

Oligodendrocyte-specific Expression and Autoantigenicity of Transaldolase in Multiple Sclerosis

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Summary

Although the etiology of multiple sclerosis (MS) is unknown, there is compelling evidence that its pathogenesis is mediated through the immune system. Molecular mimicry, i.e., crossreactivity between self-antigens and viral proteins, has been implicated in the initiation of autoimmunity and MS. Based on homology to human T cell lymphotropic virus type I (HTLV-I) a novel human retrotransposon was cloned and found to constitute an integral part of the coding sequence of the human transaldolase gene (TAL-H). TAL-H is a key enzyme of the nonoxidative pentose phosphate pathway (PPP) providing ribose-5-phosphate for nucleic acid synthesis and NADPH for lipid biosynthesis. Another fundamental function of the PPP is to maintain glutathione at a reduced state and, consequently, to protect sulfhydryl groups and cellular integrity from oxygen radicals. Immunohistochemical analyses of human brain sections and primary murine brain cell cultures demonstrated that TAL is expressed selectively in oligodendrocytes at high levels, possibly linked to production of large amounts of lipids as a major component of myelin, and to the protection of the vast network of myelin sheaths from oxygen radicals. High-affinity autoantibodies to recombinant TAL-H were detected in serum (25/87) and cerebrospinal fluid (15/20) of patients with MS. By contrast, TAL-H antibodies were absent in 145 normal individuals and patients with other autoimmune and neurological diseases. In addition, recombinant TAL-H stimulated proliferation and caused aggregate formation of peripheral blood lymphocytes from patients with MS. Remarkable amino acid sequence homologies were noted between TAL-H and core proteins of human retroviruses. Presence of crossreactive antigenic epitopes between recombinant TAL-H and HTLV-I/human immunodeficiency virus type 1 (HIV-1) gag proteins was demonstrated by Western blot analysis. The results suggest that molecular mimicry between viral core proteins and TAL-H may play a role in breaking immunological tolerance and leading to a selective destruction of oligodendrocytes in MS.

Multiple sclerosis (MS)¹ lesions are characterized by a progressive loss of oligodendrocytes and demyelination in the white matter of the central nervous system (CNS) (1). In the acute stage of disease, lesions contain macrophages, T cells, and immunoglobulin deposits suggesting that the demyelination process is mediated by the immune system. The inflammatory picture of early lesions, which is followed

by a progressive gliosis, suggested that the pathological process may be initiated by infectious agents and then self-perpetuated by a crossreactive autoimmune process (2-7). Whereas a number of myelin-derived structural proteins were shown to elicit MS-like disease in animal models, the antigen(s) driving this self-destructive process, which could account for pathogenesis of the human disease, has not been identified (1). Studies on relapsing experimental allergic encephalomyelitis (EAE) have shown that different encephalitogenic molecules or epitopes within them are selected, which is compatible with the heterogeneity of the immune response in MS, suggesting that relapse episodes are induced by different neuroantigens (8, 9). Nevertheless, oxygen radicals have been suggested to play a key role in the demyelination process. Intralésional cytotoxic T cells produce TNF- β which, in turn,

¹ Abbreviations used in this paper: ADTG, Ala-Asp-Thr-Gly; ATL, adult T cell leukemia; CNS, central nervous system; CP, chronic progressive; CSF, cerebrospinal fluid; ECG, essential cryoglobulinemia; GFAP, glial fibrillary acidic protein; GST, glutathione-S-transferase; HTLV-I, human T cell lymphotropic virus type I; MBP, myelin basic protein; MS, multiple sclerosis; PPP, pentose phosphate pathway; R/R, relapsing/remitting; SJS, Sjogren's syndrome; SLE, systemic lupus erythematosus; TAL, transaldolase; TAL-H, human TAL.

induces apoptosis, an oxidative stress-mediated programmed cell death, of oligodendrocytes (10). Macrophages and astrocytes produce nitric oxide, which can also destroy oligodendrocytes via formation of reactive oxygen intermediates (11). By contrast, cellular integrity is protected from damage of ROI by reduced glutathione which is solely dependent on NADPH produced uniquely by pentose phosphate pathway (PPP) (12). The present data provide evidence that a rate-limiting enzyme of PPP, transaldolase (TAL), is expressed in an oligodendrocyte-specific manner. Further, a subset of patients with MS has antibodies to TAL in their blood and cerebrospinal fluid. Recombinant human transaldolase (TAL-H) induces proliferation and aggregate formation of peripheral blood lymphocytes from patients with MS. Autoantigenic epitopes are contained in a retrotransposon-encoded region of the TAL-H gene (13) showing amino acid sequence homologies with viral core proteins. This study suggests that molecular mimicry inducing autoimmunity to the TAL-H protein could be involved in the selective destruction of oligodendrocytes in MS.

Materials and Methods

Human Sera. Patients included 95 with MS, 19 with Sjogren's syndrome (SJS), 25 with systemic lupus erythematosus (SLE), and 32 with essential cryoglobulinemia (ECG). All patients satisfied the criteria for a definitive diagnosis (14). Sera from 77 healthy subjects and 24 patients with other neurological diseases were used as negative control. Sera of five adult T cell leukemia (ATL) patients and a rabbit antibody raised against HTLV-I virion lysate were used as human T cell lymphotropic virus type I (HTLV-1)-specific antibodies.

Prokaryotic Expression of Recombinant Protein. A 157-amino acid long NH₂-terminal segment of TAL-H was expressed in the pEV vector system as described earlier (13). Briefly, a 474-bp EcoRI fragment, that is, the 4/2 section of the 4/2-4/1 TAL-H cDNA, which contains an uninterrupted open reading frame, was ligated into the pEV plasmid vector and expressed in *Escherichia coli* RR1 [pRK248cIts] (15). Construction of the vector is such that an ATG codon is placed before the codon corresponding to the first amino acid of the mature gene product. Bacterial cultures were grown at 30°C in M9 medium with 0.5% glucose, 10 mM MgSO₄, 10 µg/ml thiamine, 20 µg/ml thymine, 10 µg/ml proline, 1 µM CaCl₂, and 0.5% casamino acids. Expression of the recombinant protein was induced by growing the bacteria at 42°C. Bacterial lysates were resuspended in 1:10 volume of Laemmli buffer and analyzed by SDS-PAGE (16). The full-length TAL-H protein was expressed as a fusion protein with glutathione-S-transferase (GST) encoded by pGEX-2T plasmid vector (17). A BglII site was generated by polymerase chain reaction-mediated mutagenesis immediately 5' of the first methionine codon of TAL-H cDNA (13). Thus, a 1,033-nucleotide long BglII fragment of cDNA clone 4/2-4/1, between nucleotide positions 57 and 1,090, respectively, was cloned into the BamHI site of pGEX-2T, immediately downstream of the thrombin cleavage site. Optimum stimulation of expression of the recombinant fusion protein was obtained with 1 mM isopropylthio-β-galactoside (IPTG) after 2 h. By Western blot analysis of protein lysates, a 66-kD fusion protein (38 kD TAL-H + 28 kD GST) was detected using antibody 169 (13). A TAL-H/GST fusion protein was affinity-purified through binding of GST to glutathion-coated agarose beads as specified by the supplier (Pharmacia LKB,

Piscataway, NJ). TAL-H protein was cleaved from GST by 1 NIH unit of thrombin (Sigma Chemical Co., St. Louis, MO) in 1 ml of PBS containing 600 µg fusion protein. TAL-H protein was separated from the agarose bead-bound GST by centrifugation. The purified full-length recombinant TAL-H was found to be highly functional in the TAL enzyme assay by showing a specific activity of >10 U/mg protein (13, 18).

Testing of TAL Enzyme Activity. TAL enzyme activity was tested in the presence of 3.2 mM D-fructose 6-phosphate, 0.2 mM erythrose 4-phosphate, 0.1 mM NADH, 10 µg α-glycerophosphate dehydrogenase/triosephosphate isomerase at a 1:6 ratio at room temperature by continuous absorbance reading at 340 nm for 20 min (18). The assay was conducted in the activity range of 0.001–0.01 U/ml using yeast TAL as a positive control. All reagents for the TAL assay were from Sigma Chemical Co.

Retroviral Proteins. Reactivity of TAL-H-specific antibody 169 with HIV proteins was investigated by Western blot analysis of protein lysates of HIV-1-infected PBLs. Viral reagents were obtained from the National Institutes of Health (NIH) AIDS Research and Reference Program. Infectious stock of the strain HIV-1_{MB} was harvested from 24-h supernatants of freshly infected H9 cells (ATCC CRL-8543; American Type Culture Collection [ATCC], Roc) and infectious titer was determined by an *in situ* infectivity (MAGI) assay (19). Supernatants with titers of 2.1×10^5 infectious units/ml were filtered through a 0.45-micron filter and aliquots were stored at -70°C. Normal human PBLs purified from Ficoll-Hypaque gradient were prestimulated with 1% PHA (HA15; Wellcome, Beckenham, UK) and 30 U/ml human recombinant IL-2 in RPMI 1640 medium containing 20% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. After 3 d, PBL were incubated for 4 h with HIV-1 in the presence of 10 µg/ml Polybrene (Sigma Chemical Co.). Infections were standardized by incubating PBLs with cell-free virus supernatants containing 100 ng of p24 core protein/ 5×10^6 cells as measured by an ELISA following the manufacturer's recommendations (NEK-060; DuPont, Boston, MA). After virus infection, cells were washed in PBS and resuspended in 10 ml of fresh RPMI 1640 medium containing 20% FCS, 20 U/ml IL-2, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. Noninfected control PBL were cultured under identical conditions. After infection, the cells were cultured for 8 d and lysed in SDS-PAGE sample buffer at a density of 2×10^7 cells/ml. Cell lysates were boiled for 5 min and stored at -20°C until use. Recombinant HIV-1 gag proteins were obtained through the NIH AIDS Research and Reference Program. HIV-1 SF2 p25/245 gag contained the gag 24 protein (20). HIV-1/IIIB Gag4 contained the p17 COOH terminus, beginning at amino acid position 146, all of p24, and the p15 NH₂ terminus (Repligen Corp., Cambridge, MA). As positive control sera, HIV-1IIIB p17-specific and p24 specific polyclonal sheep antibodies (21), monoclonal antibodies to p24 (22), gp41 (22), and gp120 (23), and a HIV-1 gag p17-reactive human reference serum F06 from the Centers for Disease Control (Atlanta, GA) were used. Gel-purified recombinant HTLV-I gag p24 was kindly provided by Dr. Chung-ho Hung (Cambridge Biotech, Worcester, MA).

Western Blot Analysis. 500 ng of recombinant TAL-H protein in 10 µl/well was separated by SDS-PAGE and electroblotted to nitrocellulose (24). Nitrocellulose strips were incubated in 100 mM Tris, pH 7.5, 0.9% NaCl, 1% Tween 20, and 5% skim milk, with antibodies (at a 1,000-fold dilution unless otherwise indicated) for 1 h at room temperature. For detection of rabbit antibodies, after washing, the strips were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Boehringer Mannheim Corp., Indianapolis, IN). For detection of human antibodies, after washing,

the strips were incubated with biotinylated goat anti-human serum and, subsequently with horseradish peroxidase-conjugated avidin (Jackson ImmunoResearch Laboratories, West Grove, PA). In between the incubations the strips were vigorously washed in 0.1% Tween-20, 100 mM Tris, pH 7.5, and 0.9% NaCl. The blots were developed with a substrate composed of 1 mg/ml 4-chloronaphthol and 0.003% hydrogen peroxide.

Stimulation of PBLs. Peripheral blood mononuclear cells were isolated from heparinized venous blood on Ficoll-Hypaque gradient and resuspended in RPMI 1640 medium, supplemented with 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml gentamicin. 10^5 cells were incubated in each well of a microtiter plate using six parallel samples. Recombinant TAL-H was added in an optimal concentration of 1 µg/ml. Negative control and positive control cultures (containing 10 µg/ml concanavalin A) were included in each experiment. The plates were incubated at 37°C in a humidified atmosphere with 5% CO₂ for 72 h. The cultures were pulsed with 0.4 µCi [³H]TdR 8 h before termination. Cells were harvested and [³H]TdR incorporation was measured as earlier described (25). The results were expressed in cpm as mean ± SE of six parallel cultures. Statistical analysis was performed with Student's *t* test.

Immunohistochemistry. Formalin fixed (10% formaldehyde in PBS) and paraffin-embedded sections of human postmortem brain tissue without neurological disorder was stained with control preimmune rabbit serum, anti-TAL-H immune rabbit serum 169 (13), anti-gial fibrillary acidic protein (GFAP) rabbit serum (DAKO, Glostrup, Denmark) or antigalactocerebroside monoclonal antibody (Boehringer Mannheim Corp.) at dilutions of 1:5,000. Slides were developed using biotinylated goat anti-rabbit IgG or goat anti-mouse IgG, alkaline phosphatase-conjugated streptavidin, and substrate (all from DAKO).

Primary Brain Cell Cultures and Immunofluorescence Studies. Primary brain cell cultures were prepared from cerebral hemispheres of 2-d-old B10.A mice, as earlier described (26). After 7 d in culture, cells were fixed with 1% paraformaldehyde in 50 mM Tris/150 mM NaCl and permeabilized with 0.25% Triton X-100 in 0.5 M Tris, pH 7.4, and stained with Ab 169 or myelin basic protein (MBP)-specific rabbit serum (DAKO). Staining was obtained using a horseradish peroxidase-conjugated swine anti-rabbit antibody and 4-chloronaphthol substrate as described for Western blots. Cell type-specific expression of TAL and MBP was investigated by two-color immunofluorescence. TAL was detected by Ab 169 and rhodamine-conjugated anti-rabbit goat antibody while MBP was visualized using an MBP-specific rat monoclonal antibody (IgG1; Serotec Ltd., Oxford, UK) and FITC-conjugated goat anti-rat antibody. 169 preimmune rabbit serum with rhodamine-conjugated goat anti-rabbit secondary antibody and a rat monoclonal antibody to human IFN-β (IgG1; Serotec Ltd.) with an FITC-conjugated goat anti-rat secondary antibody were used as negative controls.

Sequence Analysis. Amino acid sequence homologies were analyzed using the University of Wisconsin Genetics Computer Group (GCG) Software (27). The nucleotide sequence of the TAL-H gene has been submitted to the GenBank™/EMBL Data Bank with accession number L19437 (13).

Results

Expression of TAL-H Protein in Oligodendrocytes. Expression of TAL is regulated in a developmental and tissue-specific manner (28, 29). It is a pivotal enzyme of PPP that shows

maximal activity in the developing nervous system at a period of active growth and myelination (30). Identification of TAL-producing cells has now become feasible by cloning of the human TAL cDNA, production of recombinant TAL-H protein, and generation of specific antibodies. Antibodies 169 and 170 are highly specific for the 38-kD TAL-H protein (13). Immunohistochemical analysis of postmortem sections revealed that expression of TAL-H is specific for oligodendrocytes in the human brain (Fig. 1). Localization of TAL-H to oligodendroglia was confirmed by parallel staining for galactocerebroside (concordant staining pattern with TAL-H; not shown) and GFAP (astrocyte-specific discordant staining pattern relative to TAL-H; Fig. 1 C). TAL is detectable in other mammalian species, including the mouse, by using the human TAL-H probes (13). Therefore, oligodendrocyte-specific expression was further investigated in murine brain cell cultures (26). Concordant expression of TAL-H and MBP in the oligodendroglia was demonstrated by simultaneous staining with TAL-H-specific rabbit antibody 169 and a MBP-specific rat monoclonal antibody using two-color immunofluorescence (Fig. 2, B and C). As a negative control, cultures were simultaneously stained with 169 preimmune rabbit serum and a rat monoclonal antibody to human IFN-β (Figs. 2, D and E). Anti-TAL-H and anti-MBP antibodies showed an identical staining pattern of oligodendrocytes and of their processes, indicating that myelin sheaths may also contain the TAL-H protein. No TAL-H expression was detected in neurons and astrocytes.

Detection of TAL-H-specific Antibodies in Patients with MS. Since oligodendrocytes are selectively destroyed in patients with MS, the possibility that TAL-H is involved as an autoantigen in this process was investigated. Sera from 171 patients with immune disorders and sera of 101 control blood donors was studied by Western blot analysis. Seropositivity was based on immunoreactivity to a 22-kD recombinant TAL-H protein (500 ng of gel-purified TAL-H protein/lane) at serum dilutions of 1:100 or higher. Presence of TAL-H autoantibodies was highly specific for MS. Sera of 25/87 patients with MS and of 1/32 patients with ECG reacted with recombinant TAL-H protein (Fig. 3). TAL-H-specific antibodies were not detected in other autoimmune patients including 19 with SJS and 25 with SLE and in 101 control blood donors (including 77 healthy subjects and 24 patients with other neurological diseases). Thus, detection of TAL-H autoantibodies in patients with MS is a highly significant finding as compared with control and other autoimmune disease groups ($p < 0.001$, using chi-square test). No correlation was found between TAL-H seropositivity and immunoglobulin concentrations in the sera of seven patients with MS and four control donors (data not shown).

Most patients with MS have a disease course characterized by relapses and remissions, termed relapsing/remitting (R/R) MS. A minority of patients have a primarily chronic progressive disease (CP). Many of the R/R patients will, nevertheless, eventually enter a phase of secondary progressive evolution of symptoms. As shown in Table 1, presence of TAL-H antibodies was independent of the duration or clinical phase of the disease. 13/17 cerebrospinal fluid (CSF) samples from

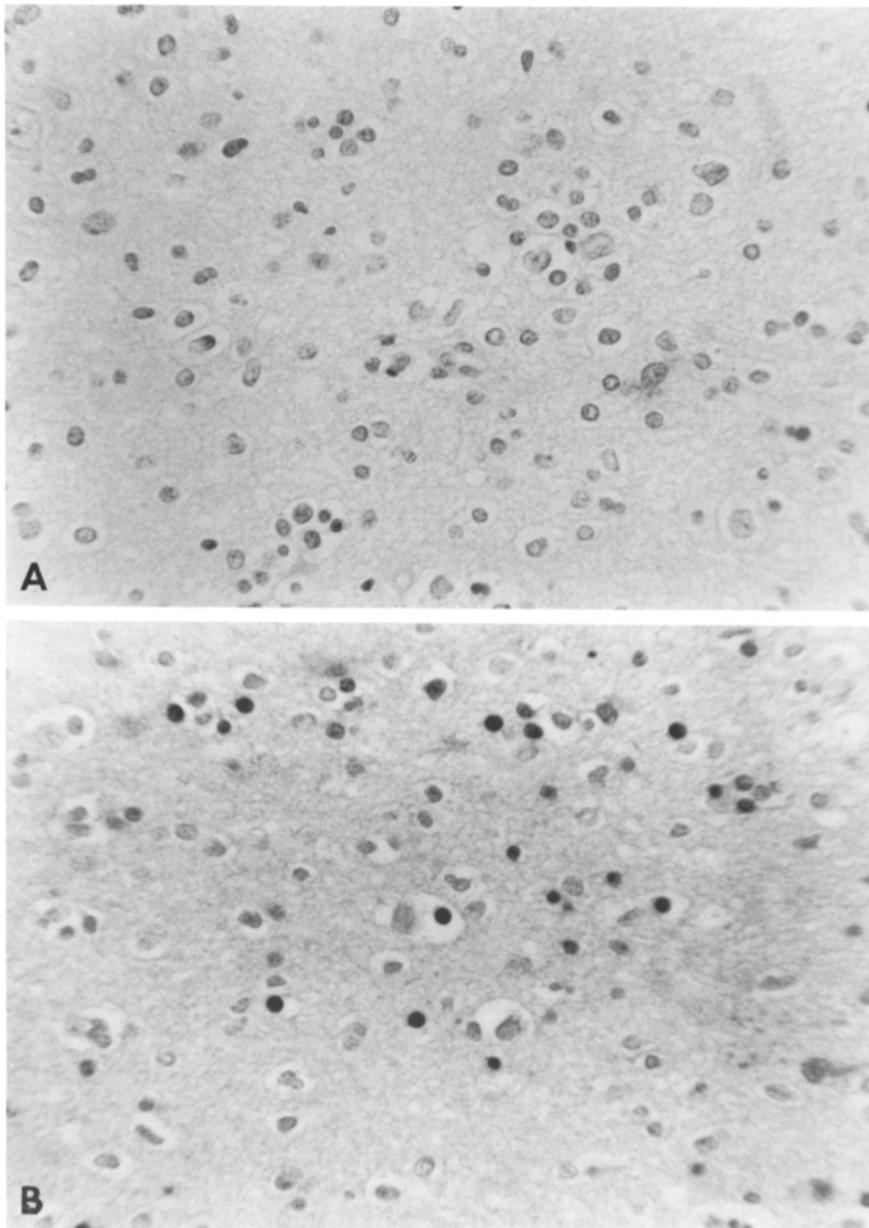


Figure 1. Frozen sections of human post-mortem brain tissue without neurological disorder was stained with control preimmune rabbit serum (A), anti-TAL-H immune rabbit serum 169 (B), and anti-GFAP rabbit serum (C). Slides were developed using biotinylated goat anti-rabbit IgG, peroxidase-conjugated streptavidin, and substrate and counterstained with hematoxylin. Formalin fixation (10% formaldehyde in PBS) causes shrinking of cytoplasm and halo formation around oligodendrocytes. While GFAP is specific for astrocytes, expression of TAL-H is specific for oligodendroglia. Original magnification, $\times 400$.

TAL-H seropositive MS patients contained antibody to TAL-H. Two of three additional CSF samples from MS patients with no available serum specimen also contained TAL-H antibodies. Thus, antibodies to TAL-H were noted in a total of 15/20 CSF samples from patients with MS. By contrast, TAL-H antibodies were absent in nine CSF samples from patients with other neurological diseases. Representative analysis of serum and CSF samples is shown in Fig. 3. While the amount of TAL-H antibodies was 5–10-fold lower in the CSF than serum of corresponding patients, the concentration of TAL-H antibodies based on the total Ig content was enriched 50–100-fold in the CSF.

Recombinant TAL-H protein used in these studies was gel-purified by electroelution in two cycles to exclude possible contamination with bacterial proteins. Antibodies 169 and

170 showed no reactivity with bacterial protein lysates. This indicated that the gel-purified recombinant TAL-H protein, used for immunization of rabbits and testing of seroactivity of the patients, was essentially free of bacterial proteins. Along the same line, TAL-H positive human sera demonstrated high affinity and specificity to the recombinant protein and showed no reactivity to bacterial proteins (Fig. 4). TAL-H specificity of MS sera was further confirmed by reactivity to a 38-kD functional TAL-H protein that had been purified by binding of a subsequently removed GST leader to glutathione-coated agarose beads (data not shown). The results suggest that TAL-H is an MS-specific autoantigen and TAL-H autoantibodies may be an important and pathogenetic factor in MS.

Stimulation of PBL Proliferation by TAL-H. In addition to increased amounts of immunoglobulins in the CSF and demye-

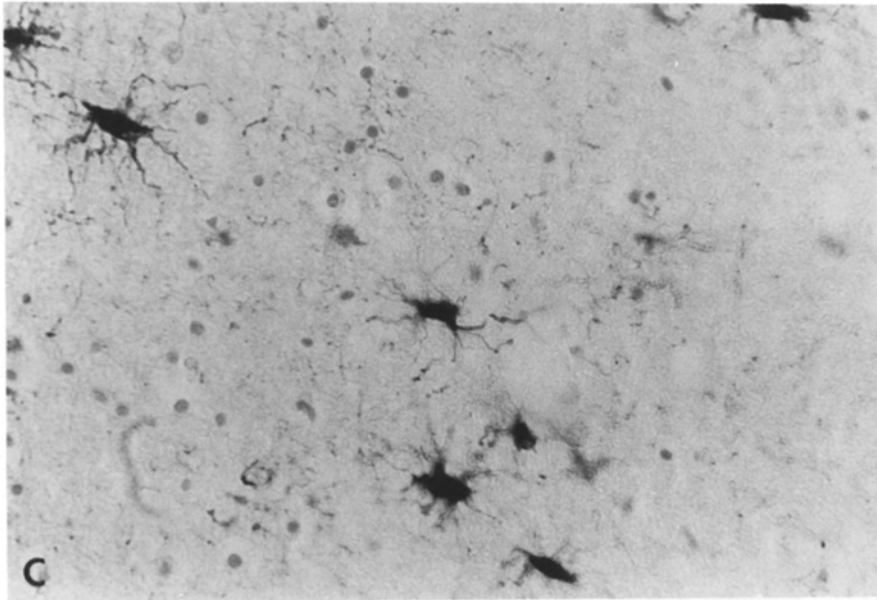


Figure 1.

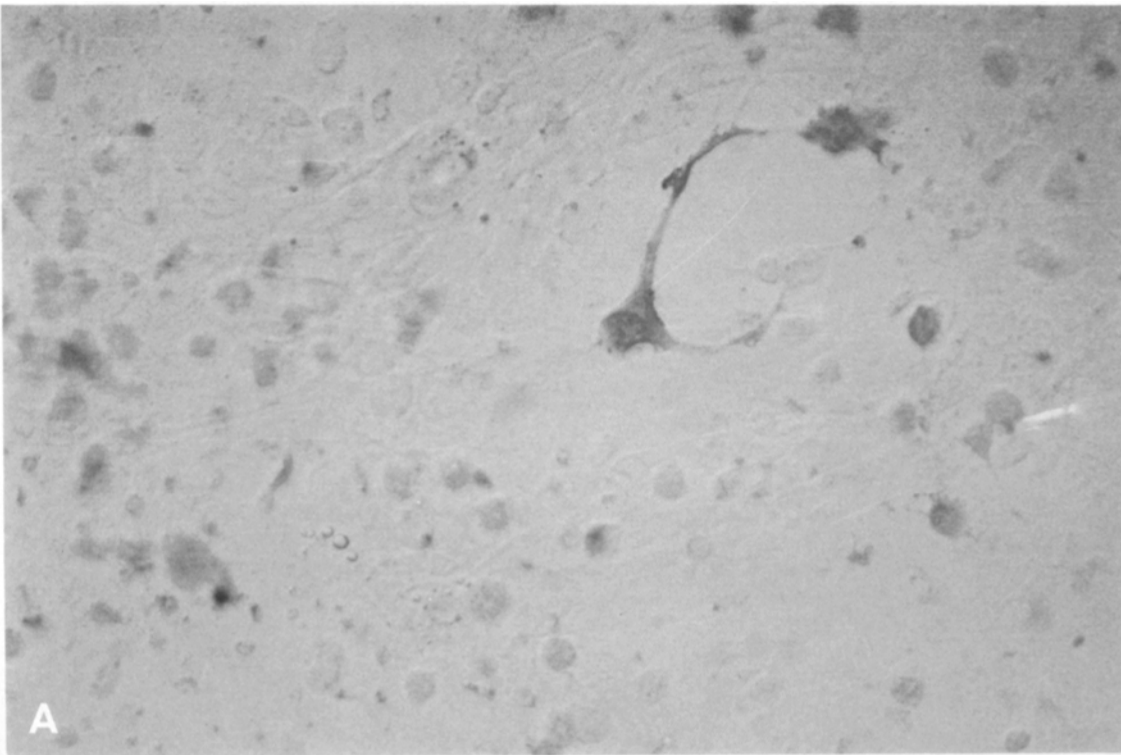
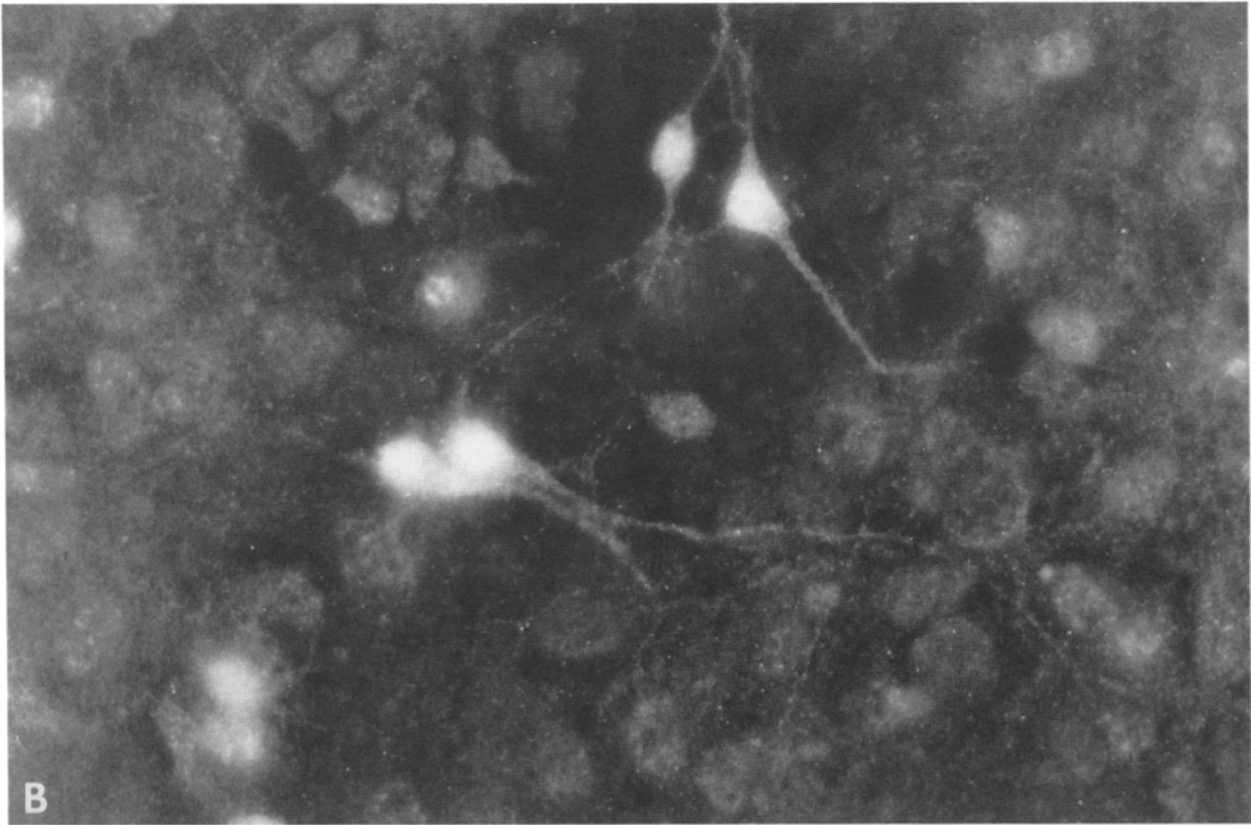
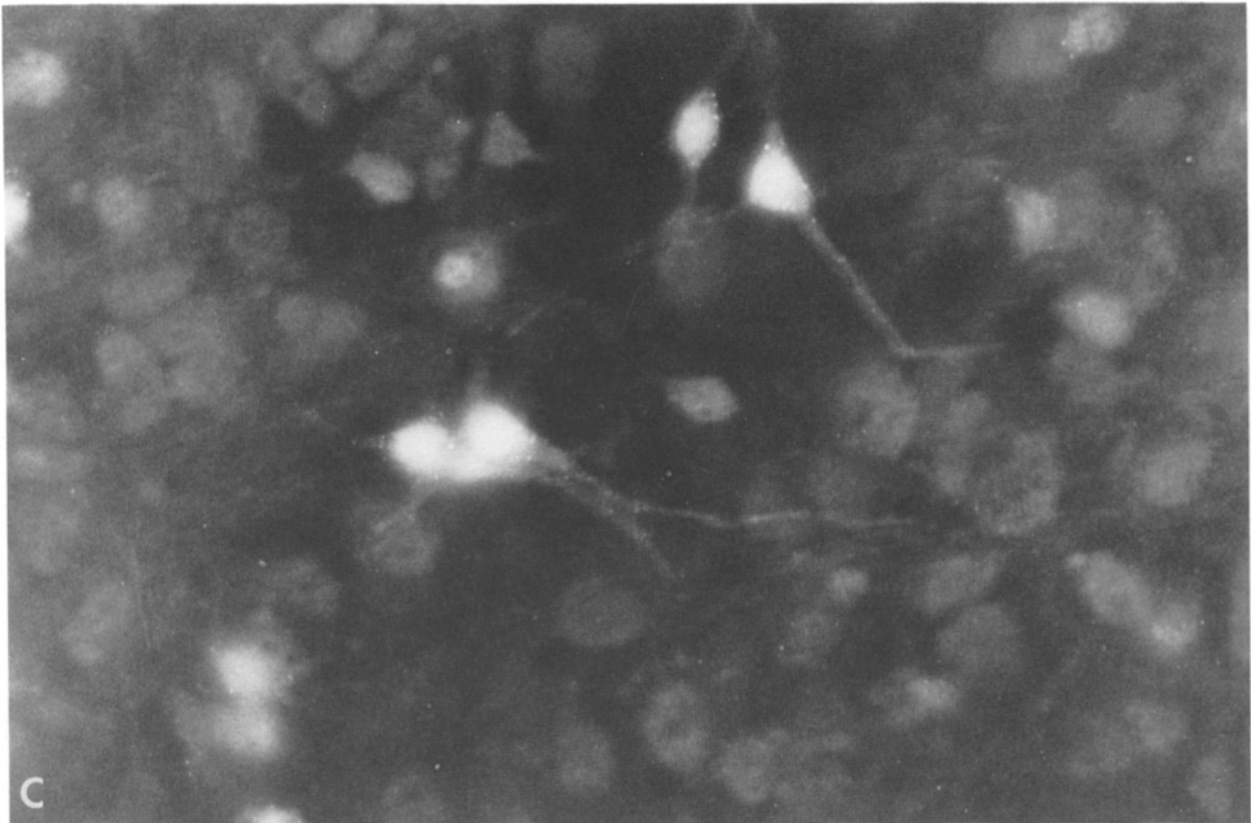


Figure 2. Detection of TAL expression in primary brain cell cultures from a 2-d-old mouse embryo with TAL-H-specific rabbit antibody 169. Expression of TAL was specific for cells with multiple arc-shaped processes, which is characteristic of cultured oligodendrocytes. (A) staining was obtained using a horseradish peroxidase-conjugated swine anti-rabbit antibody and 4-chloronaphthol substrate as described for Western blots. Using two-color immunofluorescence, an identical pattern of oligodendroglia-specific expression of TAL-H (B) and MBP (C) was noted. TAL-H protein was detected by antibody 169 and rhodamine-conjugated anti-rabbit goat antibody whereas MBP was visualized using an MBP-specific rat monoclonal antibody and FITC-conjugated goat anti-rat antibody. Red fluorescence images obtained with rhodamine conjugated secondary antibodies and green fluorescence images obtained with FITC-conjugated secondary antibodies were separately photographed. 169 preimmune serum and rhodamine-conjugated goat anti-rabbit secondary antibody (D) and a rat monoclonal antibody to human IFN- β and FITC-conjugated goat anti-rat secondary antibody (E) were used as negative control for staining of TAL-H and MBP, respectively. Original magnification, $\times 625$.



B



C

Figure 2.

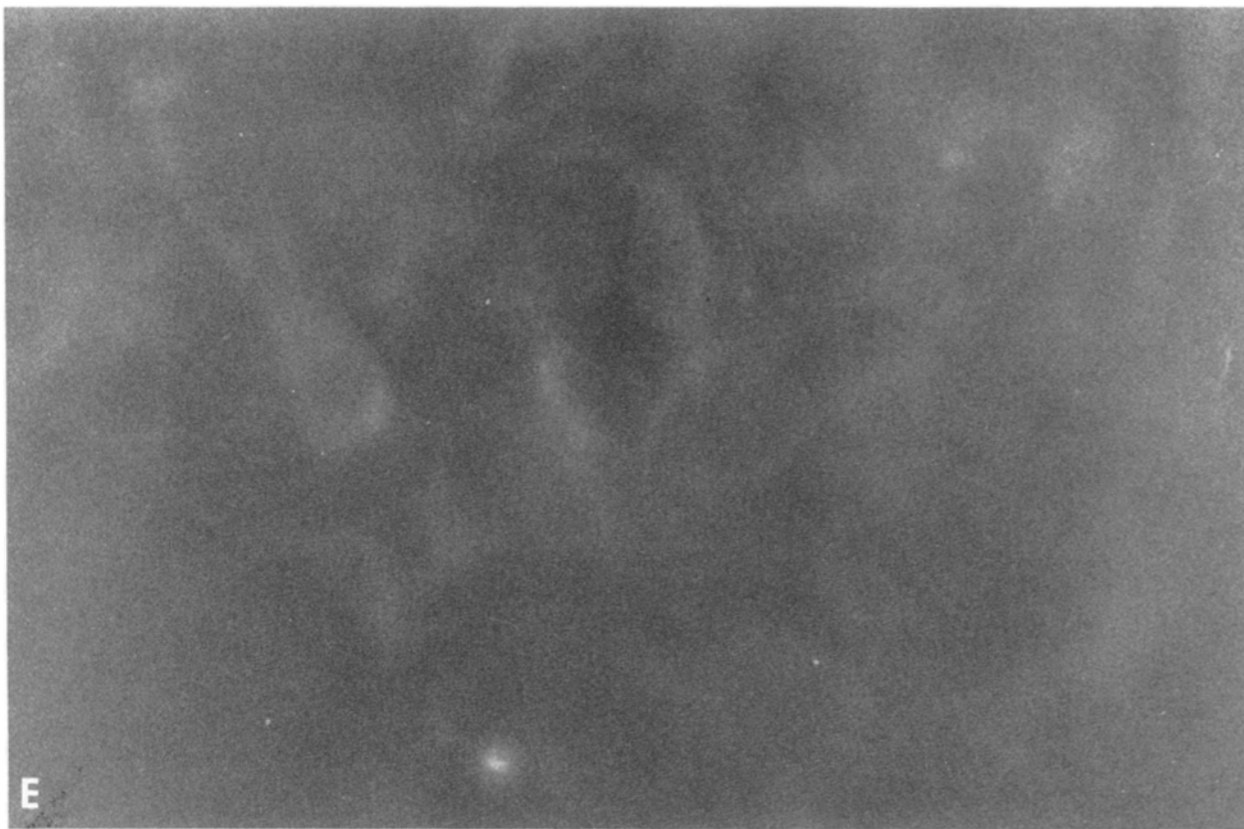
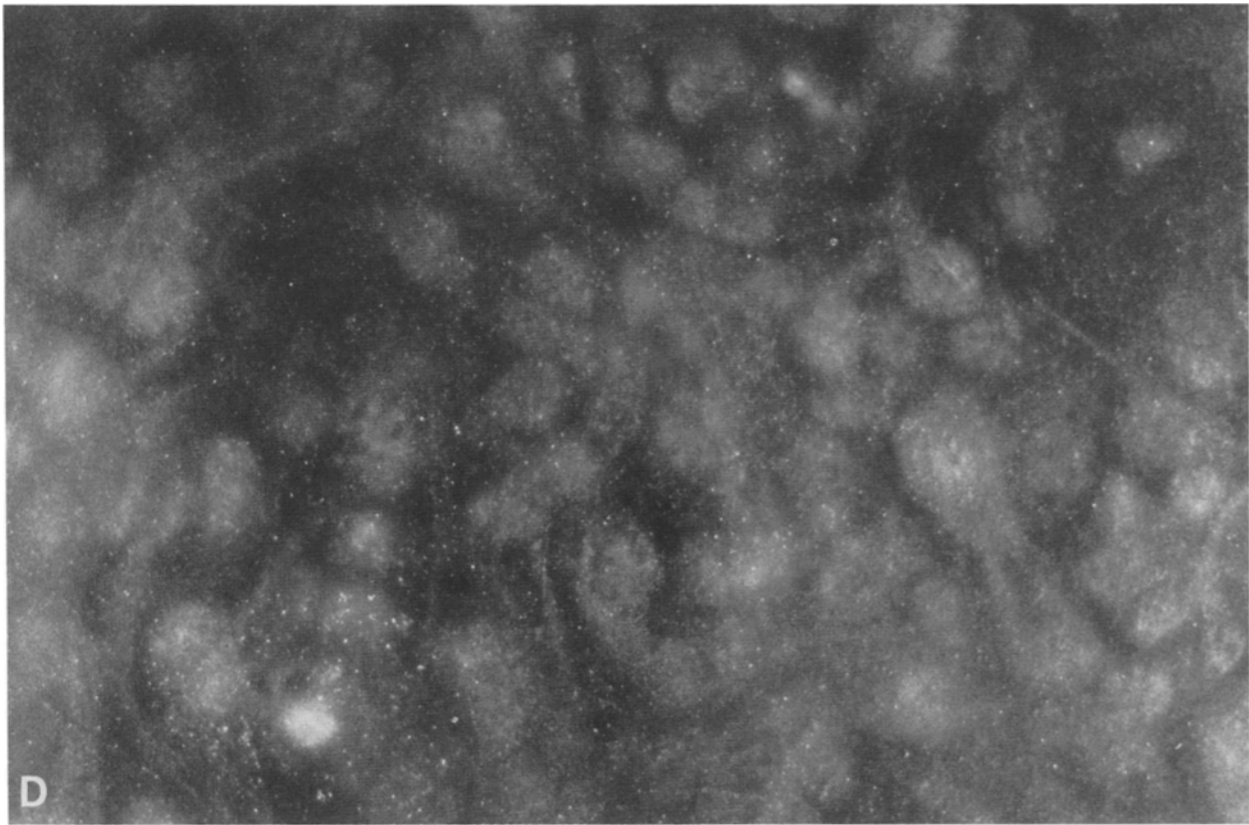


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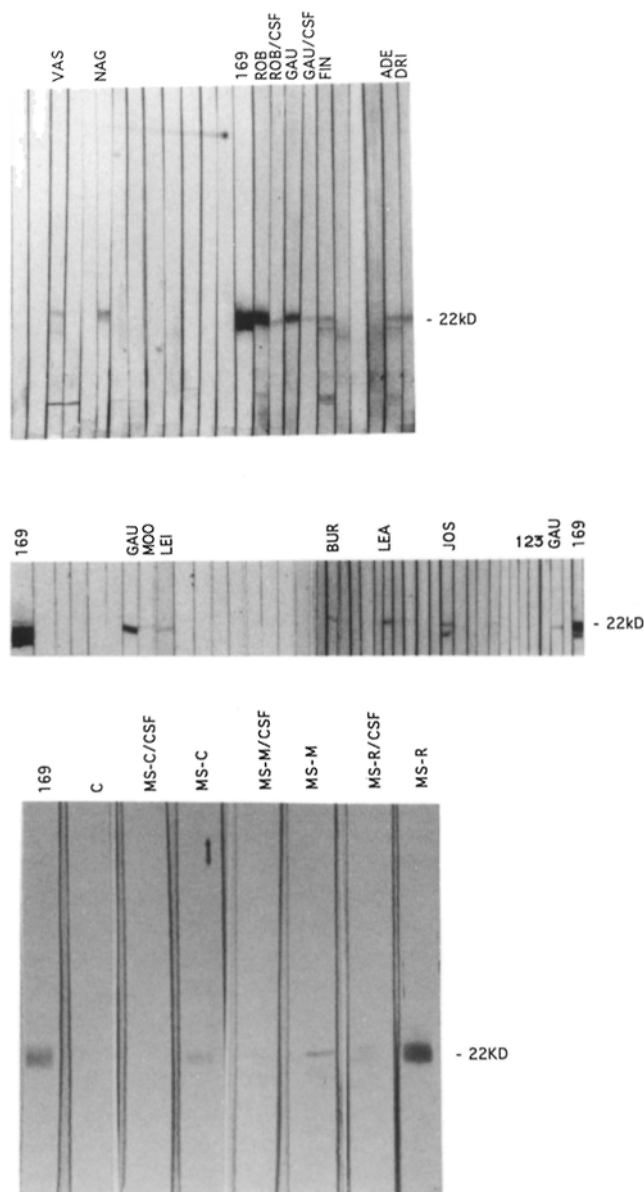


Figure 3. Western blot analysis of immunoreactivity of sera from patients with MS against recombinant TAL-H protein. TAL-H positive sera are indicated by patients' initials; 1, 2, 3, and C are normal human sera; 169, TAL-H-specific rabbit antibody; unmarked lanes indicate TAL-H negative sera of MS patients. CSF (indicated by /CSF extension) and sera were tested in parallel from patients MS-C, MS-M, MS-R, ROB, and GAU, respectively.

linating lesions, accumulation of activated T cells has been demonstrated around early MS lesions. It is generally accepted that autoreactive T cells in patients with MS recognize components of the myelin sheaths (1). To investigate whether TAL-H may be a target of autoreactive T cells, its effect on proliferation of PBLs was evaluated. Highly purified recombinant TAL-H antigen was used in these studies to ensure that the responses detected are not directed to any other myelin protein. Addition of 1 $\mu\text{g}/\text{ml}$ TAL-H significantly increased proliferation of lymphocytes from 11 patients with

Table 1. Disease Data of TAL-H Autoantibody Positive MS Patients

Patient	Age/sex	Dx duration	Dx type
VAS	26/F	4 yr	R/R
NAG	23/M	12 mo	R/R
ROB	32/M	1 mo	A
GAU	36/F	15 mo	R/R
FIN	42/F	10 yr	CP
ADE	23/F	2 mo	A
DRI	35/M	6 mo	A
MOO	45/M	12 yr	R/R
LEI	57/F	23 yr	CP
BUR	39/F	11 yr	R/R
LEA	46/M	11 yr	S
JOS	47/F	8 yr	CP
PAS	41/F	6 yr	R/R
ASH	55/F	6 yr	CP
COL	45/F	1 yr	R/R
MCG	36/F	10 yr	R/R
MCC	31/F	8 mo	R/R
DIO	22/M	3 yr	CP
GIU	53/F	2 yr	R/R
TUN	N/A	10 yr	CP
THO	33/F	2 yr	R/R
SHO	81/F	54 yr	S
GRO	82/F	25 yr	S
MS-H	38/F	8 yr	CP
MS-C	66/M	10 yr	CP
MS-R	38/F	5 yr	R/R
MS-M	N.A.	N.A.	CP
JAB	40/F	8 yr	R/R
LAK	37/F	3 yr	R/R
KUB	48/F	20 yr	CP

Dx, diagnosis; M, male; F, female; A, acute; S, stable.

MS ($p < 0.001$, Table 2). The stimulation index varied between 1.4- and 10.3-fold among the patients. Lymphocytes were incubated in the presence of 10% autologous serum. Heat-inactivation of autologous serum or use of 10% FCS had no significant effect on the proliferative responses to TAL-H. As shown in Fig. 5, the effect of TAL-H was confined to a subset of lymphocytes that showed intense blastogenesis and aggregation. By contrast, aggregation (Fig. 5 C) or proliferation of normal lymphocytes was not significantly stimulated in the presence of TAL-H (Table 2). Proliferation of lymphocytes from MS patients was not affected by 5 $\mu\text{g}/\text{ml}$ GST protein purified from the same *E. coli* strain as TAL-H (data not shown).

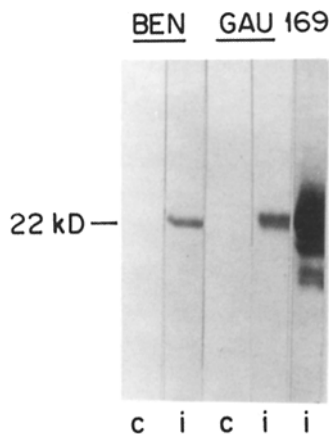


Figure 4. TAL-H specificity of human autoantibodies from patients BEN (ECG) and GAU (MS). Sera BEN and GAU strongly react with TAL-H-containing induced bacterial lysates (*i*) but do not react with control bacterial lysates (*c*).

Amino Acid Homologies and Immunological Crossreactivity between TAL-H and Retroviral Core Proteins. Molecular mimicry has been suggested to play a key role in breaking the tolerance towards self-proteins and induction of autoimmune disease (1, 31). Antibodies crossreactive with a number of viral proteins have been described in patients with MS (1).

In the amino acid sequence of TAL-H, two clusters that showed significant homology to core proteins of human retroviruses were identified (Fig. 6). An NH₂-terminal 50-amino acid long segment of TAL-H contains a region of limited homology to the HIV-1 gag p17 protein (32). The possibility of crossreactive antigenic epitopes between TAL-H and HIV-1 gag p17 was raised by immunoreactivity of antibody 169 with gag p17 and gag precursors p57 and p47 in protein lysate of HIV-1-infected PBL (Fig. 7 A). HIV-1-encoded proteins in the lysate of infected PBL were identified with a panel of HIV-1 gag p17, gag p24, env gp41, and gp120-specific antibodies (data not shown). Presence of crossreactive epitopes in TAL-H and HIV-1 gag p17 was further substantiated by binding of HIV-1 gag p17-reactive human F06 (Fig. 7 B) and gag p17-specific sheep antibodies to recombinant TAL-H (Fig. 7 C). Potential significance of four consecutive amino acid residues, Ala-Asp-Thr-Gly (ADTG), shared between TAL-H and HIV-1 gag p17 (Fig. 6) was demonstrated by use of Gag4, a recombinant protein containing the p17 COOH terminus, all of p24, and the p15 NH₂ terminus, while lacking the first 145 amino acids including the ADTG residues at positions 120–123. As shown

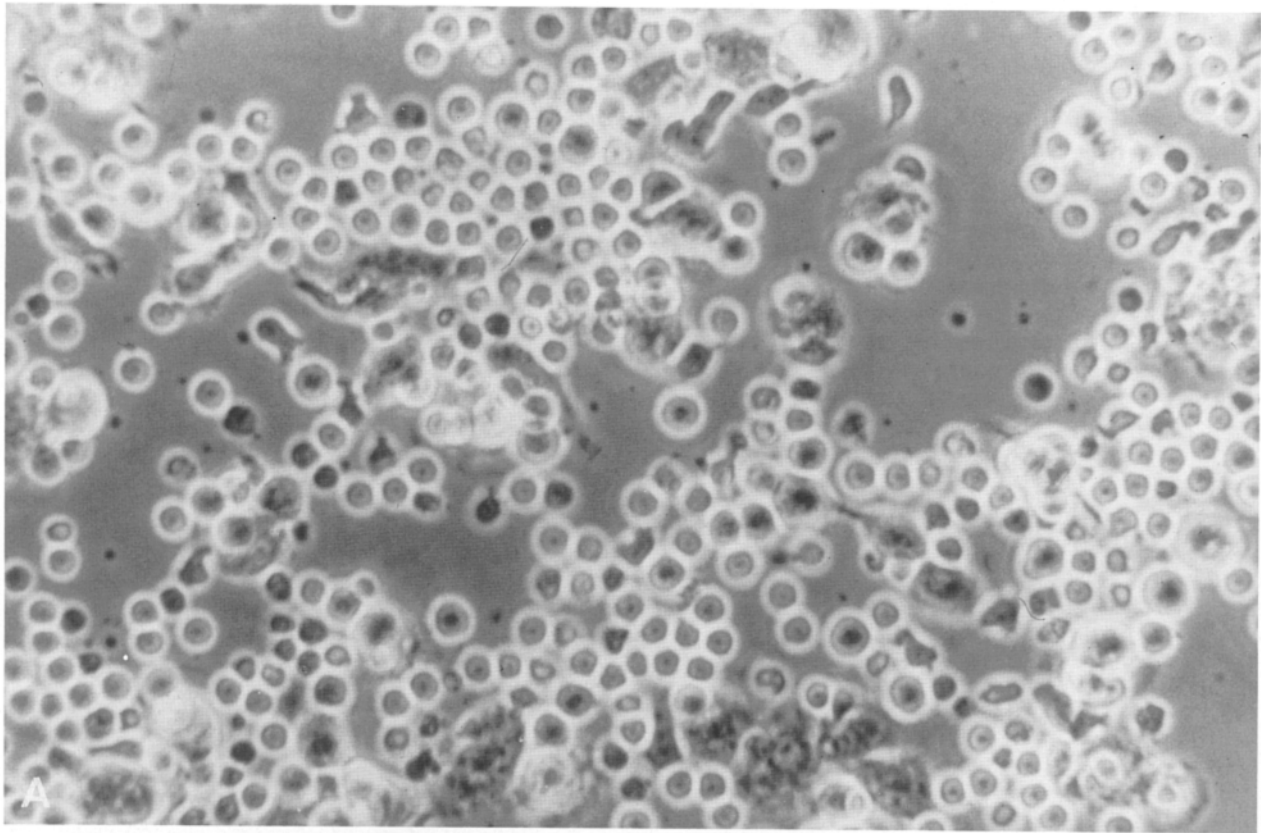
Table 2. Proliferation Responses to Recombinant TAL-H by PBLs of MS Patients and Controls

Patient	Age/sex	Dx duration	Dx type	TAL-H Ab	Proliferation response of PBLs		
					Control	TAL-H	S.I.
JAB	40/F	8 yr	R/R	+	73 ± 10	180 ± 15	2.5
LAK	37/F	3 yr	R/R	+	66 ± 13	207 ± 34	3.1
KUB	48/F	20 yr	CP	+	48 ± 3	138 ± 28	2.9
PCA	41/F	16 yr	CP	-	53 ± 6	374 ± 28	7.1
TEV	39/F	11 yr	R/R	-	49 ± 6	504 ± 47	10.3
AAB	31/M	5 yr	R/R	-	57 ± 7	178 ± 15	3.1
JWA	44/M	12 yr	R/R	-	75 ± 16	244 ± 45	3.3
ANB	57/F	26 yr	CP	-	80 ± 10	110 ± 13	1.4
GIB	49/M	19 yr	CP	-	72 ± 7	337 ± 121	4.7
CPI	28/F	3 yr	R/R	-	77 ± 7	209 ± 23	2.7
JRO	59/F	23 yr	CP	-	43 ± 6	195 ± 31	4.5
Mean ± SEM							4.15 ± 0.8*
Controls							
PRO	27/F			-	174 ± 37	177 ± 37	1.0
GIT	26/M			-	89 ± 21	209 ± 64	2.3
HAB	64/F			-	79 ± 5	79 ± 7	1.0
PEG	44/F			-	76 ± 24	55 ± 21	0.7
EST	26/F			-	42 ± 20	60 ± 15	1.4
Mean ± SEM							1.3 ± 0.3

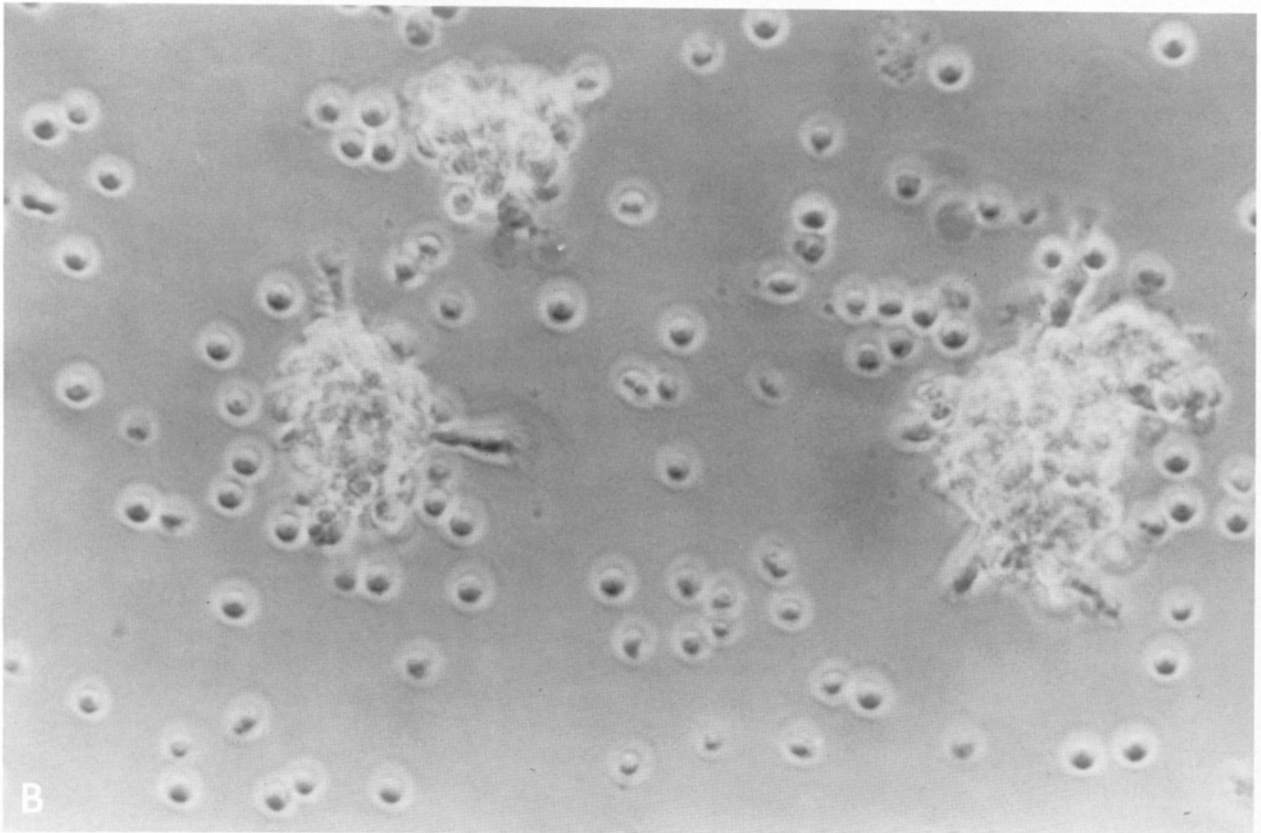
Cell proliferation was measured in the presence of 10% autologous serum without (control) or with 1 µg/ml recombinant TAL-H protein (TAL-H). Data are expressed as mean cpm ± SE of six parallel cultures.

* Significant stimulation: $p < 0.001$.

Dx, diagnosis; M, male; F, female; A, acute; S, stable; S.I., stimulation index.



A



B

Figure 5. Stimulation of aggregate formation of PBLs from a patient with MS (GIB) by recombinant TAL-H. 10^5 cells were incubated without (A) or with $1 \mu\text{g/ml}$ TAL-H for 72 h (B). C shows lymphocytes from a normal donor (BRO) incubated for 72 h with $1 \mu\text{g/ml}$ TAL-H. Original magnification, $\times 400$.

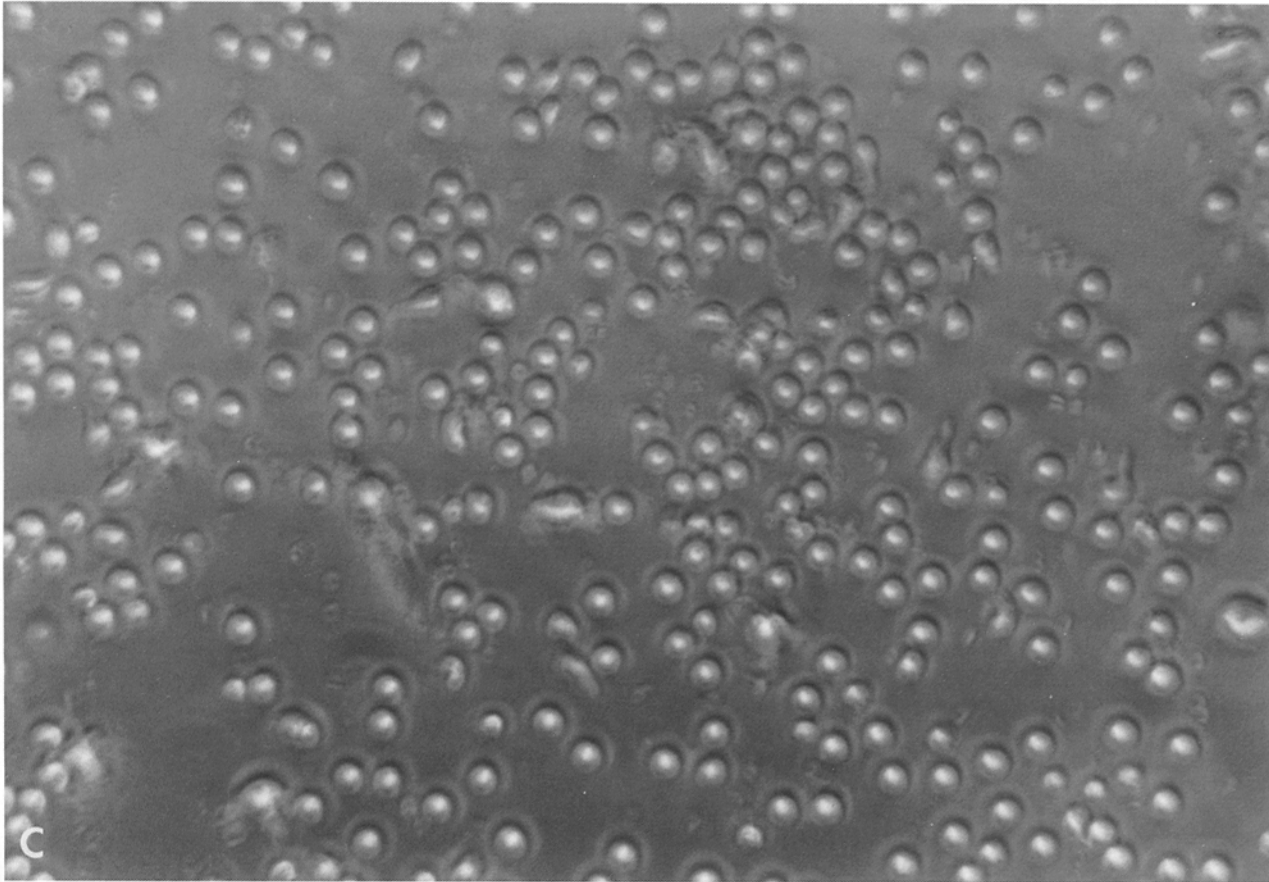


Figure 5.

in Fig. 7 C, the polyclonal HIV-1 gag p17-specific sheep antibody showed immunoreactivity with both TAL-H and Gag4 but failed to react with gag p24. Alternatively, the anti-HIV gag p24 antibody displayed specific reactivity to the Gag4 construct and gag p24 but failed to react with TAL-H. Antibody 169, recognizing the full-length gag p17 protein, failed to react with the truncated polypeptide in the Gag4 construct. These results indicate that the crossreactive epitope with TAL-H is contained within the NH₂-terminal segment of HIV-1 gag p17. The four consecutive amino acids, ADTG, present in both TAL-H and the NH₂-terminal segment of HIV-1 gag p17 are likely to represent the core of crossreactive epitopes.

To determine the significance of amino acid sequence homologies between TAL-H and HTLV-I proteins, reactivity of HTLV-I antibodies to recombinant TAL-H was evaluated. HTLV-I-specific antibodies including rabbit antisera raised against HTLV-I virion lysate and human sera from five HTLV-I-infected ATL patients reacted with recombinant TAL-H protein (representative Western blots are shown in Fig. 8 A). Conversely, TAL-H antibody 169 crossreacted with a recombinant HTLV-I gag p24 protein at a 1:1,000 dilution (Fig. 8 B). These data indicated the presence of crossreactive antigenic epitopes in TAL-H and HTLV-I gag p24. Three sets of three consecutive amino acids, Gln-Leu-Lys, containing two polar and highly charged amino acids (Gln and Lys),

present in both the transaldolase-associated repetitive element (TARE)-encoded segment of TAL-H (residues 17–19) (13) and HTLV-I gag (residues 45–47) (33), Leu-Ala-Ala (residues 50–52 in TAL-H and residues 248–250 in HTLV-I gag), as well as Lys-Leu-Leu (residues 257–259 in TAL-H and 312–314 in HTLV-I gag) are likely to be the core of crossreactive epitopes. Sera of five of the TAL-H seropositive MS patients

HTLV-I gag p19	45	QLKKFLKIALET	56	42%
TAL-H	17	QLKQFTTVVADT	28	
HTLV-I gag p24	230QANNPQQGLRREYQQLWLAAPALPGSAKDPSWASITLQGLEEPPY	274	
TAL-H	30	DFHAIDEYKPDATTNPS...LILAA.AQMPA.....YQELVEEA	65	
	275	HAFVERLNIALDNGLPETPK.....DPILRSLAYSANKECCKLL...	315	
	66	IAYGRKLGGSQEDQIKNAIDKLFVLFGAELKKIP.GRVSTVEDARLSFD	114	
	316	.QARGHTNSPLGDMLRACQWTWPKDKTKVL	344	22% (40%)
	115	KDANVARARRLIELYKEAG..ISKDRI...	139	
TAL-H	12	ESALDQLKQFTTVVADTGFHAIDEYKPDATTNPSLILAAQMPAYQELV	62	
HIV p17	106	EEQNKSKKKAQAAADTGHNSQVSNQYPIVQNIQQQMVHQAIISPTLNAMV	156	18%
HTLV-I g24	299	LRSLAYSANKECCKLLQARGHTNSPLGDMLRACQWTWPKDKTKVL	344	24%
TAL-H	243	IKALAGCDFLTITSPKLLGELLQDNAKIVPVLAKAAQASDLEKIHL	288	

Figure 6. Amino acid sequence homologies detected with the GAP program of the UWGCG Software (27) between TAL-H (13) and gag/core proteins of HTLV-I (33) and HIV-1 (32). Percent homologies and position of identical residues are shown for each sequence alignment (|). Percentage (in parenthesis) and position of functionally similar amino acids between TAL-H and HTLV-I gag p24 are also indicated (:).

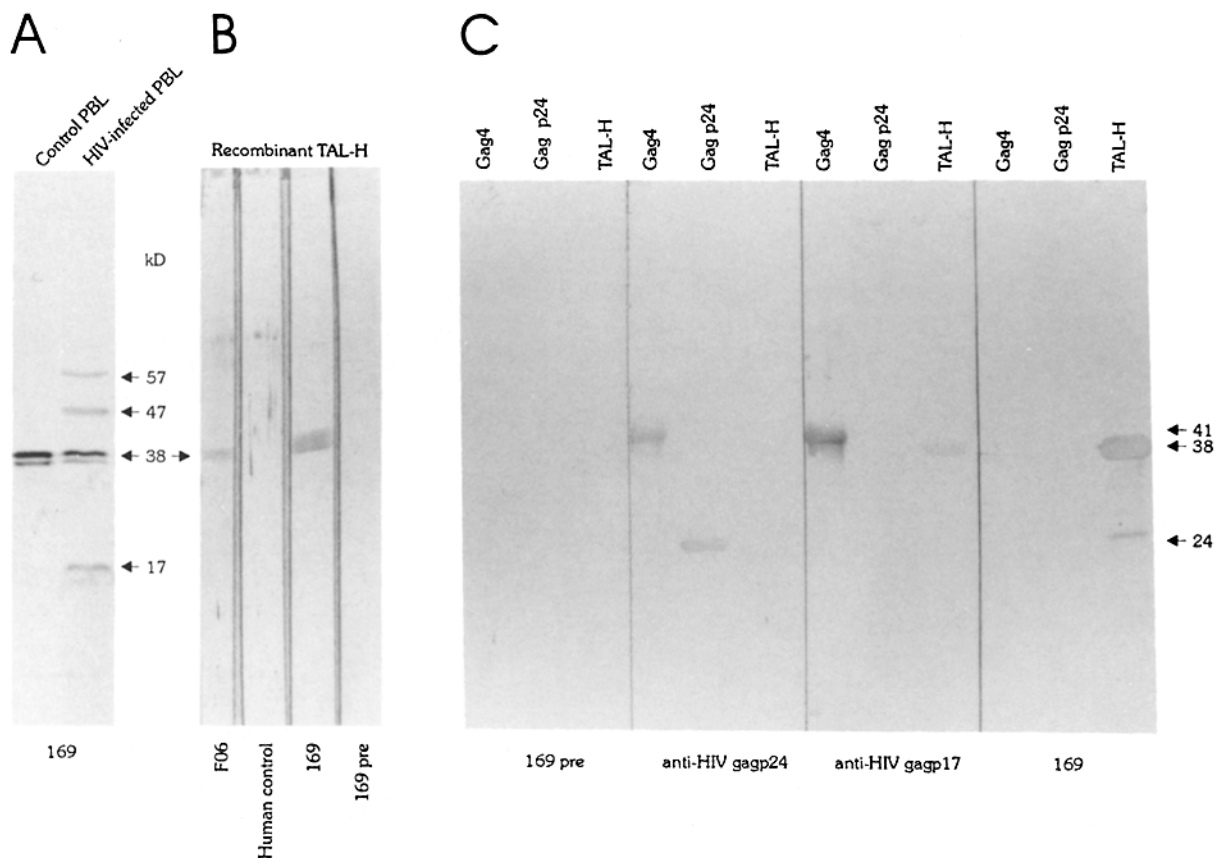


Figure 7. Immunological crossreactivity between recombinant TAL-H and HIV-1 gag p17 proteins by Western blot analysis. Protein lysates from 2×10^5 control and HIV-1-infected PBL per lane (A), 38-kD full-length affinity-purified recombinant TAL-H protein (500 ng/lane, B), and HIV-1 Gag4, HIV-1 gag p24, and TAL-H (500 ng of the indicated protein/lane, C) were separated by SDS-PAGE, transferred to nitrocellulose, and incubated with antibodies as earlier described. TAL-H antibody 169 was added at a 1:10,000 dilution to blots of A and B, while it was used at a 1:1,000 dilution in C allowing detection of TAL-H degradation products. Immunoreactivities of preimmune antibody 169, and HIV-1 gag p17- and gag p24-specific antibodies were assessed at a 1:1,000 dilution, while human control serum and F06 serum from an HIV-1-infected donor were added at a 1:100 dilution.

(ADE, BUR, JOS, ROB, and VAS) and of the one ECG patient (BEN) also reacted with recombinant HTLV-I gag p24 (data for BEN are shown in Fig. 8). Thus, autoantigenicity of TAL-H could explain the presence of HTLV-I gag-reactive autoantibodies in a subset of patients with MS (34–37) and, alternatively, molecular mimicry between HTLV-I and other retroviral core proteins may trigger autoimmunity toward TAL-H.

Discussion

Demyelination in MS results from damage to the myelin sheath which provides a multimembrane insulation for neuronal axons. Loss of the myelin cover leads to diminished conduction velocity of axons of motor and/or sensory pathways causing a wide range of neurological abnormalities (1). Myelin sheaths are formed by oligodendrocytes in the CNS. The present study demonstrates that TAL-H is expressed selectively in oligodendrocytes at high levels. TAL is a pivotal enzyme of PPP that shows maximal activity at birth and early stages of embryogenesis, coinciding with development of the nervous system at a period of active growth and myelination

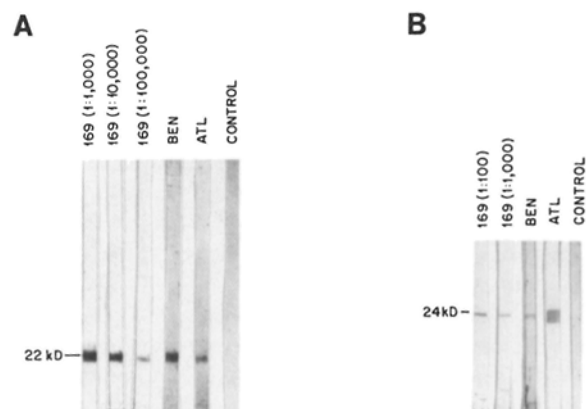


Figure 8. Detection of crossreactive antigenic epitopes between recombinant TAL-H and recombinant HTLV-I gag p24 proteins by Western blot analysis. The 22-kD recombinant TAL-H protein, composed of the NH₂-terminal 140 amino acids, was purified by electroelution from preparative SDS-PAGE in two cycles. Recombinant proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane, and incubated with antibodies as earlier described. Immunoreactivities of TAL-H Ab169 and sera from ECG patient (BEN), and a prototype HTLV-I-infected patient (ATL) with recombinant TAL-H and HTLV-I gag p24 are shown in A and B, respectively. Control serum is from a normal donor. Sera were added to Western blot strips at a dilution of 1/100 unless indicated otherwise.

(30). Another fundamental function of the PPP is to maintain glutathione at a reduced state and, consequently, to protect sulfhydryl groups and cellular integrity. Oligodendrocyte-specific expression of TAL-H is particularly interesting because of a primary involvement of these cells in demyelinating diseases of the CNS. Whereas myelin and oligodendrocytes do not show significant levels of MHC class II or class I antigen expression or surface expression of MBP (38, 39), they are sensitive to a direct attack by activated CD4⁺ T cells (40). A breakdown of the blood brain barrier may provide a route of entry for oligodendrocyte-specific antibodies. Likewise, antibodies to TAL-H may mediate demyelination by attracting microglia and macrophage through their Fc receptors and, thus, triggering phagocytosis and antibody-dependent cell-mediated cytotoxicity (41). Antibodies to TAL-H were detected in a subset of patients with MS. The fact that TAL-H antibodies were absent in controls including patients with other neurological diseases as well as in patients with systemic autoimmune diseases such as SLE and SJS indicates that autoimmune process targeting TAL-H is highly specific for MS. Further, antibody to TAL-H was detected in CSF of 15/20 MS patients. In comparison to sera, concentration of TAL-H antibodies based on the total Ig content was several-fold higher in the CSF. An increase in the amount of immunoglobulins in the CSF has been one of the earliest and most consistently reproduced findings that raised the possibility of an immune-mediated pathogenesis and is routinely used as a diagnostic criterion in MS (42). It is intriguing to consider the possibility that an intrathecal synthesis of TAL-H autoantibodies might be connected to oligodendroglia-specific expression of the protein and an eventual destruction of oligodendrocytes in MS. In contrast to the presence of high-affinity TAL-H autoantibodies, we failed to detect antibodies to purified human MBP by Western blot analysis in our patients with MS (not shown). This is in accordance with other investigators, suggesting that MBP-directed immunity is primarily T cell mediated (1, 43).

There are a number of possible mechanisms for generation of TAL-H-specific autoantibodies. First, molecular mimicry, that is, infection by an exogenous agent such as a retrovirus with crossreactive epitopes, may trigger TAL-H antibodies. We and many other investigators have not found conclusive evidence for the involvement of exogenous retrovirus in human autoimmunity (34). Nevertheless, it is possible that a retrovirus responsible for provoking autoimmunity has been cleared from the CNS, so the absence of viral particles or DNA is not conclusive (44). Core proteins are a usual target of the im-

mune response during viral infections. Because of its oligodendrocyte-specific expression and presence of crossreactive autoantigenic epitopes in its NH₂-terminal retrotransposon-encoded region, TAL-H may be a key target of HTLV-I and other retroviral-initiated autoimmunity. Since sera of all HTLV-I-infected individuals tested show crossreactivity with TAL-H, its involvement in another demyelinating disease of the CNS, HTLV-I-associated myelopathy or tropical spastic paraparesis (TSP), is also possible (45, 46). Presence of crossreactive epitopes between HIV-1 gag p17 and TAL-H may be related to neurological manifestations of AIDS (47). Thus, infections by retroviruses carrying a TAL-H related core protein may potentially trigger an autoimmune attack against oligodendrocytes. Although TAL-H is located primarily in the cytosol, antigen peptides of cytosolic proteins can be processed and associated intracellularly with MHC molecules and exported to the cell surface (48, 49). Thus, TAL-H epitopes presented on the cell surface may become targets of immune responses originally directed to a crossreactive viral core protein. The consequent destruction of target cells, such as oligodendrocytes, would release more TAL-H from the cytoplasm. The released TAL-H could then further stimulate lymphocytes already primed by the viral antigen, thus perpetuating the immune response long after the elimination of the viral infection. Since expression of TAL-H is confined to the oligodendroglia in the brain, the resultant autoimmune process could lead to a selective destruction of oligodendrocytes.

Second, autologous proteins may also trigger an immune response upon presentation in large quantities, usually accompanying extensive tissue destruction (50, 51). This mechanism, however, seems unlikely. Whereas TAL-H constitutes 1–3% of the total protein content of oligodendrocytes, MBP makes up as much as 30% of the CNS myelin (52). Thus, generation of antibodies secondary to tissue injury would just as likely result in antibodies to the highly charged MBP protein as to TAL-H. However, antibodies to MBP are absent in patients with MS.

Existence of cell-mediated immunoreactivity to TAL-H in patients with MS was substantiated by stimulation of proliferation and aggregate formation of peripheral blood lymphocytes in response to recombinant TAL-H. Levels of proliferative responses to TAL-H were higher than those in response to other myelin antigens (53). The results clearly indicate that TAL-H may be a prominent target of both cell- and antibody-mediated autoimmunity in patients with MS.

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