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Abundant GAD-reactive B cells in GAD-antibody-associated neurological disorders

Franziska S. Thaler[1], MD, Anna L. Thaller[1], MSc, Michelle Biljecki[1], MSc, Elisabeth Schuh[1], MD, PhD, Stephan Winklmeier[1], MSc, Christoph F. Mahler[1], MD, Ramona Gerhards[1], MSc, Stefanie Völk[2], MD, Frauke Schnorfeil[3], PhD, Marion Subklewe[3], MD, Reinhard Hohlfeld[1,4], MD, Tania Kümpfel[1], MD, Edgar Meinl[1], MD

[1]Institute of Clinical Neuroimmunology, University Hospital and Biomedical Center, Ludwig-Maximilians University Munich, Munich; [2]Department of Neurology, University Hospital, Ludwig-Maximilians University Munich, Munich; [3]Department of Medicine III, University Hospital, Ludwig-Maximilians University Munich, Munich; [4]Munich Cluster for Systems Neurology (SyNergy)

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Contact information of corresponding author

Franziska S. Thaler
Institute of Clinical Neuroimmunology
Klinikum Grosshadern, Ludwig Maximilians University
Marchioninistr. 15
D-81377 Munich
Tel: +49-89-4400-74435 Fax: +49-89-4400-77435
franziska.thaler@med.uni-muenchen.de

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ABSTRACT

High levels of antibodies against glutamic acid decarboxylase (GAD) are observed in patients with different neurological disorders, but cells producing these auto-antibodies are largely unexplored. We detect circulating GAD-reactive B cells in peripheral blood that readily differentiate into antibody-producing cells. These cells are highly elevated in most patients with GAD-antibody-associated disorders (n=15) compared to controls (n=19). They mainly produce GAD65-antibodies of the IgG1 and IgG4 subclasses and are as abundant as B cells reactive for common recall antigens. Bone marrow cells represent an additional source of GAD-antibodies. The identification of GAD-antibody-producing cells has implications for the selection of cell-specific biologics.

Introduction

The cytoplasmic protein glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the synthesis of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA) [1]. High serum levels of antibodies (abs) against GAD have been associated with neurological syndromes like stiff person syndrome (SPS), cerebellar ataxia, limbic encephalitis, epilepsy or oculomotor dysfunction [2-6]. A marked intrathecal antibody response against GAD or CSF oligoclonal bands support an association of GAD-abs with neurological syndromes [7] and indicate clonal B-cell-activation in the central nervous system [8]. Histologically, neuronal loss and infiltrating T cells

are seen in patients with GAD-ab-associated neurological disorders [9]. This supports the idea that immune reactions are relevant in these patients, but precise mechanisms have not been identified. GAD exists in two isoforms: GAD65 and GAD67. In patients with neurological disorders autoantibodies are mainly directed against GAD65 and less frequently against GAD67 [10, 11]. Compared to patients with neuronal surface antibodies, effective treatment in GAD-ab positive patients remains challenging [12, 13]. Therefore, determining the immune responses in these patients can help to gain pathophysiological insights facilitating therapeutic decisions. Thus, we analysed GAD-ab-producing B cells in peripheral blood cells and bone marrow (BM) cells of patients with GAD-ab-associated neurological syndromes.

Materials and Methods

Patients. 15 patients with neurological symptoms and GAD-abs detected in clinical routine along with 19 healthy controls were analysed (Table 1). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient separation. Bone marrow mononuclear cells (BMMCs) were obtained by iliac crest aspiration. BMMCs from controls included: n=1: hairy cell leukemia, n=1: MLL-rearranged acute lymphoblastic leukemia, n=1: relapsing-remitting multiple sclerosis and pancytopenia after alemtuzumab treatment. All patient and control samples were collected following written informed consent and the study was approved by the institutional review board of the Ludwig Maximilians University, Munich, Germany.

Cell culture and cell stimulation. PBMCs were stimulated with the toll-like receptor (TLR)-7/8-ligand R848 (Sigma Aldrich, 2.5 $\mu g\,ml^{-1}$) and IL-2 (R&D, 1,000 $IU\,ml^{-1}$) at a concentration of 600,000 cells ml^{-1} in 24-well plates for 11 days as described [14]. B-cell frequency was determined by flow cytometry using anti-CD19-PerCP-Cy5.5 (eBioscience; SJ2C1). To determine

frequencies of antigen-reactive cells, PBMCs were seeded in limiting dilutions at 10^2, 10^3, 10^4, $5x10^4$, 10^5 cells per 200 µl and stimulated as described above. Frequency calculation of antigen-reactive cells was performed according to the Poisson distribution as the seeded PBMC number at which 37% of the cultures were negative. BMMCs were cultured at $3x10^5$ cells per 200 µl for 5 days without addition of stimuli.

ELISAs. IgG, IgM and IgA concentrations were determined using the Human IgG, IgM and IgA ELISA development kit (Mabtech AB). GAD-abs were quantified by human anti-GAD ELISA (IgG) (EUROIMMUN). Measles virus abs (MV) were detected by Serion ELISA classic Measles IgG (Virion/Serion). Tetanus-toxoid (TT)-specific abs were determined by coating TT (Merck/ Millipore, 1 µg ml^{-1}) or bovine serum albumin (BSA) and detection with anti-human-Ig-HRP (Jackson ImmunoResearch, 109-036-003). GAD-IgG, -IgM, -IgA, -IgG1, -IgG2, -IgG3, -IgG4 were determined by coating GAD-protein (Diamyd, 2µg ml^{-1}) and detecting with anti-human-IgG-HRP (Jackson ImmunoResearch, 109-035-098), anti-human-IgA-HRP (Thermo Scientific, PA1-74395), anti-human-IgM-HRP (Zymed, 05-4920), anti-human-IgG1-HRP (Zymed, 05-3320), anti-human-IgG2-HRP (Zymed, 05-0520), anti-human-IgG3-HRP (Zymed, 05-3620), anti-human-IgG4-HRP (Zymed, 05-3820).

Cell based assay. Hek293T cells transfected with GAD65 in pcDNA3.1, GAD67 in pCMV6 (Origene) or eGFP in pMSCV were fixed and permeabilized and stained with sera diluted 1:50 and cell culture supernatants diluted 1:10. For detection anti-human-IgG-biotin (Jackson ImmunoResearch, 109-066-098) and AlexaFluor-647-conjugated Streptavidin (Jackson ImmunoResearch, 016-600-084) were applied. Anti-GAD65 (Abcam, Ab26113) and anti-GAD67 (Abcam, Ab26116) were used as positive controls.

Results

Peripheral B cells can be stimulated to produce GAD-abs. To determine the presence of GAD-ab-producing B cells in peripheral blood, we isolated PBMCs from patients #1-14 and from 19 healthy donors (Table 1), and differentiated the cells *in vitro* into Ig-producing plasmablasts [14]. After cultivation, GAD-ab-production was restricted to GAD-ab positive patients (Fig. 1A, B). Culture supernatants from healthy donors contained similar levels of IgG, IgM, and IgA (Fig. 1C-E), but no GAD-abs (Fig. 1A, B). Next, we compared the GAD-ab-levels released by blood derived B cells after *in vitro* differentiation in each patient with their respective GAD-ab level in serum (Fig. 1F). All patients with anti-GAD levels $>10^4$ IE/ml had GAD-reactive B cells in their blood, but there was no significant overall correlation suggesting different sources of GAD-abs (see also below). GAD-abs in cell culture supernatants of stimulated PBMCs and serum were mainly directed against GAD65, only in one patient anti-GAD67 reactivity was observed (Fig. 1G, H). They were mainly IgG, and only to a low extent IgA (Fig. 1I). Further, they were mostly composed of the subclasses IgG1 and IgG4 (Fig. 1J). This pattern was also observed in the serum of the corresponding patients (Fig. 1I, J).

GAD-reactive B cells in peripheral blood show comparable frequencies as MV and TT-reactive B cells. The stimulation experiments suggested high frequencies of GAD-reactive B cells ready to differentiate into GAD-ab-producing plasma cells. We further analysed 13 patients by limiting dilution assay and determined the frequencies of GAD-reactive B cells in comparison to B cells reactive for the common recall antigens MV and TT. After stimulation of PBMCs, supernatants were analysed by ELISA for the presence of GAD-abs, MV-abs and TT-abs (representative data for patient #12 in Fig. 2A). Using the Poisson distribution, we determined the frequency of GAD-reactive cells, MV-reactive cells, and TT-reactive cells (representative data for patient #12

in Fig. 2B). The frequencies of antigen-reactive PBMCs and antigen-reactive B cells were calculated for all analysed patients and revealed comparable frequencies of GAD-reactive B cells in peripheral blood to MV-reactive or TT-reactive cells (Fig. 2C).

GAD-abs are produced by plasma cells in the BM. As our comparison of GAD-ab-levels in serum with the levels of GAD-abs produced by stimulated B cells in peripheral blood indicated different sources of GAD-abs (Fig. 1F), we aimed to analyse the BM as a possible additional source of GAD-ab-production. Patient #15 (Table 1) developed relapses with seizures and cognitive impairment and showed high GAD-abs in the serum despite treatment with rituximab and azathioprine. The azathioprine dosage was therefore increased, which led to severe anaemia and leukopenia. Treatment with azathioprine was stopped, G-CSF was administered once, and a BM aspiration was performed. Here, intact hematopoiesis and an absence of CD20 positive cells were observed. After stimulation of the PBMCs with R848 and IL-2 we detected very low IgG levels and no GAD-ab-production - in line with depletion of B-cells in the peripheral blood under treatment with rituximab (Fig. 2D). BMMCs cultured for 5 days without additional stimuli spontaneously secreted GAD-abs which was not observed in BMMCs from the three control patients (Fig. 2E left panel). In contrast, total IgG production by the patient´s BMMCs was comparable to the control patients´ BMMCs (Fig. 2E right panel).

Discussion

Three theories concerning long-lasting ab-production and plasma cell generation exist [15]. First, persisting antigen stimulation would continuously generate short-lived plasma cells from memory B cells [16]. Second, activation via cytokines and TLR-ligands would give rise to plasma cells from memory cells [17, 18]. Third, plasma cells might persist in survival niches e.g. in the BM

[19]. We provide evidence that aligns with the second notion: GAD-ab-producing cells can be generated from memory B cells by cytokine-receptor and TLR-stimulation. GAD-ab-production could be achieved under minimalized culture conditions of unfractionated PBMCs stimulated with only R848 as a TLR-ligand and IL-2. This protocol was identified to efficiently and selectively activate memory B cells by directly stimulating TLR7 on memory B cells and TLR8 on myeloid cells that produce additional cytokines supporting B cell proliferation and differentiation [14]. Similar conditions could also be encountered during infections in patients. The presence of the specific antigen GAD was not required for induction of GAD-ab-producing cells. GAD-ab-production by stimulated peripheral blood cells did not correlate with GAD-ab serum levels suggesting an additional source of GAD-abs. In line with the third theory presented above, plasma cells in the BM are shown here to be a further source of GAD-ab-production.

These findings have several important clinical implications: GAD-reactive memory B cells in peripheral blood can be targeted by antiproliferative medications and CD20-directed therapies. Consequently, these treatments decrease the pool of GAD-ab-producing cells without completely abolishing GAD-ab-producing cells - as GAD-ab-producing long-lived plasma cells would not be targeted. This is in line with the observation that rituximab only moderately decreases GAD-ab levels in serum [13, 20]. Currently, the therapeutic response in many GAD-ab positive patients is not satisfying [12, 13]. Therefore, targeting both memory B cells (e.g. with rituximab) and plasma cells (e.g. with bortezomib) might be a potential treatment option, with the awareness that such treatment regimens come with strong potential side effects.

Together, our study shows a strikingly high frequency of GAD-reactive B cells in blood of most patients and demonstrates rituximab-resistant BM plasma cells as an additional source of GAD-abs. The identification of the GAD-ab-producing cells is relevant to develop strategies of cell-

specific therapies in patients with GAD-ab-associated neurological disorders. The abundance of GAD-reactive B cells extends our understanding of the dysregulated self-tolerance in these patients.

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References:

1. Erlander MG, Tobin AJ. The structural and functional heterogeneity of glutamic acid decarboxylase: a review. Neurochemical research. 1991 Mar;16(3):215-26.
2. Giometto B, Miotto D, Faresin F, Argentiero V, Scaravilli T, Tavolato B. Anti-gabaergic neuron autoantibodies in a patient with stiff-man syndrome and ataxia. Journal of the neurological sciences. 1996 Nov;143(1-2):57-9.
3. Honnorat J, Saiz A, Giometto B, et al. Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. Archives of neurology. 2001 Feb;58(2):225-30.
4. Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG. Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. Annals of neurology. 2010 Apr;67(4):470-8.
5. Peltola J, Kulmala P, Isojarvi J, et al. Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. Neurology. 2000 Jul 12;55(1):46-50.
6. Solimena M, Folli F, Denis-Donini S, et al. Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. The New England journal of medicine. 1988 Apr 21;318(16):1012-20.

7. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. The Lancet Neurology. 2016 Apr;15(4):391-404.
8. Dalakas MC, Li M, Fujii M, Jacobowitz DM. Stiff person syndrome: quantification, specificity, and intrathecal synthesis of GAD65 antibodies. Neurology. 2001 Sep 11;57(5):780-4.
9. Bien CG, Vincent A, Barnett MH, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. Brain : a journal of neurology. 2012 May;135(Pt 5):1622-38.
10. Butler MH, Solimena M, Dirkx R, Jr., Hayday A, De Camilli P. Identification of a dominant epitope of glutamic acid decarboxylase (GAD-65) recognized by autoantibodies in stiff-man syndrome. The Journal of experimental medicine. 1993 Dec 1;178(6):2097-106.
11. Gresa-Arribas N, Arino H, Martinez-Hernandez E, et al. Antibodies to inhibitory synaptic proteins in neurological syndromes associated with glutamic acid decarboxylase autoimmunity. PloS one. 2015;10(3):e0121364.
12. Arino H, Gresa-Arribas N, Blanco Y, et al. Cerebellar ataxia and glutamic acid decarboxylase antibodies: immunologic profile and long-term effect of immunotherapy. JAMA neurology. 2014 Aug;71(8):1009-16.
13. Dalakas MC, Rakocevic G, Dambrosia JM, Alexopoulos H, McElroy B. A double-blind, placebo-controlled study of rituximab in patients with stiff person syndrome. Annals of neurology. 2017 Aug;82(2):271-7.
14. Pinna D, Corti D, Jarrossay D, Sallusto F, Lanzavecchia A. Clonal dissection of the human memory B-cell repertoire following infection and vaccination. European journal of immunology. 2009 May;39(5):1260-70.
15. Radbruch A, Muehlinghaus G, Luger EO, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nature reviews Immunology. 2006 Oct;6(10):741-50.
16. Zinkernagel RM, Bachmann MF, Kundig TM, Oehen S, Pirchet H, Hengartner H. On immunological memory. Annual review of immunology. 1996;14:333-67.
17. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. Science. 2002 Dec 13;298(5601):2199-202.
18. Traggiai E, Puzone R, Lanzavecchia A. Antigen dependent and independent mechanisms that sustain serum antibody levels. Vaccine. 2003 Jun 1;21 Suppl 2:S35-7.
19. Manz RA, Thiel A, Radbruch A. Lifetime of plasma cells in the bone marrow. Nature. 1997 Jul 10;388(6638):133-4.
20. Rizzi M, Knoth R, Hampe CS, et al. Long-lived plasma cells and memory B cells produce pathogenic anti-GAD65 autoantibodies in Stiff Person Syndrome. PloS one. 2010 May 26;5(5):e10838.

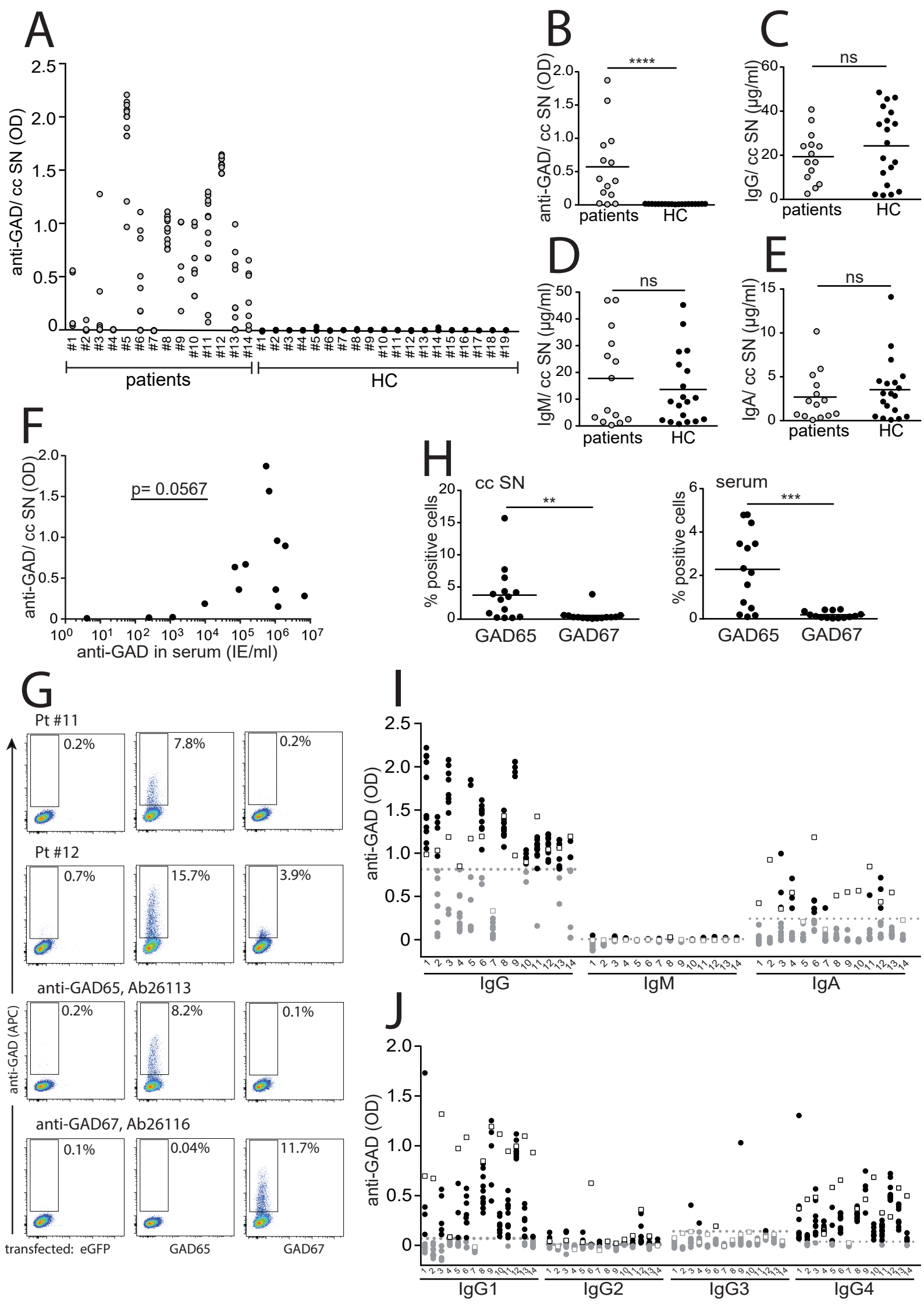
Figure legends:

Figure 1: *GAD-reactive B cells in the blood of patients with GAD-abs in the serum***.** (**A, B**) PBMCs from GAD-abs positive patients (n=14) and healthy controls (n=19) were cultured with R848 and IL-2 for 11 days and GAD-ab (IgG) levels were determined by ELISA in cell culture supernatant (cc SN); results of the stimulation of the PBMCs from patient #15 who was under treatment with rituximab and exhibited B-cell depletion in peripheral blood are not depicted in figure 1 and are presented in figure 2D. (**A**) Each circle represents one stimulated well; the number of analysed wells is as follows: patients: #1: 11, #2: 11, #3: 10, #4: 13, #5: 12, #6: 11, #7: 23, #8: 12, #9: 5, #10: 8, #11: 13, #12: 12, #13: 10, #14: 8; healthy controls: #1: 10, #2: 24, #3: 10, #4: 15, #5: 12, #6: 10, #7: 7, #8: 14, #9: 9, #10: 16, #11: 8, #12: 11, #13: 9, #14: 8, #15: 8, #16: 8, #17: 8, #18: 8, #19: 8. (**B, C, D, E**) Each circle represents the mean of all stimulated wells in one patient. Horizontal lines indicate the mean of all patients. (**B**) GAD-ab-production was significantly higher in patients than in healthy controls (Mann-Whitney test). (**C, D, E**) Total IgG, IgM and IgA levels in cc SN were determined by ELISA. IgG, IgM and IgA production was not significantly different between the two groups (Mann-Whitney test for IgG and IgA, unpaired T-test for IgM). (**F**) The mean anti-GAD reactivity of the stimulated PBMCs did not correlate with GAD-ab serum levels in the respective patients (Spearman correlation). (**G, H**) Anti-GAD65 and anti-GAD67 reactivity of cc SN of stimulated PBMCs (for each patient the SN with the highest GAD-ab levels in ELISA was selected) and sera were compared by flow cytometry using Hek293T cells transfected with plasmids coding for GAD65, GAD67 and eGFP as a control. (**G**) Results of cc SN analysis for two representative patients (patients #11 and #12) and of the control abs are depicted. (**H**) The percentage of anti-GAD65 and GAD67-stained cells is shown for cc SN (left panel) and serum (right panel). Horizontal lines indicate the mean; anti-

GAD65-reactivity was significantly higher than anti-GAD67-reactivity (Mann-Whitney test for cc SN, unpaired T-test for serum) (**I, J**) Anti-GAD-IgG, -IgM, -IgA and -IgG1-4 in the cc SN and in serum were determined by ELISA. Each circle represents one stimulated well (positive values shown in black), serum values are depicted with squares. The cut-off values for each isotype/ subclass were calculated as the mean OD of the healthy controls´ supernatants + 2 SD.

Figure 2: *GAD-ab-producing B cells in the peripheral blood show comparable frequencies as MV- and TT-reactive B cells and GAD-abs are produced by BM cells.* (**A, B, C**) Limiting dilution analysis with PBMCs from 13 patients was performed (for one patient no further PBMC samples were available). PBMCs were seeded at concentrations of 10^2, 10^3, 10^4, $5x10^4$, 10^5 cells/ well with 17 different wells for each cell concentration and cultured for 11 days in the presence of IL-2 and R848. GAD-antibody, MV-antibody and TT-antibody production was determined by ELISA. (**A**) GAD-abs, MV-abs and TT-abs in cc SN from individual wells in one representative patient (patient #12); cut-off was set as 4x SD for GAD, 1x negative control +1/2 SD for MV and at an OD of 0.2 for TT; each circle represents one stimulated well. (**B**) Depiction of the calculation of the frequencies of antigen (ag)-reactive cells in one representative patient according to the Poisson distribution. (**C**) The frequencies of ag-reactive PBMCs (upper panel) and B cells (lower panel) in all patients are depicted. Calculation of ag-reactive B cells is based on total B cell frequencies in each patient (Table 1); horizontal lines indicate the mean. No significant difference between GAD-reactive, MV-reactive and TT-reactive PBMCs as well as B cells was detected (Kruskal-Wallis test followed by Dunn´s multiple comparisons test). Calculation of ag-reactive cells was not possible in n=3 (GAD), n=3 (MV), and n=2 (TT) as no positive wells were present. **(D)** PBMCs from patient #15 who was under treatment with rituximab and exhibited B-cell depletion in peripheral blood at the time of analysis were

stimulated with R848 and IL2 for 11 days. No GAD-ab-production and only very low IgG-antibody production (ng/ml as compared to µg/ml in patients who did not receive rituximab, Figure 1) were determined by ELISA. (**E**) BMMCs of patient #15 as well as BMMCs from 3 control patients were cultured for 5 days and GAD-ab and IgG production was determined by ELISA; each circle represents one cultured well; horizontal lines indicate the mean. BMMCs from patient #15 produce GAD-abs while BM cells from control patients do not; the difference was not statistically significant with a detectable trend (Mann-Whitney test) (**E** left panel). IgG production did not differ between patient #15 and control patients (Mann-Whitney test) (**E** right panel).


A
anti-GAD/ cc SN (OD)
patients
HC
B
anti-GAD/ cc SN (OD)
****
patients
HC
C
IgG/ cc SN (µg/ml)
ns
patients
HC
D
IgM/ cc SN (µg/ml)
ns
patients
HC
E
IgA/ cc SN (µg/ml)
ns
patients
HC
F
anti-GAD/ cc SN (OD)
p= 0.0567
anti-GAD in serum (IE/ml)
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% positive cells
GAD65
GAD67
serum
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% positive cells
GAD65
GAD67
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Pt #11
0.2%
7.8%
0.2%
Pt #12
0.7%
15.7%
3.9%
anti-GAD65, Ab26113
0.2%
8.2%
0.1%
anti-GAD67, Ab26116
0.1%
0.04%
11.7%
anti-GAD (APC)
transfected: eGFP
GAD65
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I
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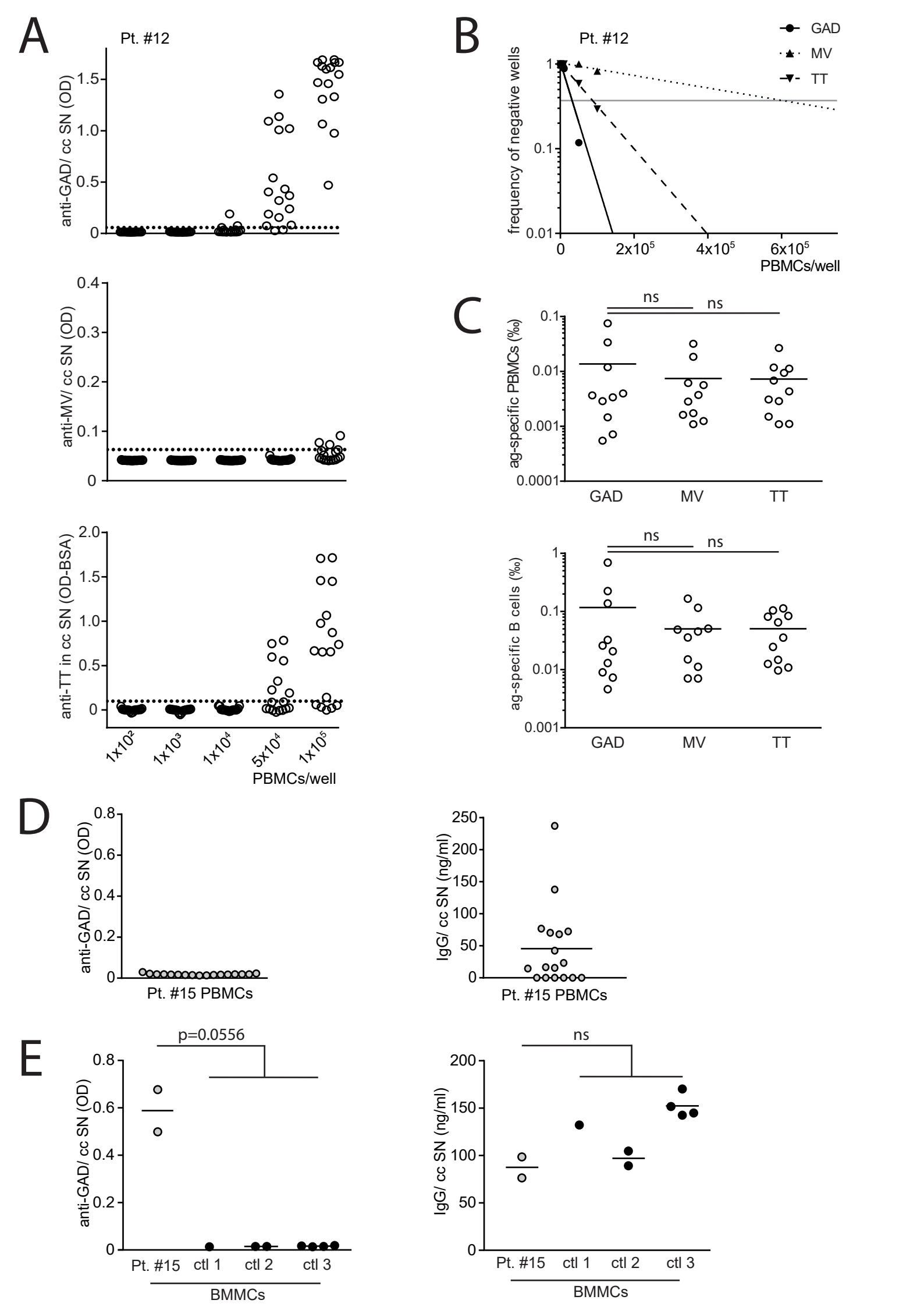
IgG4


A
Pt. #12
anti-GAD/ cc SN (OD)
anti-MV/ cc SN (OD)
anti-TT in cc SN (OD-BSA)
1x10²
1x10³
1x10⁴
5x10⁴
1x10⁵
PBMCs/well
B
Pt. #12
GAD
MV
TT
frequency of negative wells
PBMCs/well
C
ns
ns
ag-specific PBMCs (‰)
ag-specific B cells (‰)
GAD
MV
TT
D
anti-GAD/ cc SN (OD)
Pt. #15 PBMCs
IgG/ cc SN (ng/ml)
Pt. #15 PBMCs
E
p=0.0556
anti-GAD/ cc SN (OD)
Pt. #15
ctl 1
ctl 2
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BMMCs
ns
IgG/ cc SN (ng/ml)
Pt. #15
ctl 1
ctl 2
ctl 3
BMMCs

Table 1: Characterization of patients and healthy controls

Patient No.	Sex	Age at PBMC sampling (y)	Disease duration (y)	Diagnosis	IST at PBMC sampling	Anti-GAD/ serum (IE/ml)	B-cell frequency (% living cells)
# 1	f	34.3	9	SPS	sc Ig + aza	1198242	15.5
# 2	f	75.4	4	CA	MMF	1160	19.5
# 3	f	77.2	12	CA	none	9641	7.5
# 4	f	38.6	6	LE	none	244	5.8
# 5	f	61.9	0.25	CA	GCs	547972	24.5
# 6	m	59.3	0.83	CA	none	1035731	40.8
# 7	m	34.8	0.33	LE	PLEX, iv Ig*	4	10.1
# 8	f	18.6	1.67	LE	none (GCs**)	1134949	11.1
# 9	f	22.0	1.58	LE, APECED	aza	141340	11.2
# 10	f	62.4	4	CA	none	69976	11.7
# 11	f	53.6	6	SPS	none	1931662	5.3
# 12	f	28.5	0.17	LE	none	668244	10.8
# 13	m	75.6	0.67	SPS	none	91090	10.4
# 14	f	53.0	11.25	CA	none	6829116	19.2
# 15	f	48.9	6	LE	RTX + aza***	145909	B-cell depletion
	f/m	**Mean (± SD)**	**Mean (± SD)**			**Mean (± SD)**	**Mean (± SD)**
Patients	12/3	49.6 (± 18.8)	4.3 (± 3.9)			920352 (± 1678500)	14.5 (± 9.0)
HC	13/6	43.7 (± 8.9)					

Abbreviations: APECED, Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; aza, azathioprine; BMMCs, bone marrow mononuclear cells; CA, cerebellar ataxia; GCs, glucocorticosteroids; HC, healthy controls; IST; immunosuppressive treatment; iv Ig, intravenous immunoglobulin; LE, limbic encephalitis; PBMCs, peripheral blood mononuclear cells; PLEX, plasma exchange; RTX, rituximab; SPS, stiff person syndrome; sc Ig, subcutaneous immunoglobulin.

*3 weeks prior to PBMC sampling; **2 months prior to PBMC sampling; ***analysis of BMMCs.