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Selective Suppression of Maternal IgG Anti-Central Nervous System Antibody Reactivity

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Abstract. During pregnancy, it is possible that mothers may produce IgG antibodies directed against CNS antigens, which upon crossing the placenta would cause neurologic impairment in the offspring. Since anti-central nervous system (CNS) immunoreactivity is present in normal individuals, it is important to establish background rates in healthy mothers and infants. 101 mother-infant pairs were studied. Serum samples were obtained from the mothers at the time of hospital admission for delivery. Cord blood samples were taken from the infants at the time of birth. In all cases these were uncomplicated pregnancies and full-term deliveries. The serum samples were screened against Western blots of normal, human, adult, autopsy-derived frontal cortex (FC) and cerebellum (CER). To detect the presence of antibodies directed against embryonic CNS antigens, the serum samples were also screened against Western blots prepared from embryonic day (E) 17 and adult mouse cerebral cortex specimens. In the case of IgG there was no banding detected in infants, whereas in mothers the incidence of immunoreactive banding against FC was 1% and against CER was 2%. The maternal IgG anti-CNS reactivity incidence is significantly less than that seen in normal adults ($p < 0.001$). In the case of IgM, when screened against FC and CER, there was no banding detected in infants, whereas in mothers the incidence of banding was 34 and 26% respectively, a result which is in keeping with previous observations. When screened against E17 mouse CNS tissue the incidence of IgG banding in both mothers and newborns was 10%, whereas against adult mouse cortex the respective incidence were 9 and 12%. These results indicate that there is a selective suppression of maternal IgG anti-CNS antibodies during pregnancy. These results also indicate that there is a very low incidence of background IgG anti-CNS reactivity in term mothers using human CNS as substrate. Thus, these techniques are appropriate ones to use in investigating the presence of maternally derived IgG anti-CNS antibodies as potential pathogens in infants born with CNS disorders. However, results from the use of adult or fetal mouse CNS tissue as substrate would be more difficult to interpret because of the approximately 10% background IgG reactivity.

Introduction

Maternal IgG crosses the placenta by active transfer mostly in the third trimester. In maternal autoimmune diseases which are IgG mediated, pathogenic IgG may therefore cross the placenta and produce fetal and newborn diseases. These include neonatal myasthenia gravis, neonatal lupus erythematosus, fetal hyperthyroidism, fetal thrombocytopenia, herpes gestationis, pemphigus vulgaris and Behçet's disease [reviewed in 1, 2].

The majority of neurologic developmental disorders in childhood do not have a clear pathogenetic explanation. It is possible that in some cases maternal IgG antibodies may recognize developing fetal central nervous system (CNS) antigens and may thus interfere with normal brain development. This pathogenic process has been suggested in selected cases of autism [3, 4]. Furthermore, a preliminary report has demonstrated an association between the presence of maternal anti-Ro ribonucleoprotein antibodies and dyslexia in children suggesting that these specific antibodies may perturb normal CNS development [5].

A useful method to screen for anti-CNS antibody reactivity is the use of Western blots [6, 7]. This technique is reproducible and in childhood neurologic diseases has shown significant correlations between anti-CNS immunoreactivity and epilepsy, inflammatory CNS diseases, the opsoclonus-myoclonus syndrome and systemic lupus erythematosus [7]. However, the incidence of background anti-CNS immunoreactivity on Western blots is approximately 30% in older children and in adults who are neurologically normal [6, 7]. Such a high background incidence of anti-CNS immunoreactivity would make any results from maternal-fetal anti-CNS immunoreactivity investigations very difficult to interpret. During pregnancy there is a decrease in the concentration of all maternal circulating immunoglobulins (18% for IgG, 13% for IgA and 9% for IgM in the second and third trimester) [8]. Furthermore, the maternal immune system may also be attenuated thus providing fetal protection from pathogenic immunoglobulins [9]. Thus it is important to independently establish background rates of anti-CNS immunoreactivity using Western blots in healthy mothers and infants since the previously published results [6, 7] may not be appropriate for this particular clinical situation.

Materials and Methods

101 mother-infant pairs were studied. Serum samples were obtained from the mothers at the time of hospital admission for delivery. Cord blood samples were taken from the infants at the time of birth. The samples were stored at −80 °C until being used. This study was approved by hospital ethics review committees.

Brain samples were obtained at the time of autopsy from neurologically normal young adults who had died from nonneurologic causes. The time of autopsy was no later than 12 h after the time of death. Routine neuropathologic examination was normal. The brain samples were stored at −80 °C until being used.

Murine brain samples were obtained from adult mice after decapitation and from embryonic (E) 17-day-old fetal mice after the pregnant mouse was sacrificed by cervical dislocation. These brain samples were also stored at −80 °C until being used.

Western blots were made by standard techniques [10]. Homogenates of frontal cortex (FC), cerebellum (CER), E17 mouse cortex or adult mouse cortex were homogenized and boiled for 2 min in 2.5% sodium dodecyl sulfate (SDS; w/v), 7% 2-mercaptoethanol (v/v) in TBS (50 m*M* Tris-HCl, 200 m*M* NaCl, pH 7.4) and the proteins separated as a curtain by polyacrylamide gel electrophoresis (PAGE) through

a 15% acrylamide gradient. The gel loading was 10 μg protein/mm track width. The separated polypeptides were electrophoretically transferred to a cellulose nitrate sheet. To detect specific antibody binding, 3 mm wide strips from the blot were first incubated 30 min in 10% normal horse serum (NHS) in phosphate-buffered saline (PBS; 0.1 *M* phosphate buffer, 0.15 *M* NaCl, pH 7.4) to block nonspecific binding sites, then overnight in the serum sample diluted 1:100 in 10% NHS. A serum concentration of 1:100 gave optimal visualization of immunoreactive bands without significant background staining. After two 15-min washes in PBS, the blot strips were incubated for 2 h in horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG or anti-human IgM (Dako Inc.) diluted 1:100 in 10% NHS. Antibody binding was detected by washing the blot twice for 15 min in PBS and then for 15 min in 0.5 mg/ml 4-chloro-1-naphthol-0.01% (v/v) hydrogen peroxide [11]. The apparent molecular weights of antigenic polypeptide bands in kilodaltons (kD) were estimated from prestained molecular weight standards (BRL Inc.) which were blotted concomitantly. Control blots in which the serum sample was replaced by 10% NHS revealed no bands. MabN210 is a murine monoclonal antibody which recognizes the 210-kD subunit of neurofilaments [12]. Control blots in which MabN210 was substituted for the serum, and the second antibody replaced by HRP-conjugated rabbit anti-mouse immunoglobulin (Dako Inc.) diluted 1:100 in 10% NHS, consistently revealed an immunoreactive band at 210 kD.

Quantitative immunoglobulins (IgG and IgM) were determined in 98 of the maternal serum samples. These were performed in the clinical chemistry laboratory using a Beckman Array nephelometer. Normative age-related data as determined by the clinical chemistry laboratory was used for comparison.

The results of these investigations were compared to those of the author's own previously published results where identical methodologic techniques were used [6, 7]. These tests were run concurrently with those that were published in reference 7.

For statistical analysis, the χ^2 test was used.

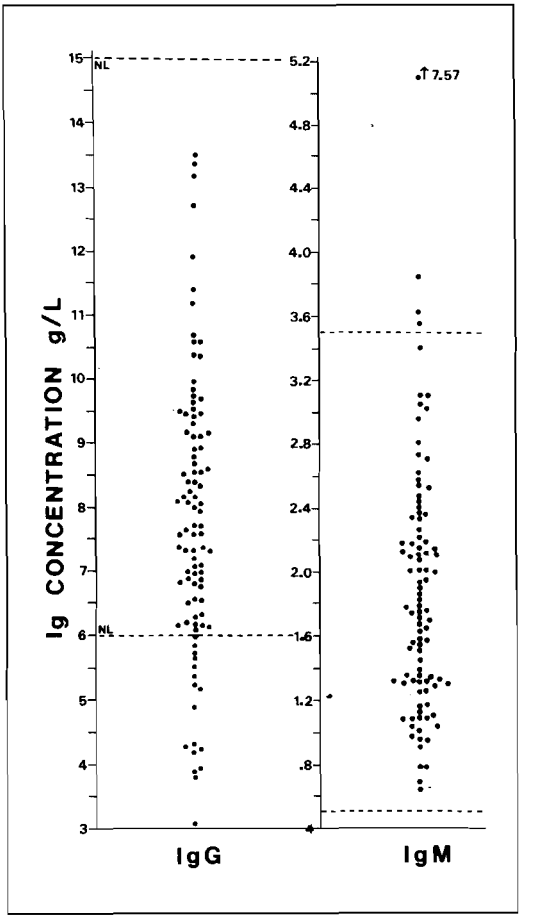

Fig. 1. Quantitative determinations of IgG and IgM concentrations in 98 serum samples from mothers at the time of delivery. Normative values are indicated by the dotted lines. For IgG, in 81 cases (83%) the results were within normal limits and in 17 cases (17%) were below normal. For IgM, in 94 cases (96%) the results were within normal limits and in 4 cases (4%) were above normal limits.

Results

In 98 of the 101 maternal serum samples, quantitative IgG and IgM determinations were performed. The results are illustrated in figure 1. Normative data is indicated by the dotted lines. For IgG, normal results were obtained in 81 cases (83%) and below normal results

in 17 cases (17%). For IgM, normal results were obtained in 94 cases (96%) and elevated results in 4 cases (4%).

All the maternal and fetal serum samples were tested twice against FC and CER Western blots. The results from the immunoblot tests were reproducible.

When tested against FC Western blots, the infant serum samples displayed absolutely no IgG banding. The maternal serum samples were positive in only 1 case (banding at 210 kD) [illustrative positive immunoblots are illustrated in 6 and 7]. There is no statistically significant difference between the infant and maternal results. However, in comparison with 99 normal adult controls where the incidence of IgG anti-FC immunoreactivity is 32% [6], there is a significant suppression of the incidence of IgG anti-FC reactivity in the mothers ($p < 0.001$). Likewise, in comparison with 199 neurologically normal children where the incidence of IgG anti-FC immunoreactivity is 24% [7], there is a significant suppression of the incidence of IgG anti-FC reactivity in the mothers ($p < 0.001$).

When tested against CER Western blots, the infant serum samples displayed absolutely no IgG banding. The maternal serum samples were positive in only 2 cases (in 1 case banding was detected at 210 kD and in the other case at 186 and 210 kD). There is no statistically significant difference between the infant and maternal results. However, in comparison with 18 adult cases of cerebellar ataxia where the incidence and anti-CER immunoreactivity is 33% [6], there is a significant suppression of the incidence of IgG anti-CER reactivity in the mothers ($p < 0.001$). Likewise, in comparison with 199 neurologically normal children where the incidence of anti-CER reactivity is 21% [7], there is a significant suppression of the incidence of IgG anti-CER reactivity in the mothers ($p < 0.001$).

In the case of IgM, when tested against FC and CER Western blots, there was no immunoreactivity detected in the infant samples. For the mothers, the incidence of positive IgM banding was 34% for FC and 26% for CER. Although no other investigations have been performed by the author assaying IgM anti-CNS immunoreactivity, these results are similar in the incidence of 32% IgG anti-FC and 33% anti-CER reactivity in adults [7].

When tested against adult mouse cortex Western blots, the infant serum samples displayed positive IgG banding in 12 cases (12%) and the maternal samples in 9 cases (9%). When tested against E17 mouse cortex Western blots, the infant serum samples displayed positive IgG banding in 10 cases (10%) and the maternal samples also in 10 cases (10%). There was no statistically significant difference between the infant and maternal results. No other investigations have been performed by the author using murine CNS as substrate. Thus, further comparisons of the incidence of anti-murine CNS immunoreactivity cannot be made.

Discussion

These results suggest that there is a selective suppression of maternal IgG anti-CNS (both anti-FC and anti-CER) immunoblot reactivity at the end of pregnancy. These results cannot be accounted for by decreased total IgG levels in the mothers since 83% had entirely normal levels and only 17% had mildly decreased IgG levels (fig. 1). Also, these

results cannot be explained by a total suppression of anti-CNS immunoreactivity since the incidence of IgM anti-CNS reactivity is still high (34% for FC and 26% for CER).

During pregnancy the maternal immune system may become attenuated [2, 9]. It is possible that the observed IgG anti-CNS immunoreactivity suppression is a selective attenuation of the maternal immune system in order to protect the developing fetal CNS.

It should be noted that this investigation was conducted at the end of pregnancy. It would be of value to investigate the incidence of maternal anti-CNS immunoreactivity longitudinally before conception and during the course of pregnancy. Since maternal IgG crosses the placenta by active transport mostly in the third trimester [2], it is possible that maternal anti-CNS IgG immunoreactivity is selectively suppressed only during the third trimester.

The incidence of anti-CNS immunoblot reactivity in infants was nonexistent for both IgG and IgM. For mothers, the incidence of IgG anti-CNS immunoblot reactivity was very low (1% against FC and 2% against CER). With such low background reactivity levels, these techniques can be used to further investigate the possibility of maternal anti-CNS IgG antibodies crossing the placenta and producing specific neurologic diseases in the fetus and newborn.

To investigate whether specific fetal CNS antigens could be detected using these techniques, the author expanded the research to include an investigation of anti-murine CNS immunoblot reactivity. With both adult and E17 murine CNS, IgG immunoreactivity was detected which did not differ significantly between infants and mothers. No specific E17 immunoblot antigens were identified by the maternal or infant serum samples. With an approximately 10% background IgG immunoreactivity rate, using adult or fetal mouse CNS as substrate would produce results which may be difficult to interpret.

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