Biotherapy **9**: 143–147, 1996.

Dialysable lymphocyte extract (DLyE) in infantile onset autism: A pilot study.

H. H. Fudenberg
NeuroImmunoTherapeutics Research Foundation Spartanburg, S.C., USA

Key words: Autism, dialysable lymphocyte extract, transfer factor.

Abstract

40 infantile autistic patients were studied. They ranged from 6 years to 15 years of age at entry. 22 were cases of classical infantile autism; whereas 18 lacked one or more clinical defects associated with infantile autism ("pseudo-autism"). Of the 22 with classic autism, 21 responded to transfer factor (TF) treatment by gaining at least 2 points in symptoms severity score average (SSSA); and 10 became normal in that they were main-streamed in school and clinical characteristics were fully normalized. Of the 18 remaining, 4 responded to TF, some to other therapies. After cessation of TF therapy, 5 in the autistic group and 3 of the pseudo-autistic group regressed, but they did not drop as low as baseline levels.

Abbreviations: AD: Alzheimer's Disease; CFIDS: chronic fatigue immune dysregulation syndrome; DLE: dialysable leucocyte extract; DLyE: dialysable lymphocyte extract; PAS: pseudo-autistic syndrome; SSSA: symptoms severity score average; TF: transfer factor; TA: true autistic.

Introduction

Many disorders of cognitive function have been found to be syndromes with multiple etiologies for each syndrome. Immuno-deficient subsets exist in chronic fatigue immune dysregulation syndrome (CFIDS)[1] and Alzheimer's disease(AD)[2,3]; and these can be markedly improved by dialysable lymphocyte extracts (DLyE) derived from household contacts. It therefore seemed not unlikely that DLyE from household donors would benefit at least some autistic patients. Preliminary results were published in 1988 [4]; this paper represents a 5-year follow-up after $3\frac{1}{2}$ years of therapy.

Materials and methods

A. Patient selection

1. All 40 patients selected were referred by Dr. Mary Coleman, Emeritus Professor of Developmental Pediatrics, Georgetown University. She deliberately selected an almost equal number of "true autistics" (TA) and of patients with pseudo-autistic syndrome (PAS), respectively, 22 in the first group, and 18 in the second group; our diagnosis was identical with hers. All patients were enrolled from 1984 through 1987 and received 3 to 3.5 years of DLyE therapy. Siblings closest in age were used as controls.

2. Histories were taken by the author from theparents with particular reference to the following:

a. Viral infection in the mother during pregnancy, especially second trimester.
b. Multiple infections, especially otitis media, in the 1st through 15 months of life.
c. Relation of onset of symptoms to immunization. Symptoms severity score average (SSSA)(with 1 normal, 5 the worst; e.g. 8–10 hours sleep=1; 0–2 hours of sleep=5) were obtained on each individual. Table 1 shows the symptoms and also the scoring criteria for severity of each symptom. All patient scores ranged between 4.5 and 5.

B. Laboratory testing

1. Immunologic testing. DNA synthesis in response to PHA, ConA, and Pokeweed, and helper and sup-

pressor T-cell functional assays were performed by standard methods [5]. IgG antibody titers to the following viruses were measured by ELISA or hemagglutination: measles, rubella, mumps, coxsackie, and polio. Antibodies to neurone axone filament proteins were measured as described elsewhere [6]. Antibodies to myelin basic protein were measured by a commercial immunologic laboratory. Status was re-evaluated in 1992.

2. Other tests. Serum levels of cortisol and of thyroid stimulating immunoglobulin were also measured. Tests for fragile X-chromosome, defect in the pyruvate lactate pathway, and in the purine salvage pathway enzyme (5'nucleotidase) were performed; none were positive. Patients were also tested for excessive toxic minerals (lead, mercury, aluminium) and deficiency or excess of trace minerals, especially zinc, magnesium, selenium, and germanium.

C. Clinical features

Fifteen of the TA patients developed symptoms within a week after immunization with the measles, rubella, and mumps vaccine (MMR): 3 had high fevers (up to 106°F.) and convulsions within one day of administration; in the other 7 TA, the symptoms gradually worsened in severity (e.g., gradual rather than sudden loss of vocabulary) with onset of clinical abnormality beginning between 15 and 18 months of age. In some PAS patients, symptoms started within the first 10 months; in others, at 2 to 3 years of age. All lacked at least one of the symptoms of TA (e.g., had not lost vocabulary, had no sleep disorder, etc.). Many of the patients had recurrent otitis media and were treated repeatedly with antibiotics. These patients, but not those without frequent antibiotic therapy, had intestinal candidiasis. They were treated with Ketoconazole for 1 month prior to other therapy (we currently use Diflucan). Eleven of the patients with TA had food and/or chemical hypersensitivity and/or allergy. Respiratory allergy was treated with Intal and gastro-intestinal allergy with Gastrocrom. Deficiencies in the enzyme pheno-sulpho-transferase or in Cytochrome P450 were found in all of the 11 patients studied, but not in their siblings of closest age. Increase in intestinal permeability was found in 10 TA and 8 PAS and were treated with commercially available rice-based food formulas.

DLyE preparation and administration.

0.1% of total body lymphocytes were collected by lymphapheresis from a parent who had been a household contact during the previous 6 months. White blood count and differential were performed and then the cells immediately frozen. Preparations with >8% granulocytes were not used [7]. DLyE was prepared as previously described [8]. Each patient received 10 Charleston Units of DLyE (equivalent to 150 International Units) every 6 weeks for three consecutive days for the first 3 visits and every 3 months thereafter with immuno function tests at each visit. If patients returned to normal (e.g. main-streaming in school, no sleep, speech, or attention problems), therapy was continued for another 6 months, then discontinued if immunologic parameters had normalized.

Physical examination

Histories were obtained from parents. Physical examination was confined to observation of attention span, hyperactivity, self-mutilation, etc.

Results

A. Laboratory

Antibodies to myelin basic protein were present in20/22 TA and in 4/18 PAS patients. 12/22 TA and 6/18 PAS patients had a decreased response to ConA and negative LIF response to PHA and decrease in suppressor functional assay [9]. (Later studies showed a good correlation of the above with low levels of CD8/CD28 and CD8/CD38 T-cells.)

Six of the TA and 12 of the PAS patients had increased toxic metal levels (usually aluminium) and decreased levels of trace minerals, as measured by hair analysis, necessary for a normal immune response.

Ten TA patients and 6 PAS patients had elevated thyroid stimulating immunoglobulin values. Titers to rubella were 10 times normal in 11/22 TA and 5/18 PAS patients. Several of the patients had had measles several times as characterized by fever, skin rash of 3-days duration, and elevated IgM levels to measles, again indicating a defect in immune regulation, as normal individuals develop measles only once.

Table 1.

Symptom Severity Score	
	Sleep hours
1	8–10
2	5–7
3	4–4.5
4	3–3.5
5	0–2.5
	Hyperkinetic repetitive movements
1	None
2	Once every 2–4 hours
3	Once every 20 to 119 minutes
4	Once every 3 to 20 minutes
5	Continually or at least every 2 minutes
	Socialization
1	Very affectionate, hugs family and physician, knows nurses
2	Normal eye contact, likes being touched
3	Moderate eye contact, tolerates being touched
4	Some eye contact, does not like being touched
5	Refuses to make eye contact, refuses to be touched
	Self-abuse
1	None
2	Sits in chair banging body against back of chair or couch
3	Bangs head against wall and/or bangs head with fists
4	Covers self with feces
5	Bangs head against wall AND covers self with feces
	Attention span
1	2 hours or more
2	30 to 119 minutes
3	2 to 29 minutes
4	30 to 119 seconds
1	0 to 29 seconds
Total	

Circle one number in each category and add those 5 numbers: Score 5 to 25

B. Clinical

Twenty one of the 22 TA improved significantly, i.e. by a decrease in SSSA of at least 2 points and 10 reached normal levels, including normal verbal I.Q. The clinical response in the PAS group was more heterogeneous. 3 with antibodies to myelin basic protein and 4 without, had a decrease of 2 points in SSSA. Dr. Coleman saw half of the patients again one year after therapy, and several patients again 2 years after therapy. She stated that the degree of improvement in those where we had indicated improvement in SSSA was dramatic.

Troubles due to candida diminished on treatment in almost all patients, and food hypersensitivity decreased with rice-based diet and/or enzyme inducers and then disappeared with DLyE therapy. Before DLyE administration, the abnormalities in trace minerals and/or toxic metals were corrected by the patient's paediatrician using I.V. infusion. The severity of symptoms or aberrations in laboratory tests normalized within 12 weeks in one severe patient; whereas, 3.5 years were necessary in another. Age, when treatment began, did not seem to affect results, since one patient whose treatment began at 15 years of age (onset of symptoms at 15 months) was essentially normal 3.5 years later, working as a painter to support himself and living in a young adult co-op where he did his share of the cooking, cleaning, yard work, etc. When last contacted, he had been off therapy for 1.5 years without any deterioration and with excellent memory. Patients were taken off DLyE 6 months after normalization. Most of them reverted, although not completely to baseline values as determined by phone follow-up.

Discussion

As they improved clinically, the TA patients first became able to type sentences and then to speak sentences. Questioning elicited the fact that prior to therapy they could understand what was said to them, even though they could not verbalize in return and that they felt "very frustrated" about this. One TA patient commented after therapy that there had been a constant "buzzing going on in his head" and that he did not "want to be like that any more." This type of block is more severe than the cognitive defect seen in severe CFIDS and severe Alzheimer's patients.

The very low IL-2 receptor/positive lymphocytesand the decrease in DR+, but not IL-2 receptor+ lymphocytes, suggest incomplete activation, a finding which is seen in other auto-immune diseases. Successful therapy with TF has been reported in other auto-immune diseases (reviewed by Fudenberg and Pizza [8]), which also suggest the possibility that TA may be and auto-immune disease. Furthermore, Strauson

reported one patient with autism who had antibodies to serotonin receptors [10]. Despite the many papers on immunologic abnormalities in autism (many cited in reference [12]), it is possible that the "auto-antibodies" are directed against various viruses and that the reaction to myelin basic protein, neuron axone filaments, one or another receptor for neurotransmitters represent molecular mimicry.

The distinction between TA and PAS is important, as the latter comprises a whole variety of conditions, some of which may be amenable to immunotherapy. However, it is impossible to draw conclusions about a given form of therapy in a heterogeneous group of patients. In TA, after correction of their clinical abnormalities, intra-muscular DLyE administration produced significant beneficial effects, including normalization in some patients. Three of the 10 patients who reached normality did not relapse when DLyE administration ceased 6 months after laboratory abnormal values had reached normal levels. Thus, as previously suggested [4], it seems that TA is probably due to adverse reactions to live virus or live virus vaccine in a genetically predisposed individual, one whose cell-mediated arm of his immune system is not yet mature, or, in a very young infant, by transplacental IgG antibodies from a mother with high titers of antibodies to one of the vaccine constituents (e.g., diphtheria toxin).

Immunologic aberrations have been found in many cognitive disorders, e.g. one subset of Alzheimer's disease [2,8], CFIDS[7]. The presence of antibodies to myelin basic proteins in autism has been confirmed recently by Singh, et al. [11]. Furthermore, auto-antibodies to serotonin receptors and other lymphocyte aberrations have been reviewed by Plioplys, et al. [11], but in none of the papers cited was the population well defined in terms of presence of all or only some symptoms, severity of symptoms, etc.. Immunologic phenomenon are associated with (and presumably are the cause of) many other neurologic and cognitive function abnormalities. Auto-antibodies (which increase calcium currents in neuronal cell lines) have recently been found in some patients with amyotrophic lateral sclerosis [13]. One subset of AD has IgG_3 auto-antibodies to cortical neurones [14] and this was also observed in a small subset of severe atypical schizophrenic patients [15] whose gene is also located on chromosome 6[16], as is the locus DA, DB, HLA-A, HLA-B, HLA-C, and DP, DQ, DR, HLA, HLB and HLD and transcription activity factor [17]. It is of interest that up to 2 years ago the vast majority of physicians, especially psychiatrists, thought that autism was a psychiatric disease.

TA is a heterogeneous population: some patients, those with food and or chemical hyper sensitivities suffer from genetically determined deficiencies (phenol-sulpho- transferase and/or cytochrome P450); others from toxic metal deposition, due for instance, to aberrant intestinal permeability and absorption or to high levels of toxic metals in drinking water; this assumption is supported by the finding that in certain very small geographic areas there is a large cluster of PAS patients.

Since auto-antibodies seem involved, the genetic predisposition is presumably related to aberrant immune response genes on human chromosome 6 [18]. Thus, studies of immune response genes extends haplotypes, in connection with response or non-response to immunotherapy, would probably identify individuals at risk for developing typical autism [19]. In those patients, rubella immunization, as well as other live virus immunizations, should be delayed until at least 3 years of age, since cell mediated immunity in many children does not reach maturity at 15 months [20], the usual time of MMR administration. We observed 2 families, each with 3 children in whom 2 of the 3 suffered from autism. The incidence of viral infections, especially otitis media, was very high in the affected children, but not in the non-affected sibling. In one family with identical autistic twins, one responded to therapy, the other did not. Thus, we contend that this syndrome occurs in congenitally predisposed individuals after exposure to a given virus, live virus immunization, or to DPT immunization at 4 weeks in those instances where the mother has a very high IgG antibody titer to, for example, tetanus toxin or diptheria toxin. Indeed, the IgG antibody crosses the placenta during pregnancy and will be present in the infant who was given these toxins at 4 weeks of age thereby precipitating reaction and subsequent autism. We have observed several infants with PAS who were given the DPT immunization at 4–6 weeks; convulsions occurred and were followed by steadily increasing autistic symptoms. Live rubella immunizations at 15 months has precipitated fever (106°F) convulsions followed by autistic symptoms; so has live hepatitis B vaccine in 2 infants at age 2 years.

The present report, anecdotal as it may seem, suggests that clinical heterogeneity exists in many disorders, currently termed "diseases", but which in reality are syndromes. Until the subset of each syndrome is defined, negative results with TF are meaningless. Furthermore, the type of TF employed (e.g., household contact, in vitro produced by lymphoblastoid cell

lines, pooled human leucocytes), and the quantity used (in autistic patients we used 20 times the conventional dose, administered in 6 to 12 week bursts) may mean the difference between success and failure. Hence, the present plea for "Syndrome" dissection and for studying groups of patients homogeneous for symptoms' severity, as wells as for the use of 10 to 20 fold greater amounts of TF in patients with diseases associated with laboratory parameters of immunologic aberrations (e.g. multiple sclerosis, Class I diabetes, etc.).

References

1. Fudenberg HH: The Florence Nightingale disease: A multisystem experiment of nature: A 50 patient five year analysis. In: The clinical and scientific basis of myalgic encephalomyelitis chronic fatigue syndrome. Ottawa, Canada: Hyde BM, 1992; 641–53.
2. Fudenberg HH & Singh VK. Implications of immunomodulant therapy in Alzheimer's Disease. Prog Drug Res 1988; 32: 21–42.
3. A five year follow-up of these AD patients has been published in Fudenberg HH: Heterogeneity of Alzheimer's Disease: An interpretive review. Mol Neurob 1994; 9: 55–71.
4. Singh VK, Fudenberg HH, Emerson D & Coleman M. Immunodiagnosis and immunotherapy of autistic children. Ann NY A, 1988; 540: 602–4.
5. Fudenberg HH & Wybran J. Experimental immunotherapy. In: Fudenberg HH, Stites DP, Caldwell JL & Wells JV, eds. Basic and Clinical Immunology. Los Altos, CA: Lange Medical Pubs 1980; 3rd Ed: 722–36.
6. Galbraith GMP, Emerson D, Fudenberg HH, Gibbs CJ & Gajdusek DC. Antibodies to neurofilament protein in retinitis pigmentosa. J Clin Inv 1986; 78: 865–9.
7. Fudenberg HH: Dialyzable lymphocyte extract in neurocognitive dysfunction diseases: Effects of granulocytes percent and degree of donor-patient contact. In: Huo BL, Wang R & Zou ZF eds. 9th International Symposium on TF. Beijing: Xueyuan Press 1992: 149–58.
8. Fudenberg HH & Pizza G. Transfer Factor 1993: New Frontiers. In: Jucker E, ed. Progress in Drug Research. Boston: Birkhauser Verlag Basel, 1994; 42: 309–99.
9. Stites DP: Clinical methods of laboratory detection of cell mediated immunity function. In: Fudenberg HH, Stites DP, Caldwell JL & Wells JV, eds. Basic and Clinical Immunology. Los Altos, CA: Lange Medical Pubs 1980; 3rd Ed.
10. Strauson DO, Walker C, et al. Mechanism of serotonin-induced lymphocyte proliferation inhibition. Cell Immun 1984; 84: 240–52.
11. Singh VK, Warren RP, Odell JD & Cole WP. Antibodies to myelin basic protein in children with autistic behavior. Brain Beh 1993; 7: 97–103.
12. Plioplys AV, Greaves A, Kazemi K & Silverman E. Lymphocyte function in autism and rett syndrome. Neuropsychb 1994; 19: 12–16.
13. Mosier D, Baldelli P, Delbono O, Smith RG, Alexianmu M, Appel S & Stefani E. Amyotrophic lateral sclerosis: immunoglobulins increase Ca2+ currents in a motoneuron cell line. Ann Neurol 1995; 37: 102–9.
14. Singh VK & Fudenberg HH. Increase of immunoglobulin G3 subclass is related to brain autoantibody in Alzheimer's Disease but not in Down's Syndrome. Autoimmun 1989; 3: 95–101.
15. Fudenberg HH, Whitten HD, Merler E & Farmati O. Is schizophrenia an autoimmune receptor disorder? Med Hypoth 1983; 12: 85–92.
16. Singh VK: Personal communication.
17. Fudenberg HH, Pink JRL, Stites DP & Wang AC. Basic Immunogenetics. New York: Oxford Univ Press, 1984; 3rd Ed.
18. Fudenberg HH, Pink JRL, Wang AC & Ferrara GB. Basic Immunogenetics. New York: Oxford Univ Press, 1984; 3rd Ed.
19. Since submission of this manuscript, we have learned that Warren, et al. found increased freauency of the null allele at the complement C4b locus of the estended immune response gene haplotype in autism. Warren RP, Singh VK, Cole P, Odell JD, Pingree CB, Warren WL & White E. Increased frequency of the null allele at the complement C4b locus in autism. Clin Exp Immunol 1991; 83: 438–40.
20. Pandey JP, Fudenberg HH, Virella G, Gotschlich EC, et al. Association between immunoglobulin allotypes and immune responses to haemophilus influenzae and meningococcus polysaccharides. Lancet 1979; 1: 190–2.

Address for correspondence: Dr. H.H. Fudenberg, Neuro ImmunoTherapeutics Research Foundation, 1092 Boiling Springs Road, Spartanburg, SC, USA; fax (803) 591 0622.