Placental Cellular Immune Response in Women Infected with Human Parvovirus B19 during Pregnancy

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Human parvovirus B19 can cause congenital infection with variable morbidity and mortality in the fetus and neonate. Although much information exists on the B19-specific antibody response in pregnant women, little information is available describing the cell-mediated immune (CMI) response at the maternal-fetal interface. The focus of this study was to characterize the CMI response within placentas from women who seroconverted to B19 during their pregnancies and compare it to controls. Immunohistochemical techniques were used to identify the various immune cells and the inflammatory cytokine present within placentals tissue sections. Group 1 consisted of placentas from 25 women whose pregnancies were complicated by B19 infection; 6 women with good outcome (near-term or term delivery), and 19 with poor outcome (spontaneous abortion, nonimmune hydrops fetalis, or fetal death). Group 2 consisted of placentas from 20 women whose pregnancies were complicated with nonimmune hydrops fetalis of known, noninfectious etiology. Group 3 consisted of placentas from eight women whose pregnancies ended in either term delivery or elective abortion. The results of the study revealed a statistically significant increase in the number of CD3-positive T cells present within placentas from group 1 compared to group 2 or 3 (13.3 versus 2 and 1, respectively) (P < 0.001). In addition, the inflammatory cytokine interleukin 2 was detected in every placenta within group 1 but was absent from all placentas evaluated from groups 2 and 3. Together, these findings demonstrate evidence for an inflammation-mediated cellular immune response within placentas from women whose pregnancies are complicated with B19 infection.

The placenta plays an integral barrier role in limiting congenital viral infections (18). However, some viruses, transmitted via the blood, are able to circumvent this barrier. Virus within maternal blood reaches the intervillous space and makes intimate contact with the placental trophoblast layer, which can lead to virus transmission across that protective barrier to the fetal side. Transmission can occur through breaks in the placental barrier, via endocytosis or receptor-mediated transfer.

The P blood group antigen globoside is the main cellular receptor for B19 (3, 4). Globoside is present not only on cells of the erythroid lineage but also on the surface of placentals syncytiotrophoblast and cytotrophoblast cells (17). The presence of the globoside receptor on trophoblast cells may play a role in transmission of the virus across the placenta.

B19 infection of pregnant women can lead to vertical transmission of virus to the fetus with outcomes that include spontaneous abortion, fetal anemia, nonimmune hydrops fetalis, and intrauterine fetal death (6, 15, 23, 26, 31, 36). Although B19 is capable of causing congenital infection, numerous clinical studies show clearly that the majority of women who become infected with B19 during pregnancy deliver healthy infants (9, 20, 27, 29, 37). The risk of fetal demise appears to be greatest when infection occurs at or before 20 weeks of gestation (23, 37). The incidence of fetal loss caused by parvovirus also varies depending upon whether the infection occurs during an endemic (approximately 1.0 to 1.5%) or epidemic (approximately 5 to 15%) period. Currently, there is no accurate way to predict fetal outcome, as maternal seroconversion has proved unreliable as a predictor.

The factors controlling B19 transmission are not well understood; however, immune factors are likely to play an important role. Neutralizing antiviral antibodies appear to represent the principal defense mechanism in B19 disease (1, 22). Although the antibody-mediated immune response in pregnant women acutely infected with B19 has been studied (10, 16, 21, 32), little information is available concerning the cell-mediated immune response in individuals infected with the virus. Von Poblotzki et al. (34) studied the proliferative response of isolated peripheral blood mononuclear cells after stimulation with B19 VP1, VP2, and NS1 recombinant proteins (34). All the adults in the study had detectable antibodies against B19 in their sera. The observed cellular immune response consisted mainly of CD4+ T lymphocytes to the recombinant B19 antigens. The data led the authors to suggest that T helper cells are important in overcoming B19 infection and for establishing long-term immunity. In a case report by Wagner et al. (35), a systemic monocyte and T-cell activation was measurable in an adult female patient with B19 infection (35). This study also demonstrated increases in mRNA levels for various inflammatory cytokines, including interleukin-1β, IL-6, and gamma interferon. Finally, in an in vitro study by Moffatt et al. (24) provides evidence for increased concentration of the secreted inflammatory cytokine IL-6 from a variety of established hematopoietic cell lines and primary human umbilical vein endothelial cells expressing an inducible form of the B19 NS1 gene (24).

Currently, information is sparse on the cell-mediated immune response occurring within placentas from women infected with B19 during their pregnancies. Garcia et al. (7)
described six cases of nonimmune hydrops fetalis due to B19. The investigators described a mononuclear cell infiltrate in the villous stroma and intervillous space in all six cases of B19 infection (7). Information on both the type of immune cells present and the inflammatory cytokines expressed in the placenta during B19 infection could provide a better understanding of the mechanism of virus transmission during pregnancy or assist in evaluating vaccines against B19 that are being developed. The focus of this study was to characterize the cellular immune response within placentas from pregnancies complicated by maternal B19 infection and compare it with controls.

**MATERIALS AND METHODS**

**Tissue procurement.** Placental tissues were obtained from pregnant women after vaginal or cesarean delivery or after spontaneous or elective abortion. Approval for use of discarded placental tissues was obtained for this study through the Human Investigational Review Board at Magee-Women’s Hospital. The placentas, derived from three different patient groups, are described in Table 1. Group 1 placentas were subdivided into those from women with good pregnancy outcomes, defined as delivery of a healthy term or near-term infant, lacking signs of anemia or nonimmune hydrops fetalis, and poor pregnancy outcomes, defined as a pregnancy ending in spontaneous abortion, nonimmune hydrops fetalis, or fetal death due to B19.

**IHC analyses.** Placental tissues fixed in 10% buffered formalin and embedded in paraffin were used in these immunohistochemistry (IHC) experiments. Tissue sections were cut 5 µm thick onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, Pa.) and incubated for 20 min at 60°C to melt the paraffin before being placed immediately into xylene three times for 10 min each. The tissues were hydrated through 100, 100, 95, and 70% alcohol for 1 min each before being placed in distilled water for 5 min followed by phosphate-buffered saline (PBS) for 5 min. Consecutive sections were analyzed for the presence of CD3, IL-2, and B19 antigens. All tissue sections were incubated for 20 min with a universal protein-blocking reagent (Shandon Lipshaw, Pittsburgh, Pa.) after any pretreatment protocols.

**TABLE 1. Study groups**

| Group | No. | Description and pregnancy outcome (no.) |
|-------|-----|-----------------------------------------|
| 1     | 25  | Maternal B19 infection; IgM seroconversion Good fetal outcome<sup>a</sup> (6) Poor fetal outcome<sup>b</sup> (19) |
| 2     | 20  | Nonimmune hydrops fetalis of known, noninfectious etiology |
| 3     | 8   | Healthy term delivery or first-trimester elective abortion |

<sup>a</sup> Defined as delivery of a healthy term or near-term infant, lacking signs of anemia or nonimmune hydrops fetalis.

<sup>b</sup> Defined as a pregnancy ending in spontaneous abortion, nonimmune hydrops fetalis, or fetal death due to B19.

**TABLE 2. IHC conditions**

| MAAb<sup>c</sup> (clone) | Tissue treatment<sup>a</sup> | Primary antibody dilution<sup>c</sup> | Primary antibody source |
|--------------------------|-----------------------------|-----------------------------------|-------------------------|
| CD3 (PS1)                | AR                          | Predilute*                         | BioGenex (San Ramon, Calif.) |
| CD4 (MAb466C)            | AR                          | 1:30<sup>b</sup>                   | Innovex BioSciences (Richmond, Calif.) |
| CD8 (MAb470C)            | AR                          | 1:30<sup>b</sup>                   | Innovex BioSciences      |
| CD20                     | None                        | Predilute<sup>b</sup>             | BioGenex                  |
| CD68 (KP1)               | AR and protease             | 1:100<sup>c</sup>                 | Dako (Carpinteria, Calif.) |
| Defensin                 | None                        | 1:8,000<sup>c</sup>               | P. Heine (personal gift)  |
| IL-2                     | None                        | 1:50<sup>c</sup>                  | Genzyme (Cambridge, Mass.) |
| B19 MAb8292 (5215D)      | None                        | 1:200<sup>c</sup>                 | Chemicon (Temecula, Calif.) |

<sup>a</sup> MAb, monoclonal antibody

<sup>b</sup> AR, antigen retrieval. Protease digestion is performed at 37°C for 1 min, using 10 µg of protease XXIV per ml. Antigen retrieval is performed in 10 mM sodium citrate buffer (pH 6.0).

<sup>c</sup> Incubated at room temperature for 30 min (+) or 60 min (†).

**RESULTS**

**Documenting B19 infection in pregnant women.** All 25 serum samples from pregnant women within group 1 had detectable levels of circulating B19-specific IgM antibodies, with 24 of 25 specimens also having detectable B19-specific IgG (data not shown). B19-specific serology data were either not obtained or not available for the pregnant women from groups 2 and 3. Placental tissue sections from all 25 B19 IgM-positive women within group 1 contained detectable B19-specific DNA by PCR. In contrast, none of the 28 control placenta from groups 2 and 3 contained detectable B19 DNA by PCR (data not shown). IHC analysis for B19 capsid proteins was also performed on every placenta within group 1. All 25 placental sections contained detectable B19 capsid proteins (data not shown).

**Increased numbers of CD3-positive T cells seen in placentas from B19-complicated pregnancies.** Table 3 illustrates the average number of CD3-positive T cells present within placental tissue sections from groups 1 to 3. Group 1 (B19-complicated pregnancies) had a mean CD3 T-cell count of 13.3 cells/200× field. Subdividing group 1 into B19-complicated pregnancies...
with good outcome \((n = 6)\) versus B19-complicated pregnancies with poor outcome \((n = 19)\) resulted in similar CD3 T-cell counts of 11.4 and 13.9/200× field, respectively. These findings from group 1 were in sharp contrast to the average CD3 T-cell counts found within placentas from groups 2 and 3, which were 2 and 1 CD3 T cell/200× field, respectively. Means were compared by Student’s \(t\) test, and these differences were determined to be statistically significant \((P < 0.001)\). The remaining IHC analyses revealed no significant differences in the levels of B cells, macrophages, or neutrophils present within placentas from groups 1 to 3, using monoclonal antibodies recognizing CD20, CD68, and defensin, respectively (data not shown).

The inflammatory cytokine human IL-2 (hIL-2) was present in placentas from B19-complicated pregnancies. Because significantly more CD3-positive T cells were found in placentas from B19-complicated pregnancies than control placentas, IHC analyses for the inflammatory cytokine hIL-2 was undertaken along with CD3 IHC staining on consecutive sections. Both activated T cells and placental syncytiotrophoblast cells have been shown to produce and secrete hIL-2 (2, 28). Figure 1 illustrates representative examples of the IL-2 IHC staining that were seen within placentas from groups 1 to 3. Figures 1A and B show examples of placentas from group 1 (B19-complicated pregnancies of poor and good outcomes, respectively). Figures 1C and D show examples of placentas from groups 2 and 3, respectively. The nonimmune control for each placental tissue section examined lacked any staining (data not shown).

Staining for IL-2 by IHC was found within the CD3-positive lymphocytes of all 25 placentas examined from group 1. In the consecutive section examined by IHC for CD3 and IL-2, over 80% of the CD3-positive lymphocytes were also positive for IL-2 (data not shown). In contrast, none of the 28 placentas from groups 2 and 3 showed any detectable IL-2 staining.

**DISCUSSION**

The focus of this study was to characterize the cellular immune response within placentas from cases of maternally acquired B19 infection. We found significantly more T cells and IL-2 production from T cells in placentas from these women compared to controls, regardless of the outcome after B19 infection. This heightened inflammatory immune response was not seen in either control group studied. Keep in mind that B19 infection within groups 2 and 3 was ruled out on the basis of PCR results alone, since serology data were not available. Because B19 infection is often asymptomatic and most infections do not have adverse effects on the fetus, it is possible, especially within group 3, that an early infection that was cleared could have been missed using this approach. However, two of the six B19 IgM-positive women within group 1 with good pregnancy outcomes seroconverted during the early second trimester and still had B19 DNA detected by PCR from their placentas at the time of their term deliveries. Therefore, the likelihood of missing B19 infection by PCR testing within group 3 is small.

Inflammatory cytokines, including IL-2, are not normally present in appreciable levels during pregnancy (19). In fact, during pregnancy there is an active suppression of inflammatory cytokine production that is postulated to be important for allograft acceptance (5, 14, 28). The data from our two control groups mirrored these findings. IL-2 appears not to be produced during normal pregnancies but only in certain pathological conditions, including infectious processes. Negishi et al. (25) published findings correlating elevated levels of IL-2 within amniotic fluid with evidence of histologic chorioamnionitis (25). IL-2 secretion has also been implicated in the pathogenesis seen in deranged vasculature of the placenta associated with preeclampsia (11).

IL-2 affects a range of lymphocyte functions. These include stimulating T-cell growth, T-cell activation, and the cytotoxic activity in natural killer cells (12). We propose that the IL-2 staining observed in placentas from B19-complicated pregnancies could help to explain the association that is known to exist between first-trimester B19 infection and spontaneous abortion. Experimental abortion can be induced in pregnant mice by IL-2 administration, presumably through placental apoptosis of villous trophoblast cells caused by activation of maternal cytotoxic T lymphocytes (30). It is possible, however, that the lack of IL-2 staining within groups 2 and 3 may be due to the small numbers of CD3-positive cells present in these placentas.

Although each placenta examined had increased numbers of T cells and IL-2 production compared to controls, there appeared to be differences in the location of the T cells and IL-2 staining between those pregnancies ending in good outcome \((n = 6)\) versus those ending in poor outcome \((n = 19)\). There was a trend revealing more CD3+ T cells and IL-2 staining on the fetal side from pregnancies with poor outcome compared to those with good outcome. In the latter case, more often the T cells and IL-2 staining were present on the maternal side within the intervillous space (Fig. 1). However, it is possible that these differences are only an artifact due to the timing of when during the pregnancy the placentas were collected. It may be that viral infection had begun to resolve in the term placentas from women with pregnancies with good outcome compared to those placentas from women with poor-outcome pregnancies who delivered during the acute phase of the infection.

We attempted to classify the nature of the T-cell response in placentas from pregnancies complicated with B19 infection as either a T-helper (CD4) or cytolytic T-cell (CD8) response using IHC. Twenty-five of 28 placentas from group 1 showed evidence of both CD4+ and CD8-positive T cells at relatively similar levels (data not shown). However, there were three exceptions to this observation. One case was an 8-week gestation spontaneous abortion which only CD4-positive staining was seen within the placenta, and two cases, a 14-week spontaneous abortion and a 37-week pregnancy with good outcome,
in which the vast majority of T cells (>90%) were CD8 in nature (data not shown).

In summary, the results presented here suggest that B19 infection is associated not only with an active maternal humoral immune response but also with an inflammation-mediated immune response at the maternal-fetal interface of the placenta. Although we clearly documented the presence of a lymphocyte infiltrate within placentas from women who became infected during pregnancy, we did not demonstrate its antigen specificity for B19. However, we believe that the importance of the T-cell immune response and inflammatory cytokine production should be considered when developing a vaccine against B19.

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