

Table S1: Clinical features of patients with MS used in this study. RR = relapsing remitting, PP = primary progressive, SP = secondary progressive, CIS = clinically isolated syndrome and NA = data not available.

Sample	Clinical Subtype	Date of diagnosis	Disease Duration from diagnosis	MRI Activity	Disease Modifying Therapy	High Dose steroids	ETX Result
MS001	RR	NA	2 months	NA	No	No	Negative
MS002	RR	NA	5 years	NA	No	No	Negative
MS003	RR	NA	3 months	NA	No	No	Negative
MS004	RR	NA	4 years	NA	No	No	Negative
MS005	RR	NA	3 years	NA	No	No	Negative
MS006	RR	NA	5 months	NA	Yes	No	Negative
MS007	RR	NA	8 years	NA	No	No	Negative
MS008	RR	NA	7 years	NA	Yes	No	Negative
MS009	RR	NA	2 months	NA	No	No	Negative
MS010	RR	NA	4 years	NA	No	No	Negative
MS011	RR	NA	1 month	NA	No	No	Positive
MS012	RR	2013	2009	NA	No	NA	Negative
MS013	RR	2001	1998	NA	Yes	NA	Negative
MS014	PP	2001	2001	NA	Yes	NA	Negative
MS015	RR	2015	2011	NA	No	NA	Negative
MS016	RR	2010	2005	NA	Yes	NA	Negative
MS017	RR	2012	2011	NA	Yes	NA	Positive
MS018	RR	2004	2004	NA	No	NA	Negative
MS019	RR	2014	2006	NA	Yes	NA	Negative
MS020	RR	2005	2004	NA	No	NA	Negative
MS021	RR	2005	2003	NA	Yes	NA	Positive
MS022	RR	2010	2010	NA	Yes	NA	Positive
MS023	RR	2014	2004	NA	Yes	NA	Negative
MS024	RR	2013	2013	NA	Yes	NA	Positive
MS025	PP	2010	NA	NA	No	NA	Negative
MS026	SP	1976	NA	NA	Yes	NA	Positive
MS027	RR	2003	NA	NA	Yes	NA	Positive
MS028	SP	2000	NA	NA	No	NA	Positive
MS029	SP	1998	NA	NA	Yes	NA	Negative

MS030	PP	1997	NA	NA	No	NA	Negative
MS031	SP	2001	NA	NA	Yes	NA	Negative
MS032	Not Known	NA	NA	NA	No	NA	Negative
MS033	RR	2007	NA	NA	No	NA	Negative
MS034	SP	1998	NA	NA	NA	NA	Negative
MS035	SP	2000	NA	NA	No	NA	Negative
MS036	PP	2009	NA	NA	No	NA	Negative
MS037	SP	1989	NA	NA	No	NA	Negative
MS038	RR	2009	NA	NA	No	NA	Negative
MS039	SP	1976	NA	NA	Yes	NA	Negative
MS040	SP	1996	NA	NA	No	NA	Negative
MS041	SP	1998	NA	NA	Yes	NA	Negative
MS042	RR	2001	NA	NA	Yes	NA	Negative
MS043	SP	1996	NA	NA	No	NA	Negative
MS044	PP	2008	NA	NA	No	NA	Negative
MS045	RR	2013	NA	NA	No	NA	Positive
MS046	RR	15/11/2012	NA	Yes	Yes	No	Negative
MS047	CIS	NA	12/08/2015	Yes	No	No	Negative
MS048	CIS	NA	12/08/2015	No	No	No	Positive
MS049	CIS	NA	2008	No	No	No	Negative
MS050	CIS	NA	2014	Yes	No	No	Negative
MS051	CIS	NA	01/01/2012	No	No	No	Negative
MS052	CIS	NA	15/04/2015	Yes	No	No	Negative
MS053	CIS	NA	15/02/2015	Yes	No	No	Positive
MS054	CIS	NA	1995	No	No	No	Positive
MS055	CIS	NA	15/09/2015	NA	No	No	Positive
MS056	CIS	NA	24/06/2014	Yes	No	No	Positive
MS057	CIS	NA	26/06/2015	Yes	No	No	Negative
MS058	CIS	NA	15/05/2015	No	No	No	Negative
MS059	CIS	NA	2013	Yes	No	No	Negative
MS060	CIS	NA	Jun-13	Yes	No	No	Negative
MS061	CIS	NA	Oct-15	No	No	No	Negative

Table S2: Clinical features of patients with CIS used in this study. CIS = clinically isolated syndrome and NA = data not available.

Sample	Clinical Subtype	Disease Duration from diagnosis	MRI activity	Disease Modifying Therapy	High Dose steroids	ETX Result
CISMS001	MS	12/08/2015	Yes	No	No	Negative
CISMS002	MS	12/08/2015	No	No	No	Positive
CISMS003	CIS	24/08/2015	Yes	No	No	Negative
CISMS004	MS	2008	No	No	No	Negative
CISMS005	MS	2014	Yes	No	No	Negative
CISMS006	MS	01/01/2012	No	No	No	Negative
CISMS007	MS	15/04/2015	Yes	No	No	Negative
CISMS008	CIS	14/08/2015	Yes	No	No	Negative
CISMS009	CIS	01/05/2014	Yes	No	No	Negative
CISMS010	MS	15/02/2015	Yes	No	No	Positive
CISMS011	MS	1995	No	No	No	Positive
CISMS012	CIS	09/06/2015	Yes	No	No	Positive
CISMS013	MS	15/09/2015	NA	No	No	Positive
CISMS014	MS	24/06/2014	Yes	No	No	Positive
CISMS015	CIS	2013	No	No	No	Positive
CISMS016	MS	26/06/2015	Yes	No	No	Negative
CISMS017	MS	15/05/2015	No	No	No	Negative
CISMS018	MS	2013	Yes	No	No	Negative
CISMS019	MS	Jun-13	Yes	No	No	Negative
CISMS020	MS	Oct-15	No	No	No	Negative

Table S3: Clinical features of patients with ON used in this study. NA = Data not available.

Sample	Date of diagnosis	Date of first symptoms	MRI Activity	Disease Modifying Therapies	High dose steroids	ETX Result
ON001	05/07/2012	28/06/2012	Yes	No	Yes	Positive
ON002	14/06/2012	09/06/2012	Yes	NA	NA	Negative
ON003	13/06/2013	08/06/2013	No	No	Yes	Negative
ON004	10/05/2012	07/05/2012	No	No	Yes	Negative
ON005	29/05/2012	19/05/2012	Yes	No	Yes	Negative
ON006	06/07/2012	03/07/2012	Yes	No	Yes	Negative
ON007	29/05/2012	19/05/2012	Yes	No	No	Negative
ON008	09/08/2012	04/08/2012	Yes	No	Yes	Negative
ON009	31/07/2012	26/07/2012	No	No	Yes	Negative
ON010	13/04/2012	04/04/2012	No	No	Yes	Negative
ON011	30/03/2012	19/03/2012	No	No	Yes	Negative
ON012	03/02/2012	27/01/2012	No	No	Yes	Negative
ON013	22/03/2012	15/03/2012	No	No	Yes	Negative
ON014	07/03/2012	23/02/2012	Yes	No	Yes	Negative
ON015	25/09/2012	18/09/2012	Yes	No	Yes	Negative
ON016	07/11/2012	01/11/2012	Yes	No	Yes	Negative
ON017	16/11/2012	11/11/2012	Yes	No	No	Positive
ON018	20/09/2012	16/09/2012	Yes	No	Yes	Positive
ON019	14/02/2013	07/02/2013	Yes	No	Yes	Positive
ON020	27/11/2012	16/11/2012	Yes	No	Yes	Negative
ON021	12/08/2013	29/07/2013	Yes	No	No	Negative
ON022	17/01/2013	09/01/2013	No	No	Yes	Negative
ON023	09/01/2013	31/12/2012	Yes	No	No	Positive
ON024	06/12/2012	26/11/2012	Yes	No	No	Negative
ON025	15/01/2013	08/01/2013	Yes	No	Yes	Negative
ON026	11/04/2013	05/04/2013	Yes	No	No	Negative
ON027	12/03/2013	28/02/2013	Yes	No	Yes	Negative
ON028	10/04/2013	29/03/2013	Yes	No	Yes	Negative
ON029	31/01/2014	20/01/2014	Yes	No	Yes	Negative
ON030	15/11/2013	06/11/2013	Yes	No	Yes	Positive
ON031	07/04/2014	25/03/2014	Yes	No	Yes	Negative

ON032	26/02/2014	18/02/2014	Yes	No	Yes	Positive
ON033	29/01/2014	24/01/2014	No	No	No	Negative
ON034	16/05/2014	07/05/2014	Yes	No	Yes	Negative
ON035	18/07/2013	16/07/2013	No	No	Yes	Negative
ON036	15/05/2013	05/05/2013	Yes	No	Yes	Negative
ON037	31/08/2012	22/08/2012	Yes	No	Yes	Negative
ON038	30/04/2013	25/04/2013	Yes	No	Yes	Negative
ON039	07/12/2012	23/11/2012	Yes	No	Yes	Negative
ON040	21/12/2012	14/12/2012	No	No	No	Positive
ON041	30/04/2013	25/04/2013	Yes	No	Yes	Negative
ON042	17/04/2013	14/04/2013	Yes	No	Yes	Negative
ON043	06/05/2014	28/04/2014	Yes	No	Yes	Positive
ON044	22/01/2014	15/01/2014	Yes	No	No	Negative

Table S4. Clinical and demographic features of patients and control used in this study. Sera that were reactive with PA83 have been excluded.

	CDMS sera	CDMS sera reactive with epsilon toxin	CIS & ON sera	CIS/ON sera reactive with epsilon toxin	control sera	Control sera reactive with epsilon toxin
N	61	14	64	16	125	13
Gender M/F	15/46	3/11	17/47	2/14	33/92	6/7
Year of Birth						
1941 - 1950	7	2	1	1	8	0
1951 - 1960	14	2	4	1	16	0
1961 - 1970	19	3	10	2	31	6
1971 - 1980	12	3	16	3	28	1
1981 - 1990	7	2	28	6	37	5
1991 - 2000	2	2	5	3	6	1

Table S5. Peptides recognised by sera using Pepscan, with s/n \geq 2.0.

Group	Serum identification	Peptides recognised ^a
Peptides recognised by rabbit sera against epsilon toxoid	Y43A-Y209A	49YDNDVDTLIE57, 68LKRMEKYYPN77, 82FDKVTINPQ90, 98NNPKVELDGEP107, 113EDVYVGKAL121, 196VPSQDILVPAN206, 249YKFSLSDTVN258, 261DLNEDGTININ271, 280MGDELIVKVRNL291, 298EYVIPVDKK306
Sera from CDMS patients, positive by Western blotting	BLT00143	170TGVSLTTSY178
	BLT00139	152TVGTSIQATAKFT165, 177SYSFANTNTN186
	309	60RYNTKYNYLKR70, 213AYLKKVNVKGN223, 306KEKSNDNIVKYRS319
Sera from MS patients, negative by Western blotting	BUH00147	175TTSYSFANTNT185
	BUH00135	285IVKVRNLNTNNVQEYVI301
	315	60RYNTKYNYLKR70, 213AYLKKVNVKGN223, 306KEKSNDNIVKYRS319, 165VPFNETGVSLTTS177
	318	60RYNTKYNYLKR70, 213AYLKKVNVKGN223, 306KEKSNDNIVKYRS319
	BUH00144	60RYNTKYNYLKR70, 213AYLKKVNVKGN223, 306KEKSNDNIVKYRS319, 128QEQKLKSQSFTCKN141
	300	185TNTNSKEITHNV196
	BUH00145	179SFANTTNTNSKEI192
	BUH00148	56IEKGRYNTKYNYLKR70, 201ILVPANTTVEVI212, 214YLKKVNVKGNVK225, 314IVKYRSLYIKAPGIK328
	316	175TTSYSFANTNT185
	BUH00137	299YVIPVDKKEK308
	BLT00133	60RYNTKYNYLKR70, 213AYLKKVNVKGN223, 306KEKSNDNIVKYRS319, 128QEQKLKSQSFTCKN141
Sera from controls, positive by Western blotting	BUH00226	90QGNDFYINNP100, 186NTNSKEITHNV196, 288VRNLNTNNVQEYV300, 294NNVQEYVIPVDKKE307
Sera from controls, negative by Western blotting	A7	180FANTTNT187, 189SKEITHNVPSQDIL202
	C6	38TVSNEMSKASY49, 59GRYNTKYNYLK69, 213AYLKKVNVKGNVK225
	A8	220VKGNVCLVG228

	D8	35ISNTVSNEMSKKA47, 61YNTKYNLYLKRMEKY74, 288VRNLNTNNVQEYVIP302
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^a Numbering indicates position in the amino acid sequence of epsilon toxin