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Inhibition of Glyceraldehyde-3-Phosphate Dehydrogenase Activity by Antibodies Present in the Cerebrospinal Fluid of Patients with Multiple Sclerosis

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We have previously shown that B cells and Abs reactive with GAPDH and antitriosephosphate isomerase (TPI) are present in lesions and cerebrospinal fluid (CSF) in multiple sclerosis (MS). In the current study, we studied the effect of anti-GAPDH and anti-TPI CSF IgG on the glycolytic enzyme activity of GAPDH and TPI after exposure to intrathecal IgG from 10 patients with MS and 34 patients with other neurologic diseases. The degree of inhibition of GAPDH activity by CSF anti-GAPDH IgG in the seven MS samples tested varied from 13 to 98%, which seemed to correlate with the percentage of anti-GAPDH IgG in the CSF IgG (1–45%). Inhibition of GAPDH activity (18 and 23%) by CSF IgG was seen in two of the 34 patients with other neurologic diseases, corresponding to the low percentage of CSF anti-GAPDH IgG (1 and 8%). In addition, depletion of anti-GAPDH IgG from CSF IgG, using immobilized GAPDH, removed the inhibitory effect of the IgG on GAPDH. No inhibition of GAPDH activity was seen with CSF samples not containing anti-GAPDH IgG. No inhibition of TPI activity was seen with any purified CSF IgG sample. These findings demonstrate an increased percentage of anti-GAPDH Abs in the CSF of patients with MS that can inhibit GAPDH glycolytic enzyme activity and may contribute to neuroaxonal degeneration. *The Journal of Immunology*, **2010, 185: 1968–1975.**

Multiple sclerosis (MS) is one of the most disabling autoimmune neurologic diseases in humans and usually starts in young adults. Its cause remains unknown. Both genetic and environmental factors have been implicated in the disease (1–3). Although attacks of inflammation in the white matter cause myelin damage, most experts agree that neuroaxonal degeneration is a major cause of permanent disability and occurs early in normal-appearing white matter (NAWM) (4–6). However, the target Ags and immunopathological processes underlying the neuroaxonal damage are unknown, making the early diagnosis of MS and prevention of disability difficult and frequently ineffective. We have previously reported that single-chain variable fragments Abs (scFv-abs) generated from the H and L chain Ig genes of clonal B cells in the cerebrospinal fluid (CSF) and lesions from patients with MS bind to GAPDH and triosephosphate isomerase (TPI) and that elevated levels of anti-GAPDH and anti-TPI Abs are seen in the CSF of the majority of individuals with MS (7).

GAPDH has historically been viewed as a housekeeping cytosol protein and plays an important role in ATP production. Recent studies have increased our understanding of the distribution and functions of GAPDH, which may play an important role in GAPDH autoimmune response-induced neuroaxonal pathological changes in MS.

GAPDH is found in several intracellular locations and has diverse activities independent of its traditional role in glycolysis (8). As a membrane protein (9, 10), GAPDH functions in endocytosis (11), although, in the cytoplasm, it is involved in energy generation, polymerization of tubulin into microtubules (12–14), and the control of protein synthesis in the endoplasmic reticulum (15); whereas, in the nucleus, it is involved in nuclear tRNA export, DNA replication, and DNA repair (16).

GAPDH and TPI are essential glycolytic enzymes with a key role in energy production. Inhibition of the glycolytic activity of GAPDH has been found to be an integral part of various forms of neuron apoptosis (17–19). Several reports suggest that GAPDH may be involved in apoptosis and age-related neuronal diseases, such as Alzheimer's disease. A reduction in GAPDH glycolytic activity is seen in cells from patients with Alzheimer's or Huntington's disease (18, 20). It has been suggested that the interaction of GAPDH with other proteins may reduce GAPDH enzyme activity and damage brain neurons (18, 21). Recently, we showed that scFv-abs generated from Ig H and L chain genes expressed by clonal B cells in the CSF and lesions of MS patients bind to GAPDH and TPI on the membrane and in the cytoplasm of axons in MS tissues (22, 23).

In the current study, we show that CSF containing anti-GAPDH IgG from MS patients strongly inhibited GAPDH enzyme activity. The percentage of anti-GAPDH IgG in the CSF IgG was significantly higher in MS patients than in patients with other neurologic diseases (ONDs). In addition, the enzyme activity inhibitory effect of the CSF anti-GAPDH IgG was removed by adsorption of the CSF with immobilized GAPDH. No inhibition of TPI activity was seen in any of the studied CSF samples. Our findings suggest

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[1]With sadness we report that Stanley van den Noort, M.D., died from complications of a brain injury on September 20, 2009. He is deeply missed.

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Abbreviations used in this paper: CSF, cerebrospinal fluid; MS, multiple sclerosis; NA, not analyzed; NAWM, normal-appearing white matter; OCB, oligoclonal bands; OND, other neurologic diseases; PBST, PBS containing 0.05% Tween-20; RT, room temperature; scFv, single chain variable fragments; scFv-abs, single-chain variable fragments Ab; TPI, triosephosphate isomerase.

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that increased intrathecal production of GAPDH-reactive Abs in patients with MS may result in widespread binding of Abs to GAPDH in axons and neurons, leading to inhibition of GAPDH glycolytic activity, neuroaxonal apoptosis, and cytotoxicity.

Materials and Methods

Patients

CSF samples were obtained from 10 patients with MS and 34 patients with OND at the Department of Neurology, University of California at Irvine, Irvine, CA. The MS patients consisted of six females and four males with a clinically or laboratory-supported diagnosis of definite MS (1) with a mean age of 48 ± 14 y. The OND patients consisted of 26 females and 8 males with a mean age of 45 ± 8 y. Laboratory tests showed oligoclonal IgG bands and/or an elevated IgG index in the CSF in seven of the MS patients and two of the OND patients (Table I).

Proteins

Human erythrocyte GAPDH and rabbit TPI were purchased from Sigma-Aldrich (St. Louis, MO) Mouse anti-human GAPDH Ab was purchased from Sigma-Aldrich.

ELISA

F-plates (Nunc, Rochester, NY) were coated overnight at 4°C with 1 μg GAPDH or TPI in PBS. All subsequent steps were at room temperature (RT). The wells were washed with PBS containing 0.05% Tween-20 (PBST) and blocked for 2 h with 3% BSA in PBS, then 35 μl CSF was added. After incubation for 3 h, the plate was washed three times with PBST and bound Abs detected by incubation for 1.5 h with HRP-coupled goat anti-human IgG Abs (1:1000 in 1.5% BSA in PBS), followed by the addition of 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid substrate solution (Bio-Rad, Hercules, CA), development for 10 min, and reading of the absorbance of each well at 450 nm. Student *t* test was used to analyze the data, with a p value <0.05 being considered significant.

Establishment of the ELISA assay for anti-GAPDH or anti-TPI Abs in CSF

To determine the appropriate dilution with a minimal background, we analyzed the limits of detection of anti-GAPDH and anti-TPI CSF IgG by ELISA. To determine the appropriate dilution of CSF to use, 0.5 μg GAPDH or TPI (7) was used to coat the plates and a linear curve was obtained with dilutions of 1:17.5–1.70 of CSF from five MS and five OND patients. The amount of GAPDH or TPI used to coat the plate was examined using 1:35 dilutions of CSF and a linear curve was obtained using 0.25–2 μg of GAPDH and TPI. The assay conditions were therefore set as 1 μg GAPDH of TPI and a 1:35 dilution of CSF. Values >2 SD above the level of controls without Ag or without CSF were considered positive.

Isolation of IgG from CSF

IgG was isolated using a protein A matrix (Sigma-Aldrich) as recommended by the manufacturer. The concentration of total IgG was determined using a sandwich ELISA.

Sandwich ELISA

F-plates (Nunc) were coated overnight at 4°C with 1 μg rabbit anti-human IgG Ab (Sigma-Aldrich) in PBS. The wells were washed with PBST and blocked for 2 h at RT with 3% BSA in PBS, then different dilutions of each CSF samples were added to the plate. As a control, several known concentrations of purified human IgG (Sigma-Aldrich) were used. After incubation overnight at 4°C, the plate was washed three times with PBST and bound human IgG detected as described. The amount of IgG in each sample was calculated from the human IgG standard curve.

Percentage of anti-GAPDH IgG and anti-TPI IgG in CSF IgG

The percentage of anti-GAPDH IgG and anti-TPI IgG in the purified CSF IgG was determined as the difference in the total IgG in an untreated sample of CSF IgG (100 ng) and the amount in the same sample after complete adsorption of specific IgG on immobilized Ag using the sandwich ELISA described above. F-plates (Nunc) were coated overnight at 4°C with 1 μg GAPDH or TPI in PBS, then the wells were washed with PBST and blocked with 3% BSA in PBS for 2 h, and 100 ng total IgG (in triplicate) was added to each well (depletion plate). After overnight incubation at 4°C, the supernatant was withdrawn and the amount of IgG remaining in the supernatant measured in the sandwich ELISA. In a parallel experiment, the supernatant was tested for anti-GAPDH or anti-TPI Abs in a regular ELISA to check that depletion was complete. Furthermore, as a second control, the "depletion plate" was washed three times with PBST and bound anti-GAPDH and anti-TPI IgG detected as described previously. The percentage of anti-GAPDH IgG or anti-TPI IgG in the total IgG was calculated using the equation

$$\frac{\text{Total IgG before depletion} - \text{Total IgG after depletion} \times 100}{\text{Total IgG}}.$$

GAPDH activity assay

The assay was performed as described previously (24) of with slight modifications. Briefly, 0.1 U human GAPDH (Sigma-Aldrich) was incubated for 10 min at RT in 0.1 M Tris-HCl, 0.5 mM EDTA, pH 8.0 (assay buffer) containing 10 mM $MgCl_2$, 0.2 mM NADH, 8 mM ATP, and 5 U phosphoglycerokinase in a total volume of 54 μl. The reaction was started by addition of 6 μl of 100 mM 3-phosphoglyceric acid and the decrease in the absorbance at 340 nm recorded for 3 min. Blanks contained no 3-phosphoglyceric acid. For inhibition assays, 0.1 U GAPDH was preincubated for 2 h at RT with different amounts of purified CSF IgG (dialyzed against assay buffer) and the assay performed as described previously. In the 100% activity control, GAPDH was incubated in assay buffer alone; whereas assay buffer without GAPDH was used in the 0% activity control. In a second negative control, different quantities of Abs were incubated without GAPDH to determine whether they affected the absorbance in the assay.

TPI activity assay

The assay was performed as described previously (24) with slight modifications. Briefly, 0.1 U rabbit TPI was incubated for 10 min at RT in the same assay buffer as above containing 0.2 mM NADH and 0.1 U α-glycerophosphate dehydrogenase in a total volume of 54 μl. The reaction was started by the addition of 6 μl of 30 mM D-glyceraldehyde-3-phosphate and the decrease in absorbance at 340 nm recorded for 3 min. Blanks contained no D-glyceraldehyde-3-phosphate. Inhibition assays were performed in the same way as the GAPDH inhibition assays.

Variances

The intra-assay coefficients of variation for the ELISA measurement of anti-GAPDH IgG and anti-TPI IgG were calculated by running 10 CSF samples three times in one assay. The interassay coefficients of variation were determined by measuring the same samples in three repeated assays. This replicate testing established the validity of the ELISA assays. The intra-assay coefficient of variation was 2.4% for anti-GAPDH IgG and 5.2% for anti-TPI IgG; whereas the interassay coefficient of variation was 5.6% for anti-GAPDH IgG and 11% for anti-TPI IgG.

Results

Anti-GAPDH and anti-TPI IgG in the CSF of patients with MS and OND

To investigate the pathobiological role of anti-GAPDH and anti-TPI IgG in the CSF of MS patients, GAPDH- or TPI-reactive Abs were measured in the CSF of 10 MS and 34 OND patients by ELISA. Increased levels of GAPDH and IgG were seen in seven MS patients and two of the OND patients and increased levels of TPI and IgG were seen in four MS patients and nine OND patients (Table I). Inhibition of GAPDH enzyme activity by CSF GAPDH IgG was observed in 7 of 10 MS patients and 2 of 32 OND patients. Fig. 1 shows ELISA results for 10 MS patients and 10 OND patients.

Increased percentage of anti-GAPDH IgG in the CSF of patients with MS

To examine whether anti-GAPDH or anti-TPI IgG was the dominant IgG in the CSF of patients, the percentage of anti-GAPDH and anti-TPI IgG in the total CSF IgG was determined using a series of ELISAs. Total IgG was purified from CSF using protein A and the amount of IgG measured from the extinction at 450 nm of 100 ng of CSF IgG from each patient was determined by a sandwich-ELISA using anti-human IgG Abs (Tables II, III, column 2). Anti-GAPDH or anti-TPI Abs were then completely removed using immobilized GAPDH or TPI and the amount of total IgG remaining

Table I. *Summary of clinical and laboratory information*

No. of Cases	Mean Age (y)	Women/Men	OCB-Positive and/or High IgG Index Yes/No/NA	GAPDH IgG$^{+/-}$	TPI IgG$^{+/-}$
MS patients					
10	48 ± 14	6/4	7/2/1	7/3	4/10
OND patients					
34	43 ± 11	26/8	2/17/15	2/32	9/25

NA, not analyzed; OCB, oligoclonal bands.

in the supernatant measured (Tables II, III, column 3). As a control of depletion, the supernatants were tested for anti-GAPDH or anti-TPI Abs using the immobilized Ag ELISA and none were found (data not shown). In the anti-GAPDH Ab-positive samples, the percentage of anti-GAPDH IgG was 1–45% in the five MS patients tested and 8% in the one OND patient tested; whereas, in the anti-GAPDH Ab-negative samples, the percentage of anti-GAPDH IgG was 5–6% in the two MS patients tested and 2–14% in five OND patients tested (Table II, column 4). In the anti-TPI Ab-positive samples, the percentage of anti-TPI IgG was 0–16% in the three MS patients tested (Table III, column 4) and 5–28% in the four OND patients tested (Table III, column 4).

Inhibition of enzyme activity by CSF GAPDH- and TPI-reactive IgG

To determine the effect of CSF IgG on the enzyme activity of GAPDH and TPI, we measured the glycolytic activity of GAPDH and TPI with and without preincubation with purified CSF IgG. Fig. 2*A* and Table II, column 5 show that, using samples from the seven MS patients with anti-GAPDH Abs, inhibition of GAPDH glycolytic activity varied from 13 to 98% and seemed to correlate with the percentage of anti-GAPDH IgG in the purified CSF IgG (Table II, column 4). The greatest degree of inhibition (>50%) was seen with samples containing a high percentage of anti-GAPDH IgG (30–45%). Fig. 2*C* and Table II, column5 show that, in the two OND patients with anti-GAPDH Abs, low inhibition of GAPDH activity (18% and 23%) was seen, corresponding to the low percentage of CSF anti-GAPDH IgG (not tested and 8%, Table II, column 4). When anti-GAPDH IgG was removed by adsorption on GAPDH, the CSF IgG failed to inhibit GAPDH glycolytic activity (Table II,column 6). Little inhibition of GAPDH glycolytic activity was seen using anti-GAPDH Ab-negative CSF samples from MS patients (0–5%) or OND patients (0–8%) (Figs. 2B,2D, Table II, column 5).

In the anti-TPI Ab-positive samples, the percentage of anti-TPI IgG was 0–16% in the three MS patients tested and 5–28% in the four OND patients tested; whereas the corresponding values in the anti-TPI Ab-negative samples were 1–9% (three tested) and 0–8% (two tested) (Table III, column 4). No inhibition of TPI activity was seen with any of the purified CSF IgG samples (Fig. 3*A–D*), irrespective of the presence or absence of anti-TPI Abs (Table III, column 5).

Degree of inhibition of GAPDH glycolytic activity

The concentration of anti-GAPDH IgG in the CSF may play an important role in inhibition of GAPDH glycolytic activity. In a model system, we added different amounts of mouse anti-human GAPDH Ab, measured the degree of inhibition of GAPDH, and found that it was dependent on the amount of mouse anti-human GAPDH Ab added (Fig. 4). The results showed 38 or 58% inhibition of GAPDH glycolytic activity after incubation of GAPDH with 2 or 4 μg, respectively, of mouse anti-human GAPDH Ab. Interestingly, 0.8 μg purified CSF IgG from a MS patient with anti-GAPDH Abs was more effective in inhibiting GAPDH glycolytic activity than 4 μg of monoclonal mouse anti-human GAPDH Ab. After exposure of GAPDH to 0.8 μg purified CSF IgG from one MS patient or one OND patient with anti-GAPDH CSF IgG, GAPDH glycolytic activity was reduced by 69 or 20%, respectively (4, 5) (Fig. 2). These results suggest that the degree of inhibition of GAPDH activity is not only dependent on the dose of IgG, but that the epitope of GAPDH recognized by the Ab may also play a role in the inhibition of enzyme activity. GAPDH-reactive CSF IgG in MS patients may be induced by epitope(s) that may play an important role in glycolytic enzyme activity.

Discussion

The main result of this study was that GAPDH activity was decreased considerably on incubation with purified CSF IgG containing a high percentage of anti-GAPDH IgG; whereas purified CSF IgG containing a lower percentage of, or no, anti-GAPDH IgG had a much lower or zero effect. The inhibitory effect of CSF IgG was

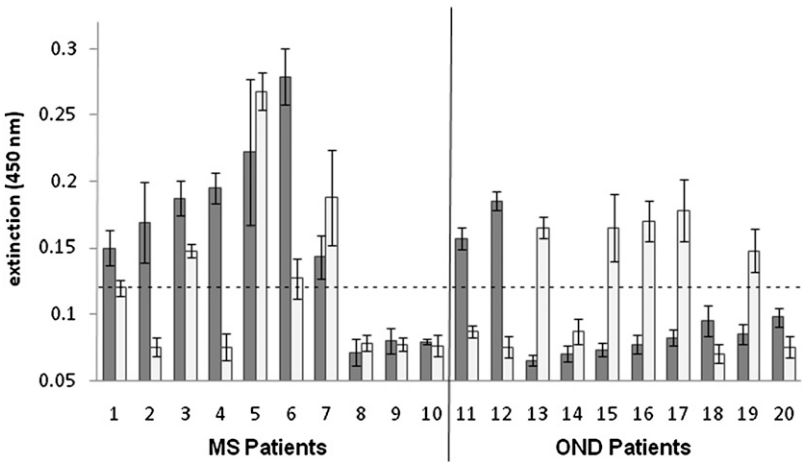

FIGURE 1. GAPDH- and TPI-reactive IgG in the CSF of patients with MS and OND. Binding of IgG from CSF samples from 10 MS patients and 10 of 34 OND patients to immobilized GAPDH (gray bars) or TPI (white bars) analyzed by ELISA. The data represent the mean and SD for three independent determinations. The line represents the cutoff level of the mean + 2 SD of the controls.

Table II. *Decrease in GAPDH activity after incubation with purified CSF IgGs*

Patient	OD_{450} in the Anti-GAPDH ELISA before Anti-GAPDH IgG Depletion[a]	OD_{450} in the Anti-GAPDH ELISA After Anti-GAPDH IgG Depletion[b]	Depletion of IgG by Immobilized GAPDH[c] (%)	Inhibition of GAPDH Enzyme Activity[d] (%)	GAPDH Inhibition after Anti-GAPDH IgG Depletion[e] (%)
Anti-GAPDH IgG-positive MS patients					
1	1.095	0.788	28	19	0.0
2	NA	NA	NA	58	NA
3	1.175	0.761	35	98	0.0
4	1.042	0.73	30	92	0.0
5	1.232	0.677	45	52	0.0
6	NA	NA	NA	39	NA
7	1.256	1.243	1	13	0.0
Anti-GAPDH IgG-negative MS patients					
8	1.101	1,046	5	5	0.0
9	NA	NA	NA	1	0.0
10	0.089	0.084	6	0	NA
Anti-GAPDH IgG-positive OND patients					
11	0.983	0.904	8	18	0.0
12	NA	NA	NA	23	NA
Anti-GAPDH IgG-negative OND patients					
13	1.026	0.882	14	6	0.0
14	1.162	1.138	2	0	0.0
15	NA	NA	NA	0	NA
16	0.915	0.819	10	8	0.0
17	0.965	0.839	13	5	0.0
18	NA	NA	NA	6	NA
19	1.246	1.182	5	0	0.0
20	NA	NA	NA	0	0.0

[a] OD_{450} of 100 ng purified CSF IgG on GAPDH ELISA plates before GAPDH depletion.
[b] OD_{450} of 100 ng purified CSF IgG on GAPDH ELISA plates after GAPDH depletion.
[c] Percentage of anti-GAPDH IgG in total purified CSF IgG.
[d] Percentage inhibition of GAPDH enzyme activity after preincubation with 100 ng purified CSF IgG before depletion of anti-GAPDH Ab.
[e] As in *d*, but after depletion of anti-GAPDH IgG.
NA, not analyzed due to an insufficient volume of CSF.

completely abolished when anti-GAPDH IgG was depleted from CSF IgG. Furthermore, anti-GAPDH CSF IgG was more effective than commercial mouse anti-human GAPDH mAbs in inhibiting GAPDH activity. In contrast, no increased percentage of anti-TPI IgG was observed in the CSF of patients with MS and patients with OND, and purified CSF IgG from these patients failed to inhibit TPI activity. Our findings suggest that increased production of anti-GAPDH IgG in the CNS of MS patients inhibits GAPDH enzyme activity, reduces ATP production, and causes other abnormalities of microtubules and mitochondria, which may result in widespread neuroaxonal apoptosis and degeneration.

Previously, we and other groups (25–29) have shown that restricted B cell clone(s) are selected and expanded in the CSF and in MS lesions. Comparison of the Ig proteome and transcriptome revealed a high degree of concordance, suggesting that the B lineage cells contained in the CSF are a source of the oligoclonal bands (30). These clonal B cells carry somatic hypermutation in their Ig variable genes, indicating an Ag-driven T cell-dependent B cell reaction in the germinal centers of secondary lymphoid tissues and in the CNS of MS patients (26, 31); these findings provide the opportunity to identify the Ags that drive B cell clonal expansion in the CSF in MS. Using in vitro recombinant scFv-ab synthesis techniques developed in the last decade (32, 33), we have shown that scFvs generated from clonal B cells in the CSF bind specifically to axons (22). The presence of an antiaxonal immune response was further demonstrated by localization of Abs secreted by plasma cells in the brain tissues from the majority of MS patients. An observation of particular relevance to the pathologic role of these Abs is their association with axonal transection in lesions (22, 23).

On testing the Ag specificity of the scFv-abs and CSF Igs from MS patients, we found that scFv-Abs generated from B cells and plasma cells in lesions bind to GAPDH and/or TPI in the majority of MS brains (22, 23). Furthermore, a recent report has shown that scFv-abs generated from clonal B cells in the CSF of patients with MS do not bind to the myelin proteins myelin basic protein, proteolipid protein, or myelin oligodendrocyte gp (34).

To gain a further insight into the role of these Abs, we analyzed the percentage of anti-GAPDH IgG or anti-TPI IgG in the total CSF IgG in individual patients using a CSF IgG depletion assay with GAPDH or TPI as Ag. The results showed that the percentage of intrathecal anti-GAPDH IgG was significantly higher in MS patients than in patients with ONDs. However, no increased percentage of anti-TPI IgG was observed in any of the CSF samples studied. These findings suggest that we have successfully identified one of the dominant target Ags in MS as GAPDH.

For a long time, neurodegeneration has been considered a major cause of functional disability in MS patients. Neuroaxonal damage was noted in the 19th century (35, 36) and axonal degeneration further demonstrated in MS lesions using silver impregnation techniques (37, 38). Magnetic resonance spectroscopy measurements of N-acetylaspartate, a marker for axons and neurons, indicate that axonal injury is widespread in MS lesions and NAWM and suggest that it occurs at disease onset (39–42). A major challenge raised by these studies is to understand the mechanisms underlying the widespread neuroaxonal degeneration and gray matter atrophy in MS.

The finding of increased intrathecal production of anti-GAPDH IgG raises the question of how Abs to GAPDH affects neuron function and survival. The involvement of GAPDH in apoptosis was first

Table III. *No inhibition of TPI activity by purified CSF IgGs*

Patient	Extinction TPI ELISA before TPI and IgG Depletion[a] (450 nm)	Extinction TPI ELISA after TPI and IgG Depletion[b] (450 nm)	Depletion of Anti-TPI IgG[c] (%)	Inhibition of TPI Enzyme Activity[d] (%)
Anti-TPI IgG-positive MS patients				
1	1.095	1.007	8	0.0
3	1.175	1.173	0	0.0
5	NA	NA	NA	0.0
7	1.256	1.055	16	0.0
Anti-TPI IgG-negative MS patients				
2	NA	NA	NA	0.0
4	1.042	0.959	8	0.0
6	NA	NA	NA	0.0
8	1.101	1.002	9	0.0
9	NA	NA	NA	0.0
10	0.889	0.880	1	0.0
Anti-TPI IgG-positive OND patients				
13	1.026	0.872	15	0.0
15	0.915	0.659	28	0.0
16	NA	NA	NA	0.0
17	0.965	0.859	11	0.0
19	1.246	1.182	5	0.0
Anti-TPI IgG-negative OND patients				
11	0.983	0.981	0	0.0
12	NA	NA	NA	0.0
14	1.162	1.069	8	0.0
18	NA	NA	NA	0.0
20	NA	NA	NA	0.0

[a] OD_{450} of 100 ng purified CSF IgG on TPI ELISA plates before TPI depletion.
[b] OD_{450} of 100 ng purified CSF IgG on TPI ELISA plates after TPI depletion.
[c] Percentage of anti-TPI IgG in total purified CSF IgG.
[d] Percentage inhibition of TPI enzyme activity after preincubation with 100 ng purified CSF IgG before depletion of anti-TPI Ab. There is no inhibition of TPI enzyme activity was observed in CSF IgG before TPI+IgG depletion. The inhibitory enzyme activity of CSF IgG after TPI depletion was not tested due to insufficient volume of CSF samples.
NA, not analyzed due to an insufficient volume of CSF.

demonstrated in primary cultures of brain neurons (43). When GAPDH enzyme activity is inhibited, neurons display chromatin condensation, internucleosomal DNA cleavage, and cytoplasmic shrinking (44), and a reduction in GAPDH activity plays an active role in various forms of apoptosis and may be involved in the neuronal death in Huntington's disease (45, 46), Parkinson's disease (47), and Alzheimer's disease (48). Inhibition of GAPDH activity has also been shown to induce overproduction of superoxide (49), and accumulation of oxidative damage in neurons has been shown to account for the increased incidence of neurodegeneration in MS and

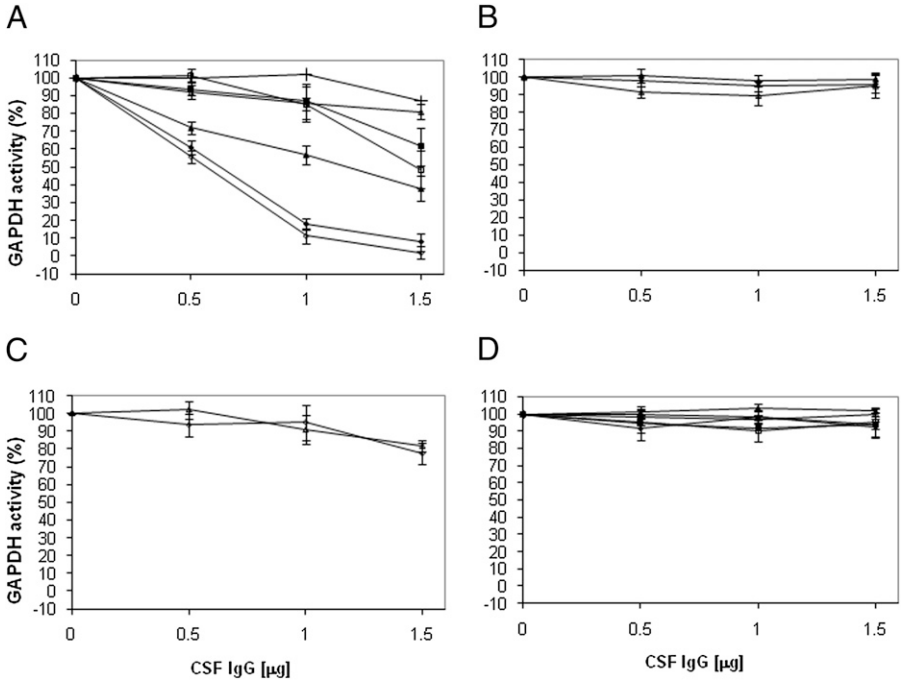

FIGURE 2. Inhibition of GAPDH glycolytic activity by purified CSF IgG containing or not containing anti-GAPDH IgG. Inhibition of GAPDH glycolytic activity by 100 ng purified CSF IgG with and without anti-GAPDH IgG. The cases are the same as those in Fig. 1. *A*, MS cases 1–7 with anti-GAPDH IgG; (*B*) MS cases 8–10 without anti-GAPDH IgG; (*C*) OND cases 11–12 with anti-GAPDH IgG; and (*D*) OND cases 13–20 without anti-GAPDH IgG.

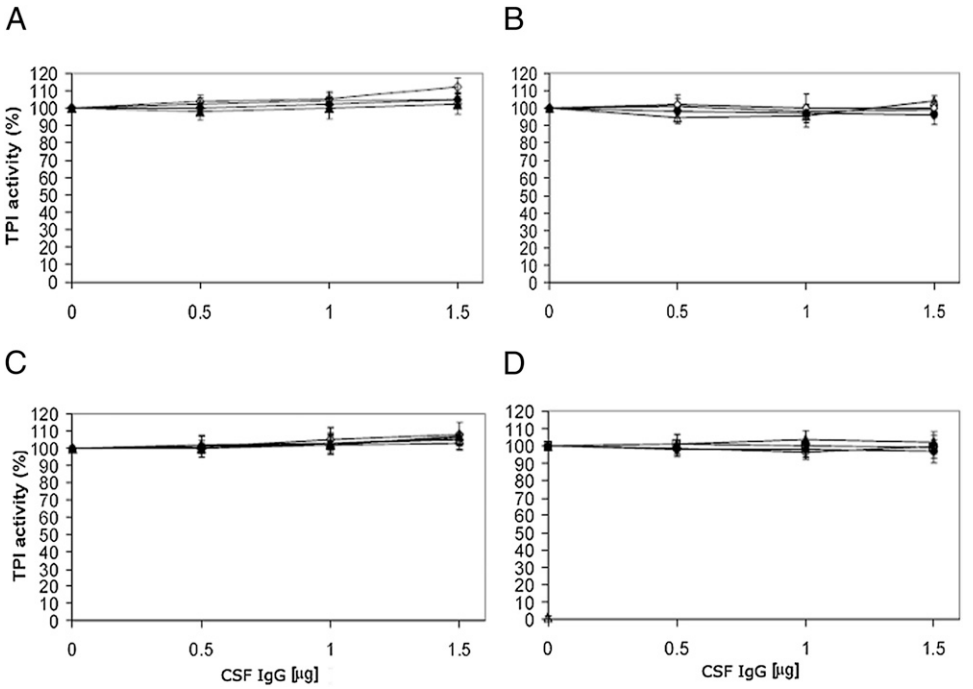

FIGURE 3. Lack of inhibition of TPI enzyme activity by purified CSF IgG containing or not containing anti-TPI IgG. Inhibition of TPI glycolytic activity by purified CSF IgG with and without anti-TPI IgG was tested as described in the *Materials and Methods*. *A*, MS cases 1, 3, 5, and 7 with anti-TPI IgG; *B*, MS cases 2, 4, 6, 8, 9, and 10 without anti-TPI IgG; *C*, OND cases 12, 14, 16, 17, and 18 with anti-TPI IgG; and *D*, cases 11, 13, 15, and 19 without anti-TPI IgG.

ONDs (50). The breakdown products of oxidative stress are present in large numbers, and the level of free radical formation corresponds to the severity of the MS attack. NO, another free radical, is found at higher than normal concentrations in inflammatory MS lesions (51).

In the current study, we showed that anti-GAPDH IgG in the CFS of MS patients inhibited the glycolytic activity of GAPDH. Inhibition of GAPDH activity varied from 13 to 98% and seemed to correlate with the percentage of anti-GAPDH IgG in the CSF IgG (1–45%). The greatest inhibition (>50%) was seen with samples containing 30–45% of anti-GAPDH CSF IgG. Low inhibition of GAPDH activity (13 and 23%) was also seen in patients with OND with a low percentage of anti-GAPDH IgG in the CSF IgG (8%).

CSF is contained in the ventricles and subarachnoid space and bathes the brain and spinal cord. Anti-GAPDH Abs in the CSF might bind to membrane, cytoplasmic, and nuclear GAPDH in neurons and axons in the areas of gray and white matter. Immune responses to these glycolytic enzymes might, therefore, damage microtubules and mitochondria, reduce energy production, and cause Ab-mediated neuroaxonal cytotoxicity. Recent reports of decreased function of the neuronal mitochondrial respiratory chain in the motor cortex of MS patients (52) and of widespread axonal injury in the NAWM (41, 42) and gray matter atrophy (53) in MS patients may be consistent with this anti-GAPDH immune response.

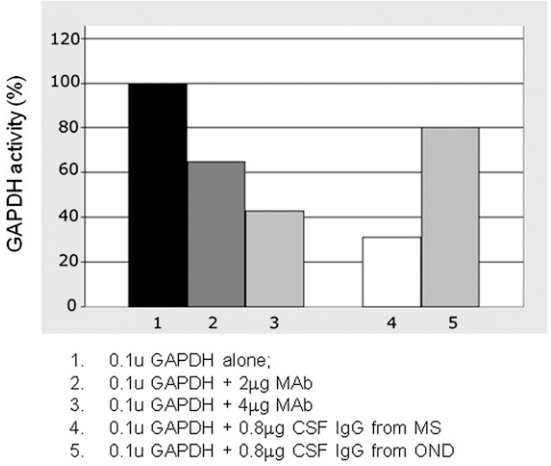

FIGURE 4. Dose-dependent inhibition of GAPDH enzyme activity by mouse anti-human GAPDH Ab and CSF IgG from a patient with MS or OND. The activity of 0.1 U of human GAPDH was measured after incubation 1) with buffer alone; 2) with 2 µg; 3) 4 µg of mouse anti-human GAPDH Ab; 4) with 0.8 µg of CSF IgG from a patient with MS; or 5) from a patient with OND; both IgG samples contained anti-GAPDH Abs.

How the anti-GAPDH immune response is initiated in MS is still unknown. Recently, Schachner's group (19) demonstrated the presence of extracellular GAPDH at the cell surface of neuronal cells, where it functions as a binding partner for the cell adhesion molecule L1. We have shown that IgG, IgA, and scFvs secreted by intrathecal GAPDH-driven clonal B cells attack membranes and cytosol of axons in patients with MS (7, 22, 23). This finding strongly indicates that anti-GAPDH Abs against surface GAPDH may contribute to a putative autoimmune pathogenesis in MS. Recently, we have developed a new animal model of experimental allergic neuroaxonal degeneration and demyelination by immunizing C57/B6 mice with GAPDH (J. Kölln, Y. Zhang, G. Thai, M. Demetriou, N. Hermanowicz, P. Duquette, S. van den Noort, and Y. Qin, manuscript in preparation). Results from mice immunized with GAPDH demonstrate: 1) infiltration of T cells and B/plasma cells into the CNS; 2) Abs localized to membrane and cytoplasmic in neurons and axons in different areas of the brain; and 3) in contrast to mice with experimental allergic encephalomyelitis induced by immunization with MOG 35–55 peptide, which show little deposition of IgG on myelin and no antineuroaxonal IgG staining, immunization with GAPDH induces a significant loss of neurons and/or other cells in experimental allergic neuroaxonal degeneration and demyelination.

The presence of autoantibodies to GAPDH has been demonstrated in the CSF of patients with lupus, a rheumatic disorder with associated neurologic abnormalities (54). This observation has led to speculation that the autoimmune response to GAPDH might be primarily initiated by foreign GAPDH, present on the surface of bacteria, viruses, and parasites, and subsequent cross-reaction with

human GAPDH (55–57). People with an increased predisposition to autoimmune diseases might have a higher risk of developing MS (1, 58). Viral infection has long been considered as one of the causes of MS (1, 58–62) and viruses, such as measles, herpes, EBV, hepatitis, and influenza, and the bacterium *Chlamydia pneumoniae* have been shown to be associated with MS (63–66). It will be interesting to determine whether CSF samples with anti-GAPDH IgG contain Abs that react with the GAPDH of these viruses. Furthermore, the development of an animal model will help us to study the role of the anti-GAPDH and anti-TPI immune responses in neuroaxonal degeneration and contribute to a better understanding of GAPDH and TPI autoimmunity in MS.

Disclosures

The authors have no financial conflicts of interest.

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