Human Transaldolase and Cross-Reactive Viral Epitopes Identified by Autoantibodies of Multiple Sclerosis Patients[1]

Maria Esposito,* Vijay Venkatesh,* Laszlo Otvos,‡ Zhiping Weng,§ Sandor Vajda,§ Katalin Banki,*† and Andras Perl[2]*

Multiple sclerosis is mediated by an autoimmune process causing selective destruction of oligodendrocytes. Transaldolase, which is expressed in the brain selectively in oligodendrocytes, is a target of high affinity autoantibodies in serum and cerebrospinal fluid of multiple sclerosis patients. A three-dimensional model of human transaldolase was developed based on the crystal structure of the enzyme from *Escherichia coli*. To identify immunodominant epitopes, 33 peptides overlapping human transaldolase by 5 amino acids were synthesized. Ab 12484, raised against enzymatically active human transaldolase, recognized antigenic determinants corresponding to linear epitopes (residues 27–31 and 265–290) and α helices (residues 75–98 and 302–329). Four immunodominant peptides harboring charged amino acid residues with topographically exposed side chains were identified by sera from 13 multiple sclerosis patients with predetermined autoreactivity to transaldolase. Autoantibodies binding to the most prominent human transaldolase epitope, between residues 271 and 285, showed cross-reactivity with Epstein-Barr and herpes simplex virus type 1 capsid-derived peptides. Molecular mimicry between immunodominant autoepitopes and viral Ags may be a decisive factor in directing autoimmunity to transaldolase in multiple sclerosis patients. ***The Journal of Immunology,*** **1999, 163: 4027–4032.**

Multiple sclerosis (MS)[3] is considered an autoimmune disease of the CNS. MS lesions are characterized by a progressive loss of oligodendrocytes and demyelination in the white matter of the central nervous system (1). 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 cross-reactive autoimmune process (2–7).

Previous studies from this laboratory, pursuing the goal to isolate autoantigens containing epitopes cross-reactive with viral proteins, demonstrated that an autoantigen, partially encoded by a retrotransposon and selectively expressed in oligodendrocytes at high levels (8), corresponds to transaldolase (9), a rate-limiting enzyme of the pentose phosphate pathway (PPP). A full length cDNA clone (4/2-4/1) was isolated and the 38-kDa protein was identified as the human transaldolase (TAL-H) enzyme (9, 10). Involvement of PPP in myelination provided a physiological explanation for the high level of transaldolase expression in oligodendrocytes (8, 11). PPP also plays an essential role in neutralization of reactive oxygen intermediates (ROIs) (12, 13). Although ROIs have long been considered as toxic by-products of aerobic existence, evidence is now accumulating that controlled levels of ROIs modulate various aspects of cellular function and are necessary for signal transduction pathways, including those mediating apoptosis (14). A normal reducing atmosphere, required for cellular integrity, is maintained by glutathione, which protects the cell from damage by excess ROIs. In turn, synthesis of glutathione from its oxidized form depends on NADPH produced by the PPP. TAL activity has a profound impact on the balance between the two branches of PPP and the ultimate output of NADPH and glutathione (14). These findings are in agreement with a dominant role of TAL within the metabolic network that controls intracellular NADPH levels and neutralization of ROIs (15). Oligodendrocyte-specific expression of TAL-H is possibly linked to production of large amounts of lipids, as a major component of myelin and vulnerability of the vast network of myelin sheaths to oxygen radicals. Although exons 2 and 3 in the 5′-region of the cDNA are highly repetitive (9), the functional TAL-H gene locus (*TALDO1*) is a single-copy element that has been mapped to the short arm of human chromosome 11 at p15.4—p15.5 (16). Based on differential segregation of the microsatellite marker, D11S922, 11p15 was identified as 1 of 8 loci linked to MS in a large scale genome-wide study of MS families with the use of 443 markers on all chromosomes with an average spacing of 9.6 cM (17). TAL-H is presently the genetic marker mapped nearest to D11S922 at 11p15. Therefore, *TALDO1* may correspond to or be located near a susceptibility gene influencing the development of MS. Patients with MS have Abs to transaldolase in their blood and cerebrospinal fluid. TAL-H autoantibodies recognize immunoblotted and three-dimensional epitopes and inhibit enzymatic activity of TAL-H (10). By contrast, TAL-H Abs are absent in normal individuals and patients with other autoimmune and neurological diseases (8, 11). In the present study, four immunodominant B cell epitopes were identified in 13 MS patients with concurrent B and T cell responses to TAL-H. Each of these peptides contain topographically exposed epitopes homologous to viral proteins. This raises the possibility that molecular mimicry may be involved in inducing autoimmunity to TAL-H in MS.

Departments of *Medicine and Microbiology and Immunology and †Pathology, College of Medicine, State University of New York, Syracuse, NY 13210; ‡Wistar Institute and the University of Pennsylvania, Philadelphia, PA 19104; and §Department of Biomedical Engineering, Boston University, Boston, MA 02215

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[2] Address correspondence and reprint requests to Dr. Andras Perl, State University of New York Health Science Center, 750 East Adams Street, Syracuse, NY 13210. E-mail address: perla@vax.cs.hscsyr.edu

[3] Abbreviations used in this paper: MS, multiple sclerosis; PPP, pentose phosphate pathway; TAL-H, human transaldolase; TAL-E, *Escherichia coli* transaldolase; ROIs, reactive oxygen intermediates.

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Materials and Methods

Patient and control sera

Serum samples were studied from 13 patients with MS previously documented to have concurrent B and T cell responses to TAL-H (11). All patients satisfied the criteria for a definitive diagnosis (18). Sera from 8 TAL-H-seronegative MS patients and 8 normal blood donors were used as negative control. Polyclonal rabbit Ab 12484 (10) was used as positive control for testing recognition of TAL-H epitopes. MS sera were tested for Abs to EBV capsid Ag with a commercial ELISA assay (SmithKline Beecham, Philadelphia, PA).

Peptide Ags

A total of 33 peptides, 32 that was 15 aa long, and 1 that was 17 aa long, overlapping TAL-H by 5 aa (Table I), have been synthesized on cellulose membranes with the use of a spot synthesizer (Abimed, Langenfeld, Germany) (19, 20). To optimally preserve natural conformation, peptides were C-terminally attached to cellulose via a $(\beta\text{-Ala})_2$ spacer (21). Before testing, the membranes were wetted in methanol for 10 min and, subsequently, in 100 mM Tris (pH 7.5), 0.9% NaCl for 10 min. The peptide-containing strips were incubated with sera of control donors and of MS patients with Ab reactivities to human rTAL-H at a 1000-fold dilution or positive control rabbit Abs 12484 and 170 at a 3000-fold dilution in 100 mM Tris (pH 7.5), 0.9% NaCl, 0.1% Tween 20, and 5% skim milk at room temperature overnight. Ab 12484 was raised against affinity-purified and enzymatically active full length recombinant TAL-H (10), whereas Ab 170 was raised against a gel-purified N-terminal 140-amino acid polypeptide fragment of TAL-H in rabbits (9). For detection of rabbit Abs, after washing, the strips were incubated with HRP-conjugated goat anti-rabbit IgG (Boehringer Mannheim, Indianapolis, IN). For detection of human Abs, after washing, the strips were incubated with biotinylated goat $(Fab')_2$ fragments directed against human IgA, IgG, and IgM and, subsequently, with HRP-conjugated avidin (Jackson ImmunoResearch, 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 incubated with chemiluminescent substrate (ECL, Amersham, Little Chalfont, U.K.), developed by exposure to x-ray film, and analyzed with a computerized automated densitometer (Bio-Rad, Hercules, CA). Cutoff levels were set at 3 SDs over reactivity of negative control sera. Preimmune rabbit sera and TAL-H-seronegative human sera were used as negative control for the rabbit and MS sera, respectively. Reactivities at or below cutoff levels were set at 0. Maximum reactivity with a single peptide was set a 2.0 for each Ab. Binding activities were considered dominant at half-maximal reactivity (1.0) or higher. Repeat experiments gave variations $<10\%$ for peptide binding values.

Three-dimensional modeling of TAL-H

Based on the crystal structure of transaldolase from *E. coli* (TAL-E) (22), a three-dimensional model of TAL-H at 1.5 Å resolution was constructed according to the Modeller program (23). A highly significant 60.3% amino acid identity was noted between residues 11 and 327 of TAL-H and TAL-E (Fig. 2). X-ray studies of TAL-E did not provide experimental data for the structure of the first 10 and last 11 amino acids of TAL-H. The solvent accessible surface areas of amino acids in the TAL-H model have been calculated by the algorithm of Lee and Richards (24), with a water radius of 1.4 Å. An amino acid residue X has been considered at least partially exposed if the solvent accessible surface area of its side chain exceeded 50% of the solvent accessible surface area of the side chain in the Gly-X-Gly tripeptide (24).

Statistical analysis

Correlations of cross-reactivities between TAL-H and viral peptides were analyzed with Pearson's multivariate χ^2 test (25). Differences were considered significant at $p < 0.05$.

Table I. *Epitope mapping of TAL-H with polyclonal rabbit Ab 12484 raised against enzymatically active full-length TAL-H*[a]

Peptide	TAL-H	Sequence	Ab 12484	Secondary Structure
1	1–15	MSSSPVKRQRMESAL	0.15	Undefined
2	11–25	MESALDQLKQFTTVV	1.82	α helix (charged residues exposed)
3	21–35	FTTVVADTGDFHAID	1.99	β sheet (charged residues exposed)
4	31–35	FHAIDEYKPQDATTN	1.97	Linear
5	41–55	DATTNPSLILAAAQM	0.64	α helix
6	51–65	AAAQMPAYQELVEEA	0.61	α helix
7	61–75	LVEEAIAYGRKLGGS	0.03	α helix
8	71–85	KLGGSQEDQIKNAID	1.85	α helix
9	81–95	KNAIDKLFVLFGAEI	1.91	α helix
10	91–105	FGAEILKKIPGRVST	0.03	α helix
11	101–115	GRVSTEVDARLSFDK	1.33	α helix
12	111–125	LSFDKDAMVARARRL	0	Inside $\alpha\beta$ barrel
13	121–135	RARRLIELYKEAGIS	0.21	Inside $\alpha\beta$ barrel
14	131–145	EAGISKDRILIKLSS	0	Inside $\alpha\beta$ barrel
15	141–155	IKLSSTWEGIQAGKE	0	Inside $\alpha\beta$ barrel
16	151–165	QAGKELEEQHGIHCN	0	Inside $\alpha\beta$ barrel
17	161–175	GIHCNMTLLFSFAQA	0	Inside $\alpha\beta$ barrel
18	171–185	SFAQAVACAEAGVTL	0	Inside $\alpha\beta$ barrel
19	181–195	AGVTLISPFVGRILD	0	Inside $\alpha\beta$ barrel
20	191–205	GRILDWHVANTDKKS	0	Inside $\alpha\beta$ barrel
21	201–215	TDKKSYEPLEDPGVK	1.00	Inside $\alpha\beta$ barrel with extruding loop
22	211–225	DPGVKSVTKIYNYYK	0.12	Inside $\alpha\beta$ barrel
23	221–235	YNYYKKFSYKTIVMG	0.36	Inside $\alpha\beta$ barrel
24	231–245	TIVMGASFRNTGEIK	0.02	Inside $\alpha\beta$ barrel with extruding loop
25	241–255	TGEIKALAGCDFLTI	0	Inside $\alpha\beta$ barrel
26	251–265	DFLTISPKLLGELLQ	0.02	Inside $\alpha\beta$ barrel
27	261–275	GELLQDNAKLVPVLS	1.25	Loop (265–290)
28	271–285	VPVLSAKAAQASDLE	0.21	Loop (265–290)
29	281–295	ASDLEKIHLDEKSFR	0	Loop (265–290)
30	291–305	EKSFRWLHNEDQMAV	0	α helix
31	301–315	DQMAVEKLSDGIRKF	0	α helix
32	311–325	GIRKFAADAVKLERM	1.06	α helix (charged residues exposed)
33	321–337	KLERMLTERMFNAENG	0	Undefined

[a] Relative binding by Ab 12484 to synthetic peptides (1–33) overlapping TAL-H residues 1–337 (GenBank accession number L19437, updated May 9, 1997; PID, g2073541) was assessed. Catalytic Lys^{142} (K142) is underlined. Based on automated densitometry, peptide reactivity with Ab 12484 at a 3000-fold dilution is shown as relative binding intensity on a scale of 0–2.0. Values represent the mean of four independent experiments.

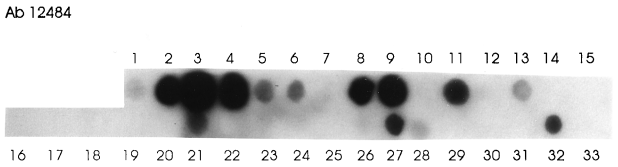

FIGURE 1. Recognition by Ab 12484 of 33 cellulose-bound synthetic peptides overlapping TAL-H (Table I). The peptides were incubated with Ab 12484 at a 3000-fold dilution. After washing, the strips were incubated with HPO-conjugated goat anti-rabbit IgG, developed with a chemiluminescent substrate, and exposed to x-ray film. Quantification of Ab reactivities with each peptide was conducted with a computerized automated densitometer (Table I).

Results and Discussion

Mapping of immunodominant B cell epitopes in TAL-H

Abs induced as a consequence of immunization with a whole protein Ag are often directed toward three-dimensional epitopes, most often composed of sets of residues that are discontinuous and brought together by folding (26, 27). Alternatively, a number of recent studies have demonstrated that discontinuous linear segments, which represent significant antigenic sites and brought together as a composite site by the folds, can be positively identified by testing antigenicity of overlapping synthetic peptides (26, 27). B cell epitopes are usually comprised of 5 aa or less in contiguity (28, 29). Peptides ~15 aa long can assume ordered conformations mimicking the native protein (30).

A total of 33 peptides overlapping TAL-H by 5 amino acids have been synthesized on cellulose membranes (Table I) (19, 20). To map epitopes exposed on native TAL-H, we used polyclonal rabbit Ab 12484, raised against the full length and enzymatically active TAL-H (10). Ab 12484 binds native TAL-H and inhibits its catalytic activity (10). Recognition by Ab 12484 of individual TAL-H peptides was assessed at a 3000-fold dilution by chemiluminescent detection (Fig. 1). Peptide reactivity with Ab 12484 was quantified by automated densitometry and expressed as relative binding intensity on a scale of 0–2.0 (Table I). Immunodominant epitopes with high binding affinity to Ab 12484 were defined as having a binding intensity ≥1.0, i.e., 10-fold or more over background (Table I).

Three-dimensional model of human transaldolase

Recognition of synthetic peptides was correlated with the three-dimensional structure of TAL-H. The functional TAL-H protein is comprised of 337 amino acids. Transaldolase from *E. coli* (TAL-E) codes for 317 amino acids. A highly significant amino acid identity of 60.3% was noted between residues 11 and 327 of TAL-H and TAL-E (Fig. 2). Based on the crystal structure of TAL-E (22), a three-dimensional model of TAL-H was constructed using the Modeller program (23). Similar to the bacterial enzyme, TAL-H forms an $\alpha\beta$ barrel with 8 α helices around 8 β sheets, 6 additional helices, and a large loop from residues 265 to 290 (Fig. 3). The catalytic site is on strand β4 around the Schiff base-forming Lys^{142} (10). Replacement of Lys^{142} with glutamine resulted in a complete loss of enzyme activity, suggesting that Lys^{142} is essential for catalytic activity of TAL-H (10). Studies of TAL-E by x-ray did not provide experimental data for the structure of the first 10 and last 11 amino acids of TAL-H. The solvent-accessible surface areas of amino acids in the TAL-H model have been calculated by the algorithm of Lee and Richards, using a water radius of 1.4 Å (24). An amino acid residue X has been considered at least partially exposed if the solvent-accessible surface area of its side chain exceeded 50% of the solvent-accessible

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TAL-H    1 MSSSPVKROR                                              10
TAL-H   11 MESALDQLKQFTTVVADTGDFHAIDEYKPQDATTNPSLILAAAQMPAYQE 60
TAL-E    1 MTDKLTSLRQYTTVVADTGDIAAMKLYQPQDATTNPSLILNAAQIPEYRK 50
TAL-H   61 LVEEAIAYGRKLGGSQEDQIKNAIDKLFVLFGAEILKKIPGRVSTEVDAR 110
TAL-E   51 LIDDAVAWAKQQSNDRAQQIVDATDKLAVNIGLEILKLVPGRISTEVDAR 100
TAL-H  111 LSFDKDAMVARARRLIELYKEAGISKDRILIKLSSTWEGIQAGKELEEQH 160
TAL-E  101 LSYDTEASIAKAKRLIKLYNDAGISNDRILIKLASTWQGIRAAEQLEKE. 149
TAL-H  161 GIHCNMTLLFSFAQAVACAEAGVTLISPFVGRILDWHVANTDKKSYEPLE 210
TAL-E  150 GINCNLTLLFSFAQARACAEAGVFLISPFVGRILDWYKANTDKKEYAPAE 199
TAL-H  211 DPGVKSVTKIYNYYKKFSYKTIVMGASFRNTGEIKALAGCDFLTISPKLL 260
TAL-E  200 DPGVVSVSEIYQYYKEHGYETVVMGASFRNIGEILELAGCDRLTIAPALL 249
TAL-H  261 GELLQDNAKLVPVLSAKAAQASDLEKIHLDEKSFRWLHNEDQMAVEKLSD 310
TAL-E  250 KELAESEGAIERKLSYTGEVKA..RPARITESEFLWQHNQDPMAVDKLAE 297
TAL-H  311 GIRKFAADAVKLERMLTERMFNAENGK 337
TAL-E  298 GIRKFAIDQEKLEKMI 313
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FIGURE 2. Amino acid sequence homologies between human (TAL-H) and *E. coli* transaldolase (TAL-E). |, Position of identical residues; :, position of functionally similar amino acids.

surface area of the side chain in the Gly-X-Gly tripeptide (24). According to this analysis, peptides recognized by Ab 12484 correspond to loops with linear epitopes and α helices in which highly charged residues are at least partially exposed (Fig. 3, Table I).

Mapping of B cell epitopes in patients with MS

Abs to TAL-H were previously detected in patients with MS (8, 11). In the present study, we examined recognition of TAL-H peptides by sera of 13 TAL-H seropositive MS patients at a 1:1000-fold dilution. All patients showed immunoreactivity to the full

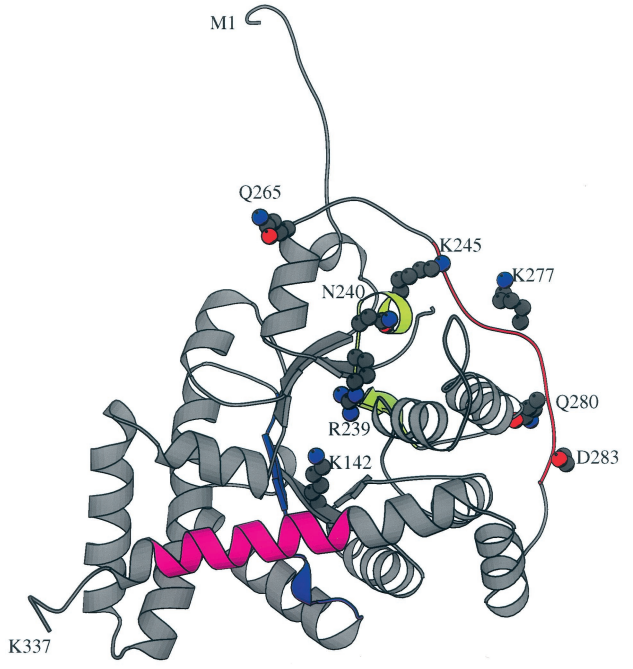

FIGURE 3. Three-dimensional model of human transaldolase (TAL-H) displayed with the Molscript program (40). TAL-H forms an $\alpha\beta$ barrel, 14 α helices (represented by spirals) around 8 β sheets (represented by wide flat arrows). The catalytic site with the Schiff base-forming Lys^{142} (K142) is on strand β4. The first and last residues (M1, K337), K142, the polar/charged residues of peptide 24 (R239, N240, K245) and peptide 28 (K277, Q280, D283), and the starting residue (Q265) of an immunodominant loop between residues 265 and 290 are shown as landmarks. Circles show the orientation of nitrogen (blue circles), oxygen (red) and carbon atoms (gray circles) of topographically exposed and charged amino acids.

Table II. *Binding of Abs from MS patients to peptides overlapping TAL-H*[a]

Peptide	AAB	ALV	JAB	JOS	LAK	LIT	MAD	PAS	PCA	PET	RYD	SOL	TEV	*N*
1	0.00	0.00	0.74	0.32	0.17	**2.00**	0.22	**1.40**	0.09	0.70	0.53	0.00	0.00	2
2	0.05	**1.57**	0.00	0.00	**1.04**	0.07	0.00	0.00	0.06	0.00	0.00	0.00	0.00	2
3	0.00	**1.62**	**1.42**	0.47	0.00	0.00	0.22	0.00	0.13	0.00	0.00	0.80	0.00	2
4	0.00	0.00	0.35	0.45	0.23	**1.17**	0.00	0.00	0.00	0.00	0.20	0.00	0.00	1
5	0.05	0.00	0.09	0.63	0.00	0.00	0.45	0.00	0.00	0.00	**1.70**	0.00	0.00	1
6	0.00	0.00	0.16	0.37	0.00	0.10	0.00	0.00	0.11	0.00	**1.70**	0.00	0.00	1
7	0.00	0.09	0.44	0.00	0.00	0.05	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0
8	0.00	0.00	0.39	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0
10	0.00	0.63	0.00	0.00	**1.07**	**1.42**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2
11	0.00	**1.28**	0.19	0.00	0.20	**1.39**	0.58	**1.97**	0.04	0.00	**1.13**	**1.72**	0.00	5
12	0.00	0.23	0.18	0.39	0.23	0.07	0.52	0.86	0.17	0.00	0.13	0.00	0.00	0
13	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0
14	0.00	**1.65**	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00	0.20	**2.00**	0.00	2
15	0.00	0.00	0.14	0.00	0.29	0.07	0.00	0.00	0.09	0.00	**2.00**	0.00	0.00	2
16	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0
17	0.00	0.00	0.42	0.00	0.00	0.00	0.09	0.86	0.00	0.00	0.00	0.60	0.00	0
18	0.05	0.34	**1.24**	0.00	0.29	0.02	0.18	0.00	0.06	0.00	0.55	0.00	0.00	1
19	0.00	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0
20	0.00	0.14	0.00	0.00	0.00	0.00	**1.04**	**2.00**	0.00	**1.17**	0.00	0.68	0.00	3
21	0.00	0.29	0.00	0.00	0.00	**1.24**	0.00	0.34	0.00	0.00	0.68	**1.84**	0.00	2
22	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
23	0.00	0.03	0.34	0.00	0.00	0.00	0.00	0.00	0.26	0.00	**1.03**	0.00	0.00	1
24	**2.00**	0.14	**2.00**	**1.53**	**1.34**	**1.59**	**1.37**	**1.40**	0.06	0.00	**1.30**	0.00	**2.00**	9
25	0.00	0.48	0.27	0.00	**1.32**	0.00	**1.37**	0.00	0.38	0.00	0.83	0.00	**1.97**	3
26	0.42	0.31	0.55	0.00	**1.37**	**1.07**	0.43	0.00	0.34	0.00	**1.03**	2.00	0.29	3
27	0.02	0.91	0.11	0.00	0.00	0.00	**2.00**	0.00	**2.00**	0.00	0.00	**1.76**	0.30	3
28	**1.47**	**2.00**	**1.65**	**1.13**	**1.09**	0.00	**1.68**	**1.07**	0.21	**2.00**	**1.28**	0.00	**1.33**	10
29	0.02	0.00	0.23	**1.50**	0.06	0.00	0.40	0.00	0.00	0.00	0.75	0.20	0.27	1
30	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.14	0.00	0.50	0.00	0.00	0.00	0
31	0.00	0.14	0.00	**1.13**	0.00	0.00	0.00	0.00	0.00	0.00	**1.70**	0.00	0.00	2
32	0.35	0.11	0.65	**2.00**	**2.00**	0.02	0.54	**1.98**	**1.30**	0.00	0.00	**1.92**	0.00	5
33	0.00	0.00	0.04	0.00	0.00	0.10	0.00	0.86	0.00	0.00	0.00	0.12	0.00	0

[a] Peptide sequences are shown in Table I. Values represent relative binding at a 1000-fold dilution on a scale of 0–2.0 as described in Table I. Baseline (=0) represents binding at or below cutoff levels. Dominant binding activities, at half-maximal reactivity (1.0) or higher, are boldface. Values represent mean of two to four independent experiments. *N* indicates number of patients showing dominant binding for each peptide.

length TAL-H protein by Western blot and ELISA. As controls, sera from eight control donors, eight TAL-H Western blot-negative MS patients, and preimmune rabbit serum for Ab 12484 showed no significant binding to the peptides. Sera of all but one patient (AAB) recognized 2 or more (maximum, 14) TAL-H peptides. Four immunodominant B cell epitopes were identified by autoantibodies of MS patients. Peptide 24 was recognized by 9 of 13 TAL-reactive sera, 10 of 13 sera recognized peptide 28, and peptides 11 and 32 were recognized by 5 of 13 sera (Table II). Each of these peptides contained topographically exposed epitopes with highly charged residues in side chains (Fig. 3, Table I). Peptides 11 and 32 were recognized by both Ab 12484 and MS sera. In contrast, peptides 24 and 28 were not recognized by Ab 12484, suggesting that these two peptides may be cryptic or nonimmunogenic in the rabbit. TAL-H, which is released from sites of demyelination in the brain of MS patients (11), may be subject to proteolytic degradation, thus exposing cryptic epitopes.

All four immunodominant peptides showed sequence homology to viral Ags (Fig. 4). To directly investigate cross-reactivity, immunodominant TAL-H epitopes and related viral peptides were tested in parallel for recognition by Ab 12484 and MS sera (Table III). Sera of eight MS patients lacking TAL-H autoantibodies also failed to recognize the viral peptides (data not shown). Binding of Ab 12484 revealed molecular mimicries between TAL-H peptide 11 (residues 101–115) and HSV1 helicase as well as TAL-H peptide 32 (residues 311–325) and HIV-1 env gp160, respectively (Table III). None of the MS sera recognized the HSV1 helicase and HIV-1 env gp160-derived peptides, which is consistent with a lack of exposure to nuclear proteins such as HSV-1 helicase and an absence of HIV-1 infection in these patients. No cross-reactivity was detected between TAL-H peptide 24 (residues 231–245) and measles envelope peptides. Recognition of TAL-H peptide 28 (residues 271–285) correlated with binding to EBV or HSV-1 capsid-derived peptides (Table III). Interestingly, sera binding to the most

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TAL-H                  101   GRVSTEVDARLSFDK
                             :||    ||||   :
HSV-1 helicase         167   ARVAEHPDARLAWAR 40% (53%)

TAL-H                  231   TIVMGASFRNTGEIK
                               ||:   : : :|||
Measles/SSPE env       281   FIVLSIAYPTLSEIK 33% (60%)

TAL-H                  271   VPVLSAKAAQASDLE
                             ||||   ||    ||
EBV capsid BOLF1       306   VPVLAFDAARLRLLE 53%

TAL-H                  271   VPVLSAKAAQASDLE
                               ||:   || |:||
HSV-1 capsid VP5       410   NPVMERFAAHAGDLV 46% (60%)

TAL-H                  311   GIRKFAADAVKLERM
                             ||: : | :: :||
HIV-1 env gp160        572   GIKQLQARVLAVERY 33% (66%)
```

FIGURE 4. Amino acid sequence homologies between immunodominant B cell epitopes of TAL-H recognized by MS patients and viral peptides. Homologies between TAL-H and viral sequences from herpes simplex type 1 (HSV-1) helicase (41), measles/subacute sclerosing panencephalitis (SSPE) virus (42), EBV capsid protein BOLF1 (43), HSV-1 capsid protein VP5 (44), and HIV-1 env gp160 (45) were detected with the UWGCG Software (46). Percent homologies, percent similarities (numbers in parentheses), position of identical residues (|), and position of functionally similar amino acids (:), as well as amino acid position of the first residue of each peptide, are indicated.

Table III. *Comparative binding by TAL-H-reactive autoantibodies from MS patients to immunodominant TAL-H epitopes and viral peptides*[a]

Peptide	Ab 12484	AAB	ALV	JAB	JOS	LAK	LIT	MAD	PAS	PCA	PET	RYD	SOL	TEV
TAL-H 11 (101–115)	**2.00**	0.00	**1.40**	0.74	0.22	0.17	**1.20**	0.42	**1.45**	0.79	0.70	**1.53**	**1.40**	0.70
HSV-1 helicase	**1.66**	0.09	0.70	0.00	0.11	0.04	0.07	0.00	0.00	0.56	0.00	0.00	0.00	0.00
TAL-H 24 (231–245)	0.45	**1.78**	0.32	**1.42**	**1.07**	**1.59**	**2.00**	**1.22**	**1.04**	0.63	0.13	**1.12**	0.80	**1.08**
Measles env	0.23	0.69	0.00	0.35	0.45	0.23	0.17	0.00	0.00	0.12	0.00	0.20	0.00	0.00
EBV capsid BOLF1	0.64	**2.00**	0.00	0.09	**1.89**	0.00	0.00	**2.00**	0.00	0.79	**2.00**	**2.00**	0.88	**2.00**
TAL-H 28 (271–285)	0.31	**1.48**	**1.89**	**1.86**	**2.00**	**1.37**	0.45	**1.07**	**1.10**	0.11	**1.66**	**1.79**	0.00	**1.91**
HSV-1 capsid VP5	0.28	0.10	**2.00**	**2.00**	0.00	**2.00**	0.05	0.40	**2.00**	0.05	0.00	0.00	0.00	0.00
TAL-H 32 (311–325)	**1.96**	0.31	0.00	0.39	**1.92**	**1.16**	0.76	0.79	**1.40**	**2.00**	0.00	0.20	**2.00**	0.00
HIV-1 env gp160	**1.91**	0.26	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.04	0.00	0.00	0.60	0.00

[a] Peptide binding by rabbit Ab 12484 is shown as a reference. Amino acid sequences of TAL-H and homologous viral peptides are presented in Fig. 4. Abs were incubated with a set of nine peptides in parallel at a 1000-fold dilution. Values represent the mean of two to four independent experiments. Data show relative binding on a scale of 0–2.0 as described in Table II.

immunodominant TAL-H epitope showed a remarkable segregation of cross-reactivities with EBV and HSV-1 capsid-derived peptides, respectively. Accordingly, 6 of 10 TAL-reactive sera (AAB, JOS, MAD, PET, RYD, and TEV) showed binding to the EBV but not to the HSV-1 peptide, whereas 4 of 10 sera (ALV, JAB, LAK, and PAS) displayed an opposing pattern (Pearson χ^2 test: $p = 0.00157$). Abs to HSV-1 have been found in sera and CSF of MS patients (31), and >99% of MS patients are infected with EBV (32). Based on an ELISA, with one exception (LIT), sera of all MS patients of this study contained Abs to EBV capsid protein (not shown). HSV-1 have been previously linked to inflammatory CNS disease (33).

The present data provide direct evidence for molecular mimicry between common viral Ags and the immunodominant epitope of TAL-H in a subset of MS patients. Cross-reactivity between a pair of related epitopes, a pathogenic determinant of a foreign (viral) Ag and the corresponding cryptic epitope of a self protein has been proposed to induce autoimmunity (34). The crypticness of self determinants, such as TAL-H peptide 28, may result in a lack of thymic tolerance. Molecular mimicry between a foreign and self-protein that is usually considered as an initiating factor in autoimmunity (8, 35–37) may lead to spreading to additional TAL-H autoepitopes (38) on release from sites of CNS injury and demyelination.

Autoantibodies are deposited around disintegrating myelin components of the MS brain (39). Patients with MS have Abs to transaldolase in their blood and CSF (8, 11). Because Ag-specific B cells can bind Ag and present it to T cells at free Ag concentrations ~1000 times lower than those required by conventional APC, Abs may be a decisive factor in directing autoimmunity to TAL-H in comparison with other oligodendroglial Ags in patients with MS. Alternatively, autoantibodies may bind to TAL-H released from sites of tissue injury (11) and form immune complexes which in turn may facilitate Ag presentation by other types of APC.

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