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Congenital Toxoplasmosis Due to Maternal Reinfection during Pregnancy

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A case of congenital toxoplasmic chorioretinitis was diagnosed (specific-immunoglobulin G [IgG] and -IgM comparative Western blot analysis) in a baby whose mother was immune during pregnancy. Maternal sera showed an increase in specific IgG and emergence of both IgM and IgA during pregnancy. The mother was probably reinfected through contact with kittens.

Congenital toxoplasmosis results mainly from primary maternal infection during pregnancy but can occasionally result from preconceptional infection (2, 7, 8) or reactivation of a latent infection in immunodeficient pregnant women (2, 9). We report a case of congenital transmission revealed by macular chorioretinitis in a 9-month-old child and confirmed by a retrospective serological analysis; the mother was immune but was probably reinfected through contacts with kittens during pregnancy. To our knowledge, only two similar cases have been previously reported (3, 4).

Case report. A 28-year-old woman tested positive for anti-*Toxoplasma* antibodies at 11 weeks of amenorrhea (w.a.); she had moderate titers of immunoglobulin G (IgG) antibodies but no IgM, thus reflecting an old infection (see Table 1). No further toxoplasmic surveillance was therefore carried out during pregnancy. Delivery occurred at 38.5 w.a., and clinical examination of the newborn was normal. However, 9 months later, the child developed divergent strabismus. Ophthalmologic examination revealed a macular chorioretinitis scar, with loss of right-sided vision. The aspect of the lesion was so typical of toxoplasmic involvement that no aqueous humor puncture was performed. Serum samples obtained on the same day from both the infant and the mother were tested for anti-*Toxoplasma* antibodies, and the first maternal serum sample was retested in parallel. Furthermore, as the mother took part in a hematological survey during pregnancy, two other stored sera were available for analysis.

Toxoplasma serology. Serology was performed by using several commercial tests. IgG antibody levels were determined by indirect immunofluorescence (Toxo-Spot IF; Biomérieux, Marcy l'Etoile, France) and by enzyme-linked immunosorbent assay (ELISA) (Platelia Toxo IgG; Sanofi Diagnostic Pasteur, Marnes la Coquette, France); IgM and IgA antibodies were detected by ELISA (Platelia Toxo IgM and Platelia Toxo IgA; Sanofi Diagnostic Pasteur) and immunosorbent agglutination assay (Immuno Sorbent Agglutination Assay IgM; Biomérieux).

Western blot analysis. The mother's and child's sera obtained 9 months after birth were comparatively analyzed by immunoblotting. Briefly, the antigenic preparation for Western blot was a sonicate of a *Toxoplasma gondii* RH lysate. After a centrifugation step (30 min, 2,000 × *g*, 4°C), the supernatant was lyophilized and the protein content was quantified. Before migration, the lyophilysate was solubilized in a sample buffer (2% sodium dodecyl sulfate, 10% glycerol, 0.5 M Tris, 5% 2-mercaptoethanol), electrophoresed through a 12% polyacrylamide gel and a 4% stacking gel (Serva, Saint Germain en Laye, France), and then transferred to a nitrocellulose membrane (Transphor; Hoefer Scientific Instruments, San Francisco, Calif.). The membrane was blocked with Tris-buffered saline (0.05 M Tris, 0.15 M NaCl) containing 2% glycine and 2.5% skim milk (Régilait, Lyon, France) and divided into strips. Two strips were incubated for 2 h with each serum diluted 1:20 in Tris-buffered saline–Régilait buffer. The protein fractions recognized by each serum were revealed as follows: one strip of each serum was incubated with either a rabbit anti-human γ-chain immunoglobulin–peroxidase conjugate diluted 1:500 or a rabbit anti-human μ-chain immunoglobulin–peroxidase conjugate diluted 1:500 (both conjugates were obtained from Sanofi Diagnostic Pasteur). The enzymatic reaction was developed by adding a substrate-chromogen solution containing 4-chloro-1-naphthol, 1 M imidazol, ethanol, and H_2O_2 (30%, vol/vol). The molecular weights of the bands were evaluated by comparison with a molecular weight ladder (Pharmacia, Saint Quentin en Yvelines, France).

Results. Serological results are reported in Table 1. Reinfection of the mother during the pregnancy was strongly suggested by the emergence of IgM and IgA antibodies and a rise in IgG titers (10-fold) between 11 and 28 w.a. Congenital toxoplasmosis was confirmed by the detection of IgM antibodies in the child's serum. The Western blot patterns obtained from the child and mother differed substantially. Common antigenic fractions corresponding to IgG transferred from the mother to her child were observed. However, the child's pattern revealed four major additional bands of about 86, 63, 55, and 42 kDa, corresponding to newly synthesized antibodies (Fig. 1). Several bands were recognized by the IgM present in the mother's serum. Two bands (of about 50 and 60 kDa) were recognized by newly synthesized IgM present the child's serum; these isotypes do not cross the placental barrier.

Discussion. These serological results point to toxoplasmic reinfection of the mother during pregnancy, leading to a probable congenital infection of the child. Infection of the child after birth seems unlikely (the macular localization of the scar being typical of congenital toxoplasmosis) but cannot be completely excluded because no child's serum was available before the ophthalmological diagnosis. The onset of chorioretinitis in the child was associated with the emergence of newly synthe-

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TABLE 1. Toxoplasma antibody analysis in the mother's and child's sera

Mo/day/yr (w.a.)[a]	Serum	IgG (IU/ml) by:		IgM result in:		IgA result in ELISA
		Indirect immunofluorescence	ELISA	ELISA	Immunosorbent agglutination assay	
03/30/92 (11)	Mother	150	223	−	−	−
05/25/92 (19)	Mother	150	204	−	−	−
07/29/92 (28)	Mother	1,200	2,060	+	+	+++
07/30/93[b]	Mother	1,200	1,060	−	−	+
07/30/93[b]	Child	600	505	−	+	−

[a] Contact with kittens 5/18/92.
[b] Nine months after delivery.

sized antibodies. Clinical examination of the child and further investigations (computerized tomography scan and cranial radiography) were normal. As the parents were opposed to all treatment, pyrimethamine-sulfadiazine could not be administered. Ophthalmologic follow-up was nevertheless performed until November 1995, with no other complications.

The maternal reinfection was probably linked to accidental ingestion of oocysts, as the mother's personal diary referred to contacts with roaming kittens on 18 May 1992 and to a flu-like illness a week later. The antibody kinetics, particularly the high IgA levels in July 1992, also suggests an infection by oocysts, as described by Fortier et al. (3) and Hennequin et al. (4). Furthermore, reactivation of latent infection in an immunocompetent patient does not usually elicit IgA or IgM antibodies. In the case described here, there were no iatrogenic or pathological factors of immunodeficiency or abnormalities in lymphocyte subsets. Congenital toxoplasmosis was detected by routine serological tests and confirmed by immunoblotting. The Western blot profile obtained with the child's serum was comparable to those obtained by Chumpitazi et al. in a study of congenital toxoplasmosis (1). The infection was subclinical at birth and became patent in the following months. This type of late clinical onset most commonly results in ocular lesions (mainly chorioretinitis) (5) and may be underevaluated because of the lack of follow-up in immune women; it may effectively underlie cases of unexplained chorioretinitis diagnosed during childhood or adolescence.

Reinfection of immunocompetent women during pregnancy is fortunately uncommon but shows that residual IgG specific antibodies do not always protect against congenital toxoplasmosis. Furthermore, it raises the hypothesis that ingestion of *Toxoplasma* cysts contained in meat does not protect against reinfection by oocysts. This could be explained by the antigenic differences between sporozoites and tachyzoites described by Kasper and Ware (6).

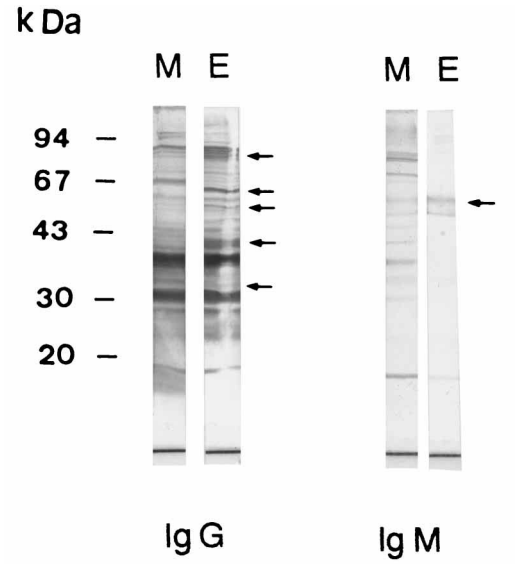

FIG. 1. Comparative immunoblot analysis of the sera of the child (lanes E) and his mother (lanes M), taken 9 months after delivery. The arrows indicate the child's neosynthesized antibodies (antigens of about 86, 63, 55, and 42 kDa for IgG antibodies and 60 kDa for IgM antibodies).

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