Supporting Information

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SI Methods

Purification and Characterization of Myelin Glycoproteins. In this study, we used lentil lectin-binding glycoproteins purified from human myelin. This protein preparation contains purely myelin proteins and proteins shared by myelin and axons (1). For characterization, individual proteins were identified by Western blotting using a range of different antibodies and antisera: mouse mAb anti-MPB (Millipore), mouse OMGP (Serotec), mouse anti-HNK-1 (anti-CD57; BD Biosciences), mouse anti-cyclic nucleotide phosphatase (Millipore), mouse anti-alpha B crystallin (Abcam), mouse anti-MOG (8–18C5), mouse anti-contactin-2 (23.4–5; Developmental Studies Hybridoma Bank, University of Iowa), rabbit anti-contactin-2 (provided by C.R.), and rabbit anti-PLP (Abcam). Bound immunoglobulins were detected using appropriate secondary antibodies, rat anti-mouse biotin, peroxidase-conjugated goat anti-mouse IgG and IgM, goat anti-rabbit IgG biotin, rabbit anti-goat biotin, and peroxidase-conjugated goat anti-rabbit IgG (all from Jackson ImmunoResearch).

ELISA. IgG isotype usage was determined using HRP-labeled mouse anti-human IgG_1 1:3,000, mouse anti-human IgG_2 1:3,000, mouse anti-human IgG_3 1:2,000, and mouse anti-human IgG_4 1:3,000 (Zymed). IgM antibodies were detected using biotin-labeled goat anti-human IgM 1:3,000 (Sigma) and streptavidin-HRP 1:5,000. All assays were performed in duplicate, and data are presented as the mean values of the difference between OD in contactin-2–coated wells and OD in BSA-blocked wells.

To determine intrathecal IgG production, CSF was diluted 1:1 and serum was diluted to the same Ig concentration. The samples were then measured in parallel as described above. The specific intrathecal IgG production antibody index (AI) was then calculated as AI = ratio of OD_{CSF} to OD_{serum}. In the case of intrathecal IgG production, the corrected AI was calculated as AI $\times$ Q_{IgG}/Q_{lim} and an AI >1.5 was considered to indicate significant and reliable intrathecal IgG production against the antigen (2). Q_{IgG} is the ratio of IgG in CSF to IgG in serum; Q_{lim} represents the IgG fraction in CSF originating only from blood; lim = limit; Q = ratio.

Deglycosylation of Recombinant Contactin-2. Contactin-2 was enzymatically deglycosylated using a deglycosylation kit (Sigma). As enzymes, PNGase F (Peptide-N4-(acetyl-β-glucosaminyl)-asparagine amidase F), neuraminidase, O-glycosidase, β-galactosidase, and β-N-acetylglucosaminidase were used. Twenty-five micrograms of contactin-2 was dissolved in 30 μL of distilled water and mixed with 10 μL of reaction buffer and 2.5 μL of denaturing solution supplied in the kit. The solution was heated at 100 °C for 5 min. After cooling to room temperature, 2.5 μL of Triton X (15% vol/vol solution) and 1 μL of each enzyme were added. The mix was incubated for 3 h at 37 °C. The deglycosylation was controlled in a silver gel by a decrease in the molecular weight of contactin-2. The deglycosylated protein was used in Western blot analysis.

FACS with Cell Lines Transfected with Human Contactin-2 or the Rat Orthologue TAG-1–Transfected Cell Line. To detect reactivity against human contactin-2, a FACS analysis was performed using a mouse myeloma cell line stably transfected with human contactin-2 (3). A total of 100,000 contactin-2–transfected cells or the same number of cells from the untransfected parental cell line were suspended in 1% FCS in PBS and seeded into 96-well plates. The cells were kept on ice during the whole staining procedure. Dilutions of immunoadsorption eluates (1:1 to 1:100) or sera (1:30 to 1:100) were incubated with the cells for 45 min on ice. The cells were washed twice in FACS buffer. Surface-bound antibodies were detected with anti-human IgG-phycoerythrin (PE) 1:1,000 (Alexis) or anti-human IgM-PE 1:10 (BD PharMingen). As a positive control, transfected cells were stained with rabbit anti-contactin-2 1:100 (Biochemistry Zurich) and goat anti-rabbit FITC 1:200 (Millipore). To detect reactivity against the rat orthologue TAG-1, cells stably transfected with rat TAG-1 were used and incubated with 10 μg/mL 4D7 (IgM) or 3.1C12 (IgG1) (both from Developmental Studies Hybridoma Bank, University of Iowa) and appropriate isotype controls and secondary antibodies.

Restimulation ELISPOT Assay and ELISA for IFN-γ and IL-17. Briefly, 2×10^5 cells were seeded in triplicate into 96-well plates and stimulated with 50 μg/mL contactin-2 on day 1 or left unstimulated. On day 5, IL-15 (7 ng/mL; eBioscience) was added at indicated samples. On day 8, cells were restimulated with 2×10^5 irradiated (30 Gy) PBMCs from the same donor and 50 μg/mL contactin-2. In addition, IL-23 (10 ng/mL; eBioscience) was added to increase IL-17 production. On day 9, cells were transferred to the precoated ELISPOT plate. The cell culture supernatant from day 9 was used for IFN-γ and IL-17 ELISAs (R&D Systems). On day 10, 21 h after restimulation, ELISPOT plates were developed and spots were counted using an automated quantitation system (Zeiss).

Histology and Immunohistochemistry. Tissue blocks from brain and spinal cord were routinely embedded in paraffin. Sections were stained with H&E, with Luxol fast blue, and by Bielschowsky silver impregnation to evaluate inflammation, demyelination, and axonal injury. For immunocytochemistry, sections were deparaffinized, nonspecific antibody binding was blocked with 10% vol/vol FCS in PBS, and endogenous peroxidase was inhibited by H_2O_2/methanol. The following primary antibodies were used: W3/13 (T cells and granulocytes; Serotec), ED1 (macrophages; Serotec), biotinylated anti-rat Ig (GE Healthcare), and a rabbit polyclonal antibody directed against complement C9 (a gift from Sara Piddlesden, Cardiff, United Kingdom). Bound primary antibodies were visualized as described before with biotinylated secondary antibodies and avidin/peroxidase (4).

Inflammation was quantified in at least 10 complete cross sections of the spinal cord and in 6 randomly selected fields of the cerebral cortex per animal by counting the number of vessels with perivascular inflammatory cuffs. The numbers of T cells and macrophages in the lesions were determined by counting them in 6 randomly sampled microscopic fields in the spinal cord and cortex per animal in the respective immunostained sections. Values given in Figure 4 represent the number of perivascular infiltrates per mm^2 for quanitification of inflammatory infiltrates and the number of cells per mm^2 for quantification of T-cells and macrophages.

1. Mathey EK, et al. (2007) Neurofascin as a novel target for autoantibody-mediated axonal injury. *J Exp Med* 204:2363–2372.

2. Reiber H, Lange P (1991) Quantification of virus-specific antibodies in cerebrospinal fluid and serum: Sensitive and specific detection of antibody synthesis in brain. *Clin Chem* 37:1153–1160.

3. Hasler TH, Rader C, Stoeckli ET, Zuellig RA, Sonderegger P (1993) cDNA cloning, structural features, and eucaryotic expression of human TAG-1/axonin-1. *Eur J Biochem* 211:329–339.

4. Storch MK, et al. (2006) Cortical demyelination can be modeled in specific rat models of autoimmune encephalomyelitis and is major histocompatability complex (MHC) haplotype-related. *J Neuropathol Exp Neurol* 65:1137–1142.

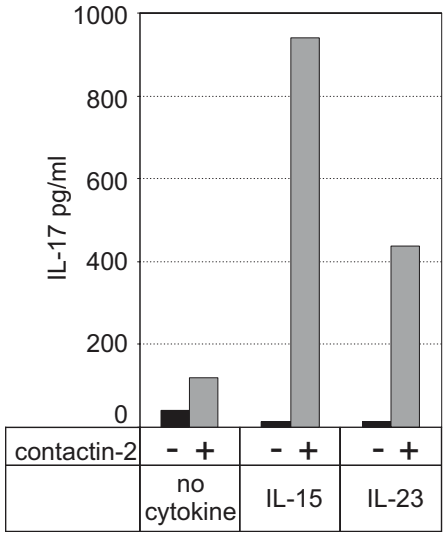

Fig. S1. Modulation of contactin-2–specific IL-17 production by IL-15 and IL-23. PBMCs of an MS patient were stimulated with 50 μg/mL contactin-2 and restimulated with contactin-2 and irradiated antigen-presenting cells after 7 days. IL-15 was added on day 5, and IL-23 was added on day 8 as indicated. IL-17 production was measured in cell culture supernatant of day 9 by ELISA. Addition of IL-15 and IL-23 led to a contactin-2–specific increase of IL-17 production. The detailed data for the other MS patients and controls are shown in Table S2.

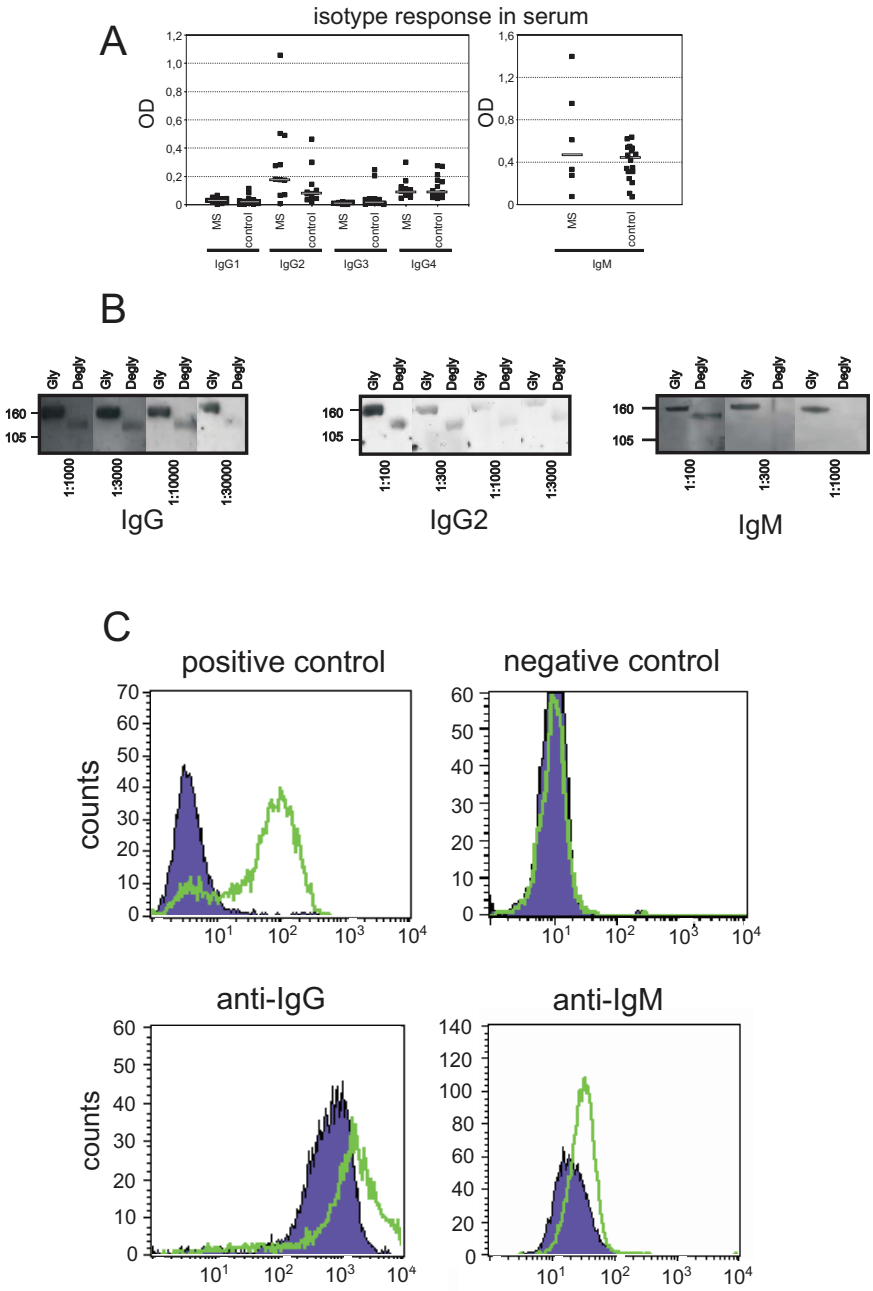

Fig. S2. Characterization of the humoral anti-contactin-2 response. (*A*) Isotype usage of the anti-contactin-2 Ig. Twenty donors (10 MS patients, 10 healthy controls and OND patients) who showed strong anti-contactin-2 reactivity in ELISAs with total IgG were further tested for the isotype usage. All sera were diluted 1:400. The ODs corrected for the individual background are shown for each tested patient. Anti-contactin-2 antibodies were mainly of IgG2 isotype, followed by IgG4 isotype. Some patients also showed an IgM serum response. There was no significant difference between the MS and control groups. (*B*) Recognition of both the glycosylated (gly) and nonglycosylated forms of contactin-2. Recombinant contactin-2 was deglycosylated (degly) using enzymatic digestion. Equal amounts of glycosylated and deglycosylated protein (1 μg of protein per lane) were separated and detected by Western blot analysis. Serum from an MS patient was used in different dilutions (1:100–1:30,000 depending on the secondary antibody). The secondary antibodies were directed against total IgG, IgG2, or IgM. Both the glycosylated form and nonglycosylated form were recognized. In particular, the IgM Abs showed a stronger recognition of the glycosylated form. Two patients were analyzed with this method and showed similar results. (*C*) Minor recognition of native contactin-2. Recognition of native contactin-2 was tested in a FACS with a cell line stably transfected with contactin-2. The closed graph shows the untransfected parental cell line. The contactin-2–transfected cell line is shown as an open graph. As a positive control, serum of an immunized rabbit was used, which strongly recognized surface-expressed contactin-2 (*Upper Left*). As a negative control, staining was performed only with secondary antibodies (*Upper Right*). Surface-bound antibodies from MS patients were detected with anti-IgG-PE (*Lower Left*) or anti-IgM-PE (*Lower Right*). In the examples shown, IgG and IgM from 2 different MS patients weakly recognized cell surface-expressed contactin-2. The immunoadsorption eluate used for IgG staining was diluted 1:3 (*Lower Left*), and the serum used for IgM staining was diluted 1:100 (*Lower Right*).

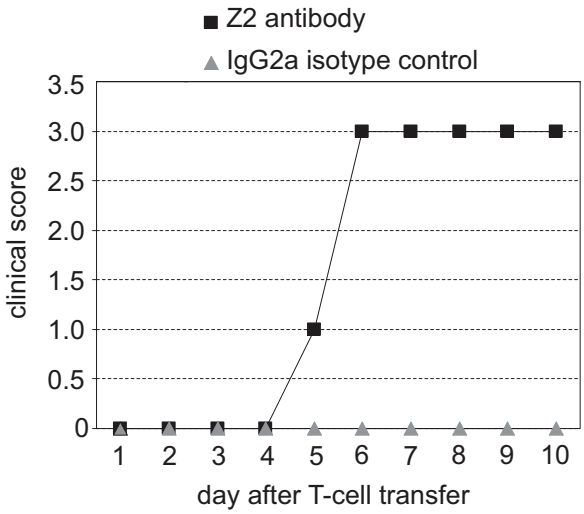

Fig. S3. Augmentation of TAG-1–mediated EAE by MOG-specific mAb. DA rats injected with 10^7 TAG-1–specific T cells were treated 4 days after transfer with 1 mg of either MOG-specific mAb Z2 ($n = 3$) or an IgG2a myeloma protein ($n = 2$). Animals treated with Z2 were killed at day 10 for histological examination.

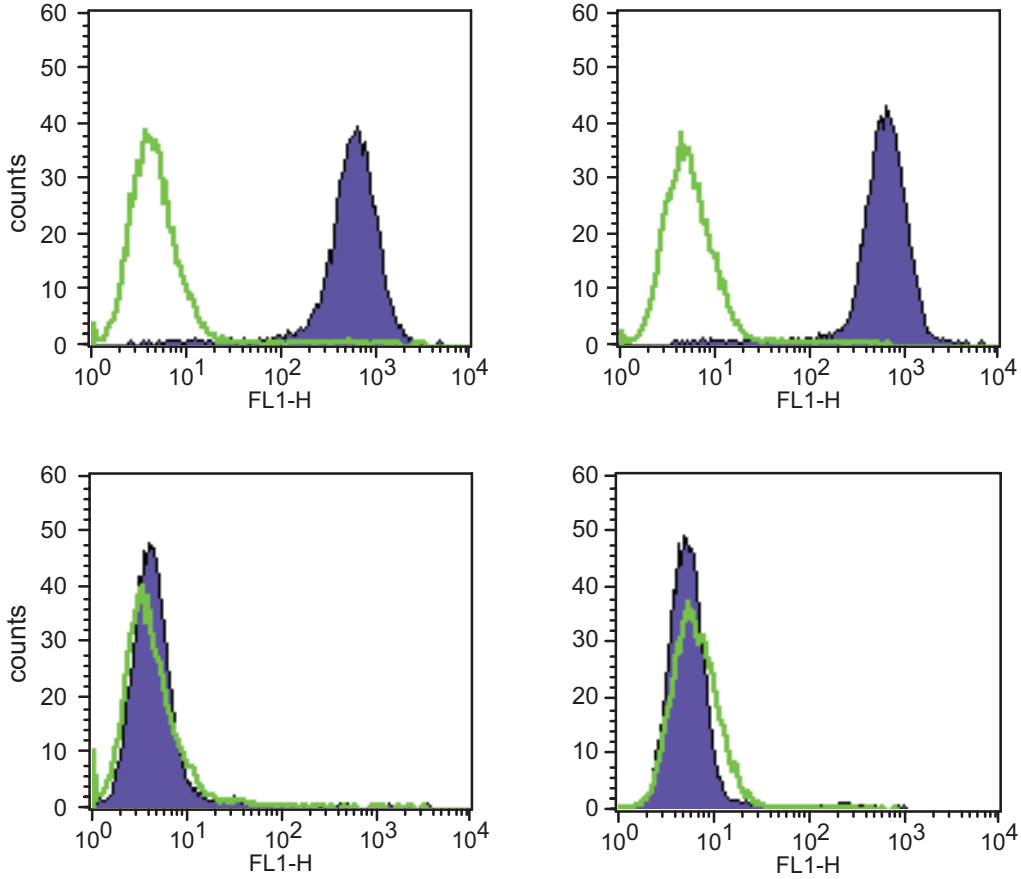

Fig. S4. The mAbs 3.1C12 (*Left*) and 4D7 (*Right*) recognize native TAG-1 as seen by a FACS. Flow cytometric analysis demonstrates that mAbs 3.1C12 (IgG1) and 4D7/TAG-1 (IgM) bind to the surface of TAG-1 transfectants (purple filled curves) but not to sham-transfected cells (green curves). The lower panels demonstrate that the myeloma proteins used as isotype controls do not bind to either cell line. Incubations with the mAbs and myeloma proteins were performed using a concentration of 10 μg/mL. FL-1H, fluorescence intensity at 520 nm representing TAG-1 staining.

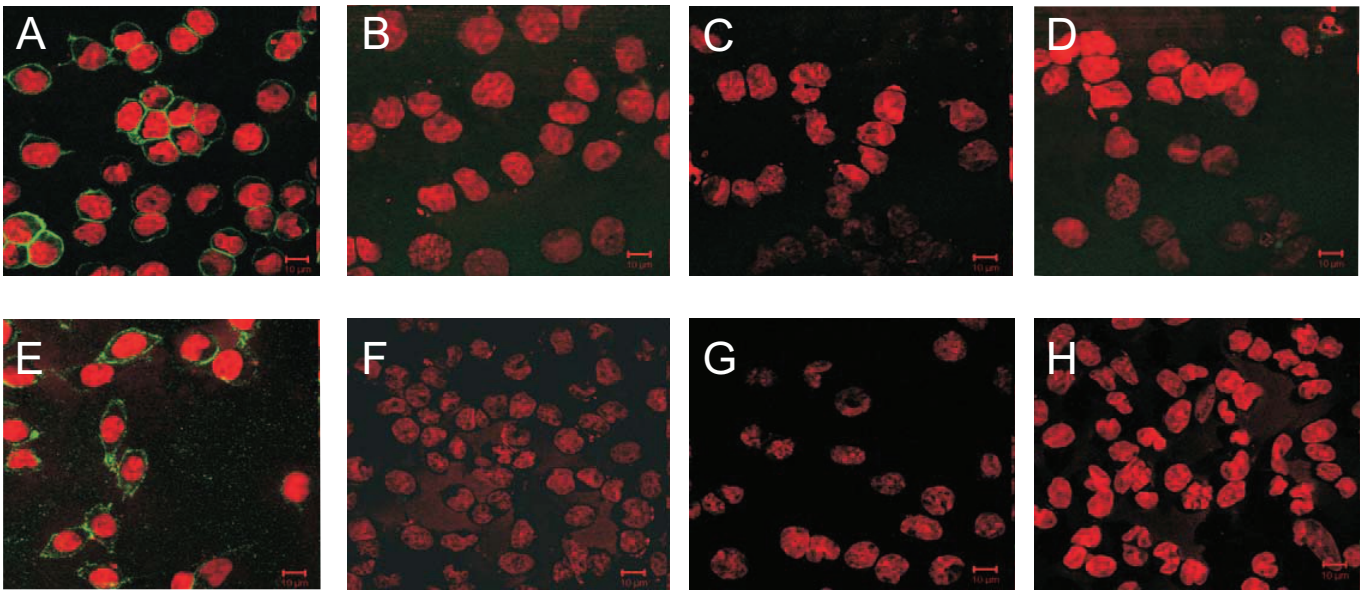

Fig. S5. The mAbs 3.1C12 and 4D7 recognize native TAG-1 as seen by immunofluorescence. The mAbs 3.1C12 (*A*) and 4D7 (*E*) bind to TAG-1 transfectants (green signals) but not to untransfected cells (*C* and G). Images demonstrate the inability of myeloma IgG1 (*B*) and IgM (*F*) isotype control antibodies, respectively, to bind TAG-1 transfectants. (*D* and *H*) These reagents also failed to bind to nontransfected cells. 4D7/TAG1 and IgM antibodies were used at 10 μg/mL, whereas 3.1C12 and IgG1 antibodies were used at 5 μg/mL. Cell nuclei are stained using TOPRO-3 and appear red. (Scale bar: 10 μm.)

Table S1. Characteristics of donors and results of ex vivo T-cell assays

Patients and controls	Age, years	Gender	Disease course	Contactin-2–specific IFN-γ spots	Contactin-2–specific IL-17 spots	Contactin-2–specific proliferation, %
Patient 1	55	F	SP-MS	4	2	3.04
Patient 2	45	M	SP-MS	15	nd	nd
Patient 3	59	M	SP-MS	7	nd	nd
Patient 4	63	M	SP-MS	8	0	0.95
Patient 5	53	M	SP-MS	6	0	3.36
Patient 6	45	M	RR-MS	17	2	0
Patient 7	57	F	SP-MS	0	1	0.41
Patient 8	49	M	SP-MS	0	1	0
Patient 9	66	F	SP-MS	8	2	2.61
Patient 10	63	F	SP-MS	24	0	8.14
Patient 11	30	M	RR-MS	nd	0	6.78
Patient 12	29	F	RR-MS	11	nd	nd
Patient 13	43	F	RR-MS	0	nd	nd
Control 1	31	F	NA	0	nd	nd
Control 2	36	M	NA	3	2	nd
Control 3	29	M	NA	12	0	nd
Control 4	42	F	NA	3	0	nd
Control 5	46	F	NA	2	0	nd
Control 6	52	M	NA	0	3	0
Control 7	55	M	NA	14	1	0
Control 8	64	M	NA	17	2	nd
Control 9	64	F	NA	nd	nd	0
Control 10	60	F	NA	nd	nd	0
Control 11	69	F	NA	nd	nd	0
Control 12	47	M	NA	nd	5	0
Control 13	22	F	NA	nd	1	5.03

All MS patients were untreated. Contactin-2–specific IFN-γ and IL-17 production was measured by ELISPOT assay. The number of cytokine-producing cells per 200,000 cells in the presence of contactin-2 after substraction of cytokine-producing cells in the absence of antigen is given. The contactin-2–specific proliferation was measured using 5-chloromethylfluorescein diacetate in FACS. The percentage of proliferating cells in the presence of contactin-2 after substraction of proliferation without antigen is shown. F, female; M, male; NA, not applicable; nd, not done; RR, relapsing-remitting; SP, secondary-progressive.

Table S2. Characteristics of donors and results of restimulation assays

Patients and controls	Age, years	Gender	Disease course	IFN-γ ELISPOT assay	IFN-γ ELISA, no antigen	IFN-γ ELISA, contactin-2 stimulation	IL-17 ELISPOT assay, contactin-2	IL-17 ELISPOT assay, contactin-2 + IL-15	IL-17 ELISPOT assay, contactin-2 + IL-23	IL-17 ELISA, no antigen	IL-17 ELISA, contactin-2	IL-17 ELISA, no antigen + IL-15	IL-17 ELISA, contactin-2 + IL-15	IL-17 ELISA, no antigen + IL-23	IL-17 ELISA, contactin-2 + IL-23
Patient 12	29	F	RR-MS	0	46	87	95	110	138	41	121	15	940	12	436
Patient 13	43	F	RR-MS	33	nd	nd	15	55	85	67	242	52	552	111	671
Patient 14	37	F	RR-MS	185	1	125	nd	0	0	nd	nd	333	155	13	73
Patient 15	81	F	SP-MS	98	42	30	nd	34	203	nd	nd	2	464	0	1,214
Control 1	31	F	NA	0	nd	nd	0	50	72	45	338	182	441	115	378
Control 5	46	F	NA	57	93	17	23	12	49	9	12	4	25	5	19
Control 12	47	M	NA	38	27	489	26	8	47	25	365	213	688	17	898
Control 14	37	F	NA	222	58	2,074	112	25	117	5	629	4	472	0	781

Donor characteristics and results of restimulation assays for IFN-γ and IL-17 in ELISA (pg/mL) and ELISPOT assay [cytokine-producing cells/200,000 cells in the presence of contactin-2 − background (cytokine-producing cells in the absence of antigen)]. All MS patients were untreated. For detection of contactin-2–specific IL-17 production, different stimulation conditions were used: contactin-2 alone, contactin-2 plus IL-15,and contactin-2 plus IL-23. In case of IFN-γ and IL-17 ELISAs, the background values without contactin-2 are shown for each stimulation condition. In case of IFN-γ and IL-17 ELISPOT assays, the respective background cytokine production has been subtracted. F, female; M, male; NA, not applicable; nd, not done; RR, relapsing-remitting; SP, secondary-progressive.