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Journal of Neuroimmunology 194 (2008) 165 – 172

Journal of Neuroimmunology

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Antibodies against fetal brain in sera of mothers with autistic children

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Received 25 September 2007; received in revised form 26 October 2007; accepted 14 November 2007

Abstract

Serum antibodies in 100 mothers of children with autistic disorder (MCAD) were compared to 100 age-matched mothers with unaffected children (MUC) using as antigenic substrates human and rodent fetal and adult brain tissues, GFAP, and MBP. MCAD had significantly more individuals with Western immunoblot bands at 36 kDa in human fetal and rodent embryonic brain tissue. The density of bands was greater in fetal brain at 61 kDa. MCAD plus developmental regression had greater reactivity against human fetal brain at 36 and 39 kDa. Data support a possible complex association between genetic/metabolic/environmental factors and the placental transfer of maternal antibodies in autism.

Keywords: Autoimmunity; Autistic disorder; Maternal antibodies; GFAP; Myelin basic protein; BDNF

1. Introduction

Autism, with an estimated incidence of 1:150, is currently recognized as the most common developmental disability among children (CDC, 2007). Clinically, a period of apparent normal neurodevelopment often precedes the identification of classical deficits in areas of social interaction, communication and language, and stereotypic behaviors (Rapin, 1997). At birth, autistic brains are typically smaller than those of healthy infants, but between 6 and 14 months of age undergo a period of accelerated growth (Courchesne et al., 2003). Genetic, biochemical, and environmental factors are most commonly mentioned as etiological mechanisms in autism (Korvatska et al., 2002; Lawler et al., 2004), but abnormalities of immune function have also been proposed (Cohly and Panja, 2005; Pardo et al., 2005).

To date, evidence that immune factors have a role in autism is primarily circumstantial. Families with autism show clustering of autoimmune disorders (Comi et al., 1999; Croen et al., 2005), evidence of immune dysregulation (Gupta, 2000), and abnormal levels of plasma immunoglobulins (Plioplys et al., 1994). Children affected with autism have serum antibody reactivity against epitopes located in both adult human and rodent cortical, subcortical, and cerebellar brain regions (Cabanlit et al., 2007; Silva et al., 2004; Singer et al., 2006), as well as against specific brain proteins, e.g., glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) (Singh et al., 1997, 1993). Based on these findings, an acquired autoimmune abnormality that affects dendritic fields and synaptogenesis has been proposed for some cases of autism. A second autoimmune hypothesis, in contrast, has suggested that the process begins in utero and is associated with the placental transfer of maternal antibodies that, in turn, interfere with fetal brain development. Evidence for the intrauterine immune hypothesis is limited. A small pilot study has identified serum antibodies against embryonic rodent brain in mothers with autistic offspring that were absent or reduced in mothers of unaffected children (Zimmerman et al., 2007). Further, animal models have demonstrated that maternal antibrain

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doi:10.1016/j.jneuroim.2007.11.004

antibodies are capable of crossing rodent placenta and causing behavioral alterations in their offspring (Dalton et al., 2003; Vincent et al., 2002).

The goal of this study is to expand our knowledge of serum antibrain antibodies in mothers of children with autistic disorder (MCAD). Western immunoblotting was performed with use of fetal and adult brain tissues derived from both humans and rodents. These tissue samples were selected in order to identify serum reactivity against fetal human epitopes, provide comparisons with prior reports (Braunschweig et al., 2006; Zimmerman et al., 2007), and evaluate differences in reactivity against developing and mature brain tissues. Adult human brain regions selected for use in this study were chosen on the basis of identified neuroanatomical abnormalities in postmortem autism brains, magnetic resonance imaging, and autoantibody studies in autistic patients (Bauman, 1991; Courchesne et al., 1988; Singer et al., 2006; Singh and Rivas, 2004; Zilbovicius et al., 1995). Results of specific antibrain antibodies were correlated with clinical histories, including family history of autoimmune disease, pregnancy, birth order, maternal and paternal ages, and evidence of developmental regression in the child with autism. We hypothesized that serum reactivity would differ in mothers of children with autistic disorder and that results obtained from use of fetal tissues would be associated with specific clinical characteristics.

2. Materials and methods

2.1. Subjects

One hundred mothers of children with autistic disorder (mean age 41 ± 6 years; range 27–66) were recruited from the Center for Autism and Related Disorders at the Kennedy Krieger Institute. The study was approved by the Institutional Review Board of the Johns Hopkins Medical Institutions. Autism was diagnosed in children by the presence of abnormalities in social and communication development, marked repetitive behavior, and limited imagination using the Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV) and Autism Diagnostic Observation Schedule-Generic (Lord et al., 2000) or Childhood Autism Rating Scale (CARS) (APA, 1994; Schopler et al., 1986). Children with diagnoses of Asperger syndrome and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) were excluded. All were judged clinically to have moderate to severe adaptive deficits, or cognitive deficits (IQ, <70) by formal testing. All affected children of mothers in the study had undergone medical evaluations and were given the specific diagnosis of autistic disorder by an expert in the field (AZ). Those with established genetic or metabolic causes of autism were excluded.

Medical data on the 100 mothers of children with autistic disorder participating in this study were obtained in personal interviews by a masters level neonatal nurse (PG) with emphasis placed on information pertaining to maternal and paternal age at the child's birth, pregnancy histories (including the total number of pregnancies before the birth of the proband), maternal, paternal and familial (in first-degree relatives) autoimmune disorders (AI) (including rheumatoid arthritis, autoimmune thyroid disease, and psoriasis, among others; for further details, see Comi et al. (1999) and Croen et al. (2005)) and the clinical course of the affected offspring.

The control group consisted of 100 aged-matched mothers of unaffected, non-autistic children (MUC, mean age 43 ± 5 years; range 27–66) from the local community. Criteria for selection included no lifetime personal history of a DSM-IV diagnosis of autism and no related disorders in their children or first-degree relatives. No ethnic or racial groups were excluded from the study. All clinical data were de-identified and entered into a database.

Serum samples were stored within 2 h of phlebotomy at −80 °C until use. Samples were coded and assayed by laboratory technicians who were blind to the diagnosis.

2.2. Serum IgG concentration measurement

Sandwich ELISA methods were used to measure serum IgG concentrations in all 200 serum samples following the methodology of Raux et al. (1999). Human IgG (Sigma) diluted in serum diluent at seven dilutions, between 3.9 and 500 ng/ml, was used to generate a standard curve. Absorbance was measured at 450 nm using a Model 680 Microplate Reader (Bio-Rad). Microplate Manager software (Bio-Rad) normalized the data, generated a linear regression curve based on standard dilutions, and calculated the serum IgG concentrations in mg/dl. All assays were performed in duplicate.

2.3. Antineuronal antibody determinations

2.3.1. Tissue preparations

Fresh, unfixed, human fetal (17-week gestation, postmortem interval less than 12 h) and adult brain tissues (postmortem intervals less than 18 h) were obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. Fresh Brodmann's Area 9 (BA9) and caudate were obtained from a 76-year-old male who died of a cardiac disorder. Fresh cerebellum (CB) and cingulate gyrus (CG) were obtained from a 36-year-old female who died of a gunshot wound to the chest. Neither individual had evidence of neurological disease. Rodent tissue included brains from adult and embryonic rats (gestational day 18).

Brain tissues were homogenized in 0.9% NaCl (2.5 g of tissue/10 ml of saline) containing protease inhibitors (1 μg/ml of aprotinin, 10 μg/ml of leupeptin, 10 μg/ml of pepstatin, and 1 mM phenylmethylsulfonyl fluoride) in a Teflon-glass homogenizer on ice. A supernatant fraction was collected and aliquots were stored at −80 °C. Protein concentrations were measured by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL).

2.4. Western immunoblotting

2.4.1. Brain tissue

Serum samples from all 100 MCAD and 100 MUC subjects were assayed against human fetal brain, adult brain areas including BA9, caudate, cerebellum, and cingulate gyrus, embryonic rat (E18), and adult rat brain.

Methodology used for immunoblotting was similar to that previously described (Singer et al., 2006). A total of 30 μg of brain tissue protein per sample was denatured, subjected to electrophoresis, and transferred to 0.45-μm nitrocellulose. After blocking, nitrocellulose was exposed to an individual's serum diluted 1:500 for 90 min at room temperature. Secondary antibody was horseradish peroxidase-conjugated sheep anti-human IgG (GE Biosciences, Pittsburgh, PA) diluted 1:3000. Membranes were developed with GE ECL reagents according to the vendor protocol, and blots were exposed to Denville Blue Bio Films (Denville Scientific, Metuchen, NJ) for 60 s. Molecular weights were estimated based on the distance migrated for seven known molecular weight standards (Bio-Rad). Digital image analysis and evaluation of Western blots were performed by Quantity One (Bio-Rad), which creates quantitative densitometric data of the blots showing the gray-intensity values (8-bit gray values) vs. Rf values. For all bands on each blot, Quantity One generates a peak intensity (pixel optical density) for each band, assigns each peak a molecular weight, and determines the peak height, defined as intensity for the entire band. All peak height measurements were corrected for the specific serum IgG content contained in each sample. The density of an anti-IgG band, identified in human brain samples at 50 kDa, was used to provide a gross measure of inter-gel variation. Bands are localized at ±2–3 kDa to allow for imprecision in scanning and extrapolation of band locations.

2.4.2. Specific antigens (GFAP, MBP)

Serum from 20 MCAD (mean age 44±3 years; range 38–52) and 20 MUC (mean age 44±4 years; range 38–51) were randomly selected for evaluation of antibody reactivity against GFAP and MBP. GFAP and MBP immunoblots were obtained using 1 μg of human GFAP (American Research Products, Inc., Belmont, MA) and 3 μg of human brain MBP (US Biological, Swampscott, MA). Proteins were denatured and electrophoresed in 15% acrylamide ready-gels (Bio-Rad). GFAP was transferred to 0.45-μm nitrocellulose and MBP was transferred to 0.45-μm Immobilon PVDF membranes. The membranes were blocked overnight, exposed to serum diluted 1:500, washed, and exposed to secondary antibody; HRP-conjugated sheep anti-human IgG (GE Biosciences) diluted 1:3000. ECL detection was performed and band presence/absence was determined visually.

To ensure the presence of specific antigens, blots were stripped of serum antibodies using Restore stripping buffer (Pierce). Membranes were then exposed to antigen-specific primary antibody; mouse anti-human GFAP monoclonal antibody diluted 1:750 (Chemicon, Temecula, CA), or rabbit anti-human MBP polyclonal antibody diluted 1:3000 (Chemicon). Secondary antibody consisted of either HRP-conjugated sheep anti-mouse IgG or HRP-conjugated sheep anti-rabbit IgG (GE Biosciences) diluted 1:3000. After washing, blots were exposed to Denville Blue Bio Films and band presence/absence was determined visually.

2.5. Serum levels of BDNF

In a post-hoc analysis, 25 MCAD (mean age 41±5 years; range 33–53) and 25 MUC (mean age 43±5 years; range 31–53) samples were selected for assay based on their Western immunoblotting results showing high levels of antibodies against human fetal brain at 61 kDa, 39 kDa, and 36 kDa.

Serum BDNF levels were assayed in 96-well microtiter ELISA plates using a sandwich enzyme immunoassay BDNF ELISA kit (USBiological). Plates were pre-coated with rabbit anti-human BDNF polyclonal antibody. Human serum was diluted 1:200 and incubated overnight at 4 °C. Secondary antibody was monoclonal, biotinylated mouse anti-human BDNF diluted 1:1000. After exposure to HRP-conjugated streptavidin diluted 1:1000 and washing, plates were developed with tetramethylbenzidine/enhancer (TMB/E) solution. Sera BDNF concentrations were measured by optical density at 450 nm on an automated Bio-Rad Model 680 microplate reader. Standards were used to determine the slope of OD (absorbances) vs. BDNF concentration. OD values (total reading minus tissue blank) were determined for each sample before statistical analysis. All samples were assayed in triplicate.

2.6. Statistical analysis

Statistical analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL) and Stata/SE 9.2 (Stata Corp LP, College Station, TX), to test the hypothesis that anti-neuronal antibody profiles differ among MCAD (*cases*) and MUC (*controls*). BDNF assay calculated concentrations were analyzed as continuous variables. Western blot analysis provided information about the presence of bands at each specific molecular weight and band density as measured by corrected peak height OD values. Band localization was assessed by comparing the proportion of case and control mothers with each band using χ^2 analysis. Fisher's exact test was used in place of the χ^2 test when the numbers were small. Simple logistic regression was

Table 1
Maternal demographics

	MCAD		MUC
Mother's age (mean±SD) (range)	41±6 years (27–66)		43±5 years (27–66)
Maternal age at 1st pregnancy (mean±SD)	28.5±6.1 years		29.5± 4.4 years
Parity, mean and (range)	2.3 (1–6)		2.6 (1–5)
Number mothers with 1 AD child	87	1st born=33 2nd born=28 3rd born=16	
Number mothers with 1AD child and 1 PDD/Asperger	3		
Number mothers with 1 AD child and 2 PDD/Asperger	1	1st born with ASD=9	
Number mothers with 2 AD children	8		
Number mothers with 2 AD children and 1 PDD/Asperger	1		
Mean interval between birth of child with AD and blood draw mean (range)	8.3 (2–21 years)		
Number AD children with regression	48		
History of autoimmune disease	24*		12

AD = autistic disorder, PDD = pervasive developmental disorder.
* $p=0.028$.

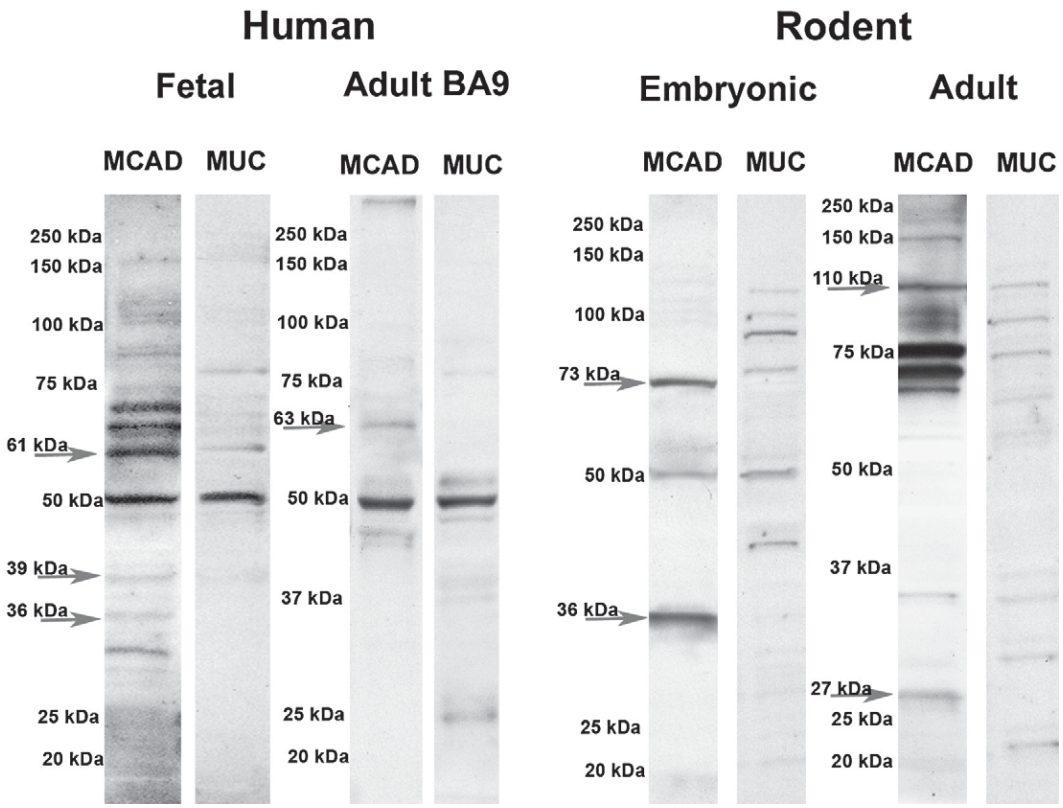

Fig. 1. Western blots from MCAD and MUC groups against human fetal and adult BA9 and rodent embryonic and adult brain tissues. Numerous bands (antigen–antibody interactions) are observed in both mothers of children with autistic disorder (MCAD) and mothers of unaffected children (MUC). Arrows identify bands showing differences between clinical groups.

also used to assess the association between the presence of each band and case-control status. Band density was corrected for serum IgG concentrations i.e., optical density (peak height) measurement divided by the serum IgG concentration expressed as mg/100 ml. Based on the presence of overlapping band densities at 50 kDa, suggesting little inter-gel variation in reactivity against human brain epitopes, no further corrections were applied. Band density was treated as continuous and quantified by calculating mean peak height. Analyses of corrected band density were not performed unless there were at least three samples in each group. The differences in band density among groups were assessed using Student's *t*-test.

3. Results

3.1. Clinical population

Information on maternal history, birth order, timing of blood draw, and offspring regression are presented in Table 1. All mothers were healthy at the time of blood draw. Eighty-seven mothers had one child with autistic disorder, nine had two affected children with autistic disorder, and four had one child with autistic disorder plus one or more with Asperger syndrome or PDD-NOS. Of 101 offspring with autistic disorder, representing one per family with the exception of two from a mother with affected monozygotic twins, 48 had a positive history for both social and language regression (Ozonoff et al., 2005), based on parents' recall of decline in previously acquired developmental milestones. MCAD had more than twice the odds of having a personal history of autoimmune disease ($n=24$) compared to MUC ($n=12$) (OR=2.32, $p=0.028$).

3.2. Serum IgG concentration

Measurements of serum IgG concentrations were similar in MCAD, 1030±260 mg/dl, and MUC, 1030±200 mg/dl, groups.

3.3. Western blots for brain proteins

Numerous bands were identified in all subjects using fetal and adult brain tissue epitopes from human and rodent sources (Fig. 1). Analyses of band specificity (number within a clinical group having a band at a specific molecular weight) as well as corrected peak height OD values identified relatively few significant differences ($p \leq 0.05$) or trends ($p \leq 0.09$). Comparisons of the total number of bands observed or the sum of all corrected peak heights showed that for each brain tissue evaluated there was no difference between MCAD and MUC (data not presented). Quantitation of the IgG band at 50 kDa, performed only with human epitopes, showed no mean differences in values in BA9, caudate, cingulate gyrus, cerebellum or fetal brain between MCAD and MUC groups. Preliminary analyses identified peak heights to have a normal distribution pattern.

Table 2
Band specificity (number of subjects with band) and band density (corrected peak height)[a]: Significant differences and trends between MCAD and MUC with human tissue as the epitope

	MCAD	95% CI	MUC	95% CI	p-values
	mean±SEM		mean±SEM		
Fetal human					
61 kDa					
Number with	30		31		ns
Peak hgt	14.1±2.0	10.0–18.1	9.2±1.2	6.8–11.6	0.037
39 kDa	14		15		ns
Peak hgt	10.9±1.3	8.0–13.8	7.5±1.3	4.8–10.3	0.085
36 kDa					
Number with	10		2		0.017
Adult human cingulate gyrus					
129 kDa					
Number with	5		0		0.059
100 kDa					
Number with	26		21		ns
Peak hgt	11.4±2.1	7.1–15.6	7.0±1.0	4.9–9.1	0.085
91 kDa					
Number with	13		20		ns
Peak hgt	15.0±2.4	9.7–20.3	8.6±5.5	6.1–11.1	0.012
Cerebellum					
100 kDa					
Number with	34		30		ns
Peak hgt	11.9±1.7	8.6–15.3	8.3±0.9	6.5–10.2	0.072
31 kDa					
Number with	25		35		ns
Peak hgt	10.7±5.5	8.4–13.0	7.6±0.9	5.9–9.3	0.028
Caudate					
155 kDa					
Number with	11		2		0.009
81 kDa					
Number with	20		21		ns
Peak hgt	10.4±1.8	6.6–14.1	7.0±0.9	5.2–8.7	0.088
BA9					
63 kDa					
Number with	39		27		0.049

Peak height values are expressed as mean±SEM. Only results with statistical differences or trends are presented. Abbreviation, hgt = height; ns = not significant.
[a] All peak height values were corrected for sample specific IgG content.

3.3.1. Human fetal brain

Results for band specificity and corrected peak height (density) expressed as mean±SEM are presented in Table 2. Only results showing statistical differences or trends are shown. At 36 kDa, 10% of MCAD had a band as compared to only 2% MUC. MCAD, at 61 kDa, had a significantly larger corrected peak heights ($p=0.037$) and at 39 kDa, corrected peak height was greater (trend, $p=0.085$) than in MUC.

3.3.2. Human adult brain

Significant differences in band specificity were identified at only two molecular weights: caudate at 155 kDa and BA9 at 63 kDa, with a trend in the cingulate gyrus at 129 kDa (Table 2). Corrected peak height differences were identified in cingulate gyrus at 91 kDa and cerebellum at 31 kDa, and trends in caudate at 81 kDa and in both cerebellum and cingulate gyrus at 100 kDa.

3.3.3. Rodent embryonic tissue

MCAD had greater band specificity at 36 kDa (MCAD, $n=48$; MUC, $n=31$; $p=0.010$) and at 73 kDa (MCAD, $n=47$; MUC, $n=31$; $p=0.015$). There were no differences in corrected band density.

3.3.4. Rodent adult brain

A significant difference in band specificity was present at only 27 kDa (MCAD, $n=22$; MUC, $n=10$; $p=0.016$). There was a trend for differences in corrected peak height at 110 kDa (MCAD, $n=29$, corrected OD 11.4±1.3; MUC, $n=34$, corrected OD 8.3±5.6; $p=0.055$).

3.4. Comparison of Western immunoblotting between fetal and adult tissues

Changes identified in MCAD showed little overlap between fetal and adult tissues in both rodent and human samples. The single exception, occurring within a ±2 kDa range, showed a larger corrected peak height against human fetal brain at 61 kDa and a greater number of reactive bands in frontal cortex (BA9) at 63 kDa.

3.5. GFAP and MBP immunoblots

There were no significant differences between 20 MCAD and 20 MUC regarding antigen reactivity: GFAP, MCAD=2, MUC=3; MBP, MCAD=1, MUC=2.

3.6. BDNF serum concentration

There was no significant difference in the calculated concentration of BDNF in serum from 25 MCAD (52.07 ng/ml±17.15) versus 25 MUC (54.87 ng/ml±17.17; $p=0.57$).

3.7. Comparisons between clinical factors and fetal brain antibody reactivity

Analyses on the subgroup of 47 maternal samples with "regression in offspring" were performed to determine whether there was an association between clinical regression and the presence of specific fetal epitopes. Results, presented in Fig. 2, show that there is a significant association between a mother having offspring with autistic disorder and their possessing a serum antibody against human fetal brain at 36 and 39 kDa, but not 61 kDa. Analyses performed to determine whether the presence of a reactive band against fetal human brain at 36, 39, or 61 kDa was predictive of multiple affected offspring (multiplex) showed that in 13 multiplex mothers the serum of five had antibody reactivity to human fetal brain at 61 kDa and one at 39 kDa. The five multiplex mothers with reactive bands at 61 kDa had at least two children with the diagnosis of autism and no unaffected offspring. A reactive band at 36, 39, or 61 kDa did not predict outcome of future offspring, since sixteen mothers had an unaffected child following the birth of a proband with autistic disorder. No positive correlations were found between the presence of reactive bands and the total number of pregnancies prior to the birth of the probands, or to the birth order of the child with autism.

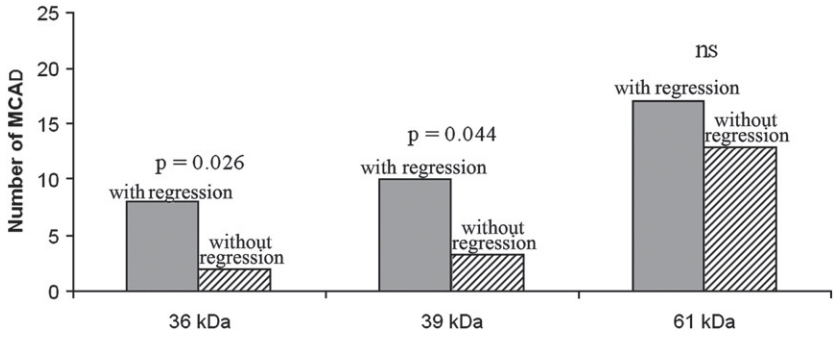

Fig. 2. Mothers with offspring having developmental regression with and without specific band reactivity. Mothers with offspring having developmental regression and reactivity to fetal human brain epitopes at 36, 39, and 61 kDa are presented as filled bar, and those without the band are shown as hatched bars. Significance is determined by odds of having regression based on presence of band using the logistic regression analysis.

A variety of statistical analyses were performed to evaluate the influence of a positive history for autoimmune disorders (AI). No difference in reactivity to any specific fetal brain epitope was identified in comparisons between MCAD with (n=24) and without (n=76) AI. Forty-seven MCAD had children with developmental regression; 13 of this group had a positive history of AI and 34 lacked this history (p=0.42). Further analyses of MCAD with offspring having regression plus the presence of specific anti-fetal brain reactivity (shown in Fig. 2) demonstrated no significant contribution from AI. In MCAD with a band at 36 kDa, 2/8 had AI; at 39 kDa, 4/10 had AI; and at 61 kDa, 9/17 had AI.

No significant correlations were identified between maternal or paternal age at the birth of the child with autistic disorder and reactivity against fetal human brain. Mothers with a band at 36 kDa were 2.2 years older on average than mothers without this band, but the association was not significant.

4. Discussion

The majority of prior studies investigating an autoimmune mechanism in autism have focused on the presence of abnormal serum antibodies in subjects diagnosed with this disorder (Cabanlit et al., 2007; Silva et al., 2004; Singer et al., 2006; Singh and Rivas, 2004). Although providing important contributions, these studies have not addressed whether the immune trigger could be the result of prenatal environmental factors, such as the maternal–fetal transfer of autoantibodies. In order to answer this question, immunoblotting and analyses of scanned blots were performed on serum from mothers of children with autistic disorder using fresh postmortem human and rodent, fetal and adult, brain tissues as antigenic substrates.

In this study, reactive bands were considered atypical if present in more individuals in one group than another, or if the optical density of the band (peak height), corrected for maternal IgG content, was greater. Results show that sera from mothers of children with autistic disorder contain antibodies that differ from controls against prenatally expressed brain epitopes. Few studies have been performed investigating maternal antibodies against fetal epitopes in autism. In a study presented in abstract form, sera from 61 mothers of children with autistic disorder had more prevalent antibody reactivity against human fetal brain tissue, with reactive bands at approximately 32, 37, 73, and 100 kDa (Braunschweig et al., 2006). Maternal autoreactivity to the band at 37 kDa was found to confer the greatest risk for autism. In a second small study, serum from mothers of children with autistic spectrum disorder (n=11) had reactivity against embryonic rat brain that differed from mothers of healthy children, with extra bands identified in the 75–100 kDa range (Zimmerman et al., 2007). Recognizing that molecular weight determination is an estimate, usually ±2–3 kDa, results in studies using human or rodent fetal brain as epitopes have areas of potential similarity. Further conclusions, however, must await the identification of the specific reactive brain epitopes.

Whether there are definite pathological consequences to the fetus secondary to the presence of maternal antibodies remains to be determined. Based on our results, however, maternal sensitization alone likely does not account for autism. For example, although mothers having autistic children with developmental regression were more likely to have serum antibody reactivity against human fetal brain at 36 and 39 kDa, mothers with unaffected children also have reactivity to similar epitopes. Additionally, despite documenting that five multiplex mothers had serum antibodies at 61 kDa and solely affected offspring, MCAD possessing similar antibodies had normal offspring following the birth of an autistic child. Hence, rather than a direct association, there is likely a complex relationship between maternal anti-fetal brain antibodies and genetic/metabolic/environmental factors. We hypothesize that the fetal brain tissue epitope(s) associated with developmental regression will be identified as an essential developmental growth factor(s), but are not GFAP, MBP, or BDNF as previously speculated (Miyazaki et al., 2004; Nelson et al., 2001; Singh et al., 1997).

Circumstantial evidence for a maternal placental–fetal transfer of IgG hypothesis in autism is expanding: HLA-DR4 has been found with increased frequency in MCAD and their sons with autism (Lee et al., 2006; Rogers et al., 1999; Torres et al., 2002); antibrain antibodies reactive against specific epitopes in fetal brain tissue are present in mothers with autistic offspring; and animal models have indicated that maternal antibodies can cross the placenta, bind to fetal antigens, and result in behavioral changes (Dalton et al., 2003; Vincent et al., 2002). Epidemiological studies, including this report, suggest an association between autoimmune disorders in mothers and the

prevalence of offspring with autism (Comi et al., 1999; Croen et al., 2005; Lee et al., 2006; Molloy et al., 2006; Sweeten et al., 2003). Nevertheless, greater serum antibody reactivity was not confirmed in this group, nor was there a correlation with offspring regression. Possible explanations include diagnoses based on history and relatively small numbers of subjects. The hygiene hypothesis, which claims a reduced incidence of infections causes the immune system to respond inappropriately to other agents (Bach, 2005; Strachan, 1989; Vercelli, 2006), has been cited to explain an increased incidence of autoimmune disorders (Fleming and Fabry, 2007) including autism (Becker, 2007). Bidirectional trafficking of cells and nucleic acids between a pregnant woman and her fetus during pregnancy has resulted in the long term persistence of fetal cells in the mother (fetal microchimerism) (Adams and Nelson, 2004; Bianchi, 2007). Whether this mechanism, however, could explain the presence of maternal anti-fetal brain antibodies that persist for years after the birth of the affected child is unknown.

Investigators have previously identified serum antibody reactivity against brain tissue in children with autism. Children with autistic disorder have more serum antibody-induced reactive bands at 100 kDa against caudate, putamen and prefrontal cortex and more dense bands at 73 kDa in the cerebellum and cingulate cortex (Singer et al., 2006), compared to controls. Although there is no direct overlap between the reactivity previously reported in children with autism and autoantibody reactivity identified in MCAD, to our knowledge, no study has simultaneously evaluated the antibody status of mothers and their affected and unaffected offspring.

This study has several weaknesses including the analysis of antibody patterns years after the delivery of the affected offspring, the selection of mothers skewed towards having offspring with developmental regression, data analysis on subjects at only a single time point, the failure to correlate antibody alterations in maternal sera to that in their offspring, and the exclusion of groups containing mothers of autistic children with established genetic or metabolic causes of autism. Additional techniques, such as measurement of actin, would be beneficial in confirming the accurate pipetting of brain proteins. We further emphasize that confirmatory evidence for a placental-transfer autoimmune mechanism in autism is lacking. More specifically, only one of five criteria deemed necessary to establish a pathogenic role for antibodies (i.e., identification, presence of immunoglobulins at the pathological site, positive response to immunomodulatory therapy, induction of symptoms with autoantigens, and passive transfer of the disorder to animal models) (Archelos and Hartung, 2000) has been established in autism. We await the results of future studies that include antibody evaluations in children with autism, their parents, and unaffected siblings, identification of specific protein epitopes, and confirmation that specific maternal IgG can induce an immune response in fetal brains, that in turn is associated with postnatal developmental abnormalities and behavioral alterations.

Acknowledgements

This research was supported in part by a grant from National Alliance for Autism Research. The authors also thank Shilpa Vernekar, M.D. for her assistance in performing laboratory assays.

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