Anti-CNS Antibodies in Childhood Neurologic Diseases

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Abstract

To study the incidence of circulating anti-CNS antibodies in childhood neurologic diseases, a population study was undertaken. Serum samples were obtained from a total of 348 children and stored at – 80 °C until being studied. The samples were collected when routine blood tests were being performed. In all cases informed consent was obtained. This study was approved by hospital ethics review committees.

One hundred and ninety-nine of the samples were from children with no known neurologic illnesses and served as the control group. One hundred and twenty-one of the samples were from children with epilepsy and the remaining 28 from a number of different neurologic conditions. The serum samples were screened against normal, adult, autopsy-derived cerebellar and frontal cortex tissue sections and Western blots. Serum immunoreactivity was revealed using HRP-conjugated anti-human IgG.

Significant findings included: (1) patients with epilepsy had an increased incidence of anti-CNS reactivity as revealed on frontal cortex immunoblots ($p < 0.05$) but not on cerebellar immunoblots; (2) there was an increase in the incidence of immunoblot reactivity with age in the controls and the neurology cases; (3) there was an increased incidence of immunoblot reactivity in those cases with a presumed inflammatory central or peripheral neurologic disease; (4) in six additional cases with opsoclonus-myoclonus there was cerebellar-specific immunoreactivity with identified antigenic molecular weights of 27 and 35, and 62 kDaltons; (5) in 31 additional cases of systemic lupus erythematosus there was significant immunoblot reactivity ($p < 0.001$) when compared to a subset of age-matched controls.

There was no difference in immunoreactivity between males and females. There was no significant increase in immunoreactivity in those children with cognitive disturbances including developmental delay and mental retardation.

Key words

Autoimmunity – Child neurology – Epilepsy – Immunohistochemistry – Neuroimmunology

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Introduction

Immune-mediated processes have been demonstrated in neurologic illnesses such as myasthenia gravis (1, 44, 59), Guillain-Barre syndrome (43, 50, 60) and multiple sclerosis (4, 61). In adult neurologic disorders, circulating antibody-mediated processes have been implicated in a number of other conditions including paraneoplastic cerebellar degenerations (19, 27, 57), familial spinocerebellar degenerations (34), paraneoplastic sensory neuronopathy (18), Sydenham's chorea (23, 24), Parkinson's disease (15, 25, 48), schizophrenia (7, 30) Creutzfeldt-Jakob disease and Kuru (2, 52).

The presence of anti-CNS antibody activity in childhood neurologic diseases has not been thoroughly studied. Children do suffer from systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis and are also subject to well documented autoimmune neurologic diseases more commonly seen in adults such as myasthenia gravis, multiple sclerosis and the Guillain-Barre syndrome.

The published literature suggests the possibility of autoimmune processes in several childhood neurologic diseases. In infantile spasms (63) precipitating antibodies to brain extracts have been described (39, 49). Furthermore, infantile spasms frequently respond to immune-modifying medications such as steroids and ACTH (33). A cell-mediated immune response to brain tissue has been described in autism (62) as well as specific defects in cell mediated immunity (17). Antineuronal antibodies have been found in children with neurologic complications from SLE (21, 26, 64). A more thoroughly studied childhood neurologic disease which may have an underlying autoimmune etiology is the opsoclonus-myoclonus syndrome (O-M) (29). This condition responds to immune-modifying medications such as steroids and ACTH (32, 45). There have been reports of increased cerebrospinal fluid immunoglobulin levels (11) and macrophage inhibition by neuroblastoma antigens (55) in this syndrome. Also, anti-neurofilament antibodies have been observed in O-M (5, 41).

To investigate whether circulating anti-central nervous system (CNS) antibodies may play a wider pathogenic role in childhood neurologic diseases, this population study was undertaken.

Serum samples were collected and screened against Western blots and sections of human CNS tissue. Immunoblots have an advantage over tissue sections in that immunoreactivity can be resolved to a finite number of bands whose molecular weights can be determined. Our results are presented in tabular form so that comparisons with other studies will be possible.

As a positive control, serum samples from patients with SLE were included since anti-CNS activity in SLE has been previously documented (8, 21, 22, 26, 64). For com-

parison, ANA-positive serum samples from other rheumatic conditions were also investigated.

The largest subpopulation within the studied neurology group was that of epilepsy. Immune system correlates of epilepsy have been previously described (9, 10, 12, 13, 28). Specific anti-CNS immunoreactivity in epilepsy appears not to have been previously reported.

Materials and methods

Serum samples were obtained from a total of 348 children and stored at −80 °C until being used. The samples were collected when routine blood tests were being performed. In all cases informed consent was obtained. This study was approved by hospital ethics review committees.

One hundred and ninety-nine of the samples were from children with no known neurologic illnesses and served as the control group. One hundred and twenty-one of the samples were from children with epilepsy (60 with generalized seizure disorders, 40 with partial epilepsy, 20 with mixed seizure patterns and one not classified). The remainder of the neurology patients included nine with developmental delay or mental retardation, eight with presumed inflammatory central peripheral neurologic diseases (three with acute cerebellar ataxia, two with Bell's palsy, and one each with transverse myelitis, Guillian-Barre syndrome and post-infectious encephalomyelitis), and 11 with a variety of other conditions (Becker's muscular dystrophy, fascio-scapular-humeral dystrophy, chronic ataxia, idiopathic 3rd nerve palsy, functional complaints, obesity, acid maltase deficiency, retinal migraine, peripheral neuropathy and leukodystrophy).

In addition, as positive controls, 31 cases of SLE and 16 cases of antinuclear antibody positive, juvenile rheumatoid arthritis (JRA) and other rheumatic diseases were also investigated. These samples were kindly provided by Dr. *E. Silverman* of the Division of Rheumatology, the Hospital for Sick Children, Toronto. Furthermore, serum samples from six children with O-M were studied. These samples were kindly provided by Drs. *Bouchard* and *Thibault* of Laval University, Quebec City. Since the SLE, JRA and O-M serum samples were obtained from other sources with different collecting and storage procedures, the results from these samples were not included in the statistical analysis of the aforementioned population study of 348 children.

Brain samples were obtained at the time of autopsy from neurologically normal young adults who had died from non-neurologic causes. They were kindly provided by Dr. *J. Deck* of the neuropathology service at the Toronto General Hospital. 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.

Western blots were made by standard techniques (58). Homogenates of frontal cortex or cerebellum were homogenized and boiled for 2 min in 2–5% sodium dodecyl sulphate (SDS; w/v), 7% 2-mercaptoethanol (v/v) in TBS (50 mM Tris-HCL, 200 mM 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 house serum (NHS) in phosphate buffered saline (PBS; 0.1 M phosphate buffer, 0.15 M NaCl, pH 7.4) to block non-specific 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 (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 (20). The apparent molecular weights of antigenic polypeptide bands in kDaltons (K) were estimated from pre-stained 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 recognises the 210K subunit of neurofilaments (31). 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 210K.

Quantitative immunoglobulins (IgG, IgA and IgM) were determined on serum samples from 117 epilepsy cases. These were performed in the clinical chemistry laboratories of the Hospital for Sick Children using a Beckman Array nephelometer. Normative age-related data as determined by the clinical chemistry laboratory, was used for comparison.

Unfixed human CNS tissue sections were cut 32 µm thick using a cryostat. The sections were placed on chrom-alum coated slides and air dried. They were fixed in acetone at −20 °C for 15 min, then rinsed in PBS. They were placed in 10% NHS for 15 min to block non-specific binding and again rinsed in PBS. The primary serum incubation was at a concentration of 1:20 in 10% NHS and took place overnight. The sections were then rinsed three times in PBS over 20 min. The secondary incubation was for 3 h in HRP-conjugated rabbit anti-human IgG (Dako Inc.) at a concentration of 1:20 in 10% NHS. The sections were again washed three times in PBS for 20 min. The HRP reaction product was revealed with a 15 min incubation in 0.5 mg/ml 4-chloro-1-naphthol-0.01% (v/v) hydrogen peroxide. When the serum was replaced with mabN210 in the primary incubation and HRP-conjugated rabbit anti-mouse immunoglobulin in the secondary incubation, the reaction product consistently had an axonal distribution compatable with that of neurofilaments.

For statistical analysis, chi-square and the Fisher exact probability tests were used.

Results

A. Reproducibility

All the serum samples were tested at least twice against Western blots prepared from adult human cerebellum and frontal cortex. The results from each immunoblot were reproducible. Similarly, tissue section immunoreactivity was reproducible.

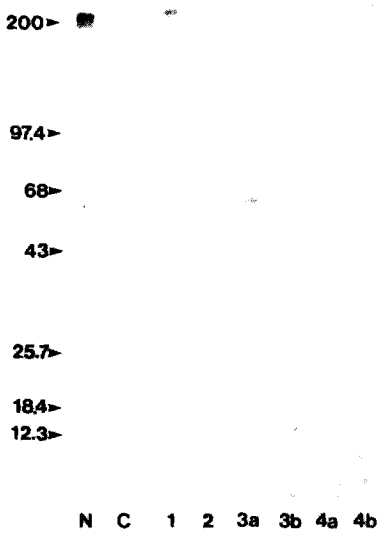

Fig. 1 Strips taken from the same Western blot of adult human cerebellum illustrating serum immunoreactive banding in children with opsoclonus-myoclonus. The protein standard molecular weights in kDaltons (K) are shown on the left. N is a positive control reacted with mabN210 which recognizes the 210K subunit of neurofilaments. C is a negative control in which the serum sample was omitted from the primary incubation. In Case 1, the serum sample was obtained two years after acute onset of symptoms and revealed a 210K band corresponding to the 210K subunit of neurofilaments. In Case 2, the sample was obtained 8 years after acute onset of symptoms and revealed no bands. In Case 3, serum samples were obtained at the time of acute presentation (3a) and 2 years and 6 months later (3b). The acute sample revealed a 62K band which was not present in the convalescent sample. In Case 4, samples were obtained at acute presentation (4a) and 2 years and 6 months later (4b). There was no immunoreactive band in either 4a or 4b.

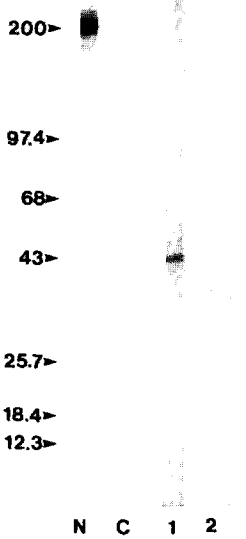

Fig. 2 Strips taken from the same Western blot of adult human cerebellum illustrating serum immunoreactive banding in two children with acute cerebellar ataxia and a clinical picture similar to that of opsoclonus-myoclonus. The protein standard molecular weights in kDaltons (K) are shown on the left. N is a positive control reacted with mabN210 which recognizes the 210K subunit of neurofilaments. C is a negative control in which the serum sample was omitted from the primary incubation. In Case 1, the serum sample was obtained during the third acute episode and in Case 2, 13 years after presentation, during convalescence. In Case 1 there is an immunoreactive band at 43K and in Case 2 there are two bands at 27K and 35K.

To test reproducibility of serum immunoreactivity over time, a subset of the controls had multiple blood tests performed. Eight cases had from two to 6 sequential blood drawings performed over periods of time ranging from one day to three months (average of 9 days). The serum samples were screened against Western blots of both frontal cortex and cerebellum and against human CNS tissue sections. Serum immunoreactivity was reproducible over time. When compared to a random distribution based upon the incidence of control serum immunoblot reactivity, there was a statistically highly significant difference with the sequentially repeated blood samples ($p < 0.005$).

B. Age related effects

Representative immunoblots are represented in Figures 1 and 2. An immunoblot was deemed to be positive when there were one or more definite immunoreactive bands detected. A tissue section was considered to be positively stained when cellular elements, either neuronal or glial, were clearly delineated. In those cases where multiple blood samples were obtained, only the results from the first sample were included in the statistical analysis. Because the SLE, JRA and O-M blood samples were obtained from other sources, their results were not included in the following population analyses.

When screened against Western blots of human frontal cortex, 16% of the controls and 24% of the neurology cases displayed immunoreactivity. When screened against Western blots of human cerebellum 21% of the controls displayed immunoreactivity and in the neurology group, it was 22%.

With age, there was a significant increase in the frequency of immunoblot reactivity. Figure 3 is a histogram of the incidence of immunoreactive banding in percent versus age in years for the control population. This figure illustrates reactivity against Western blots made from human cerebellum. There is a significant increase in the frequency of immunoblot reactivity from early childhood through the teenage years. A similar finding was made in the total neurology group when screened against Western blots of human cerebellum (Fig. 4). Figure 5 illustrates the effect of age on the entire studied population ($n = 348$). A similar age effect was seen when the serum samples were screened against immunoblots of frontal cortex in the controls, the neurology cases and subsets of the neurology group (not illustrated).

The majority of serum samples when screened against human CNS tissue sections did not reveal immunoreactive staining. When screened against frontal cortex sections, only 2% of the controls revealed immunoreactivity as did

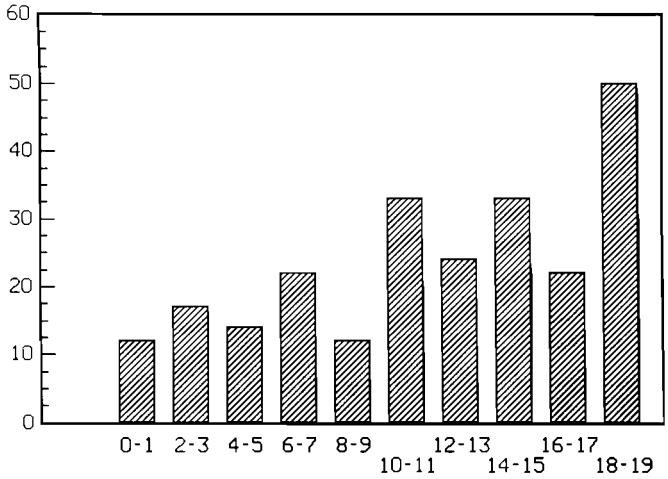

Fig. 3 A histogram of the incidence of immunoreactive banding (%) against Western blots of adult human cerebellum by age (years: 0 to 19) in the control population (n = 199). There is an increase in the incidence of banding with age.

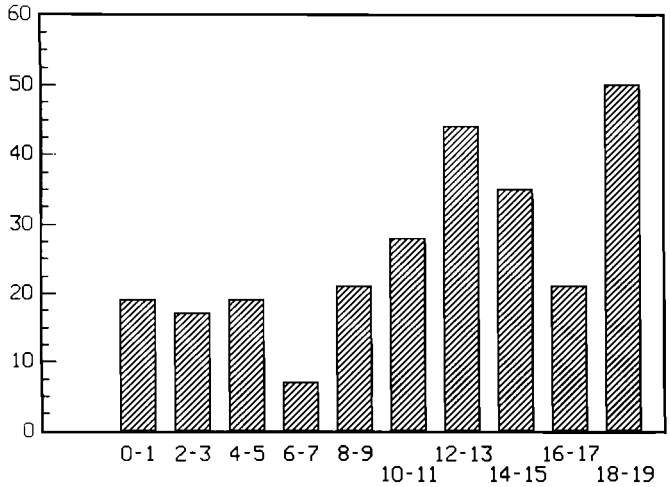

Fig. 4 A histogram of the incidence of immunoreactive banding (%) against Western blots of adult human cerebellum by age (years: 0 to 19) in the neurology population (n = 149). There is an increase in the incidence of banding with age.

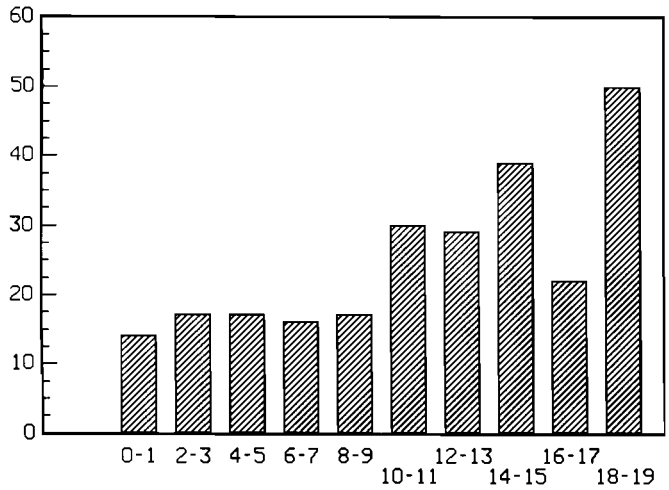

Fig. 5 A histogram of the incidence of immunoreactive banding (%) against Western blots of adult human cerebellum by age (years: 0 to 19) in the total studied population (n = 348). There is an increase in the incidence of banding with age.

3% of the neurology cases. When screened against cerebellar sections, the results were similar with 9% of the controls and 5% of the neurology group displaying immunoreactivity. In contrast to the immunoblot results, there was no increase with age in the incidence of tissue section immunoreactivity in the controls or in the neurology cases.

C. Systemic lupus erythematosus

Serum from 31 cases of SLE were studied as positive controls. The serum samples were screened against immunoblots and tissue sections of human frontal cortex and cerebellum and tissue sections of mouse CNS. The SLE results were compared to a subset of the controls (n = 89) with the same age distribution and same mean age (14 years). Only one of the SLE patients had neuropsychiatric symptoms at the time of blood sampling.

When screened against human cerebellar Western blots, 61% of the SLE cases and 28% of the controls displayed banding, a difference which was statistically significant ($p < 0.01$). Against immunoblots of human frontal cortex, 35% of the SLE cases and 18% of the controls had banding ($p < 0.05$). When the immunoblot results were tabulated for total (cerebellar and frontal cortex) reactivity, the incidence of banding was 74% in SLE and 37% in controls ($p < 0.001$). When the serum samples were screened against cerebellar tissue sections, the incidence of staining was 48% in SLE and 4% in controls ($p < 0.001$). Against frontal cortex sections, the incidence was 16% in SLE and 4% in controls ($p < 0.05$).

The immunoblot results with the SLE serum samples appear to be highly significant disease-associated ones since assays with serum samples from 16 cases with anti-nuclear antibody positive rheumatic diseases displayed no immunoreactivity when screened against Western blots of human frontal cortex and cerebellum. Details concerning SLE and JRA immunoblot and immunohistochemical results as correlated with disease activity and other immune-related laboratory parameters, are extensive and will be presented elsewhere (manuscript in preparation).

D. Epilepsy

The largest diagnostic group within the total neurology group was that of epilepsy (121 out of a total of 149). With immunoblots, there was a significant difference in the incidence of immunoreactive staining between the epilepsy cases and controls.

When the serum samples were tested against Western blots of human frontal cortex, the incidence of immunoreactive banding was 27% in epilepsy and 16% in controls, a difference which was statistically significant ($p < 0.05$). The identified molecular weights in kDaltons are presented in Table 1.

When screened against blots of human cerebellum, the incidence of banding was 24% in epilepsy and 21% in the controls, a difference which was not statistically significant. The molecular weights of the cerebellar reactivity in the diagnostic groups is presented in Table 2.

There was no significant difference in the incidence of immunoreactive staining against human CNS tissue

Table 1 Molecular weights (in kDaltons) of identified serum immunoreactive bands against Western blots of adult human frontal cortex are listed for each diagnostic group.

Controls	Epilepsy	Other neurologic diseases
210	16.6	170
140	29.1, 46.8	166
210	16	140
130, 140	155, 200	39.8, 162
26.6, 30.2	29.1	No bands in 24 cases
200	158	
200	166	
195	17.4	
29.5, 31.3	112, 148, 158, 200	
166	158	
185	102	
209	43.2	
28.8, 200, 234	214	
219	44.7	
170	38.4	
138, 166	26.6	
135	29.1	
44.7	162	
26.9	29.1, 46.8	
18.2	151	
15.8	174	
135	17	
182	126, 166	
155	166	
182	116	
204	214	
94.4, 106, 135, 166	40.7	
42.6	141	
94.4, 106	21.6	
37.6	90.2	
37.6	141	
87.1, 97.7	50.7	
No bands in 167 cases	36.7	
	No bands in 88 cases	

Table 2 Molecular weights (in kDaltons) of identified serum immunoreactive bands against Western blots of adult human cerebellum are listed for each diagnostic group.

Controls	Epilepsy	Other neurologic diseases
21.9	47.9	158
209	209	158
138	166	204
214	166	23.4
22.4, 200	224	133
22.4, 209	100, 130, 160, 179	No banding in 23 cases
37.6	158	
26	229	
170	25.7	
32.4	29.2	
30.2	158	
29.8	145	
132	151	
22.9, 214	151	
27.5	143	
136, 190	160	
132, 166	145	
27.5, 162	158	
200	22.1, 209	
132	29.1, 47.9	
27.5	195	
132	166	
18.2	130, 138, 190	
214	158	
22.9	23.4, 200	
209	22.9, 138	
34.7	90.2	
162	182, 204	
209	No banding in 92 cases	
104, 117, 209		
209		
27.5, 204		
30.9, 162, 209		
209		
43.6, 151, 209		
43.6		
209		
43.6		
151		
43.6		
151		
27.5		
No bands in 157 cases		

sections between the epilepsy cases, and the controls. When positively stained, the frontal cortex sections revealed cytoplasmic staining of small neurons. On occasion, neuronal nuclear and ependymal staining was observed. When staining occurred on cerebellar sections, it was localized primarily to Purkinje cell cytoplasm. On occasion, there was staining of ependyma and glia and diffuse staining of the molecular and granular layers. There was no significant difference in the distribution, quality and intensity of staining between epilepsy cases, other neurology cases and controls. Against frontal cortex in incidence of immunoreactive staining was 5% in epilepsy and 2% in the controls. Against cerebellum the incidence was 4% in epilepsy and 9% in controls.

Since there was an age-related effect on immunoblot reactivity (above), it was important to establish whether there was an age difference between epilepsy cases and controls. Figure 6 shows the age distributions in histograms of both the epilepsy cases and controls. There is no significant difference in the age distributions. The mean age of both populations was 8 years. There was also no significant difference in the sex distribution of the epilepsy cases as compared to the controls with 51% of both being males.

To see whether the observed anti-frontal cortex immunoblot reactivity in epilepsy may be related to medication intake, the details of medication intake were abstracted from

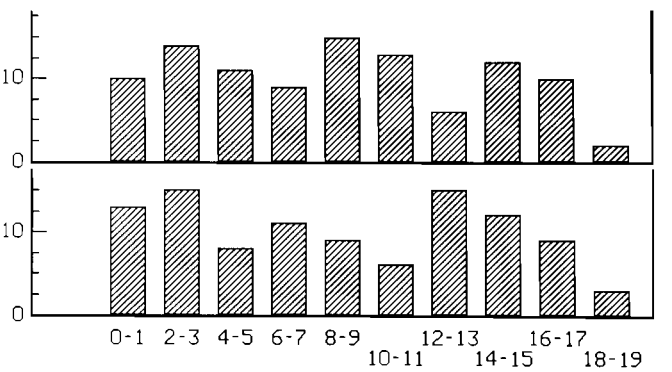

Fig. 6 Histograms of the age distribution of the studied epilepsy population (top) as compared to that of controls (bottom). The bar height indicates the percentage of the studied population in the indicated age bracket (years: 0 to 19). There is no significant difference in the age distribution of the epilepsy group as compared to that of the controls.

the medical record. There was no statistically significant difference in immunoreactive patterns between those cases who were taking and who were not taking diphenylhydantoin. Likewise, there was no statistically significant difference in immunoreactive patterns between those cases who were and were not taking carbamazepine, phenobarbital or valproic acid. There was no significant correlation between immunoreactive patterns and the intake of less commonly used medications including acetazolamide, clonazepam, ethosuximide, lorazepam and nitrazepam. There was no significant correlation between immunoreactive staining patterns and the numbers of different anticonvulsants being taken.

To see whether differences in seizure type could account for the observed immunoblot reactivity in epilepsy, details of seizure types were abstracted from the clinical records. There was no significant difference in immunoblot and tissue section reactivity between those patients having generalized seizure disorders (n = 60), partial seizures (n = 40) and mixed seizures (generalized and partial; n = 20). There was no significant difference in immunoblot and tissue section reactivity in those epileptics who had only one type of seizure disorder including generalized tonic-clonic seizures (n = 29), partial complex seizures (n = 26), focal motor seizures (n = 8), infantile spasms (n = 6), typical absence (n = 4), Lennox-Gastaut syndrome (n = 3) and myoclonic seizures (n = 2).

To see whether the immunoblot results may be a reflection of alternations in the concentration of serum immunoglobulins, quantitative immunoglobulin concentrations for IgG, IgA and IgM were performed on serum samples from 117 epilepsy cases (57 with generalized seizure disorders, 40 with partial seizures and 20 with mixed seizure disorders). In the total studied population, 4% had IgG concentrations above the normal limits (high) and 5% were below the normal limits (low). There was no significant difference between the different seizure types in whether the IgG concentration was high or low (generalized: 2% high, 5% low; partial: 8% high, 5% low; mixed: 4% high, 5% low). For IgA, however, there was a tendency for lower levels in the total epilepsy population: 3% were high and 11% were low. This finding was reflected in the individual categories of seizures (generalized: 4% high, 7% low; partial: 3% high, 15% low; mixed: 0% high, 15% low). An inverse finding occurred with IgM determinations with higher than normal levels detected in 4% of the total epilepsy population. There were no cases with below normal IgM determinations. Elevated IgM determinations occurred in all the different seizure categories: 4% in generalized; 3% in partial; 10% in mixed. There was no correlation between immunoblot or tissue section reactivity and the quantitative determination of IgG, IgA or IgM.

E. Other neurologic diseases

Eight of the neurology cases had disease processes, central or peripheral, of a presumed inflammatory nature. These included three with acute cerebellar ataxia, two with Bell's palsy, and one each with Guillian-Barre syndrome, post-infectious encephalomyelitis, and transverse myelitis. On immunoblots, three of these had anti-cerebellar banding and four had anti-frontal cortex banding. When adding both frontal cortex and cerebellar immunoblot reactivity, 75% (6 of 8) of this group displayed banding as compared to 28% in the controls.

There were a total of 49 cases with developmental delay, mental retardation or autism. Nine of these were not being treated for epilepsy, whereas the others were. There was no significant difference in the incidence of immunoblot reactivity and in immunohistochemical tissue section staining in these cases as compared to controls. There were only two cases of autism in this group, one of whom revealed a 29.1K immunoreactive band against a frontal cortex blot. This same case had an elevated quantitative IgA determination.

There were no distinctive immunoblot or tissue section staining results which typified any of the other 11 conditions studied including Becker's muscular dystrophy, fascio-scapular-humeral dystrophy, chronic ataxia, idiopathic third nerve palsy, functional complaints, obesity, acid maltase deficiency, retinal migraine, peripheral neuropathy and leukodystrophy

F. Opsoclonus-myoclonus

Immunoreactive banding of the serum samples from children with classic O-M (29) without associated neuroblastoma, are presented in Figure 1. The Western blot was prepared using a cerebellar homogenate. Case 1 was a 4-year-old male. The blood sample was obtained two years after onset of symptoms and revealed a reactive band at 210K, corresponding to the 210K subunit of neurofilaments. Case 2, a 9-year-old male whose blood sample was obtained 8 years after onset of symptoms, revealed no banding. Case 3 was a male whose blood sample taken at the time of acute presentation at the age of 1 year and 6 months (3 a) revealed a band at 62K, but none during convalescence at the age of four (3 b). Case 4 was a female whose acute blood sample at 1 year and 6 months of age (4 a) and convalescent one at four (4 b), revealed no reactivity.

When the reactive serum sample of Case 2 was tested against Western blots of human frontal cortex, occipital cortex and white matter, no banding was observed. When cerebrospinal fluid (CSF) samples from three of the opsoclonus-myoclonus patients were used instead of serum, no reactive banding was detected (not illustrated).

There were two cases whose presentation of acute ataxia and clinical course were compatable with Kinsbourne's syndrome. Since opsoclonus or myoclonus were not prominent symptoms, these two cases are presented separately in Figure 2. Case 1 was a 2-year-old male who, during an acute phase of illness, revealed on immunoreactive band at 43K. Case 2 was a 15-year-old male whose blood sample obtained after 13 years of convalescence, revealed bands at 27 and 35K. When these serum samples were tested against blots of frontal cortex, in Case 1 the same molecular weight band was revealed, but in Case 2 there was no banding detected. No reactivity was observed when blots of white matter were used. When CSF from the first case was used instead of serum, there were no bands (not illustrated). The clinical information concerning these cases and aspects of the anti-CNS reactivity have been presented previously in abstract form (56)

G. Numbers of bands and identified molecular weights

The numbers of immunoreactive bands for the total studied population, against Western blots of frontal cor-

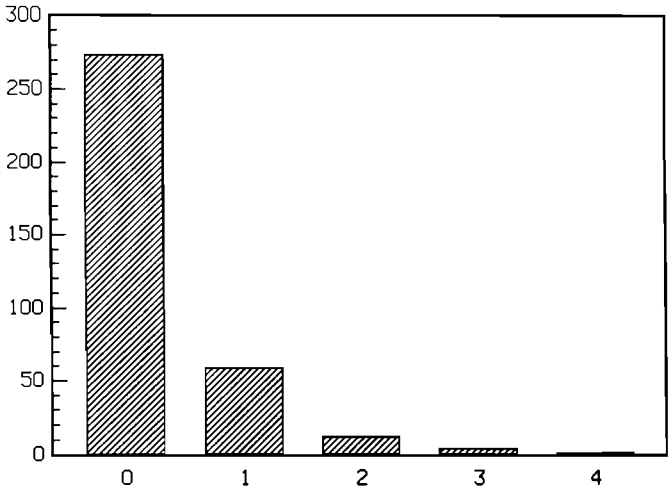

Fig. 7 A histogram of the number of immunoreactive bands against Western blots of adult human frontal cortex from each individual of the total studied population. The abscissa (0 to 4) indicates the number of immunoreactive bands on each reacted blot. The ordinate indicates the number of studied individuals with the indicated number of bands. The majority, 280 out of a total of 348 (80%) had no immunoreactive banding (column 0). Of those that had bands, 16% had only one band (column 1) and 4% had multiple bands (columns 2 through 4).

tex, are illustrated as a histogram in Figure 7. The majority, 80%, had no banding. Of those that had bands, 16% had only one band and 4% had multiple bands. A similar histogram of anti-cerebellar banding is illustrated in Figure 8. Again, the majority, 78%, had no banding. Of those that had bands 17% had only one band and 5% had multiple bands. The numbers of bands did not distinguish between controls, epilepsy cases and other neurologic diseases either on frontal cortex or cerebellar blots. The distribution in the histograms of Figures 7 and 8 are similar to those reported in the adult population (46).

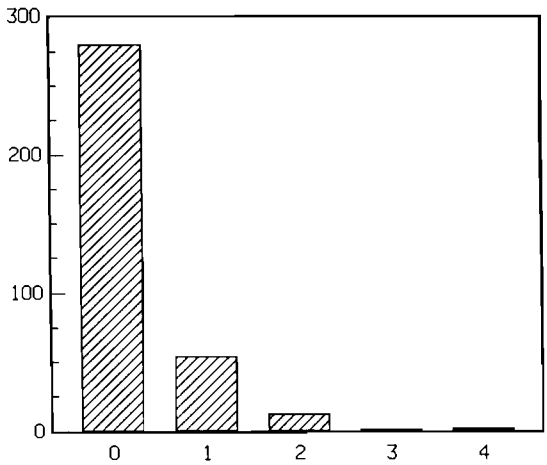

Fig. 8 A histogram of the number of immunoreactive bands against Western blots of adult human cerebellum from each individual of the total studied population. The abscissa (0 to 4) indicates the number of immunoreactive bands on each reacted blot. The ordinate indicates the number of studied individuals with the indicated number of bands. The majority, 273 out of a total of 348 (78%) had no immunoreactive banding (column 0). Of those that had bands, 17% had only one band (column 1) and 5% had multiple bands (columns 2 through 4).

No individual identified blot molecular weight, or combination of them, distinguished controls from epilepsy or other neurologic disease cases. The 210K subunit of neurofilaments is frequently recognized by human serum (46, 54). Against frontal cortex blots, the incidence of 210K reactivity (including the range of 200 to 220K) was 4% in controls and 3% in the neurology cases, with an overall incidence of 3%. Against cerebellar blots, the incidence of anti-210K reactivity was 8% in controls and 3% in neurology cases, with an overall incidence of 7%.

When the immunoreactive bands were separated into categories by molecular weight (high: greater than 90K; middle: 40 to 90K; low: less than 40K), there was no statistically significant difference between each diagnostic group, the controls and the entire studied population.

H. Sex related effects

There was no difference in immunoblot and tissue section reactivity between males and females in the total studied population and within individual diagnostic groups. In both the control and neurology groups 51% were males.

Discussion

This study has revealed a number of significant findings related to the presence of circulating anti-CNS antibody activity in childhood neurologic disorders. The methodology employed has been shown to be reproducible and effective in delineating pathophysiologic processes. The incidence of background staining on immunoblots was not high – on frontal cortex blots the overall incidence of banding was 20% for the entire studied population. Of those that had banding, 80% had only one band. Thus, for investigating anti-CNS antibody activity, whole brain Western blots are useful and reliable, without excessive background staining. It should be noted that since whole brain homogenates were used, anti-CNS immunoreactivity in general was assayed, not purely anti-neuronal activity. There is no apriori reason why neurologic autoimmune processes should be limited to neurons and not primarily affect non-neuronal cells such as glia. In a population study such as this, asssaying immunoreactivity against all the cellular elements in the CNS is advantageous.

Serum samples from patients with SLE were run as positive controls. They revealed a high incidence of anti-CNS antibody activity as compared to controls. On both immunoblots and tissue sections anti-cerebellar reactivity exceeded anti-frontal cortex activity in the SLE population. On immunoblots, the SLE population had significantly greater anti-CNS reactivity than other ANA+ rheumatic disease cases. Adding together immunoblot and tissue section results, 90% of the SLE cases had demonstrable circulating anti-CNS reactivity. Only one of the SLE cases had active neuropsychiatric symptoms. It is possible that in SLE, circulating immunoglobulins with high anti-CNS activity are constantly present and that a transient impairment in blood-brain barrier permeability (3, 8, 65, 66) or an increase in uptake and retrograde transneuronal transport (16), would allow these immunoglobulins to enter the CNS in sufficient quantity to cause neuropsychiatric symptoms. In a recent report, including seven cases of SLE with no known neuropsychiatric findings, three had significant abnormalities

noted on magnetic resonance imaging (MRI) of the brain (42). Possibly the MRI results are a consequence of the constant presence of circulating anti-CNS antibodies. Significantly one of the MRI-studied SLE cases had cerebellar vermal atrophy (42). This may be a finding which correlates with our observed anti-cerebellar immunoreactivity in SLE patients. Details concerning clinical and other laboratory findings and anti-CNS antibody reactivity in SLE and in the other rheumatic conditions studied, would be too extensive for this report and will be presented elsewhere (manuscript in preparation).

Our results with O-M are also significant. Previously serum IgG binding has been described in O-M patients to fibrillary and membrane components of guinea pig cerebellar Purkinje cells and axons of rat peripheral nerve (50). Other reports have identified serum reactivity against the 210K subunit of neurofilaments (41). In our O-M group, one of the six children displayed anti-210K serum activity. It is doubtful that the observed anti-neurofilament immunoreactivity is of pathogenic significance. In a study of 257 adults, including controls and various neurologic and psychiatric diagnoses, the incidence of anti-210K reactivity was 6% (46). Other reports have found an incidence ranging from 20% to 95% (14, 54). In the current study, the childhood incidence of anti-210K reactivity was 3% against frontal cortex blots, and 7% against cerebellar blots. Since O-M occurs in young children, in the current study in children two years of age and younger, the incidence of anti-210K activity, against either frontal cortex or cerebellar immunoblots, was 7% (5 out of 71). Thus, anti-210K immunoreactivity is common in adulthood and in childhood. Furthermore, it has been suggested that the clinical picture of O-M correlates with the intensity of serum anti-210K reactivity (41). In the current study, serum samples from three acute cases of O-M did not reveal such activity. It was a child whose disease was quiescent two years after presentation who displayed the anti-210K banding. Thus our results suggest that anti-210K activity does not play a significant role in the pathogenesis of this syndrome.

Of significance are our findings in O-M where molecular weights other than 210K were identified. In one child at the time of acute presentation, there was a cerebellar-specific band identified at 62K, a finding that disappeared 2 years and 6 months later. The anti-62K reactivity was not common to the other cases of O-M. Nevertheless, the transient nature of the anti-62K reactivity suggests possible pathogenic significance in this case. This finding is intriguing since recent reports have shown that cerebellar Purkinje cell polypeptides of 62K to 64K are the primary antigenic determinants recognized by circulating antibodies in individuals with paraneoplastic cerebellar degeneration (6). Immunoblot findings in cases with probable O-M are also intriguing. Here, an immunoreactive band at 43K was identified in an acute case as were bands at 27K and 35K in a convalescent case. In addition to the 62K to 64K range described above, in paraneoplastic cerebellar degeneration antigenic determinants with a molecular weight range of 34K to 38K were recognized (6). This corresponds to our finding of a band at 35K which was cerebellar-specific. In the current population study, the incidence of banding against cerebellar blots in the 32K to 38K range was 0.5% and in the 62K to 64K was never observed. Thus in childhood neurologic diseases, cerebellar antigenic determinants are recognized with the same molecular weights as those seen in adult paraneoplastic neurologic conditions. In addition, our results suggest that different CNS molecular weight antigenic determinants are recognized in different cases, even within a well defined diagnostic category. These results give credence to two hypotheses concerning the O-M syndrome: (1) this disease may be on the basis of an antibody-mediated autoimmune disease process (5, 11, 41) and (2) the primary CNS site that is affected may be the cerebellum (38).

Screening of the epilepsy population revealed a significantly increased incidence of anti-frontal cortex immunoblot IgG-immunoreactivity, but not of anti-cerebellar reactivity. This finding could not be accounted for by differences in age distribution, medication intake, type of seizure disorder or by abnormalities in quantitative IgG determinations. Anti-CNS antibody reactivity in childhood epilepsy appears not to have been previously reported.

There are several possibilities that may account for our finding of increased anti-CNS immunoblot reactivity in epilepsy. Possibly circulating anti-CNS antibodies, in epilepsy primarily directed against neocortical tissue, may damage CNS tissue and contribute to the development of epilepsy. Alternatively, it is possible that as a consequence of repeated seizures, CNS antigens may become exposed to the circulating immune system and elicit a secondary anti-CNS antibody response. This secondary antibody response may or may not contribute to possible further CNS insult.

There is yet a third possibility, one that is more intriguing. It is possible that the anti-CNS antibody findings are a manifestation of crossreactivity with other antigens and indicate a misregulation of the immune system in epilepsy. In idiopathic epilepsy, recent neuropathologic reports have described neocortical microdysgenesis (35, 36, 37). Intriguingly similar findings of microscopic neuronal migrational abnormalities have been described in mice with various immune system abnormalities (51, *Nowakowski, R.,* personal communication). In these dysimmune mice, microscopic cortical dysgenesis is accompanied by behavioral correlates: decreased learning abilities (40, 53). By analogy, the common finding of microscopic neocortical abnormalities in human epilepsy and in the dysimmune mice suggests that humans with epilepsy may have immune system abnormalities. Furthermore, it is possible that a biologic process or processes, yet to be defined, give rise during development concurrently to the CNS migratory anomalies and to immune system misregulation.

To address this possibility further, we investigated quantitative immunoglobulins in our studied epilepsy population. There was no significant difference in quantitative total IgG determinations, a result which is similar to that of previous reports (reviewed in 13). There have been reports of deficiencies in IgG subclasses in intractable epilepsy of childhood (9, 10), but it was not possible to perform these determinations for our study. Abnormalities in quantitative IgA and IgM in epilepsy have been previously reported (reviewed in 13). Decreased IgA levels have been an universal finding (12, 13): results which are identical to ours. There have been conflicting results reported concerning IgM determinations, with increased and decreased levels in almost equal numbers of reports (12, 13). Our results revealed increased levels of IgM. In our studied population abnormalities in IgA and IgM determinations did not correlate with medication intake or type of seizure disorder. Thus, abnormalities in circulating immunoglobulin levels substantiate the hypothesis of immune system misregulation in epilepsy.

There was an increased incidence in immunoreactive banding in those patients with presumed inflammatory central or peripheral neurologic diseases. However, the number of cases in this category was small and the number with each diagnosis was even smaller. There was no molecular weight identified which typified any of the specific diseases. More extensive immunoblot investigations of this category of childhood neurologic illnesses is warranted.

There was no increase in immunoreactivity in those children with cognitive disturbances including developmental delay, mental retardation and autism. In a previous adult population study there was no significant anti-CNS immunoreactivity detected in adults with cognitive disturbances including mental retardation and schizophrenia as compared to controls (46). Likewise, in a study of adults with Down's syndrome with and without symptoms of Alzheimer's dementia, there was no significant difference in immunoreactive patterns as compared to age and sex-matched controls (47). In our studied population, there were only two children with autism. One of these displayed anti-frontal cortex immunoblot reactivity and had elevated serum IgA levels. The number of studied autistic children was too small to draw any parallels to previously published reports of immune system abnormalities in autism (17, 62).

Other findings in our study included an increase in immunoblot reactivity with advancing age in childhood. In adults the incidence of banding on Western blots is a fairly constant 30% from the second through the seventh decades of life (46). This 30% incidence approximates the incidence of immunoreactive banding in the currently studied childhood population of those over 10 years of age (Fig. 3 through 5). A lower incidence of anti-neurofilament immunoreactivity in the first year of life has been reported (14).

The incidence of anti-210K neurofilament subunit immunoreactivity in this studied population was low: 3% against frontal cortex blots and 7% against cerebellar blots. These results are similar to a previously reported incidence of 6% in adults (46).

Our immunoreactive results have been presented in tabular form. As has been pointed out previously (46), this detailed information is of particular value when comparing molecular weights of identified CNS antigens reported in this study with those of other investigators.

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