DIAGNOSIS AND TREATMENT OF AUTISM
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Autism: Immunological Investigations

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Blood samples were obtained from a total of 17 patients with autism. There were 16 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 the DSM-III (APA, 1980) and DSM-III-R (APA, 1987) criteria for autism. 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 committies. Simultaneously drawn blood samples from healthy young adults served as controls for the lymphocyte stimulation studies and for the pokeweed mitogen stimulations. For comparison of IgG anti-central nervous system (CNS) reactivity the results from a study of 348 children were used (Plioplys, 1989). For the IgM results a comparison group of 101 normal young adults was used. For comparison of neural cell adhesion molecule (NCAM) determinations the results from seven physically and neurologically normal controls (4 male, 3 female) were used with a mean age of 19 years.

The results can be summarized in the following three areas:

LYMPHOCYTE ABNORMALITIES

Peripheral blood lymphocytes were separated on a Ficoll-hypaque density gradient. Autistic patients had normal numbers of T and B cells and T cells subsets.

However, the CD4:CD8 ratios were normal only in 4 individuals (Figure 1) while 6 patients had elevated ratios (>2.2) and 5 patients had decreased ratios (>1.5). Although the mean value of 2.09 ± 0.97 (± one standard deviation, SD) was within normal limits, there was a clustering of the CD4:CD8 ratios into two general groups: those >2 and <2 (Figure 1).

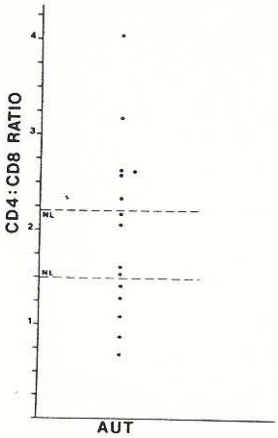

Figure 1. CD4:CD8 ratio in patients with autism. Normative values are indicated. Only 4 values are within normal limits. The CD4:CD8 distributions cluster into those >2 and <2.

There was an increased number of DR+ (activated) T cells in patients with autism (normal <2 %; Figure 2). For the group as a whole, the percentage of DR+ T lymphocytes was 7.73 ± 5.75. Within the DR+ group (11 patients) there was a decrease in percentage of DR+ T cells with increasing age (Figure 2). No patients demonstrated an increased number of interleukin-2 (IL-2) receptor positive cells.

There was an inverse correlation between CD4:CD8 ratios and number of circulating DR+ lymphocytes. In those patients with CD4:CD8<2 there was an increased number of DR+ cells (10.71 ± 2.87%) as compared to the patients with CD4:CD8>2 (5.12 ± 6.5 %; $p<0.05$).

Mitogen-induced proliferation of both concanavallin-A (5 μg/ml and 50 μg/ml) and phytametagglutinin (1 μg/ml and 10 μg/ml) in patients with autism did not differ from controls. The autologous mixed lymphocyte reaction (AMLR) was within normal limits when the group was examined as a whole. However, there was a significantly decreased AMLR (8630 ± 3045; counts per minute ± one SD) for the 5 patients with CD4:CD8<1.5 as compared to the 9 patients with a normal or elevated CD4:CD8 (15213 ± 7700; $p<0.05$). In contrast when patients were divided by numbers of DR+ (activated) T cells into normal numbers of DR+ T cells and increased number of DR+ T cells, there was no difference in AMLR (12887 ±4351 vs 12863 ± 8181).

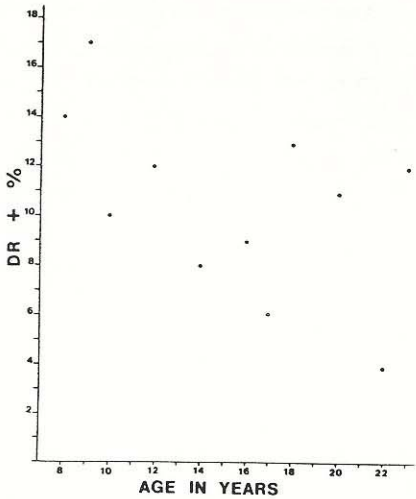

Figure 2. DR+ percentage as plotted against age for those patients with autism who had DR+ T lymphocytes. The DR+ percentages are all elevated (normal <2 %). There is a decrease in DR+ percentage with age.

Autistic patients have an abnormally increased percentage of DR+ but not IL-2 receptor ± lymphocytes suggesting "incomplete" activation, a finding which is seen in autoimmune diseases. The decrease in activated cells with increasing age suggests that there may be an autoimmune process which is more active earlier in life. Further details concerning this line of investigation may be found elsewhere (Plioplys et al., 1989).

ANTI-CENTRAL NERVOUS SYSTEM IMMUNOREACTIVITY

B cell function (proliferation and in vitro IgG and IgM synthesis in response to two different concentrations of pokeweed mitogen) was normal. Quantitative serum immunoglobulins (IgG, IgA and IgM) were normal. When tested against Western blots prepared from normal, human cerebellar tissue, there was an increased incidence of IgG anti-210K neurofilament subunit reactivity (41 % vs 7 % in 348 controls; $p<0.001$). IgM anti-210-K reactivity occurred in 53 % of the patients (25 % in 101 controls; $p<0.05$) with an overall incidence of IgM anti-cerebellar Western blot banding of 88 % (26 % in controls; $p<0.001$). IgG or IgM reactivity against frontal cortex Western blots was not observed.

There was no correlation between the anti-CNS results and abnormalities in CD4:CD8 lymphocyte ratios, AMLRs and the expression of DR surface antigen. There

was no correlation between any of these results and medication intake (one patient was taking each of the following medications: carbamazepine, haloperidol, methylphenidate and pimozide; two were taking thioridazine).

Reported cerebellar magnetic resonance imaging abnormalities in autism (Courchesne et al., 1983) may be related to this demonstrated anti-cerebellar immunoreactivity. Further details concerning this line of research may be found elsewhere (Plioplys et al., 1989).

NCAM LEVELS

The biochemical structure of neural cell adhesion molecule (NCAM) is homologous to immunoglobulins in that five extracellular NCAM domains share structural homology with members of the immunoglobulin superfamily (Berthels et al., 1987; Cunningham et al., 1987). The homophilic binding of NCAM molecules may be on the basis of complementary interactions between the immunoglobulin domains (Cunningham et al., 1987). If immunoglobulins are abnormally regulated in autism (as the above data suggests), then possibly other molecular members of the immunoglobulin superfamily, such as NCAM, may also be abnormally regulated.

The level of a NCAM serum fragment in autistic patients was determined by using an antiserum prepared with immunoaffinity purified mouse NCAM. There was a significant decrease in serum NCAM concentration in autistics as compared to controls. In autism, the mean level was 42.34 $\pm$ 8.85 optical density units per ml of serum, whereas in the controls it was 98.84 $\pm$ 16.91 ($p<0.0005$; Figure 3).

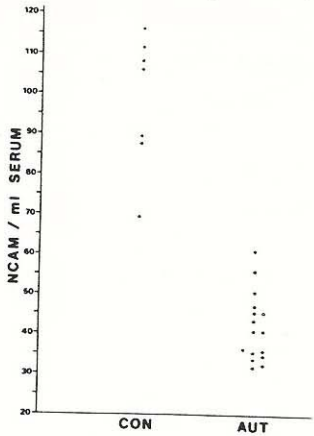

Figure 3. NCAM serum concentration expressed in optical density units per ml of serum in controls (CON) and autistics (AUT). There was a significant decrease in the mean value of NCAM levels in autistics when compared to controls (42.34 $\pm$ 8.85 vs 98.84 $\pm$ 16.91; $p<0.0005$).

When calculated as optical density units of NCAM per mg of serum protein, again there was a highly significant decrease in autistics as compared to controls. In autism the results was 0.63 ± 0.13 and in the controls 1.27 ± 0.19 ($p<0.0005$; Figure 4).

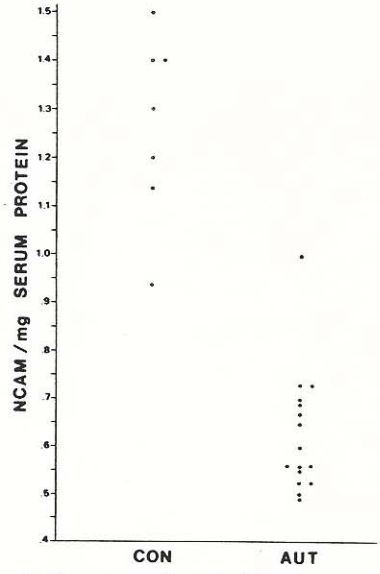

Figure 4. NCAM serum concentration expressed in optical density units per mg of serum protein in controls (CON) and autistics (AUT). There was a significant decrease in the mean value of NCAM levels in autistics when compared to controls (0.63 ± 0.13 vs 1.27 ± 0.19; $p<0.0005$).

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

Depressed serum NCAM levels in autism are distinct from schizophrenia where the levels are elevated (Lyons et al., 1988). It is possible that the elevated serum NCAM levels in schizophrenia reflect an increase in natural central nervous system synaptic turnover rate, as has been suggested (Lyons et al., 1988), whereas its depression in autism may reflect a decreased turnover rate. Furthermore, these results demonstrate that autism and schizophrenia are biologically distinct entities and suggest that circulating

NCAM levels may be useful in distinguishing autism from childhood schizophrenia within the diagnostic grouping of pervasive developmental disorders (APA, 1980; APA, 1987). Further details concerning this line of investigation may be found elsewhere (Plioplys et al., 1989).

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