Identification of Aldolase as a Target Antigen in Alzheimer's Disease

Felix Mor,[1]*† Marina Izak,*† and Irun R. Cohen[2]*

Alzheimer's disease (AD) is the most common human neurodegenerative disease, leading to progressive cognitive decline and eventually death. The prevailing paradigm on the pathogenesis of AD is that abnormally folded proteins accumulate in specific brain areas and lead to neuronal loss via apoptosis. In recent years it has become evident that an inflammatory and possibly autoimmune component exists in AD. Moreover, recent data demonstrate that immunization with amyloid-β peptide is therapeutically effective in AD. The nature of CNS Ags that are the target of immune attack in AD is unknown. To identify potential autoantigens in AD, we tested sera IgG Abs of AD patients in immunoblots against brain and other tissue lysates. We identified a 42-kDa band in brain lysates that was detected with >50% of 45 AD sera. The band was identified by mass spectrometry to be aldolase A. Western blotting with aldolase using patient sera demonstrated a band of identical size. The Ab reactivity was verified with ELISAs using aldolase. One of 25 elderly control patients and 3 of 30 multiple sclerosis patients showed similar reactivity ($p < 0.002$). In enzymatic assays, anti-aldolase positive sera were found to inhibit the enzyme's activity, and the presence of the substrate (fructose 1,6-diphosphate) enhanced Ab binding. Immunization of rats and mice with aldolase in complete Freund's adjuvant was not pathogenic. These findings reveal an autoimmune component in AD, point at aldolase as a common autoantigen in this disease, and suggest a new target for potential immune modulation. ***The Journal of Immunology,*** **2005, 175: 3439–3445.**

Alzheimer's disease (AD)[3] is the most common human neurodegenerative disease, leading to progressive decline in cognitive functions and death. As life expectancy increases, the incidence of AD and the health care expenditures for patients suffering from this incurable disease are increasing in Western societies. In the U.S., 1.9–4 million people suffer from AD, generating an annual cost of 141 billion dollars (1). AD combines with stroke as the third most common cause of death in the U.S.

The accepted paradigm for AD pathogenesis was that abnormally folded proteins (mainly amyloid-β and -τ) accumulate in the CNS and lead to progressive neuronal loss, particularly in the cortex and hippocampus (2); this manifests clinically as a relentless deterioration in cognitive functions (3). In recent years, it has become evident that local inflammation is important in AD (4–7) and in other neurodegenerative diseases such as Parkinson's disease (4) and prion disease (8). Pathologically, immune system components (Abs, complement, and T cells (9)) have been detected in the brains of AD patients. Recently, patients with AD were found to manifest T cell reactivity to amyloid-β peptide (10) and Abs to glial fibrillary acidic protein (GFAP) (11) and to other selected autoantigens (12). Other studies have detected Abs against uncharacterized Ag(s) in the choroid plexus (13), Abs to microglial cells (14), and Abs to myelin basic protein (15). Epidemiological studies have linked anti-inflammatory therapy to a lower incidence of AD (16). Moreover, recent work in animal models of AD have shown that the immune system can be recruited to clear amyloid deposits by active immunization to amyloid-β (17) or by passive transfer of Abs to amyloid-β (4).

These experimental results and the lack of any disease-modifying treatment for this devastating illness have prompted clinical trials of amyloid-β immunization in AD patients (18, 19). However, the human study of amyloid-β vaccination was stopped prematurely because some of the patients developed meningo-encephalitis, possibly as a result of the induction of autoimmunity (4, 18, 20). A recent study in mice reported autoimmune encephalomyelitis after immunization with amyloid-β peptide (21). Thus, we need to find new CNS Ags that can be used safely for immune intervention in patients with AD without risking autoimmune CNS attack. Immune mechanisms are part of the pathogenesis of AD and provide a window of opportunity in the design of therapy.

In the present study, we used immunoblotting with AD sera and identified aldolase A as a major autoantigen in AD patients. Immunization of autoimmune-susceptible Lewis rats with aldolase A did not lead to clinically or histologically detectable disease.

Materials and Methods

Patient selection

Forty-five patients with AD were randomly selected from the patients admitted to Internal Medicine A, Rabin Medical Center, Hasharon Hospital. All patients had dementia and were previously diagnosed as suffering from AD. Patients with multiple cerebrovascular accidents or Parkinson's disease were excluded. Control sera were obtained from age-matched elderly patients with preserved cognitive functions and from patients with multiple sclerosis (MS). Sera were obtained after informed consent from patients or from legal guardians.

Tissue isolation

Samples of tissues were homogenized using 1% Nonidet P-40 in 0.9% NaCl, 50 mM Tris, 1 mM EDTA, containing protease inhibitors (Sigma-Aldrich) (22). Brain, heart, liver, thymus, spinal cord, intestine, and fat tissue were homogenized with a tissue homogenizer in this buffer.

*Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel; and †Department of Medicine, Rabin Medical Center, Petach-Tiqva, affiliated with the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Received for publication March 4, 2005. Accepted for publication June 15, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Address correspondence and reprint requests to Dr. Felix Mor, Department of Immunology, The Weizmann Institute of Science, Rehovot, 76100, Israel. E-mail address: felix.mor@weizmann.ac.il

[2] I.R.C. is the incumbent of the Mauerberger Chair in Immunology and the Director of the Center for the Study of Emerging Diseases.

[3] Abbreviations used in this paper: AD, Alzheimer's disease; GFAP, glial fibrillary acidic protein; MS, multiple sclerosis.

0022-1767/05/$02.00

Antigens

Rabbit muscle aldolase and glutamic-oxaloacetic transaminase type I from porcine heart were purchased from Sigma-Aldrich. Purified human glial fibrillary acidic protein was purchased from Biodesign International. CFA was prepared with IFA, into which we added 4 mg/ml *Mycobacterium tuberculosis* H37Ra (Difco).

Western blotting

Cell suspensions of thymocytes or tissue homogenates were lysed in lysis buffer. The protein concentration was determined using the Bio-Rad Dc protein assay, based on the Lowry method (Bio-Rad Laboratories). From cell and tissue lysates, 50 μg of protein were loaded in each well. After electrophoresis in 12% SDS gel in a minigel apparatus (Bio-Rad), the gels were electrotransferred to nitrocellulose membranes (Schleicher & Schüll) (22).

The nitrocellulose membranes were washed with distilled water for 5 min and then blocked for 60 min using a blocking solution composed of 2% milk powder (blotting grade blocker, nonfat dry milk; Bio-Rad) in PBS. After 3 $\times$ 10-min washes in PBS/0.05% Tween 20 (Sigma-Aldrich), sera (diluted 1/1000) were incubated with the membranes in blocking solution for 60 min. After another series of washes in PBS/0.05% Tween 20 (3 $\times$ 10 min), the membranes were incubated with a secondary Ab (peroxidase-conjugated goat anti-human IgG H+L; Jackson ImmunoResearch) at a 1/2500 dilution in blocking solution for 60 min. After another 3 $\times$ 10-min washes, the membranes were incubated with the ECL reagent for 2 min and were exposed to x-ray film for 15–90 s. For size determination, we used prestained, broad-range protein standard markers (Fermentas).

Q Sepharose ion exchange chromatography

A brain lysate was obtained from Lewis rats. A free-flow column was packed to a volume calculated as a ratio of 10 mg of protein per 1 ml of Q Sepharose free-flow beads (Pharmacia). After equilibration of the column with 3 volumes of 20 mM Tris-HCl, the lysate was loaded and the flow-through fraction was collected. The proteins bound to the Q Sepharose column were eluted with increasing concentrations of NaCl (0.1–2.0 M). The different fractions were run on SDS-PAGE, and the protein bands were examined in Western blots. The 42-kDa band was found in the flow-through fraction. The enriched fraction was lyophilized, run in gel, and subjected to enzymatic digestion and mass spectrometry (22).

In gel digestion and mass spectrometry

The identified band was excised from the gel and subjected to trypsin digestion, and peptide fragments were analyzed by the Mass Spectrometry Unit of the Weizmann Institute using a MALDI mass spectrometer. Analysis was performed with the Bruker REFLEX reflector time-of-flight instrument with SCOUT multiprobe (384) inlet and grindless delayed extraction ion source. The protein was identified using the ProFound program (version 4.10.5; The Rockefeller University Edition).

Enzyme inhibition assay

Aldolase activity was measured after incubation of the enzyme with fructose 1,6-diphosphate and hydrazine (Sigma-Aldrich) (23). The test serum was first incubated with enzyme for 1 h at 37°C, and then the mixture was added to the hydrazine solution. The substrate, at concentrations from 12 mM to 16 μM, was added immediately before the start of readings. The product was measured by the change in absorbance at 240 nm in a PowerWave microplate spectrophotometer (Bio-Tek Instruments). Change in absorbance was measured every 6 s for several minutes in microtiter wells. Control assays were performed without serum or with control serum.

Animal immunization

Inbred female Lewis rats and mice were supplied from the Weizmann Institute animal breeding center and were used at 2–3 mo of age. The experiments were approved by the Animal Welfare Committee. Groups of eight female Lewis rats were immunized in both hind footpads with 50 μg of rabbit muscle aldolase A (Sigma-Aldrich) in CFA. The rats were observed for signs of disease from day 10 postimmunization. Twenty-five days after immunization, the rats were sacrificed and their tissues were studied by histological examination. The tissues (heart, muscle, liver, cerebrum, cerebellum, and spinal cord) were embedded in paraffin, and sections were stained with H&E. Groups of six female NOD and six female C57BL mice were injected s.c. with aldolase in CFA (50 μg/mouse). On the day of immunization and after 48 h, the mice received 200 ng of pertussis toxin i.p. The immunized mice were scored for clinical signs for 3 wk, and then they were sacrificed and tissues were analyzed for histological lesions.

Aldolase T cell line

Popliteal lymph node cells from rats immunized 25 days previously with aldolase in CFA were stimulated in vitro with aldolase (10 μg/ml) in stimulation medium, as described (24). The T cell line was expanded by stimulation every 10–12 days with Ag and irradiated thymocytes as APCs (25). Lewis rats were injected with 2×10^7 T cell line blasts, and were clinically scored for 2 wk after inoculation. Tissues were examined by histology 10 days after inoculation.

ELISAs

ELISA microtiter plates (Maxisorp, Nunc) were coated with Ags (rabbit muscle aldolase or as a control enzyme of similar molecular size, 10 μg/ml pig heart glutamic-oxaloacetic transaminase in 0.1 M carbonate buffer) for 1 h at 37°C. Next, blocking was done with 2% low-fat milk powder (Bio-Rad) for 1 h at 37°C. After washing with PBS/Tween, the sera were diluted 1/70 in 2% milk and incubated for 1 h at 37°C. After three washes (0.05% Tween 20 in PBS), a secondary Ab anti-human IgG coupled to alkaline phosphatase diluted 1/2000 in 2% milk was applied for 1 h at 37°C. In the next step, the wells were washed with 0.05% Tween 20 in PBS and incubated with an alkaline phosphatase substrate tablet (Sigma-Aldrich) in diethanolamine buffer for 20–30 min. The OD was read using an ELISA reader at 405 nm. Sera were considered positive if the OD value exceeded the mean + 2SD of control sera: mean $\pm$ SD; OD of control sera was 0.26 $\pm$ 0.075. An OD >0.41 indicated a positive result.

ELISA with addition of fructose 1,6-diphosphate

To test the effect of the enzyme substrate on the binding of Ab to aldolase, we coated test wells with aldolase in fructose 1,6-diphosphate (12 mM) in 0.1 M Tris, pH 7.3, or in 0.1 M Tris without fructose. Blocking, sera dilution, and secondary Ab were done in 2% milk (Bio-Rad) as above. Sera from AD patients were diluted 1/50 and 1/250.

Results

AD serum IgG reacts to a 42-kDa band

Lysates of rat tissues were run in PAGE, followed by transfer to nitrocellulose paper. To identify IgG Abs to tissue components, we analyzed the serum of a typical patient with AD. The serum was tested in immunoblot against a panel of rat tissues (Fig. 1). A 42-kDa band was seen in extracts of brain, heart, thymus, spinal cord, and fat, and was absent from liver and intestine.

Forty-four additional sera from AD patients were tested in immunoblot against brain and heart lysates. Twenty-two additional patients were found to be strongly positive for reactivity to the 42-kDa band. A similar analysis of 20 healthy blood donors and 25 elderly patients without dementia were negative for Abs to the 42-kDa band in Western blots (data not shown). We could not correlate the presence of the 42-kDa band with any specific clinical manifestation or severity of AD.

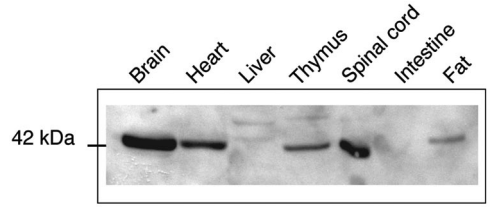

FIGURE 1. Tissue expression of the 42-kDa band. Tissue lysates (50 μg of protein) were separated on 12% SDS-PAGE and electro-transferred to nitrocellulose membranes. After incubation with the test Alzheimer's disease serum (1/1000), the membranes were incubated with secondary HRP anti-human IgG, and the Ab binding was measured using the ECL method.

Table I. *Mass spectrometry of the 42-kDa band*

Measured Mass (M)	Computed Mass	Error (%)	Residues	Sequence
1051.562	1051.577	−0.001	14–22	KELADIAHR
1064.502	1064.504	−0.000	141–149	DGADFAKWR
1106.562	1106.571	−0.001	323–331	AAQEEYIKR
1129.602	1129.624	−0.002	313–322	AWGGKKENLK
1192.592	1192.599	−0.001	140–149	KDGADFAKWR
1341.682	1341.703	−0.002	88–99	ADDGRPFPQVIK
1487.762	1487.794	−0.002	29–43	GILAADESTGSIAKR
1590.852	1590.836	0.001	319–331	ENLKAAQEEYIKR
1645.812	1645.801	0.001	43–56	RLQSIGTENTEENR
1645.812	1645.801	0.001	44–57	LQSIGTENTEENRR
1651.872	1651.838	0.002	1–14	MPHPYPALTPEQKK
1651.872	1651.823	0.003	244–258	FSNEEIAMATVTALR
1801.932	1801.902	0.002	43–57	RLQSIGTENTEENRR
1807.982	1807.944	0.002	290–304	CPLLKPWALTFSYGR
1823.952	1823.919	0.002	244–259	FSNEEIAMATVTALRR
2053.252	2053.152	0.005	23–43	IVAPGKGILAADESTGSIAKR
2122.212	2122.083	0.006	154–173	IGEHTPSSLAIMENANVLAR
2257.092	2257.028	0.003	343–364	YTPSGQSGAAASESLFISNHAY

The 42-kDa band is an autoantigen

We tested human tissues to verify that the Ab detected to the rat tissue Ag was an autoantibody. The band was clearly present in human brain lysate and was absent from human liver and skin (data not shown). Thus, the pattern of tissue reactivity was similar for rat and human tissues, indicating that the IgG Ab detected was an autoantibody.

The 42-kDa band is aldolase A

To identify the 42-kDa Ag, we used the lysate of rat brain that showed the highest level of expression of the Ag. The lysate was subjected to anion exchange chromatography and the 42-kDa protein was found not to bind to quaternary ammonium (mono Q Sepharose). The band was then collected from the flow-through and lyophilized. In the next step, the lyophilized material was separated by PAGE. The 42-kDa band was excised from the gel and was subjected to in-gel digestion and mass spectrometry. The mass spectrometry detected 18 different peptides that together covered 39% of the rat aldolase A sequence (Table I; Fig. 2). The protein was identified by searching a comprehensive, nonredundant protein database using the ProFound program (version 4.10.5; The Rockefeller University Edition). This finding identified the protein to be rat aldolase A with a high degree of probability (probability 1.0e + 000; Z score, 2.33). To confirm that the serum reacted to intact aldolase A, we used purified rabbit muscle aldolase A. Various amounts of the rabbit aldolase were run in a gel in parallel to the brain lysate. As can be seen in Fig. 3, the AD serum stained a band of identical size in both the aldolase and tissue preparations.

AD sera react to aldolase in ELISA

To simplify the assay for Ab reactivity to aldolase, we tested 45 AD patient sera, 25 control elders, and 30 patients with MS to rabbit aldolase in the ELISA. We coated the wells with 1 μg of aldolase and incubated the sera (diluted 1–70 in 2% nonfat milk (Bio-Rad) in PBS. We found that 23 of the 45 AD patients showed positive reactivity to aldolase (Fig. 4). There was a strong correlation between positivity in Western blot (42-kDa band) and positive ELISA reactivity to aldolase. Among the control elders, only 1 of 25 patients showed low aldolase reactivity (Fig. 4), and 2 of 30 MS patients showed low positive reactivity (data not shown). The differences in reactivities between the AD and control groups were highly significant (mean ± SD; AD patients, 0.719 ± 0.706; control elderly subjects, 0.26 ± 0.075; MS patients, 0.33 ± 0.146; 2-tailed *t* test, *p* value of AD vs MS was 0.00132; AD vs elder controls was 0.00121).

Anti-aldolase positive serum inhibits enzyme activity

We examined the effects of anti-aldolase Abs on aldolase activity using a previously described method (23). The reciprocal enzyme V0 was calculated and plotted against the reciprocal of the substrate concentration (Lineweaver-Burke plot), and the change in K_m was calculated. As shown in Fig. 5, sera from AD patients inhibited aldolase enzyme activity, causing an increase in K_m from 0.435 mM to 0.869 mM (with serum from AD7) and 1.239 (with serum from AD45). This increase in K_m is compatible with competitive inhibition of enzyme activity (26). A similar phenomenon

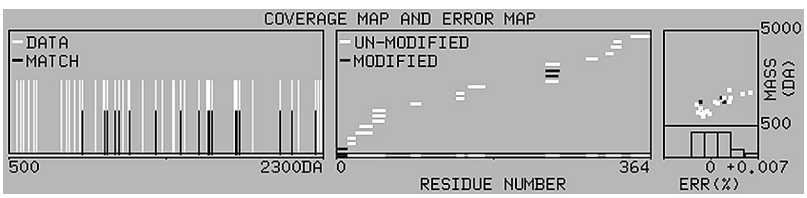

FIGURE 2. Coverage map and error map of peptides detected by mass spectrometry. The 18 peptides comprised 39% of the rat aldolase A sequence. The *left panel* shows the peptides identified in mass spectrometry (in black) relative to the potential peptides from aldolase A. The *middle panel* shows the positions of the peptides identified relative to the protein sequence. The *right panel* shows the relation between the percentage error (the difference between measured molecular peptide mass to the calculated peptide mass divided by peptide mass, expressed in percentage) to the peptide mass. Each dot represents a peptide identified.

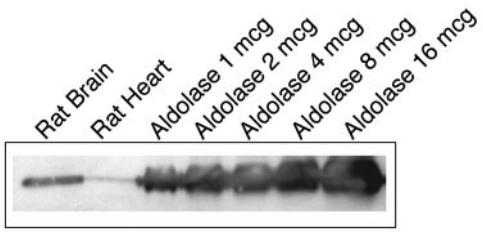

FIGURE 3. Western blot of aldolase with AD serum. Rat brain, rat heart, and rabbit muscle aldolase A (1–16 μg) were run in parallel and tested in immunoblot with a positive AD serum.

of inhibition of enzymes by autoantibodies to transglutaminase and mitochondrial M2 has previously been described (27, 28).

Enhancement of Ab binding to aldolase by added substrate

To test the effect of the fructose 1,6-diphosphate substrate on Ab binding to aldolase, we performed the ELISA coating the enzyme in the substrate solution. Fig. 6 shows that in all AD sera tested, the binding of anti-aldolase Ab was significantly enhanced by the addition of the substrate. Similar analysis of Ab binding in elderly controls did not show significant differences with relation to the presence of fructose 1,6 diphosphate. Crystallography studies have shown a significant conformational change in aldolase after binding fructose 1,6-diphosphate (29). Thus, the AD Abs may actually target the aldolase conformation induced by the binding of fructose 1,6 diphosphate. This finding can be interpreted to suggest that the in vivo immune response to the enzyme was driven by the enzyme-substrate complex.

Testing for pathogenicity of immunization

To test whether induction of autoimmunity to aldolase might be pathogenic, we immunized Lewis rats with rabbit aldolase in CFA. Lewis rats are known to be susceptible to several experimental autoimmune diseases: adjuvant arthritis (30), experimental autoimmune encephalomyelitis (31), myocarditis (32), uveitis (20), and others (33). The immunized rats did not manifest clinical signs of muscle weakness or paralysis. Histological examination of various tissues (heart, muscle, liver, cerebrum, cerebellum, spinal cord) showed normal organ architecture without inflammation. T cell proliferation from the immunized rats showed Ag-specific reactivity to aldolase A (data not shown). Moreover, immunoblot of rat tissue lysates with serum from aldolase-immunized rat detected a band of identical size (Fig. 7) to that detected with the AD serum. It is known that CNS pathology, absent in active autoimmunization, can be mediated by activated T cell lines (34). However, an anti-aldolase T cell line of the Th1 phenotype did not cause disease upon adoptive transfer (data not shown). Histological analysis of brain, spinal cord, muscle, and heart of the rats after inoculation with the aldolase-specific T cell line did not show signs of inflammation.

To further test the pathogenic potential of aldolase immunization, we inoculated six NOD and six C57BL mice with aldolase in CFA (50 μg/mouse, with 200 ng of pertussis toxin) injected i.p. on the day of immunization and after 48 h. NOD mice are known to spontaneously develop type I diabetes and thyroiditis. Moreover, both NOD (35) and C57BL (36) mice are susceptible to experimental autoimmune encephalomyelitis. None of the mice developed clinical signs of CNS or muscle disease, and histological analysis of brain, spinal cord, muscle, and heart did not reveal signs of inflammation.

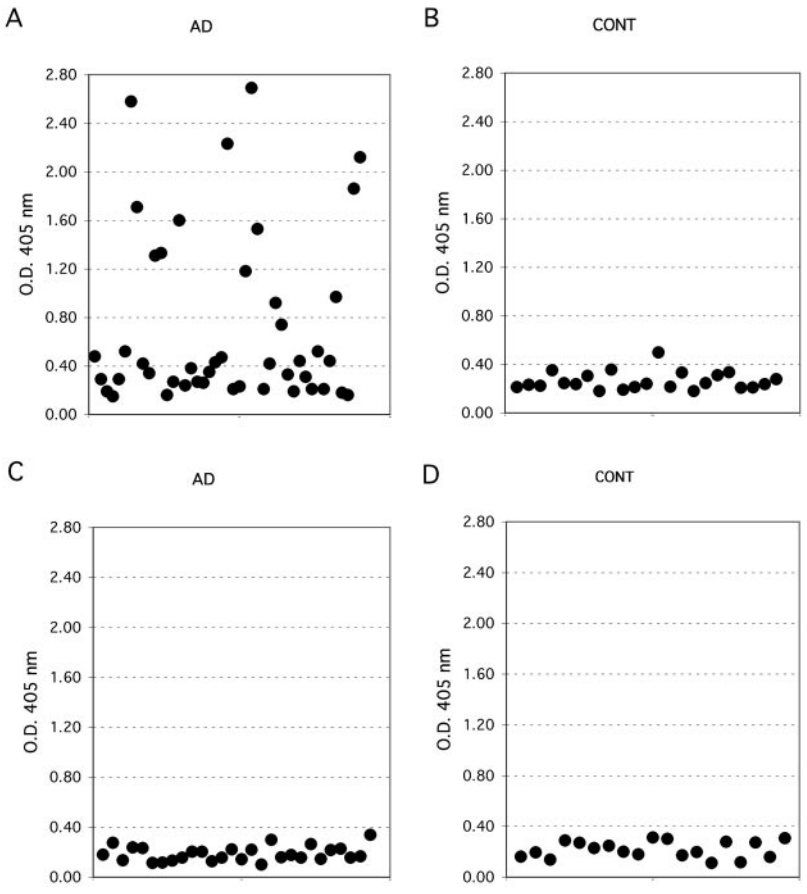

FIGURE 4. ELISA of aldolase reactive IgG Abs (*A* and *B*) and glutamic-oxaloacetic transaminase (*C* and *D*). Each dot represents a single patient serum. Forty-five sera were tested for AD, 23 from elderly controls. In the control antigen experiment (*C* and *D*), 28 AD and 19 controls were tested. Control patients were elderly persons without clinical evidence of dementia.

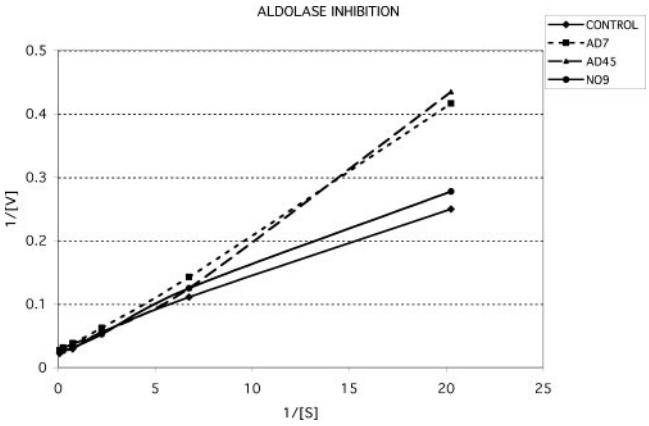

FIGURE 5. Inhibition of aldolase activity by AD serum. Aldolase activity was examined in several concentrations of fructose 1,6-diphosphate (12 mM to 16 μM) in the presence of AD serum (final dilution 1/140) or control serum. The reciprocal of V0 was plotted against the reciprocal of substrate concentration (Lineweaver-Burk plot).

Discussion

Current concepts regarding the pathogenesis of AD include the participation of the immune system (4). In contrast with the dominant role of the adaptive immune system in MS, the major players that are considered to be operative in AD belong to the innate immune system (4, 37, 38). Previous efforts to detect the involvement of the adaptive immune system in the pathogenesis of AD included the search for autoantibodies found in other autoimmune diseases (39), as well as attempts to identify novel reactivities (11, 15, 40–47). These studies sought Abs to molecules linked to AD pathogenesis, such as amyloid-β peptide (42, 46–48) or GFAP (11, 40).

Our approach to the analysis of autoantibodies in patients with AD was based on an unbiased Western blot methodology that exploited the immunoglobulins present in AD serum; the blots detected an autoantigen that was subsequently identified by mass spectrometry to be aldolase A. The Abs to aldolase were specific for AD sera. We tested the IgG reactivity of our patient and control groups to GFAP (data not shown). The reactivity to GFAP was not different in our AD patient group from the control elderly patients and MS patients (mean OD AD, 0.42 $\pm$ 0.25; control elderly subjects, 0.35 $\pm$ 0.16; MS, 0.42 $\pm$ 0.20). We previously used this

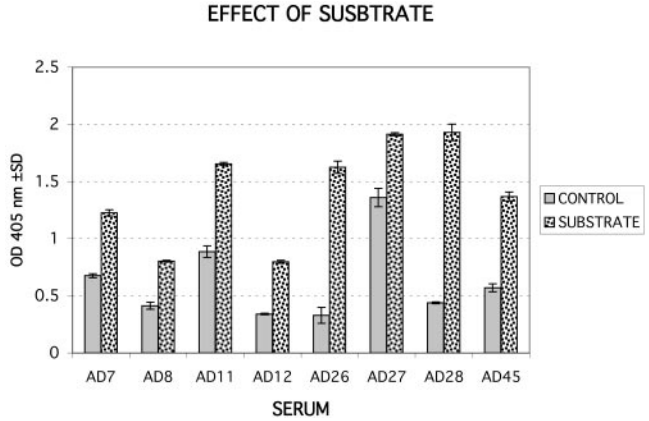

FIGURE 6. Enhancement of IgG Ab binding by addition of fructose 1,6-diphosphate. ELISA of binding to aldolase was performed in the presence or absence of fructose 1,6-diphosphate. In all dilutions the presence of substrate augmented Ab binding for all AD sera tested. The results depicted are from a serum dilution of 1/250.

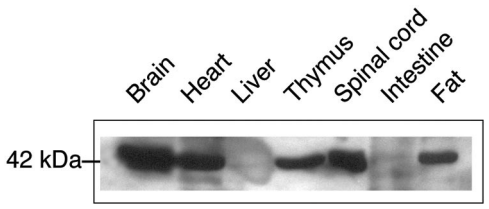

FIGURE 7. Western blot analysis of rat tissue extracts probed with day 25 aldolase/CFA immunized serum. Serum was diluted 1/1000. Aldolase-immunized rat serum IgG reactivity was similar to the AD serum IgG reactivity (Figure 1).

methodology to identify α-tropomyosin as an autoantigen in Behçet's syndrome (22).

The detection of aldolase Abs in only 50% of AD patients can have several explanations. First, the clinical diagnosis of AD is not definite and is made by exclusion of other dementing diseases; a definite diagnosis can only be made at autopsy (2). Thus, some of the patients negative for aldolase might have dementia caused by other diseases. Although the sensitivity of clinical AD diagnostic criteria is high (81%, range 49–100%) (49), mistakes in the diagnosis of AD based on clinical criteria are well known (50). The clinical criteria for diagnosing vascular dementia, frontotemporal dementia, and dementia with Lewy bodies are inaccurate (49). Thus, determination of the prevalence of aldolase reactivity in AD awaits a study of sera from pathologically proven AD. Second, it is possible that the AD population is heterogeneous immunologically. It is interesting to note that other studies that identified Abs to spectrin, amyloid-β, and GFAP in AD patients showed a proportion of positive patients similar to ours: ~50% (40, 44, 47). Third, we studied Ab reactivity at a single time point; it is possible that some of the negative patients were previously positive and may have lost reactivity as part of the determinant spreading mechanism operative in autoimmune diseases (51).

What might we learn from the discovery of aldolase as an immune target? Aldolase is a glycolytic enzyme catalyzing a reaction that converts fructose 1,6 biphosphate to glyceraldehyde 3 phosphate and dihydroxyacetone phosphate. Aldolase is conserved in evolution and exists in three types: type A is the major form found in muscle; type B in liver and kidney; and type C in brain together with type A (52). The enzyme is a tetramer of 40-kDa subunits. In SDS gels, the monomer migrates as a 42-kDa band (52). Enzymes have previously been reported as autoantigens in organ-specific autoimmune diseases: glutamic acid decarboxylase in type I diabetes mellitus (53); transglutaminase in celiac disease (54); pyruvate dehydrogenase in primary biliary cirrhosis (55); and thyroid peroxidase in autoimmune hypothyroidism (56). The functional role in disease pathogenesis of autoantibodies to enzymes is yet unclear.

One of the early findings in AD was reduced brain glucose use (57), and the activity of glycolytic enzymes was found to be reduced in the brains of AD patients (58). More recent work, however, has failed to document a reduction in the activity of these enzymes (59). Because reduced use of glucose is well documented in AD (60), it is tempting to speculate that the aldolase autoantibodies detected here might enter brain cells, bind the enzyme, and hinder its function, leading to reduced glucose use. Previous work has shown that some autoantibodies can enter living cells and have pathogenic consequences (61, 62).

What is the pathogenetic meaning of aldolase Abs? There are several possibilities. 1) The anti-aldolase Abs could represent an epiphenomenon—a result rather than a cause. For example, the toxic accumulation of amyloid and τ proteins leads to apoptosis,

and an innate inflammatory reaction causing cell damage, enhanced permeability of the blood-brain barrier (63), and release of cell contents including aldolase could result in autoimmunization. If the production of aldolase Abs is secondary to brain destruction, manipulation of the immune response to aldolase would be devoid of any therapeutic effects. 2) The anti-aldolase Abs could be a primary autoimmune factor in AD. These Abs could have pathogenic effects by inhibiting energy production and glucose use. Aldolase could represent one of several autoantigens participating in the perpetuation of autoimmune inflammation. In this case, the induction of tolerance to aldolase might help down-regulate damaging inflammation. 3) Similar to the anti-amyloid-β peptide Abs (47), anti-aldolase Abs might be found to help clear plaques and so represent a beneficial, protective immune reaction. In this regard, it is encouraging that Lewis rats and NOD and C57BL mice immunized against aldolase A did not develop signs of autoimmune disease; thus, aldolase autoimmunity might be relatively safer than autoimmunity induced to β amyloid (4, 18). Future studies will discriminate between those possibilities. Our work suggests that the presence of aldolase Abs in animal models of AD should be tested as well as the therapeutic effects of aldolase immunization in these mice.

Acknowledgments

We thank Dr. Dan Tawfik for his help and advice with the enzymatic assays.

Disclosures

F. Mor, M. Izak, and I. R. Cohen are the authors of patent application number 60-637,869 on the use of aldolase immunity in the diagnosis and treatment of Alzheimer's disease, issued to the Weizmann Institute of Science.

References

1. Clark, C. M., and J. H. T. Karlawish. 2003. Alzheimer disease: current concepts and emerging diagnostic and therapeutic strategies. *Ann. Intern. Med.* 138: 400–410.
2. Nussbaum, R. L., and C. E. Ellis. 2003. Alzheimer's disease and Parkinson's disease. *N. Engl. J. Med.* 348: 1356–1364.
3. Johnson, W. G. 2000. Late-onset neurodegenerative diseases—the role of protein insolubility. *J. Anat.* 196: 609–616.
4. Weiner, H. L., and D. J. Selkoe. 2002. Inflammation and therapeutic vaccination in CNS diseases. *Nature* 420: 879–884.
5. Hulette, C. M., and R. L. Walford. 1987. Immunological aspects of Alzheimer disease: a review. *Alzheimer Dis. Assoc. Disord.* 1: 72–82.
6. Amaducci, L., M. Falcini, and A. Lippi. 1992. Humoral and cellular immunologic repertoire in Alzheimer's disease. *Ann. NY Acad. Sci.* 663: 349–356.
7. Chorsky, R. L., F. Yaghmai, W. D. Hill, and E. G. Stopa. 2001. Alzheimer's disease: a review concerning immune response and microischemia. *Med. Hypotheses* 56: 124–127.
8. Betmouni, S., V. H. Perry, and J. L. Gordon. 1996. Evidence for an early inflammatory response in the central nervous system of mice with scrapie. *Neuroscience* 74: 1–5.
9. McGeer, P. L., J. Rogers, and E. G. McGeer. 1994. Neuroimmune mechanisms in Alzheimer disease pathogenesis. *Alzheimer Dis. Assoc. Disord.* 8: 149–158.
10. Monsonego, A., V. Zota, A. Karni, J. I. Krieger, A. Bar-Or, G. Bitan, A. E. Budson, R. Sperling, D. J. Selkoe, and H. L. Weiner. 2003. Increased T cell reactivity to amyloid β protein in older humans and patients with Alzheimer disease. *J. Clin. Invest.* 112: 415–422.
11. Mecocci, P., L. Parnetti, G. Romano, A. Scarelli, F. Chionne, R. Cecchetti, M. C. Polidori, B. Palumbo, A. Cherubini, and U. Senin. 1995. Serum anti-GFAP and anti-S100 autoantibodies in brain aging, Alzheimer's disease and vascular dementia. *J. Neuroimmunol.* 57: 165–170.
12. Ounanian, A., B. Guilbert, J. C. Renversez, J. M. Seigneurin, and S. Avrameas. 1990. Antibodies to viral antigens, xenoantigens, and autoantigens in Alzheimer's disease. *J. Clin. Lab. Anal.* 4: 367–375.
13. Serot, J. M., M. C. Bene, B. Gobert, D. Christmann, B. Leheup, and G. C. Faure. 1992. Antibodies to choroid plexus in senile dementia of Alzheimer's type. *J. Clin. Pathol.* 45: 781–783.
14. Dahlstrom, A., A. McRae, R. Polinsky, L. Nee, B. Sadasivan, and E. A. Ling. 1994. Alzheimer's disease cerebrospinal fluid antibodies display selectivity for microglia: investigations with cell cultures and human cortical biopsies. *Mol. Neurobiol.* 9: 41–54.
15. Singh, V. K., Y. Y. Yang, and E. A. Singh. 1992. Immunoblot detection of antibodies to myelin basic protein in Alzheimer's disease patients. *Neurosci. Lett.* 147: 25–28.
16. Etminan, M., S. Gill, and A. Samii. 2003. Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. *BMJ* 327: 128.
17. Schenk, D., R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, et al. 1999. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400: 173–177.
18. Dodel, R. C., H. Hampel, and Y. Du. 2003. Immunotherapy for Alzheimer's disease. *Lancet Neurol.* 2: 215–220.
19. Monsonego, A., and H. L. Weiner. 2003. Immunotherapeutic approaches to Alzheimer's disease. *Science* 302: 834–838.
20. Mor, F., F. Quintana, A. Mimran, and I. R. Cohen. 2003. Autoimmune encephalomyelitis and uveitis induced by T cell immunity to self β-synuclein. *J. Immunol.* 170: 628–634.
21. Furlan, R., E. Brambilla, F. Sanvito, L. Roccatagliata, S. Olivieri, A. Bergami, S. Pluchino, A. Uccelli, G. Comi, and G. Martino. 2003. Vaccination with amyloid-β peptide induces autoimmune encephalomyelitis in C57/BL6 mice. *Brain* 126: 285–291.
22. Mor, F., A. Weinberger, and I. R. Cohen. 2002. Identification of α-tropomyosin as a target self-antigen in Behcet's syndrome. *Eur. J. Immunol.* 32: 356–365.
23. Jagannathan, V., K. Singh, and M. Damodaran. 1956. Carbohydrate metabolism in citric acid fermentation. 4. Purification and properties of aldolase from *Aspergillus niger*. *Biochem. J.* 63: 94–105.
24. Mor, F., and I. R. Cohen. 1995. Pathogenicity of T cells responsive to diverse cryptic epitopes of myelin basic protein in the Lewis rat. *J. Immunol.* 155: 3693–3699.
25. Mor, F., and I. R. Cohen. 1993. Shifts in the epitopes of myelin basic protein recognized by Lewis rat T cells before, during, and after the induction of experimental autoimmune encephalomyelitis. *J. Clin. Invest.* 92: 2199–2206.
26. Duggleby, R. G., and R. B. Clarke. 1991. Experimental designs for estimating the parameters of the Michaelis-Menten equation from progress curves of enzyme-catalyzed reactions. *Biochim. Biophys. Acta* 1080: 231–236.
27. Esposito, C., F. Paparo, I. Caputo, M. Rossi, M. Maglio, D. Sblattero, T. Not, R. Porta, S. Auricchio, R. Marzari, and R. Troncone. 2002. Anti-tissue transglutaminase antibodies from coeliac patients inhibit transglutaminase activity both in vitro and in situ. *Gut* 51: 177–181.
28. Schmit, P., G. Gilson, and R. L. Humbel. 1999. Evaluation of an automated enzyme inhibition assay for the detection of anti-mitochondrial M2 autoantibodies. *Clin. Chem.* 45: 2287–2289.
29. Dalby, A., Z. Dauter, and J. A. Littlechild. 1999. Crystal structure of human muscle aldolase complexed with fructose 1,6-bisphosphate: mechanistic implications. *Protein Sci.* 8: 291–297.
30. Quintana, F. J., P. Carmi, F. Mor, and I. R. Cohen. 2002. Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J. Immunol.* 169: 3422–3428.
31. Mor, F., and I. R. Cohen. 1992. T cells in the lesion of experimental autoimmune encephalomyelitis: enrichment for reactivities to myelin basic protein and to heat shock proteins. *J. Clin. Invest.* 90: 2447–2455.
32. Li, Y., J. S. Heuser, S. D. Kosanke, M. Hemric, and M. W. Cunningham. 2004. Cryptic epitope identified in rat and human cardiac myosin S2 region induces myocarditis in the Lewis rat. *J. Immunol.* 172: 3225–3234.
33. Verhagen, C., F. Mor, J. B. Kipp, A. F. de Vos, R. van der Gaag, and I. R. Cohen. 1999. Experimental autoimmune keratitis induced in rats by anti-cornea T-cell lines. *Invest. Ophthalmol. Vis. Sci.* 40: 2191–2198.
34. Mor, F., M. Kantorowitz, and I. R. Cohen. 1996. The dominant and the cryptic T cell repertoire to myelin basic protein in the Lewis rat. *J. Neurosci. Res.* 45: 670–679.
35. Mars, L. T., V. Laloux, K. Goude, S. Desbois, A. Saoudi, L. Van Kaer, H. Lassmann, A. Herbelin, A. Lehuen, and R. S. Liblau. 2002. Cutting edge: Vα14-Jα281 NKT cells naturally regulate experimental autoimmune encephalomyelitis in nonobese diabetic mice. *J. Immunol.* 168: 6007–6011.
36. Mendel, I., N. Kerlero de Rosbo, and A. Ben-Nun. 1995. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor Vβ expression of encephalitogenic T cells. *Eur. J. Immunol.* 25: 1951.
37. Fassbender, K., S. Walter, S. Kuhl, R. Landmann, K. Ishii, T. Bertsch, A. K. Stalder, F. Muehlhauser, Y. Liu, A. J. Ulmer, et al. 2004. The LPS receptor (CD14) links innate immunity with Alzheimer's disease. *FASEB J.* 18: 203–205.
38. McGeer, P. L., and E. G. McGeer. 2001. Inflammation, autotoxicity and Alzheimer disease. *Neurobiol. Aging* 22: 799–809.
39. Lopez, O. L., B. S. Rabin, F. J. Huff, D. Rezek, and O. M. Reinmuth. 1992. Serum autoantibodies in patients with Alzheimer's disease and vascular dementia and in nondemented control subjects. *Stroke* 23: 1078–1083.
40. Tanaka, J., K. Nakamura, M. Takeda, K. Tada, H. Suzuki, H. Morita, T. Okado, S. Hariguchi, and T. Nishimura. 1989. Enzyme-linked immunosorbent assay for human autoantibody to glial fibrillary acidic protein: higher titer of the antibody is detected in serum of patients with Alzheimer's disease. *Acta Neurol. Scand.* 80: 554–560.
41. Schott, K., H. Wormstall, M. Dietrich, R. Klein, and A. Batra. 1996. Autoantibody reactivity in serum of patients with Alzheimer's disease and other age-related dementias. *Psychiatry Res.* 59: 251–254.
42. Myagkova, M. A., S. I. Gavrilova, N. N. Lermontova, Y. B. Kalyn, N. D. Selezneva, G. A. Zharikov, I. V. Kolykhalov, T. V. Abramenko, T. P. Serkova, and S. O. Bachurin. 2003. Content of autoantibodies to bradykinin and β-amyloid(1-42) as a criterion for biochemical differences between Alzheimer's dementias. *Bull. Exp. Biol. Med.* 136: 49–52.

43. Dicou, E., P. Vermersch, I. Penisson-Besnier, F. Dubas, and V. Nerriere. 1997. Anti-NGF autoantibodies and NGF in sera of Alzheimer patients and in normal subjects in relation to age. *Autoimmunity* 26: 189–194.
44. Fernandez-Shaw, C., A. Marina, P. Cazorla, F. Valdivieso, and J. Vazquez. 1997. Anti-brain spectrin immunoreactivity in Alzheimer's disease: degradation of spectrin in an animal model of cholinergic degeneration. *J. Neuroimmunol.* 77: 91–98.
45. Fillit, H. M., E. Kemeny, V. Luine, M. E. Weksler, and J. B. Zabriskie. 1987. Antivascular antibodies in the sera of patients with senile dementia of the Alzheimer's type. *J. Gerontol.* 42: 180–184.
46. Gaskin, F., J. Finley, Q. Fang, S. Xu, and S. M. Fu. 1993. Human antibodies reactive with β-amyloid protein in Alzheimer's disease. *J. Exp. Med.* 177: 1181–1186.
47. Hyman, B. T., C. Smith, I. Buldyrev, C. Whelan, H. Brown, M. X. Tang, and R. Mayeux. 2001. Autoantibodies to amyloid-β and Alzheimer's disease. *Ann. Neurol.* 49: 808–810.
48. Terryberry, J. W., G. Thor, and J. B. Peter. 1998. Autoantibodies in neurodegenerative diseases: antigen-specific frequencies and intrathecal analysis. *Neurobiol. Aging* 19: 205–216.
49. Knopman, D. S., S. T. DeKosky, J. L. Cummings, H. Chui, J. Corey-Bloom, N. Relkin, G. W. Small, B. Miller, and J. C. Stevens. 2001. Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 56: 1143–1153.
50. McDaniel, L. D., T. Lukovits, and K. D. McDaniel. 1993. Alzheimer's disease: the problem of incorrect clinical diagnosis. *J. Geriatr. Psychiatry Neurol.* 6: 230–234.
51. James, J. A., and J. B. Harley. 1998. B-cell epitope spreading in autoimmunity. *Immunol. Rev.* 164: 185–200.
52. Buono, P., F. P. D'Armiento, G. Terzi, A. Alfieri, and F. Salvatore. 2001. Differential distribution of aldolase A and C in the human central nervous system. *J. Neurocytol.* 30: 957–965.
53. Jun, H. S., L. Y. Khil, and J. W. Yoon. 2002. Role of glutamic acid decarboxylase in the pathogenesis of type 1 diabetes. *Cell. Mol. Life Sci.* 59: 1892–1901.
54. Reif, S., and A. Lerner. 2004. Tissue transglutaminase—the key player in celiac disease: a review. *Autoimmun. Rev.* 3: 40–45.
55. Yeaman, S. J., J. A. Kirby, and D. E. Jones. 2000. Autoreactive responses to pyruvate dehydrogenase complex in the pathogenesis of primary biliary cirrhosis. *Immunol. Rev.* 174: 238–249.
56. McLachlan, S. M., and B. Rapoport. 2000. Autoimmune response to the thyroid in humans: thyroid peroxidase—the common autoantigenic denominator. *Int. Rev. Immunol.* 19: 587–618.
57. Iwangoff, P., R. Armbruster, A. Enz, and W. Meier-Ruge. 1980. Glycolytic enzymes from human autoptic brain cortex: normal aged and demented cases. *Mech. Ageing Dev.* 14: 203–209.
58. Meier-Ruge, W., P. Iwangoff, and K. Reichlmeier. 1984. Neurochemical enzyme changes in Alzheimer's and Pick's disease. *Arch. Gerontol. Geriatr.* 3: 161–165.
59. Bigl, M., M. K. Bruckner, T. Arendt, V. Bigl, and K. Eschrich. 1999. Activities of key glycolytic enzymes in the brains of patients with Alzheimer's disease. *J. Neural Transm.* 106: 499–511.
60. Mosconi, L., A. Pupi, M. T. De Cristofaro, M. Fayyaz, S. Sorbi, and K. Herholz. 2004. Functional interactions of the entorhinal cortex: an 18F-FDG PET study on normal aging and Alzheimer's disease. *J. Nucl. Med.* 45: 382–392.
61. Yanase, K., R. M. Smith, A. Puccetti, L. Jarett, and M. P. Madaio. 1997. Receptor-mediated cellular entry of nuclear localizing anti-DNA antibodies via myosin 1. *J. Clin. Invest.* 100: 25–31.
62. Avrameas, A., T. Ternynck, F. Nato, G. Buttin, and S. Avrameas. 1998. Polyreactive anti-DNA monoclonal antibodies and a derived peptide as vectors for the intracytoplasmic and intranuclear translocation of macromolecules. *Proc. Natl. Acad. Sci. USA* 95: 5601–5606.
63. D'Andrea, M. R. 2003. Evidence linking neuronal cell death to autoimmunity in Alzheimer's disease. *Brain Res.* 982: 19–30.