kg to 280 mOsm/kg. The serum osmolality remains at this level for the remainder of the pregnancy. Serum electrolyte concentrations fall by about 5 mEq/l.

The erythrocyte sedimentation rate (ESR) rises from a normal of less than 20 mm/hr to more than 40 mm/hr. This is largely due to an expansion of fibrinogen from 250 mg/dl to 450 mg/dl. In addition, α2-globulin increases by 100 mg/dl progressively throughout pregnancy and α2-macroglobulin increases by 27%.

Serum proteins declined by 1 g/dl, most of the loss accounted for by the decline in albumin, the decline occurring during the first half of pregnancy, with a plateau reached by 24 weeks' gestation.

In spite of the expansion of blood volume there is a progressive rise in the peripheral lymphocyte (WBC) count during gestation. The WBC count rises slowly in the first trimester and reaches a peak by 30 weeks' gestation. In the third trimester, the WBC count ranges from 5000 to 12000/mm³, with counts as high as 16000/mm³ considered normal. Twenty percent of patients in the third trimester will have counts greater than 10,000/mm³. In labor there is a progressive increase in the WBC count; levels of 25,000/mm³ have been observed.

The increase in the WBC count is made up with an increase in granulocytes; peripheral lymphocytes and monocytes are unaltered. This difference is due to the selective hyperactivity of granulopoiesis. Because of this increased granulocyte production, immature forms may be released into the circulation. Therefore, the presence of myelocytes or metamyelocytes in the peripheral blood should not be considered unusual, especially in late pregnancy.

Alterations of the metabolic activity of leukocytes also occur. An increase in leukocyte alkaline phosphatase occurs progressively to term, starting at the end of the first trimester. There are increases in myeloperoxidase and a reduction in nicotinamide adenine dinucleotide oxidase levels, probably responsible for the increased bactericidal activity of leukocytes in pregnancy.

Quantitative tests of the activity of neutrophils (PMNs) were performed by Bjorksten et al. They tested chemotaxis; capacity to reduce nitroblue tetrazolium (NBT); and phagocytosis, shown by chemoluminescence. They demonstrated decreased chemo-
taxis in neutrophils from pregnant women, compared with PMNs from nonpregnant women. Gestational age did vary the effect of pregnancy on chemotaxis. Control PMNs were not influenced by serum from pregnant women, indicating that the depressed chemotaxis may be dependent on cell-mediated factors.

Phagocytosis of Escherichia coli by control PMNs was shown to be low in the presence of sera from pregnant women unless the strain of bacteria was able to activate the alternative complement pathway. These findings are at variance with those of Mitchell et al.,14 who found increased phagocytic and bactericidal activity of PMNs from pregnant women.

Pregnant women have a depressed endotoxin-stimulated (NBT) test.16 The NBT test depends on both normal neutrophil oxidative metabolism, by which NBT is reduced to formazan, and on subsequent ingestion of the extracellularly formed formazan particles. A low NBT test value may indicate a depressed phagocytic capacity in the presence of normal oxidative metabolism.

Neutrophil chemoluminescence is significantly higher in PMNs of pregnant than in nonpregnant women. The increased chemoluminescence is present in early pregnancy and has no relation to gestational age, and serum from pregnant women has no effect on the chemoluminescence of control PMNs. Chemoluminescence depends on normal oxidative metabolism, which is stimulated during phagocytosis. This information is consistent with that of Mitchell et al.,14 who reported hexose monophosphate shunt activity in phagocytizing PMNs from pregnant women to be approximately twice that found in nonpregnant women.

The alteration in the levels of complement in pregnancy serum may be important, because complement is involved in many antigen-antibody reactions. The plasma levels of C3 and C4 have been measured throughout pregnancy.17-19 Plasma levels of C3 and C4 are elevated in the second and third trimesters. The most likely mechanism for the increased levels of complement components is increased hepatic synthesis.18 Activation of the complement system by either classic or alternative pathways can be determined by the measurement of split products of C3. C3d concentrations increase in the second and third trimesters, with levels paralleling plasma C3. The elevation of C3c in the second and third trimesters is probably a reflection of the elevation of C3 and its nonspecific proteolysis, rather than complement activation. These findings suggest that neither the classic nor the alternative pathway of complement is activated in normal pregnancy.

Pregnancy-Associated Plasma Proteins, Enzymes, and Hormones

Pregnancy-Associated Plasma Proteins

The presence of unique plasma proteins during pregnancy has been demonstrated by several investigators. Smithies20 and Afonso21 described an α-globulin protein band detectable in the serum of pregnant women and not detectable in nonpregnant women or men. Several investigators demonstrated two serum proteins present in pregnancy plasma, using an antiserum to pregnancy plasma.22,23 Gall and Halbert24 described four plasma components not found in nonpregnant or male plasma. These components were characterized by Lin et al.25 PAPP-B and PAPP-C migrated as β-globulins, and PAPP-A and PAPP-D migrated as α-globulins in immunoelectrophoresis. The molecular weight for the PAPPs were determined to be 750,000 for PAPP-A, 110,000 for PAPP-C, and 20,000 for PAPP-D. The PAPPs are distinct from two other pregnancy-associated plasma proteins. One of these, the pregnancy zone protein (PZP) has been described by investigators under eight different names: α2-pregnoglobulin (Berne), SP3 (Bohn), new serum α2-macroglobulin (Stimson), PAG (Horne), Pal (Machausen), pregnancy-associated α2-globulin (Kasukawa), Xh (Dunston), and Xm (Berg).26

The other plasma-associated plasma protein (SP2, Bohn) has been identified as a sex-
immune system in normal pregnancy

binding steroid. Both PZP and SP2 are not specific for pregnancy but increase in pregnancy and in women taking steroid hormone contraceptives and in cancer patients.

Previous studies have shown that the four PAPPs increase steadily during pregnancy, particularly in the third trimester. PAPP-C and PAPP-D (human placental lactogen [hPL]) as well as PZP reach a plateau during late gestation, whereas PAPP-A continues to rise throughout the third trimester. In the postpartum period, PAPP-B and PAPP-D (hPL) rapidly disappear following delivery, when PAPP-C has a half-life of 1–2 days and PAPP-A has a half-life of 3–4 days. PZP decreases more slowly and is still detectable at 14 days after birth.

The biologic functions of the PAPPs are not well understood. PAPP-C (SPI of Bohn) appears to be essential for normal pregnancy. Antiserum to PAPP-C will induce abortion in pregnant cynomolgus monkeys, which possess an analogous cross-reactive PAPP. PZP has been reported to inhibit cell-mediated immune reactions.

Several authors have described the biologic relationship of PAPP-A to obstetric parameters as well as circulating levels in normal pregnancy. Maternal age, increased parity, and increased body weight are associated with decreased PAPP-A levels; whereas mothers carrying male fetuses, Rh-negative children, and babies with Apgar scores higher than 7 have increased levels of PAPP-A. No clear immunologic mechanism for PAPP-A has been described, although Bischof et al. have attributed an immunologic function to PAPP-A as a messenger. PAPP-A is detectable as early as the 5th week of pregnancy (10 µg/l) with a persistent rise to term. Levels at the end of each trimester were 1.7, 17, and 38 mg/l, respectively.

The PAPPs are produced by the syncytiotrophoblast in a manner similar to that of human chorionic gonadotropin (hCG) and hPL. The concentration in cord blood and amniotic fluid is very low.

Beckman et al. have shown that pregnancy-associated glycoprotein production is stimulated in the presence of genetic incompatibility between mother and fetus. This finding suggests a regulatory role in the nonrejection of the fetus. Damber et al. reported that patients with low PZP values had a higher tendency to undergo spontaneous abortion. Birkland et al. expanded the concept that PAPPs have immunologic activity by testing PZP in a system for rosette formation by T- and B-lymphocytes and in leukocyte migration inhibition tests using inhibition induced by purified protein derivative (PPD). PZP caused a dose-dependent inhibition of lymphocyte transformation after stimulation with (PHA), PPD, and mixed lymphocyte culture (MLC). The rosette tests showed PZP caused T-lymphocytes to bind to fewer sheep RBCs but had no effect on B rosettes. These results are suggestive that PZP can exert an immunosuppressive effect during pregnancy.

Recently a new additional PAPP has been described by Bohn, and maternal serum levels in complications of late pregnancy have been measured. PP-5 is described as a placent al protein with a molecular weight of 86,000, a carbohydrate content of 19%, and a B1-globulin electrophoretic mobility. Elevated PP-5 levels have been shown to be present in patients with preeclampsia, diabetes mellitus, and twins. In addition, PP-5 may have potential in predicting the occurrence of premature labor and abruptio placenta.

The entire group of PAPPs are interesting and potentially important either as markers to assess the fetal status in utero or as regulatory substances. Several investigators have presented data to support a immunologic regulatory function, but the level of research is at a descriptive rather than a mechanism-oriented level. It would be reasonable to surmise that the PAPPs may be important to regulatory substances that will contribute to the understanding of nonrejection of the fetus.

α-Fetoprotein (AFP) is a major constituent of fetal plasma and may be an immunoregulatory substance. AFP is present as early as 6 weeks of gestation and increased.
to a peak fetal serum concentration of 3 mg/ml at 13 weeks’ gestation. The maternal serum concentration is significantly less, going from a nonpregnant level of not being detectable to 500 ng/ml in the third trimester. There is no correlation between peak fetal and peak maternal levels.

The function of AFP is not known. Its molecular weight is 65,000 daltons. It has an amino acid composition similar to that of albumin and has the ability to bind estrogens but not testosterone. AFP may have an immunosuppressive role in man. Mouse studies show a suppression of T-cell-dependent immune reactions in vitro. In man AFP can inhibit PHA-induced lymphocyte transformation. Murgita et al. have suggested that AFP may induce production of suppressor T cells, which alter T-cell but not B-cell function.

AFP is currently utilized as a screening test for neural tube defects. Its role as an immunoregulating substance is suggested but not proven. Because of the very high fetal serum concentrations (up to 3 mg/ml), it may well be an important immunoregulating substance.

Effect of Placental Hormones on the Maternal Immune System

Human Chorionic Gonadotropin

hCG is produced by the syncytiotrophoblast and begins to rise immediately after conception; levels are detectable within 2 weeks of conception. Peak levels of 20,000–100,000 IU/l can be reached by 10 weeks of gestation. Values between 4000 and 11,000 IU/l are seen in the second and third trimester. Peak urine levels of 20,000–50,000 IU/24 hr occurs in the first trimester and 5000–10,000 IU/24 hr in the second and third trimesters. The placenta contains 600 IU/g weight at 10 weeks and decreases rapidly to less than 20 IU/g after the 16th week.

The concept that hCG has significant immunosuppressive activity with regard to maternal lymphocytes has been demonstrated by several investigators and refuted by others. Contractors and Davies, and Adcock et al., and Teasdale et al., have added increasing concentrations of hCG to lymphocytes and have noted an inhibition of stimulation by PHA. The inhibition occurred with concentrations of hCG as low as 1 IU/ml, marked inhibition with concentrations of 100 IU/ml (present in serum in the later part of gestation), and complete inhibition at 10,000 IU/ml, a level reached and exceeded at 10 weeks’ gestation. Hammarstrom et al. repeated the experiment in 1979 and obtained the same results.

Caldwell et al.,, Pattillo et al., and Morse have provided data that suggest that the inhibition of PHA-stimulated lymphocytes was due to crude preparations of hCG and that when purified preparations of hCG were used, no inhibition occurred until hCG concentrations were greater than 5000IU/ml. Some commercial preparations of hCG contain phenol as a preservative, and this has been shown to immunosuppressive. It would seem unwarranted to attribute non-rejection of the fetus to the effects of hCG, but it may have local regulatory function at the trophoblast site.

Human Placental Lactogen

hPL is a hormone similar to Human Growth Hormone produced by the syncytiotrophoblast of the placenta. It has been used as a measurement of placental function. The effect of hPL maternal immune reactivity has lymphocyte transformation. Contractor and Davies showed a dose-response curve in lymphocytes from three volunteers, demonstrating suppression of response of PHA-induced lymphocytes. Morse demonstrated little inhibitory effect of hPL until concentrations were greater than 1000 μg/ml (the usual maternal plasma levels are 3–5 mg/ml). Hammarstrom has demonstrated that hPL in concentrations as low as 2 μg/ml can effect almost complete inhibition of PHA-stimulated lymphocytes.

It seems clear that the difference in results may be explained by the method or the hormone preparation used. Whether the hormones have been contaminated with immunoglobulins, other proteins, or un-
known chemicals, there is a need to reevaluate the effect of hPL on immune reactivity carefully.

**Progesterone and Its Effect on the Maternal Immune Response**

Progesterone is a very important hormone in pregnancy. It is produced by the corpus luteum, which has a functional life span of 70 days. The placenta becomes the source of progesterone in pregnancy, with plasma levels rising from 2 to 12 µg/ml from the 9th to 35th week, with an increase to 17 µg/ml at term. The daily production of progesterone by the placenta increases to 300 µg/day at term. The umbilical venous blood concentration is about 500 ng/ml, and the placental blood is about 100 times higher. Clemens et al.\(^46\) has demonstrated by inhibition of mixed lymphocyte culture (MLC) reaction with progressive concentration of progesterone, estradiol, and testosterone (1-20 µg/ml). Mitogen-stimulated cultures were also inhibited by 1-20 µg/ml of progesterone or estradiol. The concentrations of hormones reported to cause inhibition are easily obtainable with progesterone at the placental site, but the placental concentrations of estradiol and testosterone are much lower. Therefore, the abrogation of the immune response at the placental site by progesterone is possible. These authors\(^46\) also presented evidence that progesterone has little effect on the activated intracellular enzyme systems involved in deoxyribonucleic acid (DNA) synthesis but has a significant effect on active transport of thymidine into the cells. The local immunosuppression by progesterone suggests that lymphocytes in the placenta are inhibited by the high concentration of progesterone but would not be inhibited by much lower concentrations of progesterone in the peripheral circulation. Estradiol has the ability to effect inhibition of MLC; but because the levels in the peripheral and placental sites are much lower, it is less likely that estrogen has a significant effect.

**Pregnancy and Alterations in Humoral Immunity**

**Immunoglobulin Levels**

Measurement of immunoglobulins, IgG, IgA, IgM, has been reported frequently; but information on IgD and IgE is more limited (Table 2). The wide range of normal values reflects a variation in antigenic exposure by the mother. The values of IgG seen in Table 2 range from 826 to 1416 mg/dl. Most authors have reported IgG values to be stable, with little variation throughout gestation. However, Studd\(^53\) and Amino et al.\(^51\) have described a progressive fall in IgG throughout pregnancy. Studd described a decrease from 1100 mg/dl at 8 weeks to 826 mg/dl at term (25% decrease); Amino showed a decrease from 1756 mg/dl at 11 weeks to 1362 mg/dl at term (22% decrease). The reason for this decrease in IgG is unclear but has been attributed to hemo-dilution, loss of IgG in the urine, and transfer to the fetus. An alternative explanation may be one of an altered regulatory mechanism for IgG. It has been shown for the nonpregnant state that the plasma IgG concentration is a key factor in determining the rate of catabolism for IgG. If the IgG concentration in body fluids drops, the catabolic rate drops accordingly.\(^53\)

**TABLE 2. Immunoglobulin Levels**

<table>
<thead>
<tr>
<th>Author</th>
<th>Maternal (mg/dl)</th>
<th>Umbilical Cord (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>Margoulis et al.</td>
<td>305</td>
<td>103</td>
</tr>
<tr>
<td>Papadatos et al.</td>
<td>929</td>
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<tr>
<td>Studd et al.(^53)</td>
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<td>Mendenhall(^50)</td>
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<tr>
<td>Amino et al.(^51)</td>
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The values of IgA and IgM remain constant throughout pregnancy. We have little information about IgD and IgE levels and their fluctuations. IgD increases from 33 mg/dl in the first and second trimesters to greater than 80 mg/dl at term.\(^5\) IgE levels have been reported as 397 μ/ml at term with the use of a radioimmunoassay kit.\(^3\) Only minor variations occurred during pregnancy.

Alterations in immunoglobulin levels have been observed in high-risk pregnancy. Increased concentrations of maternal IgG have been reported in conditions of intrauterine death or severe intrauterine growth retardation.\(^5\) Pregnancy-induced hypertension (PIH) causes changes in humoral-mediated immunity. Studd et al.\(^4\) found a significant decrease in maternal and umbilical cord IgG levels in women with pre eclampsia. Cord IgG levels are higher than maternal IgG in normal and patients with preeclampsia.

It is clear that IgG levels decrease throughout gestation, whereas IgA and IgM remain stable. The levels of IgD rise at term, but the role of IgD is unclear. Alterations of Ig occur in high-risk pregnancy, but the changes have not lent themselves to use as diagnostic measures.

**Immune Complexes**

An immune complex (IC) is a combination of antigen and antibody that is formed after the formation of antibody. The formation and disposition of ICs is a dynamic process that plays a physiologic role but which may be pathologic. The role of ICs in normal pregnancy and in preeclampsia is a matter of controversy. The relevance of circulating ICs to reproduction has been extensively reviewed.\(^6\) ICs are found when antibodies combine with their corresponding tissue-fixed antigens or antigens free in serum or other body fluids. Since pregnancy exposes the mother to a variety of fetal antigens that must be cleared by the formation of ICs and removed by the maternal reticuloendothelial system. The confusion arises because of a variety of methods and interpretation of results (Table 3). A large number of methods to detect ICs have been derived but are based on different principles; so their sensitivity and specificity with regard to pregnancy is difficult to evaluate. The majority of IC assays are complement-dependent and therefore will only detect complement-fixing ICs. It is clear that the presence of ICs in normal pregnancy as measured by current techniques is not increased, but that on occasion IC are present in preeclampsia. The exact role of ICs in preeclampsia is unknown.

Recently, additional information regarding ICs and the presence of rheumatoid factors in pregnancy has been published.\(^5\) Pope et al. found that immune complex concentrations in normal pregnancy serum as measured by four antigen-nonspecific radioimmunoassays were not elevated, but assays for IgG rheumatoid factor (RF) and IgM RF were increased. This finding may help explain the previously reported increases in IC, because RF interferes with IC detection by latex agglutination inhibition techniques. These elevated RFs in pregnancy do not represent increased levels of IgG and IgM, because no increase of either class of immunoglobulin is observed. Therefore, the increases in RF detected in pregnancy reflect true elevations of RF.

**Cellular Immunity in Pregnancy**

**T- and B-lymphocytes**

The total number of lymphocytes remains unchanged throughout pregnancy at 1500–3000/mm\(^3\). The usual percentage of T-lymphocytes is 70%; B-lymphocytes, 25%; null cells, 10%. There is a B/T cell ratio of 1:3. Strelkaukas et al. described an inversion of the usual B/T cell ratio in early pregnancy. They described a decrease in the percentage of T cells to 25% and an increase of B cells to 70% at 10–13 weeks of pregnancy and a return to the normal ratio after 20 weeks. It is suggested that this inversion of T and B cells may represent a physiologic depletion of suppressor T cells, which allows the number of B cells to rise. The
TABLE 3. Immune Complexes in Normal and Preeclamptic Pregnancies (Number Abnormal per Total)

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Assay Method</th>
<th>Normal Pregnancy</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masson</td>
<td>pRF-LI</td>
<td>55/55</td>
<td>ND</td>
</tr>
<tr>
<td>Gleicher</td>
<td>RAjI Cell</td>
<td>0/20</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/23 (AMFL)</td>
<td>ND</td>
</tr>
<tr>
<td>Knox</td>
<td>RAjI Cell</td>
<td>0/18</td>
<td>0/34</td>
</tr>
<tr>
<td></td>
<td>Ciq-B -A</td>
<td>0/18</td>
<td>2/34</td>
</tr>
<tr>
<td>Stirrat</td>
<td>Ciq-LI</td>
<td>6/16</td>
<td>13/16</td>
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<tr>
<td>Shena</td>
<td>Ciq-B -A</td>
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<td>15/21</td>
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<td>S'Amelio</td>
<td>PEG</td>
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<td></td>
<td>Ciq-LI</td>
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<td>PEG-CC</td>
<td>0/15</td>
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<tr>
<td></td>
<td>Ciq-B -A</td>
<td>0/15</td>
<td>0/25</td>
</tr>
<tr>
<td></td>
<td>MCT</td>
<td>0/15</td>
<td>5/25</td>
</tr>
</tbody>
</table>

Ciq-B -A, Ciq-binding assay; PEG, polyethylene glycol; PEG-CC, polyethylene glycol-complement consumption; MCT, microcomplement consumption; pRF-LI, polyclonal rheumatoid factor latex agglutination inhibition assay; Ciq-LI, Ciq latex agglutination inhibition assay; Ciq-SP, solid phase Ciq.


increase in B cells may assist in the production of antibodies that function as blocking factors allowing the allograft to be accepted.

The inversion of the B/T cell ratio in early pregnancy has been studied in more detail. This inversion was confirmed with the use of a fluorescence-activated cell sorter to detect fluorescent antibodies to T-cell surface antigen, B-cell surface antigen, and surface immunoglobulin. Bulmar and Hancock showed a depletion of T cells both by percentage and absolute number of E-rosette-forming cells with a concomitant rise in IgG-bearing cells (B cells). The inversion of the B/T cell ratio was not observed.

The changes described in T and B cells have been more definitely studied with the use of monoclonal antibodies. By the use of monoclonal antibodies, T-cell subset abnormalities of mononuclear cells in peripheral blood from normal pregnant women, post-partum women, and newborns were studied. Murine monoclonal antibodies were used: OKT3 (all peripheral T cells), OKT4 (helper/inducer T-cell subset), OKT8 (suppressor/cytotoxic T-cell subset), OKM1 (monocyte/null cell), and OKla (B cells, monocytes, and activated T cells). A significant decrease in the percentage and absolute number of helper lymphocytes were found in normal pregnant women throughout pregnancy and during labor. The decrease was progressive throughout gestation. In addition, the percentage and absolute number of T cells (T3 + cells) were significantly decreased in the second and third trimesters of pregnancy. The percentage and absolute number of suppressor cytotoxic T cells (T8 + cells) and B cells were unchanged from normal controls. The percentage of monocytes was increased throughout pregnancy.

The decrease in helper T cells may be operative with other mechanisms of non-rejection. Several autoimmune decreases have been associated with a high ratio of helper/ suppressor cells. In pregnancy a lower helper/suppressor ratio occurs with a decrease in antibody production and clinical amelioration of disease. A low helper/suppressor ratio is probably normal for the maintenance of pregnancy.

The cause of alterations of helper T cells in pregnancy is not known but may be a consequence of the hormonal changes associated with pregnancy. Immunosuppressive effects have been shown during in vitro studies for hCG, estrogen, progesterone, corticosteroids, α-fetoprotein, prolactin, and α-globulin.
Natural Killer (NK) Cells in Pregnancy

Natural killer cells are a recently discovered subpopulation of lymphoid cells that are present in most normal individuals. NK cells have spontaneous cytolytic activity against a variety of tumor cells and some normal cells, and their activity can be augmented by interferon. These cells have characteristics distinct from other lymphoid cells and are associated with large granular lymphocytes. NK cells comprise about 5% of blood and splenic leukocytes. The function of NK cells is to mediate natural resistance against tumors in vivo, certain viruses, and other microbes and may be an important factor in immune surveillance. An excellent review has recently been published. Alanen and Lassila have measured the NK function of peripheral blood lymphocytes from nonpregnant women, normal pregnant women, and pregnant women with toxemia. The NK activity was found to be significantly lower in patients with pre-eclampsia than in either normal pregnant women or nonpregnant women. In addition, the NK activity was less in normal pregnant women than in nonpregnant women. The addition of interferon to enhance the effect of NK activity was shown to be as effective in blood during pre-eclampsia as in normal peripheral blood. This suggests that patients with pre-eclampsia have active NK cells but may be inhibited from reacting.

Cellular Function in Pregnancy

HLA and Normal Pregnancy

The uniqueness of human tissue can be measured in various ways. The system for the characterization of unique tissue antigens and specificity is via the major histocompatibility complex (MHC) antigens. In man the MHC consists of the HLA antigens, HLA-A, HLA-B, HLA-C, and HLA-D/DR antigens. Therefore, because the fetus received half its HLA antigens from the paternal source and half from the maternal source, it is logical to question whether the HLA antigens and antibody formation are important in the nonrejection of the fetus and whether abnormalities of the system cause reproductive complications and losses.

Antibodies to HLA antigens are present in approximately 20% of multiparous women. Since the trophoblast is of fetal origin and in contact with the maternal circulation, it is important to determine what MHC antigens are expressed. This has been a controversial area, but most investigators now are convinced that there is a lack of MHC antigens on the trophoblast. Data have shown the presence of β_2_ microglobulin and HLA on cells and vascular endothelium of the mesenchymal stroma of the chorionic villus but an absence of either β_2_ microglobulin or HLA on trophoblasts. These findings were true for both immature and mature placental tissue. HLA antigens are present on endothelial cells of the placenta and umbilical cord. Galbraith et al. extended the findings by examining chorionic villi for the presence of MHC antigens, using both conventional and monoclonal antisera. Cells of the mesenchymal stroma within the chorionic villus as well as trophoblastic cell membranes were studied by immunofluorescence for major histocompatibility antigens. The stroma cells were positive for HLA and H-Y antigens as well as DR and DC gene products. Trophoblast cells and membranes were consistently negative for MHC antigens. It is difficult to escape the conclusion that transplantation antigens are not expressed on the trophoblast. However, the mechanism responsible for this nonrepresentation is not known. Additional immunologic studies have identified proteins and their location in the mature villus. Actin, plasminogen, and transferrin have been identified on trophoblastic cells with C2, IgG, fibrinogen, and collagen on trophoblastic membranes. Many proteins were present in the stroma with all four IgG subclasses present (IgG1 and IgG3 most prominent) as well as β_2_ microglobulin, collagen, actin, plasminogen, α_2_ macroglobulin, and C4.
Contributions of the Fetus to Alterations in Maternal Immunity

The knowledge of the transplacental passage of fetal red blood cells (FRBCs) into the maternal circulation to cause maternal isoimmunization has stimulated an interest in transplacental passage of lymphocytes. This was initially demonstrated by Desai and Creger, using an atabrine technique of cell labeling. Shroder and Chapelle studied the presence of human Y-chromosome-bearing lymphocytes from the peripheral blood of women who delivered male infants. Seven women who gave birth to male infants had 0.05–0.20% lymphocytes with a Y body. They calculated that on the basis of the incidence of Y-body-containing lymphocytes in males (35–66.5%), approximately 0.1–0.5% of the lymphocytes in the peripheral blood of pregnant women with a male fetus are of fetal origin. This amount is greater than that found for fetal erythrocytes by several orders of magnitude and suggests that fetal lymphocytes actively cross into the maternal circulation. Olding, Oldstone, and colleagues have provided excellent data pertaining to the functioning of the fetal lymphocytes in the maternal circulation. It was shown that fetal lymphocytes will cause an inhibition of mitosis of stimulated lymphocytes from not only the mother but also unrelated women. Additional studies have suggested two mechanisms for the suppression of maternal lymphocytes. These authors demonstrated the presence of a fetal suppressor T-cell population, which These authors demonstrated the presence of a fetal suppressor T-cell population, which specifically affects maternal B cells by decreasing immunoglobulin production. The second proposed mechanism is via the release of a low-molecular-weight suppressive factor(s) from proliferating newborn lymphocytes. Therefore, it seems reasonable to suggest that either fetal suppressor T cells or a soluble suppressive factor(s) produced by fetal T cells, or both, is operative in modulation of the maternal immune response. The primary focus seems to decrease the functioning maternal B cells and diminished immunoglobulin production. If B cells are diminished, there is a lowered B/T ratio, which is beneficial to the fetal allograft. The concept of fetal participation in the immune regulation of pregnancy has been strengthened by the findings of Mather et al. They identified the fetus as the producer of cytotoxic antibodies to adult T-lymphocytes by finding these antibodies in umbilical artery and vein blood but not in maternal peripheral vein blood. In addition, their data indicate that the fetal cytotoxic antibody is not directed against known HLA antigens and that these antibodies are primarily IgG followed by IgM. The purpose of the fetal production of cytotoxic antibodies to maternal T-lymphocytes is unknown but may be a selective suppression or masking of T-cell recognition of fetal antigens by the maternal immune system. This may lead to a suppression of maternal T-lymphocytes in general or a specific subset. It has already been pointed out that women in early pregnancy have reduced T-lymphocyte counts and lack a specific subset of regulatory T cells. Durandy et al. expanded the knowledge of the effect of fetal suppressor T cells. They demonstrated that impaired newborn B-cell maturation is due to both an immaturity of lymphocyte subsets and an increased suppressor T activity. They also demonstrated that prostaglandin E₂ (PGE₂)-dependent monocyte suppressive activity does not play a role in the suppression observed in the newborn.

The concept of fetal regulation of immune factors that affect the mother has long been suspected but only recently proven. At this time it would appear that this regulatory process is of considerable importance in the nonrejection of the fetus.

Clinical Effects of Impaired Cell Mediated or Humoral Function

It has been a general presumption that the alterations in the immune system that occur during normal pregnancy, allowing the fetal allograft to be successful, lead to an increase in disease states dependent on cell-
mediated immunity. A corollary to this presumption has been that certain abnormal conditions of pregnancy such as habitual abortion may have a direct immunologic basis.

Mild depression of reaction to tuberculin testing occurs in the second half of pregnancy, with a return to normal after delivery.\textsuperscript{75,76} This may add to confusion in the diagnosis of suspected cases of tuberculosis. Therefore, appropriate x-ray studies are indicated in patients in whom tuberculosis is suspected.

Decreased resistance to a number of viral diseases has also been reported, indicating that pregnant patients have less ability to control diseases depending on cell-mediated functions. This deficiency has been shown for influenza, varicella, hepatitis, polio, and Coxsackie viruses.\textsuperscript{77-81} In addition, while not causing fetal maternal infection, several viruses produce congenital and/or neonatal infection, i.e., cytomegalovirus, herpes simplex virus, and rubella. More recent evidence concerning cytomegalovirus (CMV) infection in pregnancy shows significant depression of the ability of peripheral blood lymphocytes from pregnant women to respond to specific CMV antigens.\textsuperscript{82} The investigators also demonstrated an increased suppressor effect during pregnancy and postulated that CMV-specific suppression of cellular immunity plays an important role in reactivation of CMV in pregnancy.

There is no doubt that viral infections are more frequent and more severe and take longer to resolve during pregnancy than in the nonpregnant patient. However, it is also recognized that cell-mediated immunity is active. In many ways the pregnant patient resembles a patient with selective immunosuppression, i.e., diminished ability to defend against certain viruses.

Exciting new information is available pertaining to habitual abortion and the immune system.\textsuperscript{83-85} Rocklin et al.\textsuperscript{83} used an in vitro technique to detect cellular immune reactivity of multiparous women to their spouses with the use of their cells as antigen. The method used was inhibition of macrophage migration which measures the production of a soluble factor, migration inhibition factor (MIF) by sensitized lymphocytes. Lymphocytes from multiparous women make this inhibitory factor in response to paternal antigens, indicating a presensitized state, whereas lymphocytes from women with a history of habitual abortion fail to make the factor in response to paternal alloantigens. A blocking factor, an IgG antibody, is present in the serum of normal multiparous women that prevents production of MIF by maternal lymphocytes. The blocking factor has been shown not to be directed against HLA-A, HLA-B, or HLA-C antigens. Other work has demonstrated no correlation between lymphocytotoxic antibodies (anti-HLA-A, -B, -C and anti-B-lymphocyte anti-HLA-DR) studied before 20 weeks' gestation, from 21 to 30 weeks, and between 31 and 40 weeks and obstetric complications such as fetal wastage or problems with placental or infant birth weight. Beer et al.\textsuperscript{4} studied a group of patients with habitual abortion and no apparent cause. They demonstrated an increased sharing of HLA antigens at the A, B, C and D/DR loci with spouses. The MHC homozygosity was usually associated with the female responder and male stimulator hyporeactivity in mixed lymphocyte reactions. Unander and Olding showed that the lymphocytes from women who are habitual aborters are suppressed by their husbands' lymphocytes and lack cytotoxic antibody against their respective husbands' lymphocytes.

It is now recognized that significant changes occur in the maternal immune system during pregnancy. The alterations are initiated at the placental site by the hormones of pregnancy. Production of immunoregulatory agents by the mother and the fetus modulate the immune response, and blocking antibodies prevent a cytolytic attack. Undoubtedly the puzzle of the non-rejection of the allograft fetus will soon be solved.
IMMUNE SYSTEM IN NORMAL PREGNANCY

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