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***Mycobacterium tuberculosis* Infection of the Placenta: A Study of the Early (Innate) Inflammatory Response in Two Cases**

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Abstract

Infections with *Mycobacterium tuberculosis* (MTb) are globally prevalent in many countries, yet descriptions of placental pathology in tuberculous patients are scanty. The usual necrotizing granulomatous response associated with tuberculous infections requires an activation of the adaptive immune system. However, before this system is turned on, the 1st encounter with the tubercle bacillus is mediated by the innate immune system. This pathway utilizes innate surface receptors in neutrophils and histiocytes predominantly and does not produce a granulomatous pattern of inflammation. In this report we describe 2 cases of placental involvement with MTb in which an acute abscess-like inflammatory response with Myeloperoxidase and CD68-positive neutrophils and histiocytes causing acute villitis and intervillitis, with abundant acid-fast mycobacteria, were identified. Other cellular markers consistent with adaptive immunity were negative. These nongranulomatous lesions are seen in primary tuberculous infections occurring in a naïve woman and, obviously, a naïve fetus. These cases with early response inflammation in the placenta are frequently missed precisely because the mother is not known to be infected or has been recently diagnosed and because the symptoms in the newborn may not develop for several weeks, by which time the placenta may have been discarded. This report also shows that the differential diagnosis of acute villitis and intervillitis in the placenta should include tuberculosis aside from the more common bacterial infections such as listeriosis.

Keywords

innate immunity; placenta; tuberculosis

Introduction

Tuberculosis (TB) affects some 2 billion patients worldwide. Despite those numbers, it appears that only about 400 cases of congenital or perinatal TB have been reported

throughout the last century, and very few of these have involved adequate placental evaluations [1]. The delay in clinical onset of disease and diagnosis of the newborn (and often the mother) is the most likely explanation for the scanty reports of placental pathology in congenital TB, as this organ would likely have been discarded when the diagnosis is finally considered several weeks postpartum. In this report we present 2 cases of maternal tuberculosis with placental involvement showing an early nongranulomatous inflammatory reaction.

Case Reports

Patient 1

An appropriate-for-gestational age newborn female was delivered at term by elective cesarean section, with good Apgar scores. The infant's initial evaluation was unremarkable. The mother was a healthy 31-year-old African from Liberia with good prenatal care, and the pregnancy was uncomplicated. However, intractable postpartum bleeding led to a hysterectomy, with a diagnosis of retained placenta. The placenta and uterus were examined, with extensive sampling of the placenta, and a diagnosis of placenta accreta was rendered.

The newborn developed fever and leukocytosis in the 1st week of life and she was treated for bacterial sepsis, although all cultures were eventually negative. Her physical examination was unremarkable except for slight hepatomegaly. A TORCH and other viral and malaria studies were negative, but serum immunoglobulin M was 250 mg/dL (normal: up to 25 mg/dL), consistent with a congenital infection. Head imaging was negative, but an eye exam showed chorioretinitis. In the first 3 weeks of life she appeared mildly ill, with lethargy and poor feeding.

At day 20 postpartum, the mother developed a respiratory infection with pleural effusion, and her clinical studies led to the diagnosis of pulmonary TB. On this basis, a similar diagnosis was entertained in the newborn when she developed classic miliary disease in her lungs and hepatosplenomegaly. A purified protein derivative of tuberculin (PPD) skin test showed 6 mm of induration, and a gastric lavage was positive for *Mycobacterium tuberculosis*. Treatment of the child with Isoniazide, Rifampin, and Streptomycin was administered. Because of persistent liver dysfunction, a liver biopsy was performed. The patient continued to improve, although mild liver dysfunction, mild hypersplenism, and poor weight gain persisted, and was discharged at 2 months of age. At 9 months of age she had improved substantially and had gained weight, and toward her 2nd year of life she had recovered completely, with no residual deficits. Anti-TB therapy was discontinued at 1 year of age.

Patient 2

A 22-year-old, Guatemalan woman with poor prenatal care was admitted 10 days before delivery to an outside hospital with a 1-month history of fever and a 14-kg weight loss. She had no respiratory symptoms, but her chest X-ray showed diffuse micronodular infiltrates. Sputum and bone marrow biopsies were positive for acid-fast bacilli and were confirmed as *M. tuberculosis*.

The infant was delivered at 25 weeks in gestation, weighing 757 g, with Apgar scores of 6 and 8. The newborn developed respiratory distress, and a chest X-ray was consistent with respiratory distress syndrome, which prompted transfer to our neonatal unit. Except for a bilirubinemia of 6.9 mg/dL (direct: 0.4), all laboratory tests were unremarkable, and all samples, including gastric lavage, blood, and endotracheal tube, obtained to identify mycobacteria were negative. The child was treated with intravenous and subsequently with oral Isoniazid and never developed signs or symptoms of active TB infection. The infant was ultimately discharged to the care of the mother to complete the course of therapy under supervision of the local health department.

The mother was treated with Rifampin, Isoniazid, Pyrazinamide, and Ethambutol, and the father and 1 sister, both of whom were PPD positive, were treated with Isoniazid.

Pathology Studies

A review of the many samples of placenta from patient 1, which had been obtained to address the issue of postpartum hemorrhage, was undertaken. A cluster of acute villitis and intervillitis, with a predominant neutrophilic and histiocytic response and no evidence of granulomatous inflammation, was identified in only 1 of the tissue blocks (Fig. 1). An Auramine-O fluorescent stain showed many acid-fast bacteria in this lesion. The liver biopsy revealed non-caseating granulomatous hepatitis with positive acid-fast bacteria on microscopic examination and infection with *Mycobacterium tuberculosis* (MTb) on culture, despite several weeks of anti-TB therapy.

The placenta from patient 2 showed multiple foci of acute villitis and intervillitis in 15 blocks, and a Ziehl-Neelsen stain showed abundant acid-fast mycobacteria (Figs. 2,3A).

Immunohistochemical studies on both placentas, conducted to identify histiocytes (CD68), neutrophils (Myeloperoxidase), T and B lymphocytes (CD3 and CD20), interdigitating reticulum cells (CD21), and natural killer cells (CD56), were performed using standard methods with an automated Dako Autostainer Plus machine (Dako, Carpinteria, CA, USA).

Both placenta specimens showed intense and diffuse staining of inflammatory cells with CD68 and Myeloperoxidase (Fig. 3B,C). Adjacent villi to these inflamed areas showed apparent proliferation of Hofbauer cells in the CD68-stained slides (Fig. 3B). The placenta from patient 1 showed some CD3-positive cells in the mixed cellular reaction and apparent activation of CD3 cells in adjacent villi (Fig. 3D). None of the specimens showed CD20-, CD21-, or CD56-positive cells.

Discussion

Tuberculosis is one of the most globally prevalent diseases, with some 2 billion patients affected worldwide and with 9 million new cases reported in 2005. However, it appears that only about 400 cases of congenital or perinatal TB have been reported throughout the last century, and very few of these have involved adequate placental evaluations [1].

Perinatal TB had been rarely reported in developed countries until more recently, as immigration from the developing world has increased [2]. The AIDS epidemic has also caused a spike in maternal TB infections and, thus, its perinatal version [1]. In a South African study [3], of 107 women with TB (77% HIV co-infected) during pregnancy, mycobacteria were isolated in 16% of the newborns at 1–3 weeks postnatally.

A fetus-newborn may be infected with TB by 1 of 4 routes: (1) transplacental or hematogenous transmission, the hallmark of which is hepatic lesions derived from mycobacteria in umbilical vein blood; (2) inhalation of infected amniotic fluid, usually manifested by pulmonary infiltrates; (3) infection from the maternal genital tract at delivery; and (4) postnatal infection from contact with the mother, other relatives, or health care providers.

Infection of the fetus or newborn is even less frequent if the mother is known to have chronic TB and especially if she has received treatment. The untreated chronic TB patients have low fertility associated with their weakened state, and their lesions consist of chronic necrotizing granulomatous inflammation with variable fibrosis and low levels of mycobacteria in the tissue, blood, and genital secretions. Therefore, many cases of perinatal TB seem to be associated with a concomitant acute or new infection in the mother, in whom mycobacteremia is more likely; this is a necessary requirement for hematogenous transplacental transmission. Our cases reflect reports in which the mother was apparently healthy or just recently sick, without family history of TB or other infectious problems. Indeed, it was the mother's pre- or postnatal acute onset of fever, lung disease, and subsequent TB diagnosis that led to the eventual diagnosis in the child, who may otherwise have remained a clinical puzzle.

In addition, as in our cases, these perinatally infected newborns do not manifest signs or symptoms for several days to weeks. At first they appear to be septic, with fever, neutrophilic leukocytosis, and lethargy. Standard antibacterial medications have no effect, and eventually other signs start to appear, such as hepatosplenomegaly and pulmonary infiltrates. For several reasons, the skin test may not be positive in the newborn for weeks or months, but gastric lavage provides a good yield for TB diagnosis [4].

Placental findings in perinatal tuberculosis include chronic necrotizing granulomas in the decidua or endometrium, if the uterus is also examined. These lesions are rare in this location and tend to reflect a more established or chronic maternal infection. If sufficiently active and productive of mycobacteria, they are a potential source of fetal infection in the birth canal. A necrotizing granulomatous inflammation was not seen in the many sections of placenta or the uterine lining of our 1st case.

As seen with other transplacental infections, acute or chronic villitis, often accompanied by intervillitis, is the hallmark lesion. While granulomatous inflammation is to be expected and has been reported [4,5] in cases of congenital tuberculosis, an acute neutrophilic histiocytic (nongranulomatous) reaction was seen in our 2 placentas. In our 1st case, acute villitis-intervillitis (Fig. 1) was seen only focally in the many blocks of placenta. This focality of placental lesions may be another reason for the negative placental findings in many previous

reports, in which the placentas may have been undersampled. On the other hand, some of the older reports, including the 1st confirmed cases of congenital tuberculosis in 1891 [6] and in the early 20th century [7,8], indicate the presence of acid-fast bacteria within the placenta and fetus, without an inflammatory reaction. In those pre-antibiotic days, many patients suffered fulminant TB infections, causing rapid death of the fetus and mother, in whom an inflammatory reaction may not have had time to evolve.

Why a nongranulomatous, acute neutrophilic-histiocytic inflammatory response?

It is very likely that an acute reaction to TB, as seen in these placentas, represents a 1st contact response to the mycobacteria. This is a rarely witnessed morphological event in the usual setting of TB of the lung. However, it has been described in some of the pathology textbooks throughout the 20th century. For example, Forbus, in his 1943 text *Reaction to Injury* [9], shows a photomicrograph of a hepatic neutrophilic abscess from a patient with tuberculous miliary disease (his fig. 373, p. 582). In the same text, the author states that inoculation of mycobacteria in rabbits results in a situation in which “The bacilli (in the lung) are quickly phagocytosed in the capillaries by the polymorphonuclear leucocytes. Within from 18–24 hours, the mononuclear wandering cells, the macrophages, accumulate within the capillaries, and from that time on they play the most conspicuous role” (p. 583).

In *Pathology of the Human Placenta* [10], the authors state that “In other cases we have identified acute granulomas (consisting mostly of neutrophils) adjacent to villi (sic)” In another report [11] of 2 patients with perinatal TB, the authors found no lesions in the placenta but state that the lesions in 1 of the newborns at autopsy were “non-granulomatous.”

It is important to mention that a neutrophilic-histiocytic inflammatory response in the villous placenta includes in the differential a bacterial infection caused by *Listeria monocytogenes*; pyogenic Staphylococci, Streptococci, or Pseudomonas; and a rare case of transplacental *Herpes simplex* virus infection. It is easy to understand that encountering a placental abscess would trigger a search for those microorganisms rather than mycobacteria.

An acute, neutrophilic-histiocytic inflammatory reaction in TB is likely mediated by the innate immune system. The role of the innate immune system in many conditions, such as asthma, atopy, and other inflammatory disorders, has been recently emphasized [12]. Innate immunity has served the animal kingdom well. It is the only system in invertebrates, who certainly have survived successfully. The acquired or adaptive immune system evolved with vertebrates.

In TB infections, the adaptive immune system is predominantly responsible for development of granulomas and caseating necrosis, both hallmarks of the pathology of TB [13]. In patients with a defective immune system, a granulomatous reaction either does not develop or is poorly formed. An autopsy study [14] of TB infection in 20 AIDS patients with low CD4 counts showed acute, neutrophilic tuberculous meningitis and poorly formed granulomas elsewhere. Patients with genetic immunodeficiencies also show diffuse histiocytic or poor granulomatous responses when inoculated with live *Mycobacterium bovis* in BCG vaccines [15,16].

The innate immune system [12] has several components: the physical barrier system (skin, intestinal mucosa, gastric acid, and lysozyme in tears and saliva). The innate system proper relies on a limited repertoire of receptors in neutrophils, histiocytes, and dendritic cells, among other cells, and humoral factors, which target conserved cell surface molecules that are common to many pathogens.

The neutrophilic and histiocyte reaction to TB seen in our cases seems to reflect an innate immune response of the mother in her 1st encounter with this pathogen and of the fetus in his 1st encounter with any pathogen. Upon birth, or if congenitally infected, the fetus-newborn is suddenly exposed to foreign antigens, which he deals with by using his innate immune system and a rapidly turned-on adaptive immunity [17,18]. Indeed, the apparent focal presence of CD3 positive cells in adjacent villi of the 1st case (Fig. 3D) indicates an early adaptive 271 immune intervention.

A neutrophilic and histiocytic response in the innate immunity phases of TB infections is documented in several studies in humans, as mentioned, and in experimental animals [19–23]. However, the clinical and experimental literature shows conflicting results with regard to whether the innate response is actually protective or whether it contributes to tissue injury. The findings in our 1st index case indicate that the encounter with mycobacteria, presumably in that placental focus, was not a protective one, though it may have been in the 2nd case.

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Figure 1. From patient 1, the placenta showed a focal cluster of acute villitis and intervillitis, which was positive for mycobacteria with an Auramine-O acid fast fluorescent stain (not shown). Hematoxylin and eosin, magnification $\times 400$.

Figure 2.

The placenta from patient 2 showed multiple foci of acute villitis and intervillitis, with no evidence of granulomatous reaction. Hematoxylin and eosin, magnification $\times 200$.

Figure 3.

(A) A Ziehl-Neelsen (Z-N) stain reveals multiple acid-fast mycobacteria within areas of inflammation in the placenta of patient 2 (Z-N stain, magnification $\times 600$). (B) The inflammatory infiltrate in both placentas contained abundant histiocytic cells, demonstrated by CD68 immunostains. Adjacent villi show an apparent proliferation of Hofbauer cells (arrow) (CD68, magnification $\times 200$). (C) The presence of abundant neutrophilic cells is confirmed with immunostains using Myeloperoxidase antibodies (Myeloperoxidase, magnification $\times 400$). (D) The inflammatory lesion in the placenta of patient 1 showed a small component of CD3+ T cells, also seen in adjacent chorionic villi (arrow). This influx of T cells may represent an early activation of the adaptive immune response (CD3, magnification $\times 200$).