Expression of a Neural Cell Adhesion Molecule Serum Fragment Is Depressed in Autism

Audrius V. Plioplys, M.D., F.R.C.P.(C)
Susan E. Hemmens, M.Sc.
Ciaran M. Regan, Ph.D.

The level of a neural cell adhesion molecule (NCAM) serum fragment in autism was determined by using an antiserum prepared with immunoaffinity purified mouse NCAM. Autistic patients (N=16) had statistically significantly decreased serum NCAM levels compared with age-matched controls ($p<0.0005$). This observation could not be attributed to a medication-induced effect. Depressed serum NCAM levels in autism are distinct from schizophrenia, in which serum NCAM levels are elevated.[1]

(The Journal of Neuropsychiatry and Clinical Neurosciences 1990; 2:413–417)

Autism is a syndrome characterized by social and communicative deficits of early onset, accompanied by abnormal behaviors. There are many different biomedical causes underlying autistic symptomatology, including genetic, metabolic, and infectious ones, as reviewed by Coleman and Gillberg 1985.[2] However, in the majority of autistic patients no clear etiology is ascertainable.

In the past, autism was considered to be a form of schizophrenia. Indeed, the term *autism* itself was coined by Bleuler[3] in 1911 to designate a category of thought disorder present in schizophrenia. In 1943, Kanner[4] described infantile autism as a distinct diagnostic entity, but still closely related to schizophrenia. Subsequently, diagnostic criteria for infantile autism have been developed that clearly distinguish it from schizophrenia and other psychoses.[5–8] However, accurate distinctions in disturbed young children are frequently difficult to make.

It has been suggested that schizophrenia comprises two potentially overlapping syndromes: type I and type II.[9] Type I schizophrenia is characterized by positive symptoms, which include delusions, hallucinations, and thought disorders. Type II schizophrenia is associated with negative features, such as loss or absence of affect, poverty of speech, and loss of volition; it is more closely associated with intellectual impairment and structural brain changes.[10,11]

Received January 31, 1990; revised March 12, 1990; accepted March 14, 1990. From the Department of Pediatrics, University of Toronto, Toronto; and the Department of Pharmacology, University College, Belfield, Dublin, Ireland. Address reprints requests to Dr. Plioplys, Department of Neurology, Michael Reese Hospital, Lake Shore Drive at 31st Street, Chicago, IL 60616.

Schizophrenia has been suggested to occur by alteration of natural synaptic turnover rates.[1,12] This could arise by hyperinnervation following a nearby primary lesion,[13,14] or it may be inherent. This would be consistent with the observation of elevated serum levels of a neural cell adhesion molecule (NCAM) fragment in schizophrenia.[1] NCAM is a morphoregulator capable of detailing the synaptic connections that are critical to the development of the central nervous system.[15] Also, in the adult, NCAM plays a role in neuronal reinnervation of experimentally denervated neuromuscular junctions.[16–18] NCAM is comprised of three immunologically related polypeptides of approximately 120, 140, and 180 kd,[19,20] which are highly conserved[21] and which were originally identified by an antiserum raised against a 65 kd soluble fragment.[22] NCAM is believed to mediate its action by a homophilic binding mechanism, the strength of which is inversely proportional to the amount of sialic acid.[23] The sialic acid content decreases during development,[24] thereby increasing adhesion and promoting the final stabilization of the synaptic network. NCAM is shed and/or secreted from the cell,[25,26] and soluble forms have been identified in cerebrospinal fluid and serum,[27] which in the latter appears as a 70 kd fragment.[1]

Autism is characterized by social withdrawal, by isolation, and—in over 80% of cases—by mental retardation. Structural brain abnormalities have been described in autism.[28–30] These autistic symptoms and findings are reminiscent of those of type II schizophrenia. Elevated serum levels of an NCAM fragment have been positively correlated with the negative clinical features of type II schizophrenia.[1] This investigation was undertaken to see whether serum levels of NCAM fragments would distinguish patients with autism from controls.

METHODS AND MATERIALS

Blood samples were obtained from a total of 16 patients with autism. There were 15 males and 1 female. The age range was from 8 to 23 years, with a mean age of 17. The diagnosis of autism conformed to DSM-III[7] and DSM-III-R[8] criteria. There were no identified neurobiologic causes of autism in any of the studied population. Parental signed consent was obtained prior to phlebotomy. This study was approved by ethics review committees. The controls were seven physically and neurologically normal students (4 males, 3 females) with a mean age of 19 years (range 18–19).

The NCAM antiserum was prepared using immunoaffinity purified mouse NCAM. A monoclonal antibody that recognizes all three mouse polypeptides—H28[31]—was coupled to cyanogen bromide activated agarose polymer (Sepharose) (Pharmacia; 6–7 mg/ml gel) as per manufacturer's instructions. Whole adult mouse brain first was homogenized (10% wt/vol) in 20 mM Tris · HCl, pH 7.4, containing 2.5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulphonylfluoride (PMSF), 5 μg/ml aprotinin, and 1% octylphenol-ethylene oxide condensate (Nonidet) P-40 (NP-40), and then solubilized at 4°C for 30 min. Samples were clarified (20,000 g for 1 hour) and applied to the column in 5-mg aliquots of solubilized protein, which was operated with a flow rate of 5 ml/hour using the above solubilization buffer. Bound NCAM was eluted by changing the column buffer to 1 of 50 mM diethylamine, pH 11.5, containing 1 mM PMSF and 5 μg/ml aprotinin. The diethylamine fractions were neutralized with solid glycine and assayed for protein content by the method of Lowry et al.,[32] and for NCAM by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).[33] The combined NCAM fractions were further purified by making them 0.1% with casein (Marvel) and refractioning on the column which had been preequilibrated with 1% casein. The eluting buffer was as before and contained no casein.

The antiserum was prepared by intramuscularly injecting New Zealand white rabbits with 100-μg aliquots of purified protein mixed with Freund's complete adjuvant on day 1 and with Freund's incomplete adjuvant on days 14 and 28. The animals were then totally bled by cardiac puncture. Antibody specificity was evaluated by previously described immunoblotting techniques,[34] using mouse anti-NCAM (diluted 1:20) followed by a sheep anti-rabbit immunoglobulin G (IgG), A and M peroxidase conjugated second antibody (diluted 1:100; Serotec).

NCAM levels were estimated blindly, using an enzyme-linked immunosorbent assay. The details essentially were as described previously,[1] but included the following modifications. The blocking buffer contained 5% casein in Dulbecco's phosphate-buffered saline (DPBS). Anti-mouse NCAM was diluted 1:20 and the second layer sheep anti-rabbit IgG, A and M peroxidase conjugate was diluted 1:250, each in DPBS containing 0.5% casein. The NCAM amount was expressed as optical density units per ml serum or per mg protein. Statistical differences were estimated using an unpaired Student t test and expressed as means± SD.

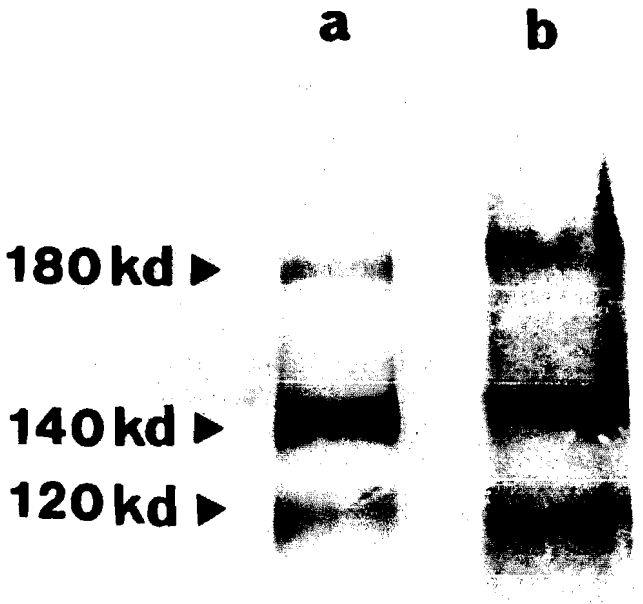
RESULTS

Immunoaffinity purified mouse NCAM was comprised of three immunologically related polypeptides of 120, 140, and 180 kd, as expected (Figure 1a). Further, immunoblotting procedures demonstrated that anti-mouse

NCAM specifically recognized all three polypeptides in whole-brain homogenates (Figure 1b).

There was a significant decrease in the serum NCAM concentration in autistic subjects compared with controls. In autistic subjects, the mean level was 42.34±8.85 optical density units/ml, whereas in the controls it was 98.84±16.91 ($p<0.0005$, Table 1 and Figure 2). When calculated as optical density units of NCAM per mg of serum protein, again there was a highly significant decrease in autistic subjects compared with controls. In autistic subjects the result was 0.63±0.13, and in the controls it was 1.27±0.19 ($p<0.0005$, Table 1 and Figure 3).

FIGURE 1. Evaluation of immunoaffinity purified mouse NCAM and anti-mouse NCAM reactivity. Samples of purified mouse NCAM (20 µg) were separated on 7.5% acrylamide gels and visualized using a silver stain (a).[35] Whole brain homogenate (100 µg) was separated on 7.5% acrylamide gels, transferred to nitrocellulose sheets, and probed with anti-NCAM (diluted 1:20), followed by peroxidase conjugated, anti-rabbit IgG, A and M (diluted 1:100) (b).



The difference between autistic and control subjects could not be attributed to a medication-induced effect in the autistic serum samples. Approximately one-third of the autistic population was receiving medications at the time of phlebotomy. Two patients were receiving thioridazine, one haloperidol, one carbamazepine, one methylphenidate, and one pimozide. When these patients were grouped and compared with the control population, the significant difference in NCAM serum levels remained when expressed as either NCAM/ml serum or NCAM/mg protein (in both cases $p<0.0005$, Table 1). There was no statistically significant difference between NCAM serum levels (either as NCAM/ml or NCAM/mg protein) in autistic subjects taking medications compared with autistic subjects not taking medications.

The difference in NCAM serum levels was not sex-linked, although that of the male controls was significantly higher than that of their female counterparts when expressed as NCAM/ml serum ($p<0.005$), but not as NCAM/mg protein ($p>0.05$). Within the autistic population there was no correlation between NCAM serum levels and age.

DISCUSSION

These results indicate that NCAM serum levels can distinguish between patients with autism and normal individuals. The serum fragment of NCAM has a molecular weight of 70 kd[1] and is similar to the 65 kd fragment reported to be generated by the inherent proteolytic activity of purified NCAM.[36] Given that the 65 kd fragment contains determinants which mediate cell-cell adhesion,[36] it is reasonable to assume that it represents the functional amino terminal portion of the molecule and therefore would be intimately involved in synapse selection and maintenance. If NCAM is released during natu-

TABLE 1. Serum NCAM levels in autistic and normal individuals

	Normal Subjects	Autistic Subjects		
	All (N=7)	All (N=16)	Drug-free (N=10)	Drug-treated (N=6)
Serum NCAM units				
NCAM/ml serum	98.84±16.91	42.34±8.85*	43.82±10.35*	39.87±5.50*
NCAM/mg protein	1.27±0.19	0.63±0.13*	0.66±0.15*	0.59±0.09*

Note: NCAM amount is expressed as optical density units per milliliter serum or per milligram serum protein. Values are means±SD.
*$p<0.0005$

ral synapse turnover, then the serum fragment may represent the extent of this process.

Schizophrenia and autism have several neurobiologic similarities. Both have been suggested to be neurodevelopmental disorders,[37,38] a feature which makes analysis of NCAM serum fragments most appropriate. Further, the finding of enlarged ventricles in schizophrenia[11] and in autism[28,29] may reflect a common basic feature of neuroanatomical damage or abnormal early development. However, ventriculomegaly simply may be the result of a variety of congenital and acquired diseases. It is possible that the elevated serum NCAM levels in schizophrenia reflect an increase in natural synaptic turnover rate, as has been suggested,[1] whereas their depression in autism may reflect a decreased turnover rate.

A decreased complement of synaptic connections also might account for the depressed NCR levels in autism. However, the volume of brain tissue in autism is minimally decreased due to ventriculomegaly,[28,29] a finding which is quantitatively similar to that in schizophrenia.[11] A Golgi impregnation study of cerebral cortical neurons in autism did not reveal a significant difference in dendritic branching and spine distribution, suggesting that the number of synaptic connections is not significantly disturbed.[39] Furthermore, in one case in that same report, where the cerebral cortex was examined electron-microscopically, there did not appear to be a deficiency of synaptic contacts.[39] None of the patients in our investigation had an identified biomedical cause for his or her cognitive impairment that would have produced an atrophic central nervous system. Also, none of our patients was microcephalic. Thus, it is unlikely that decreased NCAM levels in autism are due to a decreased complement of synapses.

If autism is characterized by a decreased synaptic turnover rate, then a defect in the ongoing synaptic reorganization may be a neurobiologic explanation for the cognitive impairment seen in this condition. These investigations should be extended to other causes of mental impairment associated with mental retardation to see whether decreased serum NCAM levels are specific to autism.

Concern has been expressed that the elevated serum NCAM levels in schizophrenia may reflect long-term neuroleptic-induced release of neuronal or muscle NCAM.[1] However, in this study, medications, including neuroleptics, had no significant effect on NCAM serum levels.

FIGURE 2. **NCAM serum concentration expressed in optical density units per milliliter of serum in control (CON) and autistic (AUT) subjects. There was a significant decrease in the mean value of NCAM levels in autistics when compared with controls (42.34±8.85 vs. 98.84±16.91; p<0.0005).**

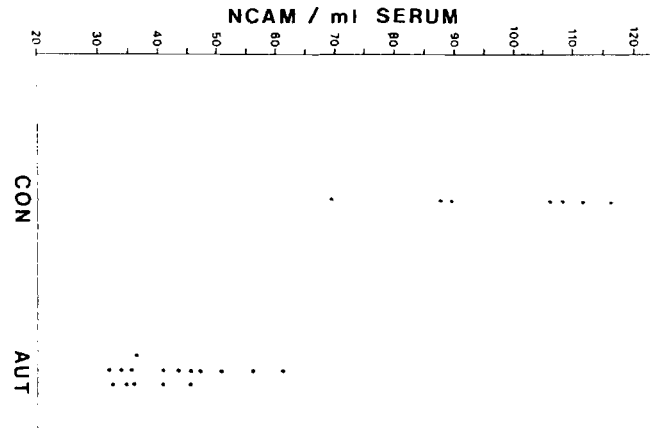
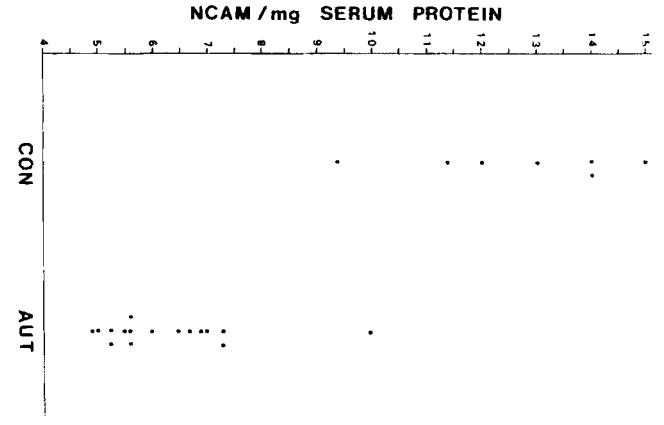


FIGURE 3. **NCAM serum concentration expressed in optical density units per milligram of serum protein in control (CON) and autistic (AUT) subjects. There was a significant decrease in the mean value of NCAM levels in autistics compared with controls (0.63±0.13 vs. 1.27±0.19; p<0.0005).**



This work was supported in part by the Irish Science and Technology Agency, EOLAS (Grant 88/86; CMR) and in part by a grant from Physicians' Services Incorporated Foundation (AVP).

The authors thank the staff members of The Geneva Centre and of the Thistletown Regional Centre for their assistance, Ms. Adonna Greaves for technical assistance, and Mr. Roddy Monks and Ms. Pat Waugh for preparing this manuscript. Monoclonal antibody H28 was provided by F. Walsh.

References

1. Lyons F, Martin ML, Maguire C, et al: The expression of an N-CAM serum fragment is positively correlated with severity of negative features in type II schizophrenia. Biol Psychiatry 1988; 23:769–775

2. Coleman M, Gillberg C: The biology of the autistic syndrome. New York, Praeger, 1985
3. Bleuler E: Dementia Praecox or the Group of Schizophrenias. Translated by J. Zinkin. New York: International Universities Press, 1950 (originally published in Vienna, 1911)
4. Kanner L: Autistic disturbances of affective contact. Nervous Child 1943; 2:217–250
5. Rutter M: Diagnosis and definition of childhood autism. Journal of Autism and Childhood Schizophrenia 1978; 8:139–161
6. Wing L: Early childhood autism. 2nd edition. Oxford: Pergamon Press, 1980
7. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition. Washington, DC, American Psychiatric Association, 1980
8. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Revised. Washington, DC, American Psychiatric Association, 1987
9. Crow TJ: Molecular pathology of schizophrenia: more than one disease concept? Br Med J 1980; 280:66–68
10. Crow TJ, Corsellis JAN, Cross AJ, et al: The search for changes underlying the type II syndrome in schizophrenia, in Biological Psychiatry. Edited by Perris C, Struwe G, Jansson B. Amsterdam, Elsevier/North-Holland Biomedical Press, 1981, 727–731
11. Johnstone EC: Structural changes in the brain in schizophrenia, in Psychopharmacology: Recent Advances and Future Prospects. Edited by Iversen SD. Oxford, Oxford Medical Publications, 1985, 196–203
12. Haracz JL: Neural plasticity in schizophrenia. Schizophr Bull 1985; 11:191–229
13. Hokfeldt T, Ljundahl A, Fuxe K, et al: Dopamine nerve terminals in the rat limbic cortex: aspects of the dopamine hypothesis of schizophrenia. Science 1974; 184:177–179
14. Coyle JT, Johnston MV: Functional hyperinnervation of cerebral cortex by nonadrenergic neurons results from fetal lesions: parallels with schizophrenia. Psychopharmacol Bull 1980; 16:27–29
15. Edelman GM: Cell adhesion molecules in the regulation of animal form and tissue pattern. Annual Review of Cell Biology 1986; 2:81–116
16. Covault J, Sanes JR: Neural cell adhesion molecule (N-CAM) accumulates in denervated and paralyzed skeletal muscles. Proc Natl Acad Sci USA 1985; 82:4544–4548
17. Rieger F, Grumet M, Edelman GM: N-CAM at the vertebrate neuromuscular junction. J Cell Biol 1985; 101:285–293
18. Daniloff JK, Levi G, Grumet M, et al: Altered expression of neuronal cell adhesion molecules induced by nerve injury and repair. J Cell Biol 1986; 103:929–945
19. Chuong C-C, Edelman GM: Alterations in neural cell adhesion molecules during development of different regions of the nervous system. J Neurosci 1984; 4:2354–2368
20. Sheehan MC, Halpin CI, Regan CM, et al: Purification and characterization of the D2 cell adhesion protein. Analysis of the postnatally regulated polymorphic forms and their cellular distribution. Neurochem Res 1986; 11:1333–1346
21. Rutishauser U, Jessell TM: Cell adhesion molecules in vertebrate neural development. Physiol Rev 1988; 68:819–857
22. Thiery J-P, Brackenbury R, Rutishauser U, et al: Adhesion among neural cells of the chick embryo, II: Purification and characterization of a cell adhesion molecule from neural retina. J Biol Chem 1977; 252:6841–6845
23. Hoffman S, Edelman GM: Kinetics of homophilic binding by embryonic and adult forms of the neural cell adhesion molecule. Proc Natl Acad Sci USA 1983; 80:5762–5766
24. Meier E, Regan CM, Balazs R: Change in the expression of a neuronal surface protein during development of cerebellar neurones in vivo and in culture. J Neurochem 1984; 43:1328–1335
25. Annunziata P, Regan CM, Balzas R: Development of cerebellar cells in neuron-enriched cultures: cell surface proteins. Developmental Brain Research 1983; 8:261–273
26. Gower HJ, Barton CH, Elsom VL, et al: Alternative splicing generates a secreted form of NCAM in muscle and brain. Cell 1988; 55:955–964
27. Ibsen S, Berezin V, Norgaard-Pedersen B, et al: Enzyme-linked immunosorbent assay of the D2-glycoprotein. J Neurochem 1983; 41:356–362
28. Damasio H, Maurer RG, Damasio AR, et al: Computerised tomographic scan findings in patients with autistic behaviour. Arch Neurol 1980; 37:504–510
29. Campbell M, Rosenbloom S, Perry R, et al: Computerised axial tomography in young autistic children. Am J Psychiatry 1982; 139:510–512
30. Courchesne E, Yeung-Courchesne R, Press GA, et al: Hypoplasia of cerebellar vermal lobules VI and VII in autism. N Engl J Med 1988; 318:1349–1354
31. Hirn M, Pierres M, Deagostini-Bazin H, et al: Monoclonal antibody against cell surface glycoprotein of neurons. Brain Res 1981; 214:433–439
32. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin reagent. J Biol Chem 1951; 193:265–275
33. Liu AYC, Greengard P: Regulation by steroid hormones of phosphorylation of specific proteins common to several target organs. Proc Natl Acad Sci USA 1976; 73:568–572
34. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76:4350–4354
35. Switzer RC III, Merrill CR, Shifri S: A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Anal Biochem 1979; 98:231–237
36. Hoffman S, Sorkin BC, White BC, et al: Chemical characterisation of the neural cell adhesion molecule purified from embryonic brain membranes. J Biol Chem 1982; 257:7720–7729
37. Murray RM, Lewis SW: Is schizophrenia a neurodevelopmental disorder? Br Med J 1987; 295:681–682
38. Volkmar FR, Cohen DJ: Neurobiologic aspects of autism. N Engl J Med 1988; 318:1390–1392
39. Williams RS, Hauser SL, Purpura DP, et al: Autism and mental retardation: neuropathologic studies performed in four retarded persons with autistic behavior. Arch Neurol 1980; 37:749–753