The Maternal Immune Response in Coccidioidomycosis*

Is Pregnancy a Risk Factor for Serious Infection?

Robert A. Barbee, M.D., F.C.C.P.; Mary Jane Hicks, M.D.; David Grosso, Ph.D.; and Carolyn Sandel, R.N.

Seven subjects with prior coccidioidal disease and three with active *Coccidioides immitis* infection during their first trimester were studied during pregnancy and postpartum to determine their general and antigen-specific cell-mediated immune status. All ten were white and carried their pregnancies to term without incident. Decreases in total lymphocytes and T-helper and T-suppressor subsets were noted during the third trimester, presumably secondary to an increase in plasma volume. Lymphocyte responses to the mitogens phytohemagglutinin, concanavalin A, and pokeweed were mildly decreased late in pregnancy, with significant intrasubject and intersubject variation. Responses to tetanus antigen were consistently and significantly lower as pregnancy progressed, rising above first trimester levels by 12 weeks postpartum. A similar pattern of response was noted with spherulin antigen for the seven subjects with previously demonstrated coccidioidal immunity. The three subjects with active coccidioidomycosis either failed to mount a significant spherulin immune response or demonstrated an early response that fell as pregnancy progressed. This antigen-specific immune suppression continued for up to 16 months postpartum despite the fact that there was no clinical evidence of coccidioidal activity beyond the first trimester. Thus, while all three completed pregnancy without complication, the data suggest that significantly increased maternal risk may be present when active coccidioidomycosis and pregnancy occur together. This risk may be greatest among darker-skinned individuals who become infected during the latter half of pregnancy. (Chest 1991; 100:709-15)

CMI = cell-mediated immunity; Con-A = concanavalin A; FH = Ficoll-Hypaque; LSM = lymphocyte separating media; PHA = phytohemagglutinin; PWM = pokeweed mitogen

For many years it has been clear that a number of alterations of the maternal immune system must occur to permit the fetal allograft to survive in utero for the duration of pregnancy. The precise nature of these alterations, or immune modulations, have not been fully explained, although a large number of both cellular and noncellular mechanisms have been proposed. Whatever the mechanisms, the intensity of the immunosuppression at the fetal-maternal interface must be much more intense than that which occurs systemically. Without such a differential, maternal mortality from infections which require cell-mediated immune host defenses would be devastating. Even with a modest depression in cell-mediated immunity (CMI), maternal morbidity and mortality from a variety of viral infections is said to be increased. The possibility of an increase in maternal susceptibility to systemic fungal infections, in which host CMI response is also vital, was initially raised in 1946 by Mendenhall et al when they reported the first case of maternal mortality from coccidioidomycosis. This was followed shortly thereafter by several similar reports.

Records from Kern County, California, in the heart of the endemic coccidioidomycosis area, during the 1950s and 1960s indicated that this disease was the second leading cause of maternal death. In 1975, Purtilo provided further evidence that pregnancy was a significant risk factor for severe coccidioidal infections. In his review of maternal deaths from systemic fungal infections, coccidioidomycosis was the second leading cause, and the only one in which maternal immunosuppression was not present prior to the pregnancy. He speculated that a specific mechanism might exist during pregnancy that made infections with *Coccidioides immitis* more lethal than in the nonpregnant state. Several years later, Drutz et al provided evidence for such a mechanism. In a series of studies, they noted that the serum concentrations of progesterone and 17β-estradiol that are achieved during the latter half of pregnancy are capable of stimulating the growth and maturation of *C immitis* in vitro. Also, they identified a specific binding site for progesterone on the fungal cell wall. Despite these clinical and basic studies, the incidence of serious pregnancy-related infections in the endemic coccidioidomycosis area have remained quite low. In a retrospective study, Wack et al could identify only ten instances of maternal coccidioidomycosis among 47,000 pregnancies in the Tucson area during a ten-year period.

Clinically, the documentation of a CMI response against *C immitis* has been dependent on the presence of a delayed skin reaction to coccidioidin. In his initial studies, Smith reported that a positive reaction to

---

*From the University of Arizona, College of Medicine, Tucson. Supported by BRSG grant 328672. Manuscript received October 22; revision accepted February 13. Reprint requests: Dr. Barbee, Division of Respiratory Sciences, University of Arizona College of Medicine, Tucson 85724*
this antigen could be expected in more than 90 percent of infected individuals within two weeks of the onset of illness. Subsequent reports have placed this percentage at only 60 to 70 percent.\textsuperscript{26-28} Recently, a number of investigators have described an \textit{in vitro} antigen-specific lymphocyte blast transformation test that is both quantitative and more sensitive than the coccidioidin skin test in assessing antigen-specific CMI in this disease.\textsuperscript{29-33} Using this test, it has been possible to distinguish immune from nonimmune individuals, and, in those with active coccidioidal infections, to determine the adequacy of their immune response.\textsuperscript{34}

In the current study, maternal mitogenic and antigen-specific lymphocytic responses were measured in ten subjects throughout pregnancy and the postpartum period. In seven of the ten, specific CMI against \textit{C immitis}, as measured by the coccidioidin skin test, was evident prior to or early in pregnancy. For the other three, active coccidioidomycosis was present during the first trimester of their pregnancy. Our objective, in addition to documenting the changes in maternal CMI that occur during the course of pregnancy, was to compare the maternal immune responses to \textit{C immitis} in previously immune subjects with those of subjects with active coccidioidomycosis during their pregnancy. In so doing, we hoped to provide data concerning whether pregnancy is a potential maternal risk factor for serious coccidioidal infection.

**Materials and Methods**

**Study Population**

Following informed consent, subjects were identified at their initial prenatal visit and divided into two groups: Group 1 consisted of seven women with either a documented history of coccidioidomycosis or a positive coccidioidin skin test indicating prior coccidioidal exposure. Group 2 included three subjects with active coccidioidomycosis. Disease activity was confirmed by the presence of a compatible clinical history, positive serologic blood tests for either precipitins or complement-fixing antibodies against \textit{C immitis}, and positive coccidioidin skin tests. All ten subjects were white with a mean age of 26.8 years. None gave a history of respiratory disease or chronic ill health prior to the onset of pregnancy.

**Skin Test Antigens**

On entry into the study and in most subjects also at 36 weeks' gestation, delayed skin test reactivity was assessed with the following skin test antigens: coccidioidin (1:100), spherulin (usual dose, equivalent to coccidioidin 1:100), Candida (1:1,000), and tetanus toxoid (purified ultraflaen, Wyeth-Ayerst). Reactions were read at 48 hours and were considered positive when the area of induration was \(\geq 5 \times 5\) mm.

**Hormone Assays**

Serum 17B-estradiol and progesterone concentrations were measured by standard double-antibody radioimmunoassay techniques (using kits obtained from Leeco Diagnostics, Southfield, Mich). When necessary, samples in which hormone levels exceeded the range of the standard curve were diluted with steroid-depleted serum provided for that purpose. Intra-assay and interassay coefficients of variation were, respectively, 5.8 percent and 7.3 percent for estradiol and 7.3 percent and 8.6 percent for progesterone.

**Lymphocyte Function Studies**

**Lymphocyte Separation:** Blood was collected in sodium heparinized specimen tubes. Lymphocytes were separated by density gradient (specific gravity, 1.077) centrifugation procedures as previously described.\textsuperscript{33} Briefly, blood was carefully layered over Ficoll-Hypaque (FH) or lymphocyte separating media (LSM) (Litton Bionetics, Kensington, Md) and centrifuged at 22°C for 20 minutes at 400 g with the brake off. The discrete band of mononuclear cells at the plasma/FH or LSM interface was aspirated and washed in a sifid to eightfold volume of phosphate-buffered saline solution (400 g centrifugation for 30 minutes) and diluted to the appropriate concentration for subsequent testing in RPMI-1640 medium containing 20 percent plasma. Viability counts, performed by trypan blue dye exclusion, ranged from 98 to 100 percent.

**Lymphocyte Transformation**

Lymphocyte blast transformation was performed by a microculture technique.\textsuperscript{36} Antigens included preservative-free spherulin (Berkeley Biologicals, Berkeley, Calif) and tetanus toxoid (Wyeth-Ayerst, Philadelphia). The three mitogens employed were phytohemagglutinin (PHA), concanavalin A (Con-A), and pokeweed mitogen (PWM) (Gibco, Grand Island, NY). Antigen and mitogen concentrations used were determined by dose-response curves to give maximum transformation response, using lymphocytes from skin test reactive, healthy donors for the spherulin and tetanus antigens. With both antigens, 10-, 20-, and 30-μL volumes were used for testing to provide a dose-response curve. Each dose of antigen was set up in triplicate, using 20 percent fresh autologous serum. Unstimulated cell controls containing autologous plasma alone were run to assess spontaneous transformation. The microtiter plates were incubated at 37°C in a humidified incubator containing 5 percent CO\(_2\). On day 3, mitogen-stimulated cells were pulsed, and on day 5 antigen-stimulated cells were pulsed with 50 μL of tritiated thymidine (New England Nuclear, Boston). Eighteen to 24 hours after pulsing, the cells were harvested onto glass fiber filters using a harvester (Skatron, Sterling, Va). The filters were dried at room temperature, punched out, and counted in a scintillation counter. The result of each lymphocyte-induced transformation was expressed as the mean of the triplicate counts per minute. The highest value in response to three different doses of antigen in each test system was used in the analysis of the data. There were no significant differences in the control values during the study period. When more than one set of stimulation data was available during a trimester, a mean value for that trimester was determined to provide one set of values for each trimester. In addition to the 6- and 12-week postpartum values, subjects in group 2 (active disease) also underwent transformation studies at various postpartum periods beyond 12 weeks. These late postpartum values are reported individually in the text.

**Statistical Methods**

Differences in test variables during pregnancy and postpartum were tested for significance using a program (Statview, Abacus Concepts, Berkeley, Calif) designed for a specific computer (Macintosh). Significant differences were determined by the paired \(t\) test. Because of missing data points, some analyses were carried out on an \(n\) of 8 or 9, rather than 10. P values less than or equal to 0.05 were considered significant.

**Results**

**Clinical Status**

**Group 1:** All seven subjects were in excellent health. Four of the seven had prior diagnoses of coccidioi-
mycosis that had resolved uneventfully long before onset of pregnancy. Three had no prior coccidioidomycosis but reacted strongly to coccidioidin and spherulin skin tests at their initial prenatal visit. Positive skin reactions to tetanus antigen were also present in all seven subjects. Six subjects were retested with coccidioidin and tetanus antigens during their third trimester. Positive reactions were again noted, with no significant change in reaction size from their previous test.

**Group 2:** Prior to their initial prenatal visit but within two to six weeks from the onset of their pregnancies, the three subjects with active disease noted the typical symptoms of acute coccidioidomycosis. These included cough, low-grade fever, chest pain, malaise, and, in one case, erythema nodosum. Except for the continuing presence of excessive fatigue, all acute symptoms had resolved by the time of their first prenatal visit. Chest roentgenograms taken either at the time of their acute illness or during this visit were within normal limits. Coccidioidal serologic tests (precipitins and/or complement fixation antibody titers), obtained by their private physicians while they were acutely ill, were positive in all three subjects. Repeated serologic testing during the latter half of pregnancy and also at 12 weeks postpartum in one subject was negative. Thus, there was no evidence of continuing disease activity beyond the first trimester of pregnancy. As was the case in group 1, delayed skin reactions to coccidioidin, spherulin, and tetanus antigens were assessed on several occasions during and following pregnancy. Initially, positive responses were elicited in all three subjects. During the third trimester, two of the three subjects continued to demonstrate positivity (one significantly reduced in size) to coccidioidin; the third was negative. Postpartum testing at 12 weeks in two subjects and at 16 months in the third subject revealed two of the three to be negative to coccidioidin. All three continued to react strongly to tetanus. Despite this skin test reversion in two of the three subjects, there was no clinical or serologic evidence of active disease during postpartum follow-up of six to 18 months.

**Lymphocyte Subsets**

Serial measurements of total lymphocytes, T helpers (CD4), and T suppressors (CD8) are shown in Figure 1. Throughout pregnancy there was a progressive fall in all three, with a return to first trimester levels or above at six weeks postpartum. Because the relative decline in the level of the subsets was similar, the ratio of the two remained constant.

**Mitogen Blast Transformation**

The pattern of change of tritiated thymidine uptake by PHA-, Con-A-, and PWM-stimulated lymphocytes was virtually the same in all subjects. These changes are represented by the PHA data shown in Figure 2. Because of the amount of both intrasubject and intersubject variation, a large standard error of the mean is evident, with a barely significant decline during the second trimester compared with 12 weeks postpartum. It should be noted that all values are well above 20,000 cpm, the lower limit of normal stimulation for this mitogen.

**Tetanus-Antigen Blast Transformation**

In an attempt to determine whether any decline in spherulin-induced blast transformation is an isolated phenomenon or one that also occurs with other antigens, stimulation studies were carried out with tetanus, an antigen to which all subjects showed delayed skin reactivity. Serial data with this antigen in all subjects are shown in Figure 3, with the dotted line at 7,000 cpm indicating the lower limit of a positive immune response in our laboratory. A progressive and statistically significant decline was noted compared with the group mean at 12 weeks postpartum. All values are well above the lower limit of normal response (20,000 cpm).
The normal groups are plotted (vertical bars) for all subjects during pregnancy and postpartum. The dotted line at 7,000 cpm represents the lower limit of response in tetanus-immune subjects. A significant decline in response is apparent during the second and third trimesters. Compared with 12 weeks postpartum, decreased response continues through the immediate postpartum period, but the difference is not statistically significant because of the smaller n at six weeks.

Spherulin-Antigen Blast Transformation

Data for all ten subjects are shown in Figure 4a (upper), and those for the two groups separately in Figure 4b (lower). The dotted line indicates the lower limit of the normal immune response as determined in prior studies.38 For the entire group, the pattern is similar to that which was noted for tetanus antigen, except that the magnitude of the suppression of the spherulin-induced transformation in the third trimester vs 12 weeks postpartum is somewhat greater. As was seen previously, values at 12 weeks postpartum exceeded those for the first trimester. When the values for the previously immune and active disease subjects are plotted separately, differences between the two groups become apparent. While the immune subjects show changes that are similar to those for tetanus antigen, the active disease subjects never achieved normal immune status during the study period. The subject who developed coccidioidomycosis earliest (within two weeks of the onset of pregnancy) developed a normal initial spherulin response, with 23,000 cpm early in the second trimester. Later in pregnancy, that value had fallen to 4,000 cpm, and remained at that level when last tested at 16 months postpartum. Her coccidioidin skin test was also negative at that time. The two other active disease subjects who had the onset of symptoms at approximately six weeks' gestation never developed a significant spherulin response during pregnancy or in the immediate postpartum period. However, both had positive responses when last tested, at 36 and 40 weeks following delivery. At that time, one reacted positively to coccidioidin skin testing while the other remained nonreactive.

Serum Hormone Levels

Both progesterone and 17β-estradiol levels during each trimester of pregnancy and postpartum were within the expected physiologic ranges. For progesterone the mean values were 30.6, 71.1, and 158.6, and finally, less than 0.1 ng/ml at six weeks postpartum. Comparable estradiol values were 4.38, 19.30, 35.10, and 0.3 × 10⁻⁹ mol. In general, there was an inverse relationship between the hormone levels and both the tetanus and spherulin lymphocytic responses. However, this correlation was not consistent for individual subjects. Nor was it evident six weeks postpartum,
when hormone levels had returned to nonpregnant levels while immunosuppression continued in group 2 patients.

**Autologous Mixing Studies**

In an attempt to determine whether the pregnancy-related suppression of spherulin-induced blast transformation in the group 2 subjects was dependent on plasma suppressive factors or a cellular immune defect, two of the three underwent autologous mixing studies several months postpartum, when their spherulin stimulations were in the normal immune range (>10,000 cpm). Their fresh immune lymphocytes were mixed with previously frozen plasma from each trimester of pregnancy. In one instance, pregnancy plasma suppressed spherulin stimulation to nonimmune levels that were only slightly higher than they had been during pregnancy and well below current immune levels. Such a result indicates significant plasma-induced immune suppression. In the second subject, plasma that had been obtained during pregnancy failed to suppress her normal immune postpartum lymphocyte transformation response. Stimulation responses using plasma saved from each trimester of pregnancy were in the normal immune range in contrast to the nonimmune (ie, “suppressed”) values that had been obtained previously (during pregnancy) with the same plasma. This indicates that her previous nonresponsive state was probably due to a specific cellular defect. Hormone levels during pregnancy were comparable in both subjects.

**DISCUSSION**

Among the three common systemic mycoses, coccidioidomycosis, histoplasmosis, and blastomycosis, the former has an incidence of acute clinical disease that is many times greater than the other two. Approximately 40 percent of inhalation exposures to *C immitis* are followed by a clinically recognizable illness. Despite this relatively high clinical attack rate and a number of reports of maternal death from coccidioidomycosis, the recognition of active coccidioidomycosis as a significant maternal risk factor during pregnancy has been a matter of some debate. If such a risk exists, one would expect, in the endemic area, to see many instances in which coccidioidomycosis and pregnancy occur together among the child-bearing population. Such has not been the experience of physicians treating this population.

The discovery of three otherwise healthy patients with active coccidioidomycosis while they were in the early weeks of pregnancy provided a unique opportunity to study this problem. In addition to assessing their clinical outcome, it was possible to compare their CMI response to coccidoidal spherulin antigen with that of seven subjects who had documented immunity against *C immitis* prior to their pregnancy. More generalized changes in maternal CMI responses during pregnancy were also assessed. Despite the presence of active infection, the course of pregnancy was the same in the three group 2 subjects as it was in group 1 subjects. Serologic evidence of disease activity was no longer present when they were retested during the second trimester, and all subjects successfully carried their pregnancies to term. Such an outcome may explain, at least in part, the apparent rarity of the simultaneous occurrence of coccidioidomycosis and pregnancy. In most cases, the acute symptoms of coccidioidomycosis are nonspecific. Sixty percent of infections are discovered only in retrospect by the presence of a positive delayed skin reaction to coccidiolin. This, coupled with the fact that most of the reported maternal deaths have occurred among dark-skinned individuals who acquired their disease in the latter half of pregnancy, suggests that many mild coccidioidomycosis infections, especially when they occur early in pregnancy, are not diagnosed.

The overall status of maternal CMI during pregnancy has been the subject of many studies. Among the more intriguing observations has been the apparent clinical improvement in women with rheumatoid arthritis during pregnancy, with relapse postpartum. In addition to evidence suggesting that a variety of both hormonal and nonhormonal plasma factors are capable of suppressing maternal immune function, investigators have reported changes in either the total number or specific lymphocyte subsets during pregnancy. Others have failed to substantiate these findings and have noted, as we have, that such changes, since they occur during the second half of pregnancy, are more likely secondary to an increase in plasma volume that is corrected shortly following delivery. Thus, although the absolute numbers of both T-helper and T-suppressor cells appear to fall, the ratio of the two remains quite constant.

A number of authors have also described T-cell function during pregnancy. Early reports indicated that there was a significant fall in mitogen stimulation that was most evident during the third trimester. Subsequently, Gehrz et al found that such responses fell within 2 SDs of the values in age-matched nonpregnant controls. Our data with PHA, Con-A, and PWM confirm the conclusions of Gehrz et al. While a significant difference (p<0.05) was noted between the second-trimester and 12-week postpartum values, all were well above the lower limit for mitogen lymphocyte stimulation in normal populations.

In contrast to the mitogen, stimulation with tetanus antigen was significantly lower during the third trimester, compared with 12-week postpartum values. Again, these data are in agreement with those of Gehrz et al, who found statistically significant depressions in...
in lymphocyte stimulations, not only to tetanus, but also to SK-SD, Candida, and cytomegalovirus during the third trimester. Within 90 days postpartum, the antigen responses had returned to prepregnancy levels.

Previous reports from our laboratory and that of Cox and others have demonstrated that healthy coccidioidin immune subjects and those with mild self-limited infections develop vigorous T-cell responses when exposed to coccidioidal antigens in vitro, not unlike those that have been described with tetanus antigen. The seven subjects in group 1 demonstrated such responses early in pregnancy and especially when tested 12 weeks postpartum. Their stimulation data parallel those of all subjects with tetanus, with a third-trimester decline and postpartum rise. The reason for this pattern of third-trimester suppression is unclear. Kasakura in 1971 and more recently Sütteri and Stites have described plasma inhibitory factors that are capable of suppressing maternal CMI responses. The latter authors were able to induce significant mitogen suppression with concentrations of estradiol and progesterone that are achieved during the latter half of pregnancy. Csapo and colleagues made similar observations on the immunosuppression capacity of progesterone and its importance in the modulation of the maternal immune response that allows pregnancy to be maintained.

In contrast to the transient, primarily third-trimester suppression of CMI that was evident in the subjects in group 1, the three group 2 subjects had a prolonged immune suppression to spherulin despite the fact that their disease was mild and self-limited. As noted above, from previously published data, all three would have been expected to develop and maintain both clinical (positive coccidioidin skin tests) and in vitro evidence of coccidioidal CMI. In fact, all three did have positive skin tests when initially seen, and one of the three had stimulation values well above 10,000 cpm early in her pregnancy. Despite the fact that all three cleared their infection effectively with no clinical evidence of disease beyond the second trimester, they failed to mount significant spherulin-induced immune responses during the latter half of pregnancy or for months afterward.

Finally, at 36 and 40 weeks postpartum, respectively, two of the three showed normal spherulin immune responses with positive coccidioidin skin tests. One of the two had previously reverted from skin test positive to negative during her third trimester. The third continues to be nonresponsive on in vitro testing with a negative skin test 16 months postpartum. Positive tetanus skin reactions are present in all three.

While the data support the fact that the normal development of coccidioidal immunity was suppressed in these three subjects, the mechanisms responsible

are not clear. In one of the three, humoral suppression from one or more plasma factors appeared to be responsible, but no such factor was longer present six months following delivery. Cox and Pope, and others have noted similar suppression in patients with progressive pulmonary and disseminated coccidioidomycosis. However, there was no evidence of continuing disease activity in any of the three subjects. Gehrz et al have speculated that a maternal suppressor cell population may prevent appropriate macrophage T-cell interaction during pregnancy. Such a mechanism could account for the type of cellular immune defect that appeared to be present in the second mixing study subject. Whatever mechanism was operative during pregnancy prevented the development of the normal spherulin immune response in these three subjects, not only during pregnancy, but for many months afterward. Whether the three would have been susceptible to a second infection with C. immitis during that time is not known.

On the basis of the small number of subjects in this study, it is not possible to reach definite conclusions concerning the degree of risk involved when coccidioidomycosis occurs during pregnancy. In this instance, the subjects with active infections had mild disease early in the first trimester, and encountered no difficulty, despite the blunting of their immune response. Given the lack of specificity of their symptoms, one can well imagine that their disease might have gone unrecognized. It is possible that a more serious infection, occurring later in pregnancy, especially in darker-skinned individuals, who are known to be more susceptible to dissemination, might result in significant maternal risk. Confirmation of such a conclusion must await the study of a larger, more varied maternal population with active coccidioidomycosis.

ACKNOWLEDGMENTS: The authors gratefully acknowledge the laboratory expertise of Pamela Schubart, Amy Horn, Thuy Nguyen, David Pena, and James Logan. Special thanks to Patricia King for preparation of the manuscript.

REFERENCES

4. Check W. Suppressor cells may help prevent fetal rejection. JAMA 1979; 240:1226