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Syphilitic placentitis: demonstration of *Treponema pallidum* by immunoperoxidase staining

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Summary. We report a case of early congenital syphilis in which the placenta showed diffuse proliferative villitis and *Treponema pallidum* was identified by indirect immunoperoxidase stain in formalin-fixed paraffin-embedded placental tissue. This is the first report demonstrating *T. pallidum* in placental tissue using an immunohistochemical method.

Key words: Congenita syphilis – Villitis – Immunoperoxidase

Introduction

Since the advent of penicillin, the reported incidence of congenital syphilis has fallen to such an extent that it has become an almost forgotten disease. However, it is anticipated that the resurgence of syphilis in women of child-bearing age (Hira et al. 1985) will renew the interest of medical personnel, especially those involved in perinatal medicine. We report a case of early congenital syphilis confirmed by the direct demonstration of *Treponema pallidum* by a immunoperoxidase method in formalin-fixed, paraffin-embedded placental tissue.

Materials and methods

A female infant weighing 960 g was born at 28 weeks gestation to a 19-year-old primiparous woman. At birth the baby was asphyxiated and required intubation for resuscitation. She was transferred to the neonatal intensive care unit of the Kanagawa Children's Medical Center. On admission the infant had severe respiratory distress without hepatosplenomegaly or petechiae. The chest roentgenogram showed a reticular pattern with air-bronchograms. C-Reactive protein was 3.6 mg/dl and non-specific immunoglobulin M (IgM) was elevated at 340 mg/dl. She was mechanically ventilated and given cefotaxim sodium and amikacin sulphate with a diagnosis of congenital pneumonia. Bacterial cultures, including *Ureaplasma urealyticum*, and viral cultures of the nasopharynx were negative. Posterior nasal smear specimens were negative for *Chlamydia trachomatis* by both enzyme immunoassay and the direct fluorescein antibody method. Specific IgM antibodies to rubella virus and cytomegalovirus were not elevated by enzyme immunoassay. One month after admission, the attending neonatologist was informed that the mother had untreated syphilis. Subsequent studies of both the mother's and infant's serum revealed strongly positive results for the serological test for syphilis (STS). The infant's serum was positive for *T. pallidum* haemagglutination (TPHA)-IgM by the passive haemagglutination method. Her STS and TPHA-IgM became negative following two courses of ampicillin sodium. Bone roentgenographic series on the 33rd day of life showed a bone-in-bone appearance suggesting intrauterine malnutrition. She had two episodes of transient ileus on the 30th and 45th days of life. She developed a conjugated hyperbilirubinaemia during the 2rd week of life with its peak values occurring in the 2rd month of life. After 4 months the patient was discharged from the neonatal intensive care unit without neurological or respiratory abnormalities.

The placenta, submitted 5 h after delivery, was fixed in neutral-buffer-formalin for 4 days and prepared for histological sections. After paraffin embedding, 3-μm-thick sections were prepared for the immunoperoxidase method. The positive control used was a syphilitic skin lesion (condylomata lata). Negative controls consisted of three placentas with villitis of unknown aetiology, three normal placentas, and autopsied materials of lung, adrenal and liver tissues without pathological findings.

The sections were deparaffinized, and incubated with methanol containing 0.3% hydrogen peroxide for 20 min, followed by incubation in 5% normal goat serum (Dako, Denmark) for 10 min. The effect of proteinase-K was checked with serial preparations of slides with 0.1% proteinase-K/0.01 M phosphate buffered saline (PBS) pH 7.4 at 37° C for up to 20 min. The anti-*T. pallidum* rabbit serum (The Japan Lyophilization Laboratories, Kiyose, Tokyo) was diluted 1:300, 1:900, and 1:2700 with 0.01 M PBS pH 7.4 containing 1% bovine serum albumin. After soaking with the above-mentioned anti-*T. pallidum* serum for 60 min, each section was washed and incubated with a peroxidase-labelled anti-rabbit immunoglobulin (Fab fragment, MBL, Nagoya, Japan, diluted 1:50). Counterstaining was performed with haematoxylin.

Results

The 335-g placenta was moderately enlarged with a pale maternal surface. The fetal surface was cloudy and had

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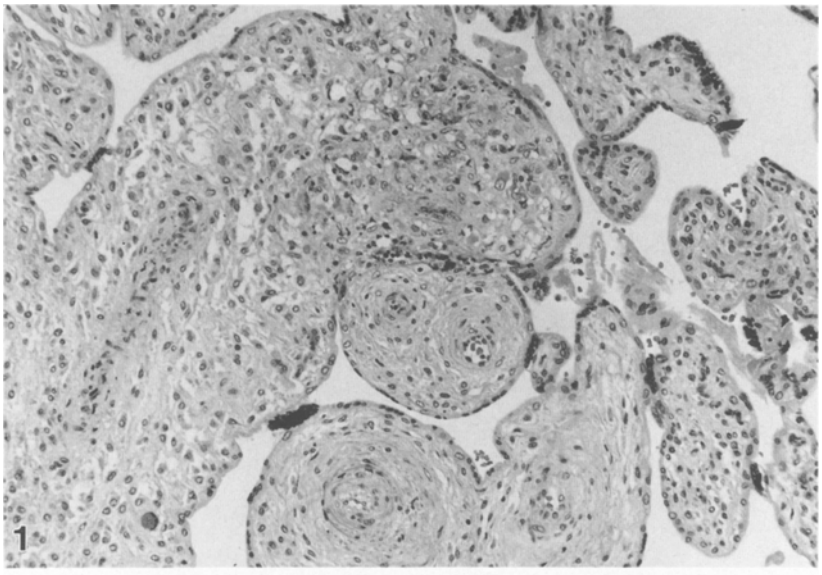

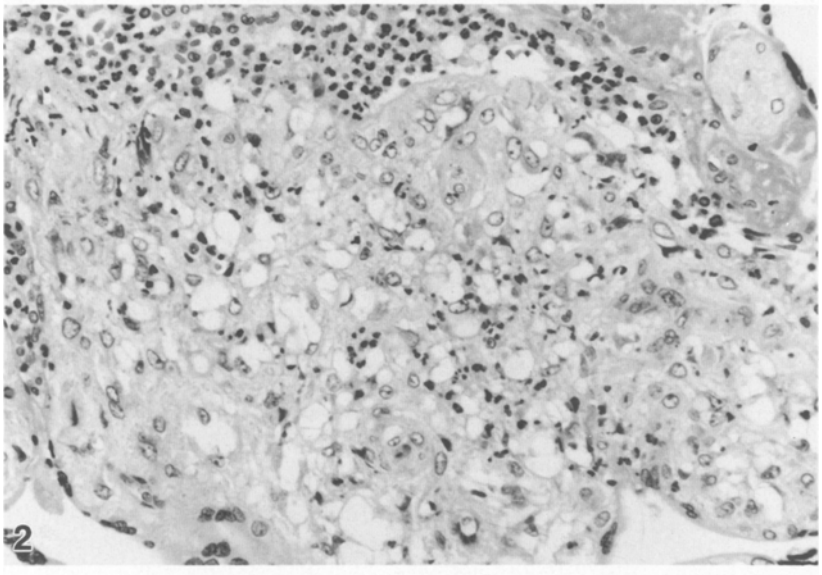

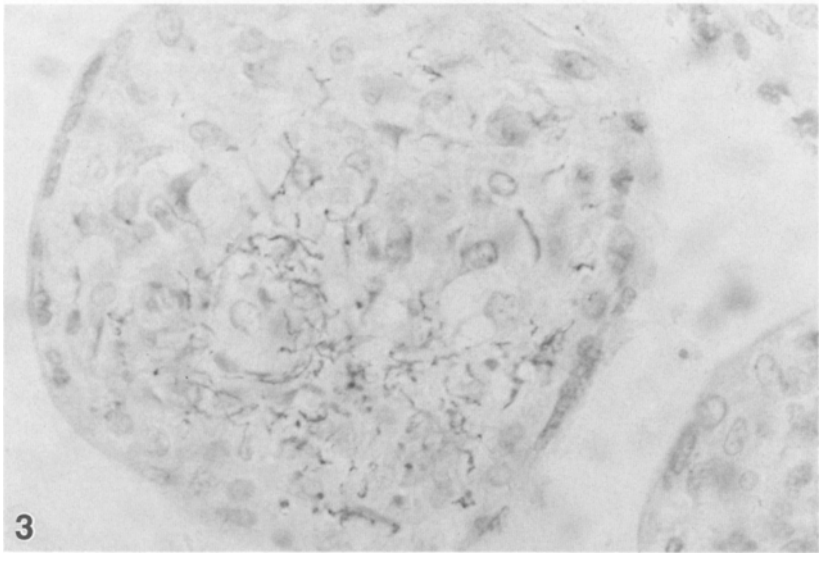

Fig. 1. The placenta. The villous stroma was hypercellular. Fetal vessel lumens showed varying degrees of narrowing with endovascular and/or perivascular proliferation. Haematoxylin and eosin, original magnification ×172

Fig. 2. High power view of the placenta. A focus of necrotizing villitis is shown. Note infiltration of mononuclear cells and polymorphonuclear leucocytes. Haematoxylin and eosin, original magnification ×343

Fig. 3. Immunoperoxidase stain with anti-*Treponema pallidum* antibody demonstrated numerous spirochaetes in the villous stroma. Original magnification ×343

sporadic string-like areas. The most significant microscopic findings was villous hypercellularity with histiocytic and Hofbauer cell proliferation (Fig. 1). There were sporadic small foci of necrotizing villitis, including polymorphonuclear and lymphoplasmacytic components (Fig. 2). The vessels of terminal and stem villi, as well as the chorion, often showed varying degrees of narrowing of lumens with endovascular and/or perivascular proliferation. Mild lymphoplasmacytic vasculitis was seen in umbilical vessels and in the chorionic plate. Mild lymphocytic and polymorphonuclear deciduitis was occasionally encountered. The amnion revealed no significant lesions except for polyp-like projections of the anion with occasional squamous metaplasia.

In immunocytochemistry the proteinase-K preparation reduced the immunoreactivity. Best results were obtained at a dilution of 1:900 anti-*T. pallidum* serum, without proteinase-K preparation. Positive controls revealed numerous spirochaetes. No reactivity was found on the negative control slides, including the three placentas with villitis of unknown aetiology. Our case showed numerous spirochaetes in the villous stroma with and without necrosis (Fig. 3).

Discussion

The diagnosis of congenital syphilis is usually made by a maternal history of syphilis, persistently elevated STS or elevated specific IgM for syphilis in a infant's serum, and clinical signs, especially roentgenographic bone findings. However, direct demonstration of *T. pallidum* is preferable for a definite diagnosis (Ingall et al. 1990). Placenta appears to be a good tissue for the direct demonstration of *T. pallidum* because it can be obtained noninvasively and syphilitic skin lesions, which usually are the easiest sites to demonstrate the organism, are reported to develop in only one-third of patients with early congenital syphilis (Hira et al. 1985).

Although several methods, including silver staining, have been introduced to detect *T. pallidum*, they are technically difficult to perform and are subject to misinterpretation because of non-specific staining of other tissue components (Ito 1987). Immunofluorescence methods to identify, *T. pallidum* are now applied, not only to frozen sections, but also to formalin-fixed, paraffin-embedded section (Takeshita et al. 1980; Chung et al. 1989). We demonstrated *T. pallidum* using the indirect immunoperoxidase method in formalin-fixed, paraffin-embedded sections. This method does not require a cryostat or a fluorescence microscope, and preservation of results is easier than with immunofluorescence methods. These points indicate that this method is also applicable for retrospective evaluation. To our knowledge, immunohistochemical documentation of *T. pallidum* has not previously been reported in placental tissue.

According to correspondence with N. Fukushima (Doai Memorial Hospital, Tokyo), in February 1990, the anti-*T. pallidum* rabbit serum used in this study has weak cross-reactivity with *Candida albicans*, *Aspergillus*, and some species of bacteria. However, it is easy to discriminate the treponemes from the above-mentioned cross-reactive organisms. The anti-*T. pallidum* rabbit serum was reported not to react with *Leptospira*, which would be morphologically difficult to differentiate (Tateshita et al. 1980).

Walter et al. (1982) reported that the histological changes in syphilitic placentitis developed as early as 4 months of gestation and inflammatory infiltrates, with either a predominantly lymphoplasmacytic infiltration or a mononuclear-phagocytic cell (Hofbauer cell and monocyte) reaction, corresponding to the fetal reactive phase. Although there is no pathognomonic finding for syphilis (Walter et al. 1982), the following three points are considered to be basic pathological changes in syphilitic placentitis (Blanc 1981; Russel and Altshuler 1974; Walter et al. 1982): Villous hypercellularity; focal villitis, with or without necrosis, infiltrated by polymorphonuclear leukocytes, lymphocytes, or plasma cells; and fetal vessel obliteration with or without peri- and/or vascular proliferation. The histological features of our case included all three of these basic points.

Retrospectively, our case had characteristic placentitis, as well as some suggestive clinical findings of congenital syphilis, such as conjugated hyperbilirubinaemia and skeletal changes. The diagnosis of syphilis was delayed due to an inappropriate maternal history and an erroneous interpretation of the early neonatal imflammatory response. The latter was thought to represent a nonspecific congenital pneumonia, which is very common in extremely premature infants.

For rapid diagnosis of early congenital syphilis, placentas from premature births should be examined routinely and the accuracy of the maternal history should be confirmed. Once chronic placentitis is discovered, the immunoperoxidase stain for detecting *T. pallidum* is useful in the diagnosis of congenital syphilis.

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References

Blanc WA (1981) Pathology of the placenta, membranes, and umbilical cord in bacterial, fungal, and viral infections in man. In: Naye RL, Kissane JM, Kaufman N (eds) Perinatal diseases. Williams and Wilkins, Baltimore, pp 67–132

Chung KY, Lee MG, Chon CY, Lee JB (1989) Syphilitic gastritis: demonstration of *Treponema pallidum* with the use of fluorescent treponemal antibody absorption complement and immunoperoxidase stains. J Am Acad Dermatol 21:183–185

Hira SK, Bhat GJ, Patel JB, Din SN, Attili RV, Patel MI, Baskarnathan S, Hira RS, Andu NN (1985) Early congenital syphilis: clinical-radiologic features in 202 patients. Sex Transm Dis 12:177–183

Ingall D, Dobson SM, Musher D (1990) Syphilis. In: Reminton JS, Klein JO (eds) Infectious diseases of the fetus and newborn, 3rd edn Saunders, Philadelphia, pp 367–394

Ito F (1987) Fluorescent antibody study on the demonstration of *Treponema pallidum* in the tissue (in Japanese). J Nippon Med School 54:54–62

Russell P, Altshuler G (1974) Placental abnormalities of congenital syphilis. Am J Dis Child 128:160–163

Tateshita T, Asakuma Y, Saito T, Masumura T, Fukushima N (1980) Fluorescent antibody technique for demonstrating *T. pallidum* in the paraffin section (in Japanese). Med Technol 8:647–655

Walter P, Blot P, Ivanoff B (1982) The placental lesions in congenital syphilis. A study of six cases. Virchows Arch [A] 397:313–326