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Heterogeneity of Alzheimer's Disease*

An Interpretive Review

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Introduction

Many neurologic diseases (e.g., Myasthenia Gravis *[1]*, Guillian-Barre Syndrome *[2,3]*, multiple sclerosis *[4a,b]*) are immunologic in origin and respond to immunotherapy with Dialyzable Leucocyte Extract (DLE) or Dialyzable Lymphocyte Extract (DLyE) containing Transfer Factor (TF). We have found that cognitive function disorders (e.g., autism *[5]* and myalgic enkephalitis *[6]*) are also immunologic in origin and corrected by TF. The beneficial effect of DLE in myalgic encephalitis (chronic fatigue immune dysregulation syndrome), using oral DLE, has been confirmed by Pizza, Viza, and Fudenberg *(7)*.

In 1982 and 1984, we published evidence that schizophrenia is not one disorder but could be subdivided into several *(8)*, confirmed in 1992 by Kildreas et al. *(9)*; a small group of which had immunologic aberrations *(8,9)*. Two in a group of ten such patients with prolonged severe atypical disease of 10 yr duration later had a dramatic therapeutic response to DLyE enriched in TF *(10)*. In 1984, we demonstrated immunologic heterogeneity of Alzheimer's "Disease" (AD) *(11)*, another disorder of cognitive function with an immunologically aberrant subset that responded to therapy with pyrrolidone (namely, Piracetam) and extended this in 1988 *(12)*. Immunologic heterogeneity was subsequently demonstrated by Torack in 1986 *(13)* and by others shortly thereafter (e.g., McRae et al. *[14]* and Miller et al. *[15]* in 1987). We herein report therapeutic trials of forty patients with AD, none of the familial variety, most with marked impairment, and also of five patients with mild AD.

Methods

Patient Selection and Evaluation

Forty patients aged 52–89 at onset of the disease were studied. All had severe disease with marked impairment of daily function *(see below)*. Patients were classified into stages 1–12. Stages 1 and 12 matched two Reisberg's *(16)* criteria, 1 and 7; namely, (1) normal and (7) completely nonfunctional, curled up in a fetal position. The difference in Reisberg stages 2–6 was expanded so that inability to recognize spouse, inability to speak understandably, loss of rectal and bladder sphincter control, loss of ambulation, and so on, each counted as one stage (Table 1). All forty subjects were in our stages 5–12; five additional subjects with mild disease (stages 3–5) were also investigated. All subjects were given our Daily Function Tests (DFT) (Table 2), as were the five early stage subjects. The usual daily functions were scored as: "1" no problem; "2" needs reminding; "3" needs supervision; "4" needs assistance; and "5" cannot do, even with assistance; and assessed each Saturday by a spouse or offspring. An expanded Hopkins' minimental exami-

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Table 1
Scale for Evaluation of Clinical Status in Alzheimer's Disease

Stage	Cognitive decline	Clinical characteristics	Psychometric concomitants
1	None	Clinical interview does not elicit evidence of memory deficit.	The normal individual
2	Very mild	Forgetting where familiar objects have been placed; Continually forgetting names they formerly knew; Objective evidence of memory deficit; Individual displays appropriate concern about symptoms.	Patient performs below average for age.
3	Mild	Objective evidence of memory deficit obtained only through intensive interview; Concentration deficit may be present; Decreased facility in remembering names of people just introduced to; Coworkers become aware of patient's relatively poor performance; Patient denies problem.	Patient performs one standard deviation or more below average for age.
4	Moderate	Clear-cut deficit is apparent in clinical interview; Concentration deficit when patient requested to do serial subtractions; Ability to travel alone is notably curtailed; Difficulty managing personal finances; Patient remains well oriented to time and person; Generally no deficit in patients' ability to travel to familiar locations; Denial often becomes dominant defense mechanism.	Always 3 or more errors on Mental Status Questionnaire.
5	Moderately severe	Early dementia; Incorrect responses when asked about address or telephone number and close family members; Invariably know own name and generally that of spouse and children; Require no assistance with toileting or eating; Difficulty choosing proper clothing; Wrong for weather Shoes on wrong feet More than one set of clothing (2 pair of pants, and so on).	Deficits are evident on brief Mental Status Questionnaire assessment.
6	Severe	Occasionally forget name of spouse; Generally unaware of their surroundings, year, or season; May have difficulty counting from 10 backward, sometimes even forward; Require substantial assistance with daily living activities; Require assistance in traveling; occasionally will display ability to travel to familiar locations—however, cannot be trusted to do so; May be able to distinguish between familiar and unfamiliar people.	
7	Severe	Loss of rectal and bladder sphincters.	
8	Severe	Delusional, paranoia, obsessive symptons; Anxiety symptoms and congnitive dysfunction easily recognized.	
9	Severe	Inability to speak understandably.	
10	Severe	Inability to recognize spouse.	
11	Severe	Inability to walk.	
12	Severe	Curled up in a fetal position.	

Table 2
Alzheimer's Disease Daily Function Test (Fudenberg, 1984)

Name				Date	
	1	2	3	4	5
Function	No difficulty, no reminding, supervision, or assistance	Minor difficulty, needs reminding	Moderate difficulty, needs supervision	Much difficulty, needs assistance	Cannot do, even with assistance
Feeds self					
Shave/makeup					
Brush teeth					
Groom hair					
Wash/bathe					
Dress/manage clothes					
Take medicine					
Do home chores					
Exercise					
Reading: a. comprehension b. attention span					
TV					

nation (Table 3) was also evaluated by myself at each visit and by spouse or offspring weekly. In addition, pictures of Presidents (from Hoover through Reagan initially; subsequently, Roosevelt through Bush) were presented to see if verbal and visual recall differed (they did in two non-AD dementia subjects).

The diagnosis of AD was made by the following criteria: normal serum B_{12} and folic acid levels, negative serologic tests for syphilis, no clinical or CAT scan evidence of tumor of the brain obtained more than 2 yr after onset of symptoms. Frontal lobe atrophy on CAT scan taken at least 2 yr after preceding CAT scan was a definite requirement; subjects without such cortical atrophy were excluded, as were subjects with hypothyroidism, internal carotid artery stenosis by auscultation, or any chronic disease requiring medication (e.g., hypertension, diabetes).

Controls for immune function tests consisted of 40 subjects, matched for sex and for age (54–82); ten of these had other dementias (carotid artery stenosis, small multi-infarct dementia, normal pressure hydrocephalus, and so on); ten were spouses of the affected patients with no illnesses and taking no prescription or over-the-counter medications (environmental control); ten with primary depression with cognitive dysfunction; and the other ten normal, age matched (5 males, 5 females).

Immunologic Tests

These included lymphocyte DNA synthesis in response to PHA, Con A, pokeweed mitogen, and α_1 acid glycoprotein (the latter is an immunologic

Table 3
Alzheimer's Disease
Hopkins Expanded Mini-Mental Status

Name		Date
Maximum score	Score	Mental Task
12		Orientation What is the (year) (season) (date) (day) (month)? Where are we: (country) (state) (county) (city) (hospital/doctor) (floor)? Where do you live?
4		Registration Tell the patient your name. Ask him to repeat it. (1 pt) Tell the patient the names of 3 objects. (Allow 1 second to say each.) Then ask the patient to say them back to you. (1 point for each correct.)
9		Trials (Attention and calculation) Count by 7s (1 point for each correct answer. Stop after 5 answers.) Spell "WORLD"; spell "WORLD" backwards. Count 1–15; count 15–1.
15		(Recall) Immediate: Ask to name the 3 objects mentioned earlier. (Give 1 point for each correct answer.) Ask patient if s/he remembers your name. Past: Presidents: (current) (one just before him) (last one remembered) Do you recognize: (show pictures of:) Roosevelt? Truman? Eisenhower? Kennedy? Nixon? Carter? Bush? Reagan?
9		(Language) Ask patient to name a pencil and watch. (2 pts) Repeat after me: "No ifs, ands, or buts." Do as I say: "Pick up this paper in your right hand, fold it in half, then put it on the floor." (3 pts)
3		(Reading/Writing) Read and obey: "Close your eyes" (1 pt) Ask patient to write a sentence. (1 pt) Draw a simple design on a piece of paper. Ask patient to copy it. (1 pt)
Total Score 52		Assess level of consciousness along a continuum: Alert Drowsy Stupor Coma

modulator) *(17)*, and in autologous mixed leukocyte reaction (AMLR) *(18)*. Lymphocyte (and subsets thereof), monocyte, and natural killer cell enumeration were performed using a fluorescent activated cell sorter and monoclonal antibody. Leukocyte migration inhibition factor (LIF) production in response to PHA and percent T-cells positive for surface membrane orosomucoid were observed as discussed elsewhere *(19)*. Antibodies to neuron axon filament proteins (Ab-NAFP), were detected as described elsewhere *(20)*. Interactive T-cell function (this measures T-cell ability to react with antigen presented by B cells) was evaluated by the method of Goust and Fudenberg *(21)* (Fig. 1). IgG subclass levels were measured by indirect diffusion in agar, and antibodies to cerebral cortical cells were measured by immunofluorescence.

Therapies

Pyrrolidones (Fig. 2)

Piracetam, a nooleptic designed for treatment of dyslexia (thought by us to represent a mirror image

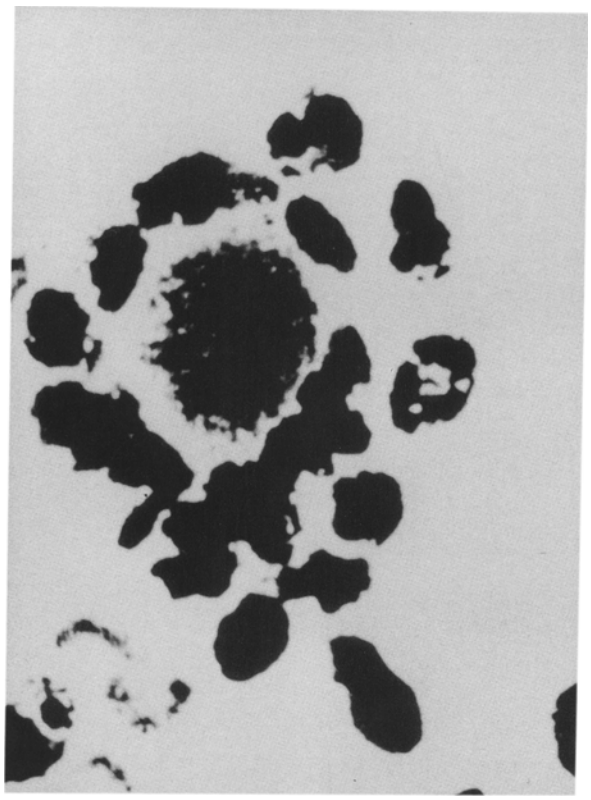

Fig. 1. Interactive T-cells surrounding a β-cell from the RAJI lymphoblastoid cell line to form an interactive "rosette."

of GABA) (GABA is thought by many to inhibit normal cognitive cerebral function and for 5–10 yr was thought by many to be the cause of AD) was used as the first therapy *(11)* since our in vitro studies showed that it had immune modulating properties *(22)*. This was used in a single-blind control placebo trial described below.

Subsequently, a double-blind placebo crossover study was carried out with Aniracetam, another pyrrolidinone with a different sidechain.

DLyE

Dialyzable lymphocyte extract (DLyE), prepared from purified mononuclear cells, was obtained by lymphopheresis of spouse or other household contact (i.e., someone in close proximity to the patient for at least 6 mo) *(10,23)*. We have shown that with AD patients, DLyE made from leukocyte preparations containing >20% granulocytes are usually ineffective *(10,23)*. DLyE contains TF, which transfers the cell-mediated immunities (CMI) of a normal donor to a normal or diseased recipient in vitro (Fig. 3) or in vivo (Fig. 4A,B). (An example of the therapeutic effects of antigen specific TF is shown in Fig. 5; namely, the hands of a patient with familiar chronic mucocutaneous candidiasis before and 2 mo after such therapy.) We assumed that in any disease caused by an unknown infectious agent, household contacts of at least 6 mo duration presumably exposed to the same infectious agent and who did not develop the disease, despite presumable exposure to the same infectious agent, had good CMI to the infectious agent *(6,10)*. The donor immune cells (approx 1×10^{11}, or 0.1% of the body's total immune cells) were collected in the cold, counted, frozen, and DLyE prepared by vacuum micropore filtration (12 KD exclusion size), then concentrated by lyophilization three times in distilled water. The resultant preparation was administered subcutaneously as 0.3×10^8 lymphocyte equivalents (one South Carolina unit) for three consecutive days with immunologic function measured at time 0 on d 1 and also on d 4. DLyE injection and immunologic evaluations were performed every 6 wk for 12 mo, then every 3 mo for several years. In occasional instances, much greater amounts were administered and the patient seen at 4–10 d *(vide infra)*.

Immunosuppressant Therapy

Immuran—200 mg daily for 4 wk followed by 1 mo rest, then repeated for 2 mo—was administered to six patients in Group III *(see below)* and for 4 mo to four patients in each of the other groups.

Patient Survival

Three AD patients died prior to completion of this study—one committed suicide when he relapsed after switched to "blind" placebo therapy, one had a stroke, and one had a coronary. Of the control patients, two died—one of cerebral thrombosis, and the other of a coronary.

Experimental Design

Pyrrolidones

The first study was a single blind Pyrrolidone placebo crossover where patients received either active medication or a placebo for 3 mo, then after 1 mo washout were switched; the responders in either arm were given active medication for an additional 6 mo. Immunologic tests were performed every 6 wk. These tests made it possible to evaluate responses at 3 mo (active agent) or 6 mo

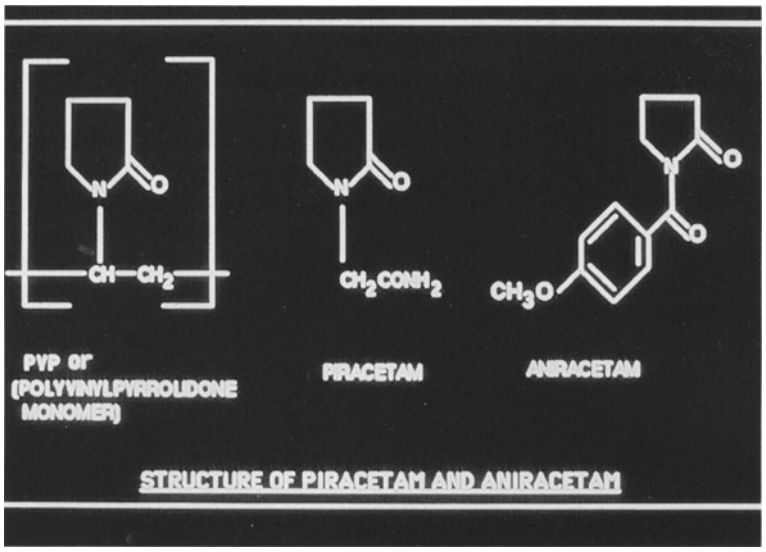

Fig. 2. Structure of the pyrrolidones Piracetam and Aniracetam.

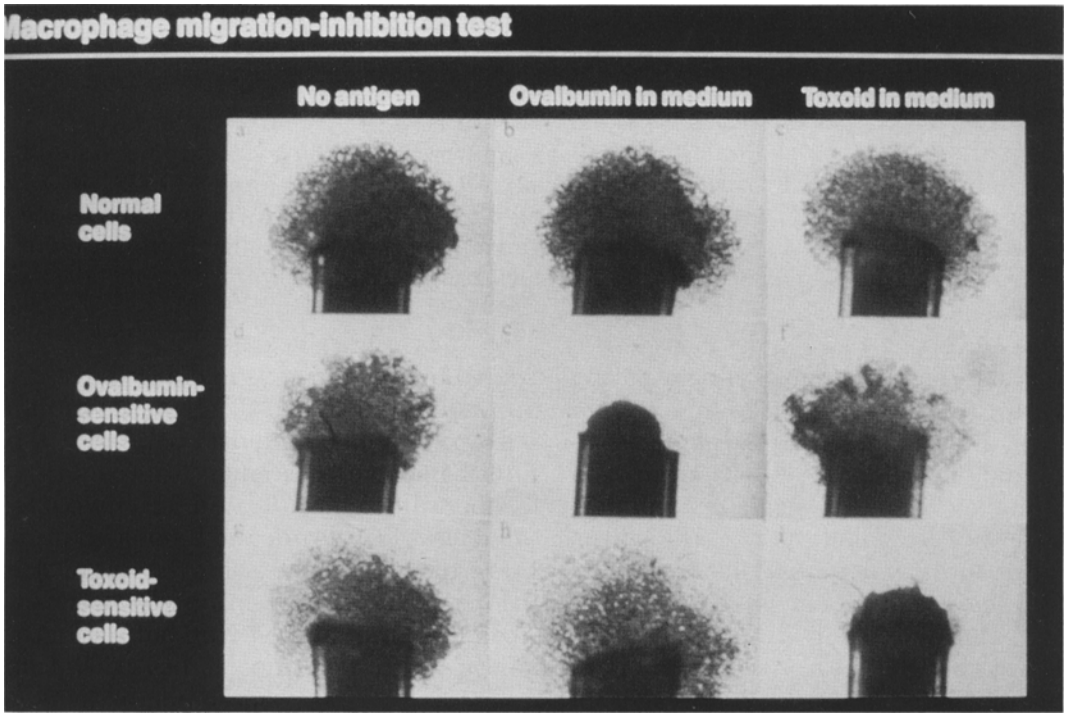

Fig. 3. Effect of TF in vitro. Note that neither of the two antigens (ovalbumin nor tetanus toxoid [TT], respectfully) inhibit migration of macrophages (MM) from a capillary tube when the macrophages are obtained from an individual sensitized to neither antigen. In contrast, ovalbumin inhibits MM when exposed to ovalbumin but not to cells sensitized to TT; in individuals previously sensitized to TT, but not to ovalbumin, MM inhibition occurs when TT is added.

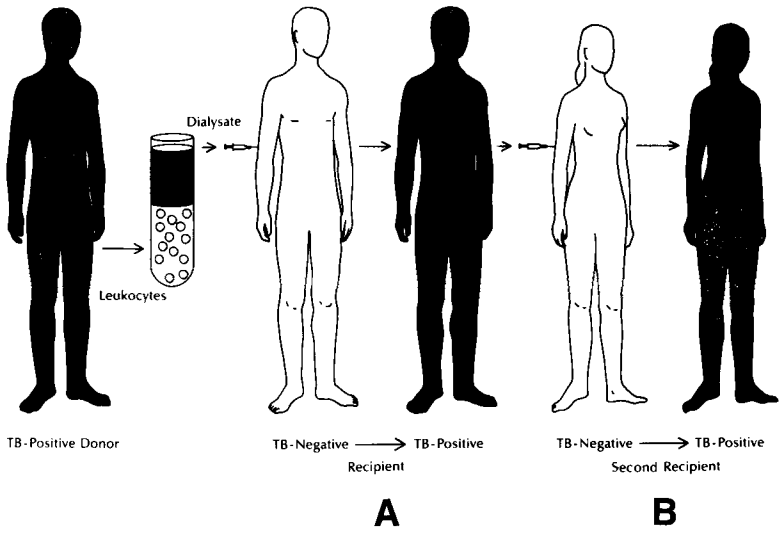

Fig. 4. **(A)** Effect of TF in vivo. Leukocyte of a TB positive donor, as shown by skin test, can transfer via DLE immunity to a donor previously negative to this antigen by skin test. The dialysate converts skin tests in this primary recipient from PPD (purified protein derivative of TB) negative to PPD positive. This primary recipient remained skin test positive for 6 mo. **(B)** If during that time, 20 mL of blood is taken from the primary recipient and the white cells separated, washed, disrupted, and then similarly dialyzed, the blood of this primary recipient (secondary donor) can convert a secondary recipient (normal or diseased) previously negative by skin test to PPD to PPD positive. If the primary donor was negative for a second antigen (e.g., coccidiomycosis), the primary recipient and secondary recipient are also negative to that antigen by skin test after the serial transfers.

(active agent/placebo/additional active agent) vs response at 9 mo (placebo/active agent, additional active agent). A 1 mo-washout period was observed prior to the first switch. In trial 2, the aniracetam trial, the agent was given orally in a dose of 0.5 g two times daily. This was a double-blind placebo crossover study with active drug for 6 mo in one arm and the placebo in the other arm, with a 1 mo washout, then crossover for 6 mo. Six additional months of active medication were given for those who had responded in either arm (Table 4).

DLyE

Mononuclear cell equivalents (0.3×10^8) were given consecutively for 3 d every 6 wk for at least 12 mo and on some occasions, later in therapy, at more frequent intervals and/or at greater dosages to observe the effects since cognitive functions of some patients improved markedly for the first 3 or 4 wk then began deteriorating to baseline. Placebo control was not used in the DLyE-TF study of the patients since no placebo effect was observed with AD patients on pyrrolidones or with DLyE-TF in 50 patients with Florence Nightingale Syndrome (Chronic Fatigue Immune Dysregulation Syndrome) *(6,24,25)* and since placebo effects last no more than 1 wk but beneficial effects were still present, at last check, 5 yr after treatment.

Immuran

Informed consent could be obtained from the guardians of only 6 of the 16 patients with IgG_3 antibodies to cerebral cortical cell neurons. Six patients with autoantibodies to cerebral cortical cells and unresponsive to pyrrolidones and DLyE were given 200 mg of immuran daily for 1 mo, rested for 1 mo, then 200 mg daily for 6 wk, rested 1 mo, then 200 mg daily for another 6 wk. Insofar as immuran immunosuppressive therapy is concerned in the group with antibodies to cerebral cells and aberrant DNA synthesis, the trial was too short to warrant conclusions.

Four patients each in Groups I and II were also treated in the same fashion but showed no clinical improvement. The four patients in Group IV lacked all three biological markers described above.

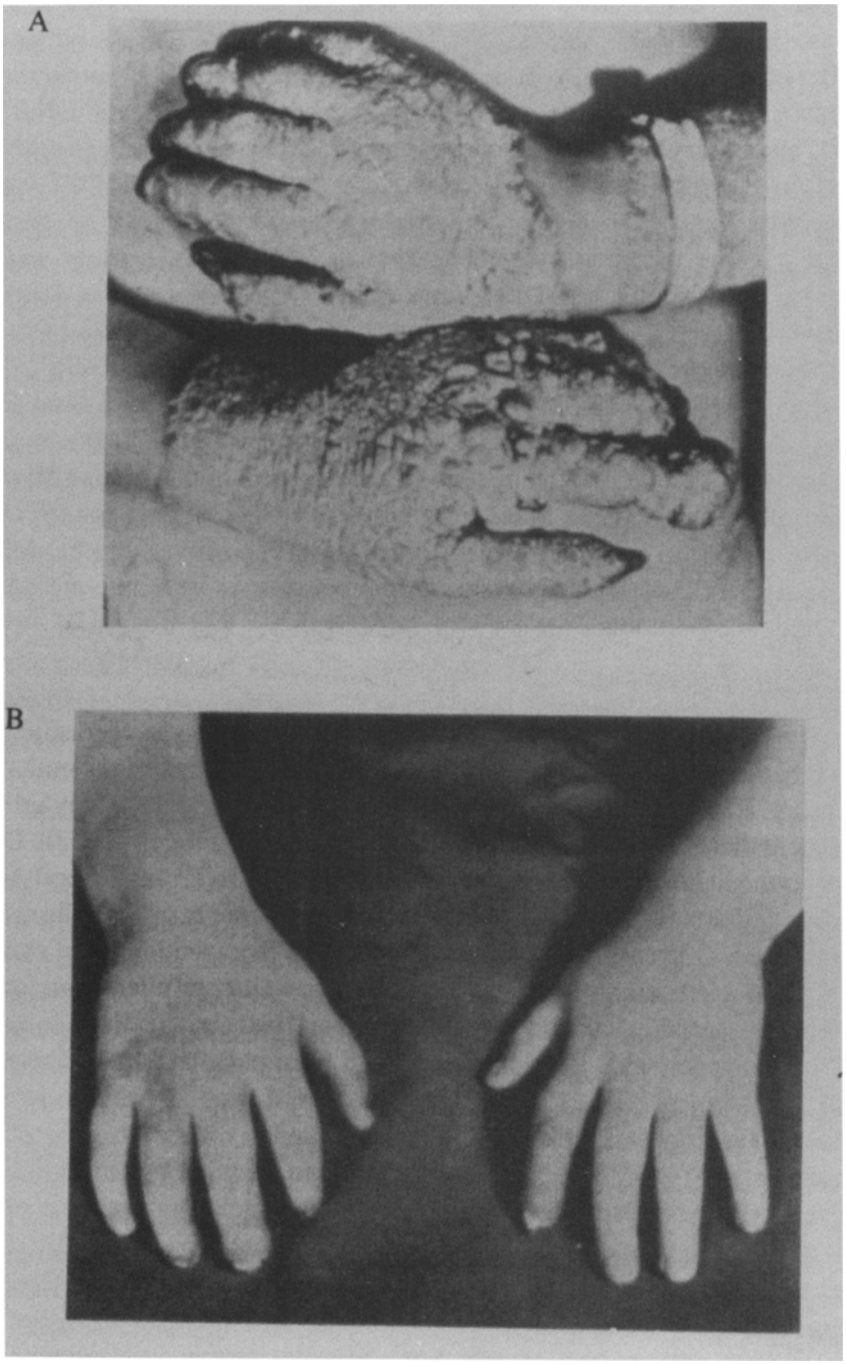

Fig. 5. Dramatic response of patient with familiar mucocutaneous candidiasis to DLE-$TF_{a.s.}$ for candida.

Results

Pyrrolidones

Piracetam

Nine of the forty patients responded clinically to Piracetam with regain of at least one stage and, in some cases, mild improvement of the minimental within 3 mo; some gained two stages (laboratory improvement was evident at 6 wk). When placebo was substituted for the next 3 mo, patients returned to baseline levels. In those that received Piracetam in the first arm, the additional 6 mo following the

Table 4
Pyrrolidone Trials

GROUP # RESPONSE	PIRACETAM (Single Blind)								
1 R	●	●	●	⟷	□	□	□	⟷	♦ ➡ ♦
1 N	●	●	●	⟷	□	□	□	⟷	□ ➡ □
2 R	□	□	□	⟷	●	●	●	⟷	♦ ➡ ♦
2 N	□	□	□	⟷	●	●	●	⟷	□ ➡ □
Month →	1	2	3	4	5	6	7	8	9 ➡ 14

● = piracetan; □ = placebo; R = responders; N = nonresponders; ↔ = washout period; ◆ = 12 mo additional therapy.

GROUP # RESPONSE	ANIRACETAM (Double Blind)														
1R	●	●	●	●	●	●	⟵	—	⟶	□	□	□	□	□	□	* ♦ ➡ ♦
1N	●	●	●	●	●	●	⟵	—	⟶	□	□	□	□	□	□	* □ ➡ □
2R	□	□	□	□	□	□	⟵	—	⟶	●	●	●	●	●	●	* ♦ ➡ ♦
2N	□	□	□	□	□	□	⟵	—	⟶	●	●	●	●	●	●	* □ ➡ □
Month →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	* 16 ➡ 27

● = aniracetam; □ = placebo; R = responders; N = nonresponders; ↔ = washout period; ◆ = 12 mo additional therapy; * = Code broken at this point.

placebo resulted in clinical improvement averaging "3" stages. In those who received Piracetam in the second arm, followed by an additional 6 mo (e.g., 9 consecutive mo), clinically increased by "3" to "5" stages. All responders to these had decreases in interactive T-cells prior to therapy, normalizing on therapy.

In the five early stage patients, four became worse; in one patient with severe disease who failed to respond to Piracetam, patients declined to baseline or below by 6–9 mo after therapy was discontinued. Nonresponders declined by 3 stages after 18 mo of trial in each patient with one exception.

After the Piracetam and a subsequent 3-mo washout, a more potent pyrrolidone (namely, Aniracetam) about five times as potent, shown by in vitro tests (27), was administered. Aniracetam or placebo were usually administered for 6 mo before crossover. Those who received placebo for 6 mo declined 1 to 2 (1.6 ± 0.8) stages; those who received the active agent for 6 mo had gradually increasing improvement in laboratory tests and a 2- to 3-stage

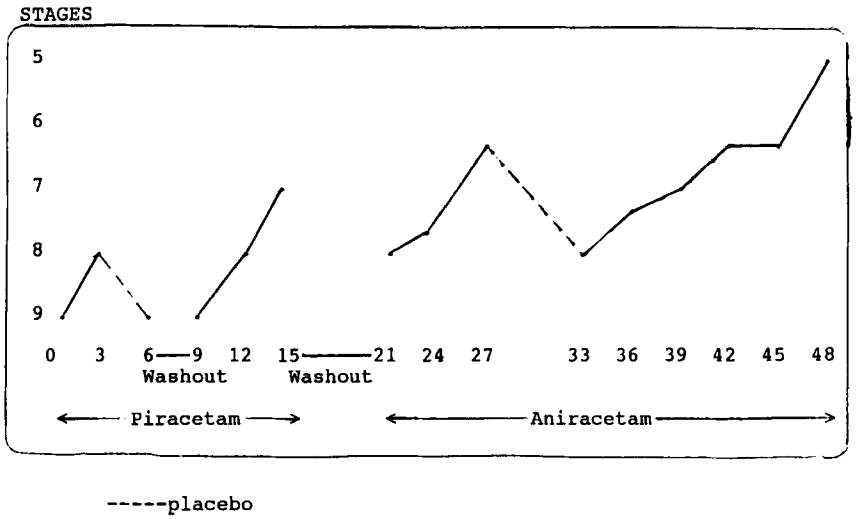

Fig. 6. Response of a representative AD patient to Piracetam, indicating improvement (1 stage) of patient at 3 mo, but decline to baseline on 3 mo of placebo. When given an additional 6 mo of Piracetam, he had a two-stage response; after a washout period of 6 mo, he was placed on Aniracetam for 6 mo and had a two-stage response equivalent to that of Piracetam at 6 mo, but starting at a higher baseline. On 6 mo of placebo, he had dropped back to baseline. On 15 mo of Aniracetam, he gained three additional stages.

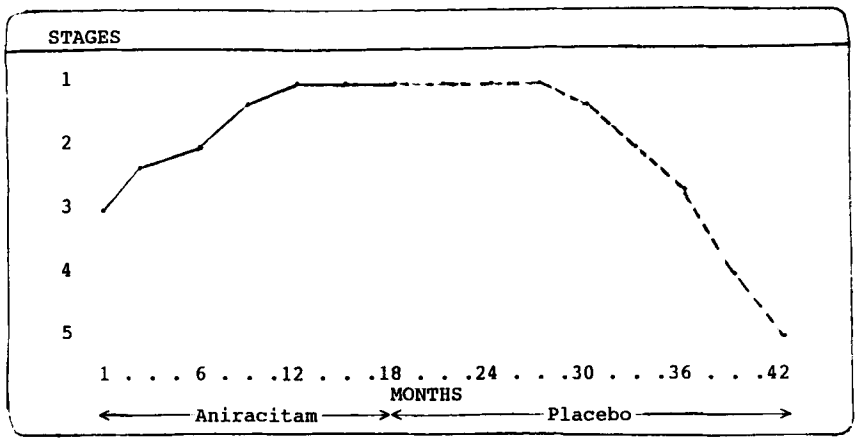

Fig. 7. Response of a mild AD patient (stage 3) who responded to Grade 1 on 18 mo of Aniracetam (stage 1—completely normal). He stayed this way for an additional 9 mo without Piracetam (on placebo but after another 16 mo declined to stage 5).

improvement on our scale. Those who received placebo for the first 6 mo, then Aniracetam for 12 mo (total 18 consecutive mo), had a 3 to 4+ stage improvement (3.6 ± 1.3).

Aniracetam

Laboratory values (especially interactive T-cells) improved prior to clinical improvement on Aniracetam. The same patients who responded to Piracetam responded to Aniracetam (Fig. 6) with one exception. The clinical response lag time was about 4 wk with Aniracetam and about 6 wk with Piracetam. Response mirrored the response to Piracetam, but some of the responders gained 5–6 stages, especially in those who received 18 consecutive mo (the difference in the Aniracetam trial was that there was 6 mo, rather than 3 mo, before crossover). One mild patient (stage 3), E. S., who had been fired from his supervisory job because of memory loss, became completely normal and was able to return to work and was then promoted! (About 1 yr after trial ended, patient relapsed to stage 5; *see* Fig. 7). The patient had deficient

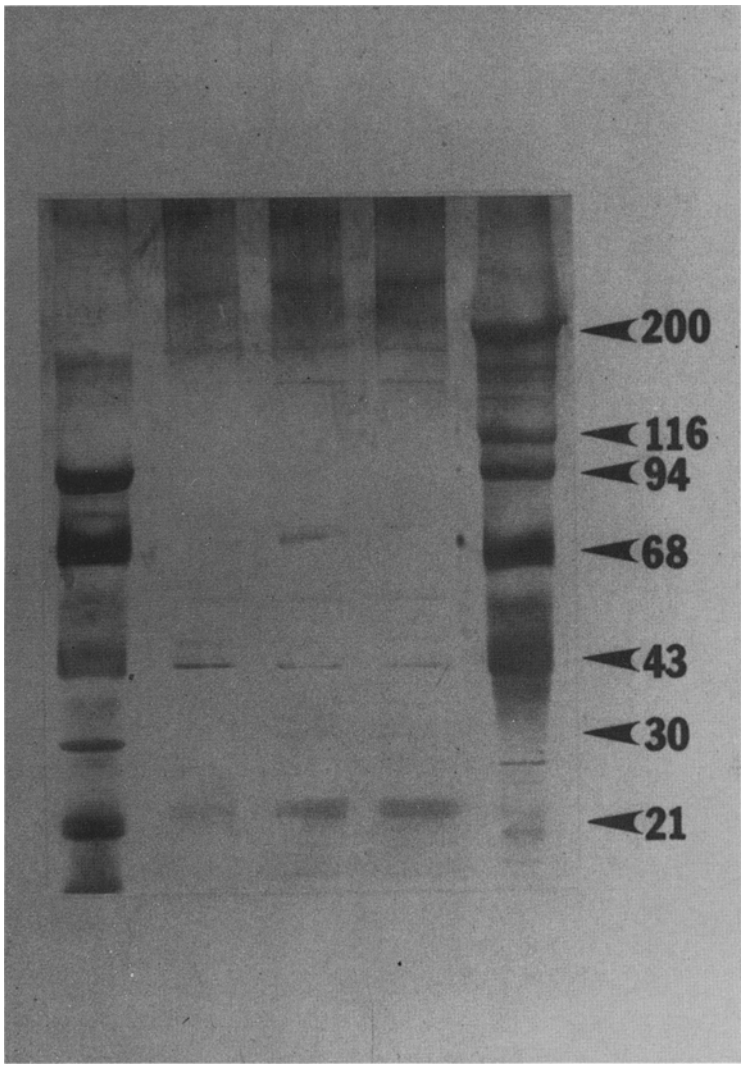

Fig. 8. Antibodies to neuronal axon filament proteins as determined by immunoblot. Exterior lanes 1 and 5 are normal serum. Lane 4 shows CJD control. Lanes 2 and 3 show AD patients.

LIF response to PHA, which was corrected by Aniracetam.

DLyE Enriched in Transfer Factor (TF)

The patients in this group had abnormal lymphocyte DNA synthesis in response to PHA, α_1 acid glycoprotein and in AMLR; most had abnormal percent T-cells with surface membrane orosomucoid and deficient LIF production in response to PHA. Both immunologic responses and clinal status improved in 11 patients. In these 11, DLyE containing TF obtained from household contacts was effective after 12–24 wk in almost all cases except when a viral infection in either the donor or recipient had occurred 3 wk prior to donation or administration *(10)* or 3 wk after general anesthesia or severe painful stress (e.g., auto accident) *(10)*; some patients gained six stages. In several instances, DLyE obtained from normal volunteers or from relatives living away and seeing the patient for 1–3 wk yearly was ineffective *(23)*. (Clinical improvement varied after 18 wk and gained an average of 3 stages.) Half of these patients had 70, 160, and/or 200 kDa antibodies to NAFP in high titers (Fig. 8), similar to those seen in Creutzfeldt-Jakob disease *(28)* and one subset of retinitis pigmentosa *(29)* and of autism *(5)*; the other half had such antibodies in low titers (1:10 or 1:20) (Table 5). Most of the patients in this subset (but not other subsets) had

Table 5
Serum Antibodies to NAFP
by Immunoblot Technique

Disease	No. tested	No. positive
Alzheimer's disease	40	16
Parkinson's disease	11	2
Senile dementia	10	0
Down's syndrome	10	0
Autism[a]	38	20
Creutzfeldt-Jakob disease	3	3

[a]Usually to 70 or 160 kDa rather than 200 kDa.

abnormal percent T-cells with surface membrane orosomucoid.

Many spouses stated that after the DLyE patients often did extremely well for 3–4 wk, then relapsed to baseline during the following 2–3 wk. Hence, on several occasions, patients were given three times the usual dose of DLyE, and I visited their homes (or they visited me) several days thereafter. Results were dramatic. For example: One white male, E. D., 67-yr-old, former New York advertising executive, was disoriented as to time and place, unable to walk, unable to control rectal and bladder sphincters; one week later was oriented to time and place, walked through two buildings by himself to find my office, and had regained rectal and bladder sphincter control. When I gave him a copy of the Sunday *New York Times*, he read and, at my request, explained the economic jargon in one article. When given the Book Review section and asked to read full page ads, 5 min later he was able to recall title, author's name, and the publisher when I pointed to the appropriate page.

One of the five minimally affected AD patients (stage 4) responded to DLyE and was completely normal in 12 mo.

Group III—Patients with Antibodies to Cerebral Neurons

Sixteen patients had IgG_3 antibodies, to cerebral cells *(30,31)*. These patients should theoretically respond as do patients with other autoimmune diseases to immuran and prednisone in high doses; we did not use prednisone because we feared complications, especially since the patients were unable to communicate. Therefore, with spouse's permission (6 patients), we tried immuran alone, as described above. Mild improvement was noted in three of the six patients in this group whom it was given (1.1 ± 0.6) and in none of the 12 other patients to whom it was not given.

Group IV

In four patients, no abnormalities were noted in any of the laboratory tests outlined above, and no clinical response to any of the medications listed above. We assumed that in this group the cause was an amino acid substitution in either the neurotransmitters or the receptors and hence would not respond to any of the above therapies.

Nonfamilial AD appears to be not a single disease, but rather a syndrome of different etiologies with the same clinical course *(32,33)*. Hence, hope for one drug to treat all cases, unless it acts on a final common pathway, seems unreasonable. As in the case of anemia, the proper medication depends on etiology (e.g., iron efficiency, B_{12} deficiency, autoimmune, hemolytic anemia, and so on). In the case of the pyrrolidones, the pyrrolidinone pathway (Fig. 9) starts from putrescine and after many metabolic steps, liberates succinic acid used in glucose metabolism that furnishes energy *(34)* to maintain membrane flexibility. Presumably pyrrolidone bypasses an enzyme abnormality in this pathway. (Pyrrolidinone concentrations are fivefold higher in lymphoid and cerebral cells than in any other body cells *[35]*). Another subset of AD, characterized by deficient function of subpopulations of T-cells, responded well to DLyE as is the case in viral, mycobacterial, fungal, protozoa, and parasitic diseases *(10)*. Patients with subsets of other cognitive function disorders (e.g., Florence Nightingale Syndrome *[6]*, autism *[5]*, and so on), in which viruses appear to be involved in pathogenesis, also responded well. Leszek et al., have demonstrated heterogeneity in AD *(36)* and the efficacy of TF inducers (e.g., peat extract) *(37a)* in AD and other immunotherapy in some patients with schizophrenia *(37b)*.

These data again clearly demonstrate that at least four different subsets of "Alzheimer's Disease" exist (although clinically indistinguishable), three definable by laboratory tests, and each of the three responding to a different therapeutic agent. (We thus far have no biologic markers for the fourth subset.) In the 40 patients studied, 36 fell into one of the three categories. No nonfamilial patients were studied. Actually, cases of familial AD may be caused by continued exposure to a slow virus; i.e., sporadic and familial AD may share only a common environment since we have seen four out of five individuals working on the second floor in a clothing

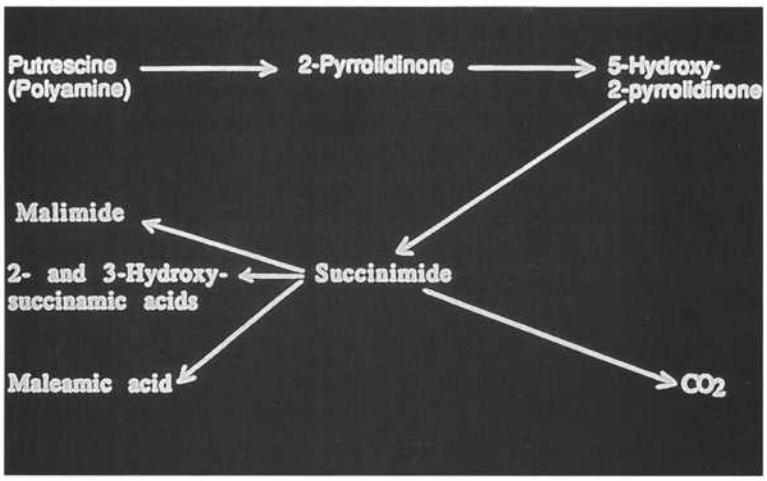

Fig. 9. Pyrrolidinone pathway. Presumably pyrrolidinones bypass a metabolic block. The succinic acid breakdown products produce compounds that provide energy via the Dickens' shunt for neuronal and lymphoid cells; we believe part of the energy is used to maintain membrane flexibility.

store in Beaufort, SC, develop AD. One of the four had two sisters who subsequently developed AD as well. None of the five individuals working on the first floor developed AD. The interval between exposure of the three coworkers to the affected sister and onset of symptoms was 12–15 yr *(38)*, so perhaps this AD cohort represents an environmental rather than a genetic etiology, or perhaps prions themselves are transmitted in AD, since their structure is so markedly different from conventional infectious agents *(28)*, their biological activity and transmission may vary in ways as yet unknown and perhaps vary from one disease to another. The possibility that AD may be viral in origin has been suggested by others *(39)*, especially since the β-amyloid in Parkinson's Disease in Guam *(39a)* is identical in amino acid sequence to the amyloid in AD *(40b)*.

The first subset is characterized by a decrease in interactive T-cells (a measure of T-cell function, not numbers); presumably this represents a defect in membrane flexibility so that the receptors for neurotransmitters cannot maximally bind the neurotransmitters in vitro. These responded to pyrrolidinone membrane modulators as shown by the increased uptake of neurotransmitters (approx 35%) in patients with decreased membrane flexibility in the presence of pyrrolidones and increased production of interleukin-1 *(11)* in half of a subset of AD patients in whom IL-1 production by monocytes is deficient *(41)*. Again, the subset presumably is deficient in an enzyme in the pyrrolidinone pathway responsible for the energy glucose generation that provides the energy necessary for maintenance for normal membrane flexibility. An in vitro test can demonstrate which pyrrolidones (whether those synthesized by pharmaceutical firms or those synthesized by ourselves) will be most effective in a given patient and the optimal concentrations *(27)*. Such defect was present in only one of the 40 control subjects including those who had dementia from another cause.

Patients with abnormal PHA response and abnormal AMLR and deficient LIF production, often associated with Ab-NAFP, indicate immune deficiency, perhaps selective for prions. If so, this immune deficiency could be either a cause of or a result of the prions but, in any event, could explain the long lag phase observed between exposure and development of symptoms (up to 15 yr) in some cases of AD; i.e., similar to the lag phase in Creutzfeldt-Jakob disease. These autoantibodies are not caused by autoimmune disease, but rather a consequence thereof, since most household contacts (spouses, offspring, and so on) of such patients also have such antibodies (unpublished observations), presumably because of alteration of NAF membranes by a virus or toxin so these membranes are no longer recognized as self; and these Ab-NAFP household contacts are the best donors for dialyzable lymphocyte extract *(22)*.

The mechanism responsible for improvement in the subset responding to DLyE is unknown, but perhaps reflects the effect of DLyE on a virus that has homed to the brain (as does lymphocytic chorio-

Table 6
Four Subsets of Alzheimer's Disease

Group	No. of patients	Biologic marker	Effective medication
I	11	Decreased IAT	Pyrrolidones
II	9	Ab-NAFP; aberrant lymphocyte DNA synthesis	DLyE-TF
III	16	Antibodies to cerebral cortical neurons	Immuran (?)
IV	4	None	None (all tried)

meningitis virus *(42a)* in one strain of inbred rats but not others) (reviewed in M. Oldstone: The Rous Whipple Lecture American Association of Pathologists. Viral Diseases of the 24th Century, 1993); or one that crossreacts immunologically with brain proteins ("molecular mimicry") *(42b)*. The usual duration of benefits is about 4 wk with decline to baseline in 6 wk because of the limitations in the amount of lymphocytes and frequency of donations that may be obtained from a single donor (because of FDA regulations). The maximum amount provided material sufficient for 3 mo of therapy except in two cases where two household contacts had the antibodies described above. Response of one of them is described below.

Insofar as immuran immunosuppressive therapy is concerned in the group with antibodies to cerebral cells and aberrant DNA synthesis, the trial was too short to warrant conclusions. We hesitated to treat such patients with doses that would be optimal for autoimmune disease, especially since they could not speak understandably and would be unable to describe adverse effects, and since leucopenia occurred in two patients after only a month of therapy. Furthermore, high doses of prednisone may cause severe complications, and aged persons catabolize medications far less rapidly than younger, therefore the usual doses given systemic lupus patients could cause marked toxicity in these aged AD patients; therefore, corticosteroids were not used. A mild response was noted in two of the six patients treated for 6 wk with immuran. As noted above, clinical responses to immunosuppressive agents have been observed by Leszek in some AD patients but not others. The mechanism(s) whereby antineuronal antibodies are involved in pathogenesis may be similar to those observed by others in that they may produce memory dysfunction *(43)* in rats similar to that found in AD or by mechanisms similar to those described by Bradford et al., who found that antibodies in serum of AD patients can cause immunolysis of cholinergic terminals from rat's cerebral cortex *(44)*.

As noted in the Introduction, heterogeneity of AD, based on laboratory results without clinical differences, was first described by us in 1984 and confirmed by others. We found that at least four groups exist (Table 6). Based on laboratory abnormalities in three groups, two responded to pyrrolidones, and dialyzable lymphocyte extract by laboratory results and patients, respectively, produced dramatic laboratory and clinical responses. Patients in a third group probably would respond to the sort of therapy used in severe auto immune disease, such as lupus erythematosus. So far we have found no biologic markers for this fourth group.

With regard to Group I, it is of interest that the longer the therapy, the better the results. For example, after 6 wk, laboratory results improved in this subset. Clinical benefits were marginal but at 3 mo were definite. After that, patients relapsed to near baseline when given placebo for 3 mo. Those who received active agent in the first arm, then placebo, were back at baseline. After a subsequent 6 mo of therapy, improvement averaged 2 stages on our scale of a 12 stage total. Those who received placebo first and thereafter Piracetam (9 mo total) had a 2–3+ stage benefit (Table 7).

Again, those receiving DLyE enriched in TF every 6 wk improved for about 4 wk and descended back to baseline for about 2 wk; but with 12–18 mo of therapy, gradually improved, gaining 2–3 stages for 3–4 wk in most instances.

The data presented above indicates that AD is heterogeneous in etiology and/or pathogenesis as measured by different responses to different therapeutic agents. Whether the amyloid deposition in an AD brain represents a final common pathway is unknown in view of the failure of symptoms of AD to develop in Down's Syndrome despite identical pathological features to those of AD (e.g., *45*) However, it is of considerable interest that increased amyloid production in familial AD has been associated with amino acid substitutions determined by genes on chromosomes 14, 19, and 21 *(46)*, and with two different sites within one of these chromo-

Table 7
Effect of Therapy with (1) Pyrrolidones (a) Piracetam, (b) Antiracetam, (2) DLyE-TF, and (3) Immuran in the First Three Subsets of Alzheimer's Disease[a]

	(1) Pyrrolidones				
Month	(a) Piracetam	(b) Aniracetam	(2) DLyE-TF (H.C.)[b]	(3) Immuran	Nonresponders
3	1.2 ± 1.6	1.4 ± 1.8	0.6 ± 1.1	(2 mo) 0.6	–0.6
6	2.7 ± 1.4	2.8 ± 1.6	1.7 ± 1.9	ND	–0.8
9	3.6 ± 2.1	3.8 ± 2.3	2.6 ± 2.2	—	–1.8
12	3.9 ± 2.0	4.1 ± 2.4	2.9 ± 2.4	—	–1.8
15	4.2 ± 2.2	4.6 ± 2.2	3.2 ± 2.6	—	–2.8

[a]Figures indicate stages gained or, in nonresponders, lost at 3-mo intervals ranging from 3–15 mo on therapy.
[b]Household contact.

somes. Furthermore, the β-amyloid present in nonfamilial AD appears to differ in sequence from that in familial AD *(47)*. In any event, our data indicate that there are at least four different etiologies for nonfamilial AD. If a therapeutic agent that treats the final common pathway is discovered, this will simplify therapeutic approaches. However, the only agent to date that may do so is Tacrin *(48a)*, which appears to be hepatotoxic in many patients in doses necessary for clinical improvement. If final common pathway agents are not developed, then individualization of therapy for each patient will be necessary, as is the case of various types of anemia (e.g., iron deficiency, B_{12} deficiency, autoimmune). Our data suggest that the first generation agents are already available.

In any event, these results suggest that at least until late stages, neurons are not dead in AD but merely atrophic. To the best of my knowledge and that of a skilled neuropathologist whose primary interest is AD *(48b)*, histologic examinations have been performed only in postmortem patients and one biopsy of a stage 12 patient. Obviously, if function improves with immunologic or other therapies, the atrophy of the cerebral neurons is secondary to disuse as a result of defects in other cells.

The similarities between the immune system (IS) and the central nervous system (CNS) have been known for a decade (e.g., *see* ref. *32*) (e.g., microglia and astrocytes are functionally and antigenically identical to two of the four subsets of monocytes *[49,50]*). Furthermore, peripheral blood immune cells in AD have low binding of CRF *(51)*, implying an effect in number or function of receptors for this hormone; it is noteworthy that deficient CRF receptors are also present in AD brains *(52)*. The data also confirm that peripheral blood immunocytes can be used to measure functions of the corresponding CNS cells and that a correlation exists between severity of AD and severity of immunologic defect in the immunologic subset and in the interactive T-cells (when brain equivalent is probably a subset of astrocytes) *(11)*.

AD appears to be a regression from normal adult life to childhood, infancy, and eventually fetal life. The anterior, ventromedial parts of the temporal lobe are affected first, with other higher association cortices being affected subsequently and primarily motor and sensory regions being affected last, and often least (*see* ref. *53*); the layer II pre-alpha neurons of the transentorhinal region of the medial temporal lobe have recently been identified as the neurons first affected by tangle formation within the brain. Entorhinal neurons give rise to some of the fibers in the perforans pathway that innervate the hippocampus, an anatomical pathway involved in higher order cortical functions, particularly memory. Hence, malfunction may be caused not by neuronal loss but rather by a secondary disuse atrophy.

Moreover, it has been observed that in humans of 50+ yr, a phase of resprouting, dendritic growth, and renewed synaptogenesis occurs that may ameliorate some of the effects of age-related neuronal attrition. A similar resprouting process has been observed in AD *(53)*.

In another context, AD has been regarded as a disease of aging; however, we found evidence only of an "aged" brain, which in reality has caused gradual regression from adult life to infancy (loss of speech, loss of sphincter control, loss of ability to ambulate, and so on). Skin elasticity and other parameters (e.g., bone density) were normal for the age in the vast majority of subjects *(54)*. Indeed, in some AD patients, although various immune responses were less than in normal control subjects (age 25–35), they were far better than those in age-

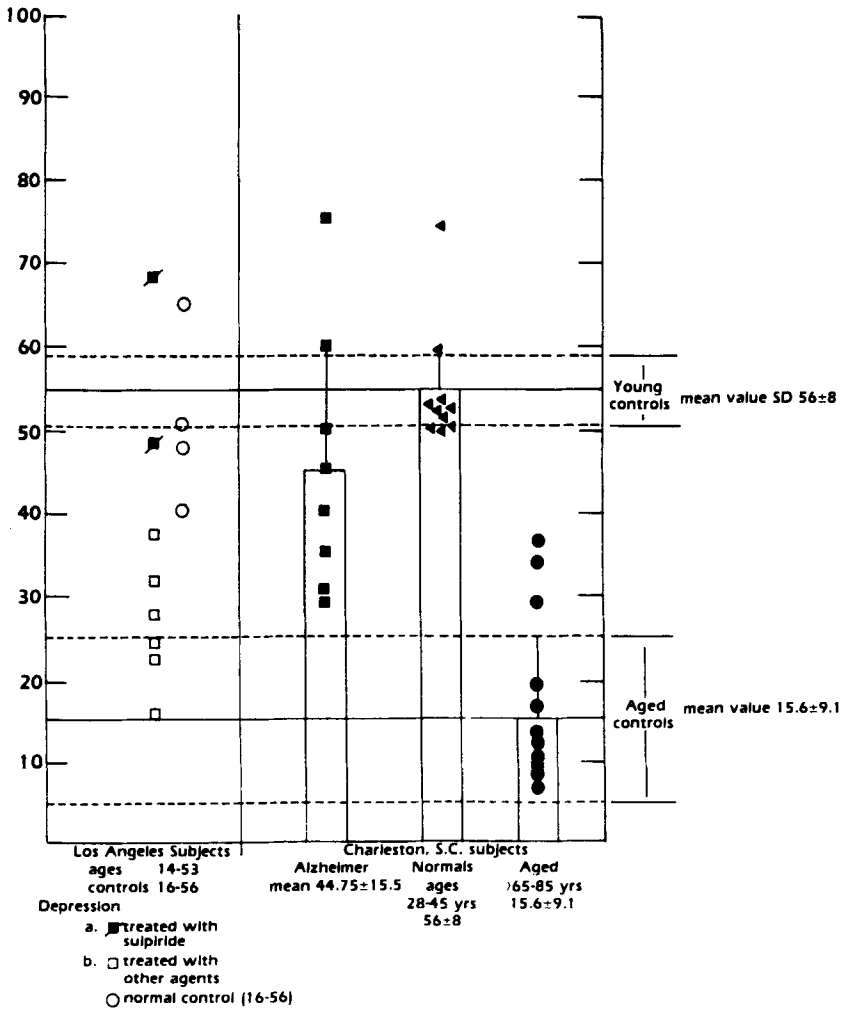

Fig. 10. Note for AD subjects percent T-cells positive for α_1 acid glycoprotein is closer to normal young people than to normal aged control.

matched controls *(17)* in some assays, but not in others (e.g., percent T-cells with surface orosomucoid) *(54)* (Fig. 10).

References

1. Yang B. Z., Li Z. L., Cui Z. Y., and Luo Y. M. (1991) *Int. J. Immunopharmacol.* **13,** 825–829.
2. Goust J. M., Chenais F., Carnes J. E., Hanes C. G., Fudenberg H. H., and Hogan E. L. (1978) *Neurology* **28,** 421; and unpublished observations.
3. Baoqun L., Jianyang X., and Chengguanliang (1990) in *Recent Advances in Transfer Factor and Dialyzable Leucocyte Extracts.* Maruzen, Tokyo, Japan, 339.

4a. Pekarek J., Cech K., Nevsimal O., Barnet K., Doutlik S., and Cervenkova O. (1990) in *Recent Advances in Transfer Factor and Dialyzable Leucocyte Extracts.* Maruzen,Tokyo, Japan, 326.

4b. Salier J. P., Goust J. M., Link H., Pandey J. P., Daveau M., and Fudenberg H. H. (1983) *J. Immunogenet.* **10,** 311.

5a. Fudenberg H. H., Coleman M., Rosenberger D., and Fudenberg H. L. (1989) *Clin. Res.* **37,** 556a (Abstract).

5b. Singh V. J. and Fudenberg H. H. (1988) *Ann. NY Acad. Sci.* **504,** 602.

6. Fudenberg H. H. (1992) in *The Clinical and Scientific Basis of Myalgic Enkephalitis/Chronic Fatigue,* Hyde B., ed., Ottawa U. Press, Ottawa, Canada, and Ogdensburg, NY, pp. 644–653.
7. Visa D., Pizza G., and Fudenberg H. H. (1994) in preparation.

8a. Fudenberg H. H., Whitten H. D., Arnaud P., and Khansari N. (1984) *Clin. Immunol. Imunopathol.* **32,** 127–131.

8b. Fudenberg H. H., Whitten H. D., Chou Y. K., Arnaud P., Shums A. A., and Khansari N. K. (1984) *Biomedicine and Pharmacotherapy* **38,** 285–290.

9. Kilidreas K., Latov L., Strauss D., Gorig A., Hashim

G., Gorman J., and Sadiq S. (1992) *Lancet* **ii,** 571–572.
10. Fudenberg H. H. and Pizza G. (1993) in *Progress in Drug Research.* Birkhuser, Basel, Switzerland.
11. Fudenberg H. H., Whitten H. D., Arnaud P., Khansari N., Tsand K. Y., and Hames C. G. (1984) *Biomed. Pharmacol.* **38,** 290–297.
12. Fudenberg H. H. and Singh V. (1988) *Drug Dev. Res.* **15,** 165–174.
13. Torack R. M. (1986) *Neurosci. Lett.* **71,** 365–369.
14. McRae-Degueurce A., Booj S., Haglid K., Rosengren L., Karlsson J. E., and Karlsson I. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9214–9218.
15. Miller C. A., Rudnicka M., Hinton D. R., Blanks J. C., and Kozlowski M. (1987) *Proc. Natl. Acad. Sci. USA* **84(23),** 8657–8661.
16. Reisberg B. and Ferris S. H. (1988) *Psychopharm. Bull.* **24(4),** 629–636.
17. Dirienzo W., Steffanini G. F., Miribel L., Paulling E. E., Canonica G. W., and Fudenberg H. H. (1986) *Cell. Immunol.* **103,** 65–72.
18. Nel A. E., Dirienzo W., Stefanini G. F., Wooten M. W., Canonica G. W., Latanze G. R., Stevenson H. C., Miller P., Fudenberg H. H., and Galbraith R. M. (1986) *Cell. Immunol.* **103,** 65–72.
19. Singh V. K. and Fudenberg H. H. (1987) *Clin. Immunol. Newslett.* **8,** 165–166.
20. Galbraith G. M. P. and Fudenberg H. H. (1984) *Clin. Immunol. Immunopathol.* **31,** 254–260.
21. Goust J. M. and Fudenberg H. H. (1983) *J. Immunol. Meth.* **59,** 29–38.
22. Fudenberg H. H. (1982), unpublished observations.
23. Fudenberg H. H. (submitted) Influence of per cent granulocytes and degree of donor-patient contact or clinical efficacy of transfer factor.
24. Fudenberg H. H. (1994) Florence Nightingale Disease (FND): A multisystem experiment of nature. Observations and speculations on etiology and pathogenesis, in press.
25. Fudenberg H. H. (1990) First International Symposium on Chronic Fatigue Syndrome, Los Angeles, CA.
26. Deleted in proof.
27. Fudenberg H. H. (1984) *In vitro* test for evaluation of possible therapeutic agents in Alzheimer's Disease. US Patent Office.
28. Gadjusek D. C. (1993) Plenary Lecture at Univ. Göteborg Int. Symposium on Neurodegenerative Diseases, Jamaica.
29. Galbraith G. M. P., Emerson D., Fudenburg H. H., Gibbs C. J., and Gadjusek D. C. (1986) *J. Clin. Invest.* **78,** 865–869.
30. Singh V. J. and Fudenberg H. H. (1989) *Autoimmunity* **3,** 945–1001.
31. Singh V. K., Fudenberg H. H., Emerson D., and Coleman M. (1988) *Ann. NY Acad. Sci.* **540,** 602–604.
32. Fudenberg H. H. and Singh V. K. (1988) in *Immunological Aspects of Alzheimer's Disease and Brain Amyloidosis.* IPSEN Foundation, Paris, publ.
33. Fudenberg H. H. and Singh V. J. (1988) *Progress in Drug Research,* **32,** 21–42.
34. Lundgren D. W. and Fales H. M. (1980) *J. Biol. Chem.* **217,** 2163; **255,** 4481–4486.
35. Lawson A. M. and Chalmers R. A. (1980) *Adv. Mass Spect.* **8B,** 1306–1313.
36. Leszek J. and Kowal-Gierczak B. (1989) *Psychiatr. Pol.* **23 (5–6),** 373–378.
37a. Leszek J. (1992) personal communication.
37b. Leszek J., Inglot A. D., Cantell K., and Wasik A. (1991) *Eur. J. Immunol.* 555–563.
38. Fudenberg H. H. and Gadjusek D. C. (1980) unpublished observations.
39. Hasse A. T., et al. (1986) *Can. J. Neurol.* **13 (Suppl. 4),** 449–450.
40a. Gadjusek, D. C. (1984) personal communication.
40b. Guiroy D. C. (1987) *Proc. Natl. Acad. Sci.* **7,** 2073.
41. Khansari N., Whitten H. D., Chou Y. K., and Fudenberg H. H. (1985) *J. Neuroimmunol.* **7,** 279–285.
42a. Laing P., Knight J. G., Hill J. M., Harris A. G., Oxford J. S., Webster R. G., Markwell M. A. K., Paul S. M., and Peri C. B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1998–2002.
42b. Oldstone M. (1993) *Am. J. Path.* in press.
43. Chapman J., Alroy G., Weiss Z., Faigon M., Feldon J., and Michaelson D. (1991) *Neuroscience* **40,** 297–305.
44. Bradford H. F., Foley P., Docherty M., Fillit H., Luine V. N., McEwen B., et al. (1988) *Can. J. Neurol. Sci.* **16,** 528–534.
45. Singh V. K. and Fudenberg H. H. (1987) in *Alzheimer's Disease: Advances in Basic Research and Therapies.* Wurtman R. J., Corkin S. H., and Growdon J. H., eds., pp. 597–602. Proceedings of the fourth Meeting of the International Study Group on the Pharmacology of Memory Disorder Associated with Aging. Zurich, Switzerland.
46. Marx J. (1992) *Science* **258,** 550.
47. Beyreuther K. (1993) in discussion at Göteborg Symposium on Neurodegenerative Disorders.
48a. Emerling M. (1993) in discussion at Göteborg Symposium on Neurodegenerative Disorders.
48b. Wisniewki H. (1993) personal communication at Göteborg Symposium on Neurodegenerative Disorders.
49. Khansari N. and Fudenberg H. H. (1984) *Scand. J. Immunol.* **19,** 337–342.
50. Chou Y. K., Stanley W., Virella G., Kilpatrick J. M., and Fudenberg H. H. (1987) *Immunobiol.* **174(4–5),** 444–459.
51. Singh V. K. and Fudenberg H. H. (1986) *J. Clin. Psychiat.* **47,** 592–595.
52. Whitehouse P. J., et al. (1987) *Neurology* **3,** 905–909.
53. Roberts G. W., Nash M., Ince P. G., Royston M. C., and Gentleman S. M. (1993) *Neuroreport* **4,** 7.
54. Fudenberg H. H. and Pitts F. (1985) unpublished observations.