The Different Clinical Effects of Anti-BLyS, Anti-APRIL and Anti-CD20 Antibodies Point at a Critical Pathogenic Role of gamma-Herpesvirus Infected B Cells in the Marmoset EAE Model

Jagessar, S. Anwar; Fagrouch, Zahra; Heijmans, Nicole; Bauer, Jan; Laman, Jon D.; Oh, Luke; Migone, Thi; Verschoor, Ernst J.; 't Hart, Bert A.

Published in:
Journal of neuroimmune pharmacology

DOI:
10.1007/s11481-013-9448-6

Publication date:
2013

J Neuroimmune Pharmacol (2013) 8:727–738
DOI 10.1007/s11481-013-9448-6

ORIGINAL ARTICLE

The Different Clinical Effects of Anti-BLyS, Anti-APRIL and Anti-CD20 Antibodies Point at a Critical Pathogenic Role of γ-Herpesvirus Infected B Cells in the Marmoset EAE Model

S. Anwar Jagessar · Zahra Fagrouch · Nicole Heijmans · Jan Bauer · Jon D. Laman · Luke Oh · Thi Migone · Ernst J. Verschoor · Bert A. 't Hart

Received: 19 February 2013 / Accepted: 27 February 2013 / Published online: 19 March 2013

Abstract The robust and rapid clinical effect of depleting anti-CD20 monoclonal antibodies (mAb) in multiple sclerosis (MS) demonstrates a critical pathogenic contribution of B cells. The clinical effect of anti-CD20 mAb has been replicated in a relevant preclinical MS model, experimental autoimmune encephalomyelitis (EAE) in marmoset monkeys (*Callithrix jacchus*). By contrast, treatment with mAbs against two essential cytokines in B cell activation growth and survival, i.e. BlyS/BAFF and APRIL, was only partially effective. All three mAbs induced depletion of CD20+ B cells from the circulation, albeit with different kinetics and based on distinct mechanisms of action. In the current study we analyzed whether the different clinical effect of anti-CD20 mAb or the anti-BLyS and anti-APRIL mAbs is due to different depletion of B cells infected with the EBV of marmosets, CalHV3. Employing a novel PCR-based assay, half of the colony of group-housed marmosets was tested positive for CalHV3 DNA in secondary lymphoid organs. The same prevalence was observed in placebo-treated monkeys. In marmosets treated with anti-CD20 mAb the load of CalHV3 DNA in lymphoid organs was substantially reduced, while this was not observed in the monkeys treated with anti-BLyS or anti-APRIL mAbs. To examine the pathogenic role of virus-transformed B cells, we infused EBV-transformed B lymphoblastic cell (BLC) lines presenting the immunodominant MOG34-56 peptide. We observed in the recipients of MOG34-56 pulsed BLC, but not in their fraternal siblings infused with non-pulsed BLC, activation of anti-MOG34-56 T cells and meningeal inflammation. Collectively, the data show that among CD20+ B cells, the herpesvirus-transformed subset has a particularly important pathogenic role in the marmoset EAE model.

Keywords EBV · CalHV3 · Marmoset · Experimental autoimmune encephalomyelitis · Multiple sclerosis · B cell

S.A.J. and Z.F. share first authorship, E.J.V. and B.A.'t H. share senior authorship.

S. Anwar Jagessar · N. Heijmans · B. A. 't Hart (✉)
Department Immunobiology, Biomedical Primate Research Centre, PO Box 3306, 2280 GH Rijswijk, The Netherlands
e-mail: hart@bprc.nl

S. Anwar Jagessar
e-mail: jagessar@bprc.nl

S. Anwar Jagessar · J. D. Laman · B. A. 't Hart
MS Centre ErasMS, Rotterdam, The Netherlands

Z. Fagrouch · E. J. Verschoor
Department Virology, Biomedical Primate Research Centre, Rijswijk, The Netherlands

J. Bauer
Department of Neuroimmunology, Medical University Vienna, Center for Brain Research, Vienna, Austria

J. D. Laman · B. A. 't Hart
Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

L. Oh · T. Migone
Human Genome Sciences, Rockville, MD, USA

B. A. 't Hart
Department of Neuroscience, University Medical Centre Groningen, Groningen, The Netherlands

Introduction

The common marmoset (*Callithrix jacchus*) is a small-bodied Neotropical primate that shares important genetic and immunological similarities with humans. These features make the marmoset a useful preclinical model for translational research into pathogenic mechanisms and therapy of multiple sclerosis (MS). Sensitization of marmosets with myelin, myelin proteins or peptides induces models of experimental autoimmune encephalomyelitis (EAE), which recapitulate clinical and pathological aspects of the human disease (’t Hart and Massacesi 2009). The marmoset EAE model typically displays MS-like primary demyelination of white (WM) and grey matter (GM) in the brain and spinal cord (Pomeroy et al. 2005; Merkler et al. 2006; Kap et al. 2011b).

We have used the EAE model in marmosets, for modeling the role of B cells in MS. The study is relevant because the surprising clinical effect of B-cell depleting monoclonal antibodies in MS without the expected ablation of demyelination-mediating autoantibodies hints at elusive immunopathogenic functions of B cells in the disease (Barun and Bar-Or 2012). The studies were performed in a well-validated EAE model induced by immunization with recombinant human myelin/oligodendrocyte glycoprotein (rhMOG) in complete Freund’s adjuvant (CFA).

The rhMOG/CFA EAE model recapitulates many of the clinical and pathological features of MS. Full clinical development of the model involves two distinct pathogenic pathways (’t Hart et al. 2011). The initiation of EAE involves synergy of T helper 1 cells specific for MOG epitope 24–36 inducing inflammation and B cells producing autoantibodies that mediate demyelination by opsonization of myelin sheaths. This EAE initiation pathway essentially recapitulates the situation of classical mouse EAE models (Genain et al. 1995; Brok et al. 2000; Villoslada et al. 2001). The response to immunomodulation confirms the Th1 signature of this pathway as early treatment with the anti-human IL-12/IL-23p40 monoclonal antibody (mAb) ustekinumab prohibited the development of clinically evident EAE (Brok et al. 2002), whereas an anti-IL-17A mAb only delayed the time of disease onset (Kap et al. 2011a). It is not clear whether this Th1/autoantibody pathway has a correlate in MS as a clinical trial with the same antibody failed because of lack of activity (Segal et al. 2008).

The progression of EAE to severe neurological impairment in the rhMOG/CFA model is driven by a different immunopathogenic mechanism, as was illustrated by the higher activity of anti-IL-17A and the lower efficacy of the anti-IL-12/IL-23p40 mAb in late-stage disease (’t Hart et al. 2005; Kap et al. 2011a). The development of clinically evident EAE via this non-classical progression pathway involved IL-17 producing and cytotoxic effector memory T (Tem) cells specific for epitopes comprised within $MOG_{34–56}$, without a detectable (ELISA) contribution of autoantibodies. In the EAE model induced with rhMOG/CFA these strongly pathogenic T cells are engaged relatively late in the disease process (Kap et al. 2008, 2010), presumably as a response to the release of antigens from primary CNS injury inferred by the initial autoimmune attack (’t Hart et al. 2009). Direct activation of the EAE progression pathway could be achieved by immunization with the $MOG_{34–56}$ peptide in IFA (Jagessar et al. 2010, 2012c). The ensuing EAE model recapitulates immunological and pathological aspects of progressive MS, such as the widespread demyelination and microglia activation with the cortical grey matter and the dominance of MHC class I restricted T cells, although their exact function is still unknown (Jagessar et al. 2010; Kap et al. 2010, 2011b). A likely human correlate of these T cells has recently been documented in MS (Zaguia et al. 2013).

B cells seem to have different roles in different EAE models. In the rhMOG/CFA model B cells produce demyelination-enhancing anti-MOG autoantibodies (Genain et al. 1995; Menge et al. 2007). In the novel EAE model induced with $MOG_{34–56}$/IFA B cells are intimately involved in the activation of cytotoxic cells that cause CNS demyelination without involvement of anti-MOG antibodies. In EAE induced with rhMOG/CFA (Kap et al. 2010, 2011b) or $MOG_{34–56}$/IFA (Jagessar et al. 2012a) we observed similar robust suppression of MS-like pathology and disease when CD20+ B cells were depleted starting 21 days after EAE. Intriguingly, late stage depletion of naïve and memory B cells by antibodies capturing cytokines which B cells need for their survival and differentiation, i.e. BLyS and APRIL (Liu and Davidson 2011), induced only a moderate albeit significantly delayed disease onset in the rhMOG/CFA model but not complete abrogation of EAE onset (Jagessar et al. 2012b).

The current study was undertaken to analyze in more depth the effects of treatment with mAb against CD20 versus mAbs against BLyS or APRIL on the B cell compartment. We report here that in monkeys treated with anti-CD20 mAb, but not in those treated with the anti-BLyS and anti-APRIL mAb titers of CalHV3 are reduced in lymphoid organs. CalHV3 is a γ-herpesvirus of marmosets that shares many functional similarities with EBV, including the transformation of B cells (Cho et al. 2001). The current results therefore suggest that the anti-CD20 mAb, but not the anti-BLyS and anti-APRIL mAbs had induced the ablation of γ-herpesvirus transformed B cells.

To test directly whether γ-herpesvirus transformed B cells are capable of activating MOG_{34-56} reactive T cells in vivo we set up an experiment in five marmoset twins. Autologous B lymphoblastoid cells (BLC) were generated by ex vivo infection of B cells with the EBV laboratory strain B95-8. One sibling of each twin was infused with non-pulsed BLC (BLC_0)

and the other with BLC pulsed with the immunodominant $MOG_{34–56}$ epitope of rhMOG (BLC_{MOG}). We observed activation of $MOG_{34–56}$ specific T cells and meningeal inflammation in some monkeys infused with peptide-pulsed BLC, but not in recipients of empty BLC.

Collectively, the reported data suggest that among CD20+ B cells, the γ-herpesvirus infected subset may have a particularly important pathogenic role in the marmoset EAE model. We like to postulate that these data are relevant for MS, as they may provide a mechanistic explanation for the remarkable clinical effect of B cell depleting anti-CD20 mAbs in relapsing-remitting disease and for the still elusive association between MS and EBV (see also (’t Hart et al. 2012)).

Materials and methods

Animals

Adult common marmosets were purchased from the purpose-bred colony of the Biomedical Primate Research Centre. As both genders are equally susceptible to EAE, male and female animals were used. All study protocols and experimental procedures were reviewed and approved according to the Dutch law on animal experimentation.

Tissue samples

For this study we have used tissues from three previous EAE experiments for preparation and quantification of CalHV3 DNA: *1*. Treatment with anti-BLyS and anti-APRIL mAb of marmosets in the EAE model induced with rhMOG/CFA. Efficacy data have been reported elsewhere (Jagessar et al. 2012b); *2*. Treatment with anti-CD20 mAb of marmosets in the EAE model induced with rhMOG/CFA. Efficacy data have been reported in (Kap et al. 2010) and (Kap et al. 2011b); 3. Treatment with anti-CD20 mAb of marmosets in the EAE model induced with $MOG_{34–56}$/IFA. Efficacy data have been reported in (Jagessar et al. 2012a). A summary of the previously reported clinical data is given in Fig 1.

PCR for CalHV3

DNA was isolated from tissue using the QIAamp DNA mini kit (QIAGEN Benelux B.V., Venlo, The Netherlands). For diagnostic purposes two different PCR assays were carried out on each DNA sample. The first assay was a pan-herpes PCR described by DeVanter et al. (VanDevanter et al. 1996). The second assay was specific for CalHV3 and made use of the outer primer set CalHV3-outF1 (5′-GGTGTCGACCTTG TCAG-3′), and CalHV3-outR (5′-CCGGCGGTGAAGT TTTGAA-3), and the inner set CalHV3-inF (5′-ACGC GGTGTAGACGGGTTTTG-3′) CalHV3-inR (5′-AAGGGC TGTCTCGGGGTTATTTC-3′). This PCR amplifies a 509 base pair fragment from the viral DNA polymerase gene. The outer amplification reaction was performed in a 50 μl volume using 1 μg of DNA, 2 units Truestart™ Hot Start *Taq* DNA polymerase (Fermentas GMBH, St. Leon-Rot, Germany), 5 μl 10× Hot Start PCR buffer, 50 pmol of each primer, 2 mM $MgCl_2$, and 200 μM of each dNTP. Cycling conditions were 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. In a second amplification reaction, 2 μl of the PCR product of the outer PCR was used as template. Inner PCR conditions were identical to those for the outer PCR, except that an annealing temperature of 52 °C was used. The PCR fragments were analyzed by electrophoresis on a 1 % agarose gel.

Real-time quantitative PCR assay

Similar to EBV, CalHV3 can be found in lymphoid tissue (Cho et al. 2001). CalHV3 loads in marmoset lymphoid tissue were determined using real-time PCR. DNA polymerase gene-specific primers and probe were designed based on the published sequence of CalHV3 (GenBank accession number NC004367). The primers used were CalHV3-For (5′-CACTC GCAAATCTGAAATGG-3), and CalHV3-Rev (5′-GACA CTGCAGGATATTTGCC-3′).

The probe (5′-CGCCACAATATACCGATAGCCGTG-3′) was labeled at the 5′ end with the FAM reported dye, and at its 3′ end with Black Hole Quencher 2. The PCR was carried in a 25 μl volume using Brilliant III Ultra Fast QPCR Master Mix (Agilent Technologies Netherlands B.V., Amstelveen, The Netherlands) with the final concentrations of 150 nM for each primer, 200 nM for the probe, 5.5 nM $MgCl_2$, and using 10 μg DNA. Target DNA was amplified for 40 cycles, consisting of 20 s denaturation at 95 °C, followed by a 20 s annealing-extension step at 60 °C. All the reactions were carried out with an iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories BV, Veenendaal, The Netherlands).

Titration of BLyS and APRIL on BLC

To test the effect of anti-BLyS and anti-APRIL on the growth and survival of the BLC, a titrating dose of the antibodies was added to the BLC in a 96-well U bottom plate (100,000 cells/well), and after 6 days incubation the total number of viable cells were counted.

Preparation of BLC and in vivo administration

An autologous adoptive transfer experiment was set up to test whether BLC presenting $MOG_{34–56}$ in vivo induce the activation of $MOG_{34–56}$ specific T-cell responses and CNS inflammation. The experiment comprised five male twins; due to the bone chimerism one twin sibling lacks alloreactivity against

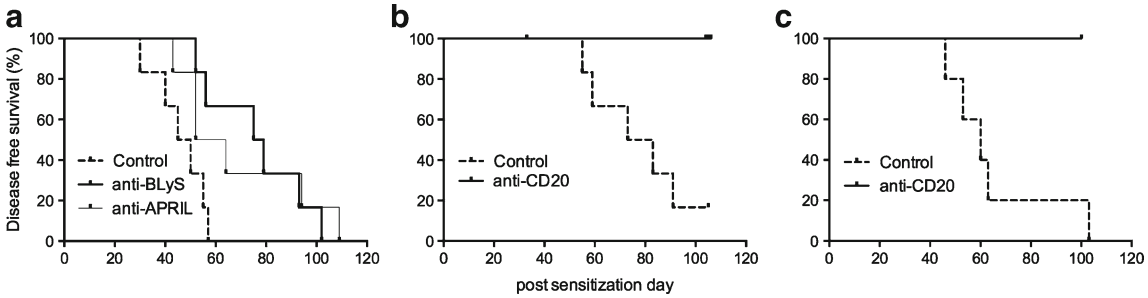

Fig. 1 Survival curves of B cell targeting immunotherapies in prior marmoset EAE studies. **a** Marmosets were treated weekly with anti-BLyS or anti-APRIL mAb from day 21 after immunization with rhMOG in CFA. The original clinical data have been published elsewhere (Jagessar et al. 2012b). **b** Marmosets were treated weekly with the anti-CD20 mAb HuMab7D8 from day 21 after immunization with rhMOG in CFA. The original data have been published elsewhere (Kap et al. 2010a). **c** Marmosets were treated weekly with the anti-CD20 mAb HuMab7D8 from day 21 after immunization the first immunization with MOG34-56 in IFA. The original data have been published elsewhere (Jagessar et al. 2012a)

immune cells of the other (Haig 1999). Details of the individual animals are summarized in Table 1. For preparation of B lymphoblastoid cells (BLC) 1.5 ml venous blood sample was collected from the vena femoralis into heparinized vacutainers (Becton Dickinson). Mononuclear cells (MNC) were isolated by density gradient centrifugation and infected with supernatants from the EBV-producing cell line B95-8 for immortalization (Antunes et al. 1998). The B95-8 strain used for B cell transformation has been derived from an EBV isolate from human infectious mononucleosis that has been maintained for several decades in cottontop tamarin cells (Raab-Traub et al. 1980).

Stably transformed BLC were grown for at least 1 month to obtain high cell numbers. Just prior to the experiment BLC of both twins were mixed (ratio 1:1) and subsequently divided into two equal portions, one portion (1.10^7 cells in 1 ml) was incubated overnight at 37 °C with 5 mg/ml $MOG_{34–56,}$ and will be indicated henceforth as BLC_{MOG}. The other portion (1.10^7 cells in 1 ml) was identically treated with medium and will henceforth be indicated as BLC_0. After incubation, the cells were harvested, washed once with buffered saline and injected at a dose of 1.10^7 BLC_0 or BLC_{MOG}. Each twin received three BLC injections, at days 0, 28 and 56. Note that the chimeric state renders twin siblings tolerant for alloantigens expressed on the mixed BLC (Niblack et al. 1977).

Clinical and post mortem examination

Marmosets were examined twice daily by visual inspection in the home cage for the expression of neurological deficits. Clinical signs of EAE were recorded using a semi-quantitative scoring developed for the marmoset EAE model (’t Hart et al. 1998). As an objective surrogate clinical marker, body weights were measured three times per week. The clinical end-point for each monkey was EAE score 3, representing complete paralysis of the hind part of the body. Monkeys reaching this score were first deeply sedated by intramuscular injection of Alfaxan (10 mg/kg) (Vétoquinol S.A., Magny-Vernois, France). After collection of the maximum venous blood volume, they were euthanized by infusion of sodium pentobarbital (Euthesate®; Apharmo, Duiven, The Netherlands). Monkeys not reaching the clinical end-point were humanely killed at the predetermined endpoint of the study, i.e. around psd 112.

Table 1 individual data of monkeys in the BLC infusion study

Monkey[a]	Twin	Treatment[b]	Age[c]	Exp. end (psd)	Clinical EAE	Meningeal inflammation[d]
M07022	1	BLC_0	33	112	no	0
M07023	1	BLC_{MOG}	33	112	no	0
M07038	2	BLC_0	33	114	no	0
M07039	2	BLC_{MOG}	33	114	no	+
M07043	3	BLC_0	33	112	no	0
M07044	3	BLC_{MOG}	33	112	no	+++
M07082	4	BLC_0	29	115	no	0
M07083	4	BLC_{MOG}	29	115	no	0
M07105	5	BLC_0	27	114	no	0
M07106	5	BLC_{MOG}	27	114	no	0

[a]All animals were male, [b]All animals received three times the same BLCL suspension, [c]Age in months at the start of the experiment; [d] 0 = no infiltrated cells detectable; + few numbers of infiltrated cells; ++ moderate numbers of infiltrated cells; +++ many of infiltrated cells

At necropsy, brain and spinal cord were collected for analysis with immunochemistry, histology and magnetic resonance imaging. Secondary lymphoid organs, axillary (ALN), inguinal (ILN), lumbar (LLN) and cervical (CLN) lymph nodes as well as spleen were aseptically collected for preparation of MNC (Jagessar et al. 2010).

Blood collection

EDTA or heparinized blood was collected every 2 weeks from the femoral vein.

Mononuclear cells (MNC) and plasma were separated by density gradient centrifugation (Brok et al. 2000). Plasmas were stored at −20 °C until further analysis.

Mononuclear cell (MNC) phenotyping

MNC subsets were phenotyped with commercially available mAbs preselected for cross-reaction with marmoset MNC subsets (Brok et al. 2001) as previously described (Jagessar et al. 2012c). Phenotyping of proliferated cells for specific markers in the CFSE dilution assay was performed as previously described (Kap et al. 2010). Flow cytometric analysis was performed on a FACS LSRII flow cytometer using FACSDiva software 5.0 (BD Biosciences) for analysis.

Cytokine production

Supernatants of PBMC cultures were collected after 48 h stimulation with rhMOG or a panel of 23-mer MOG peptides overlapping by 10 and covering the rhMOG sequence (Brok et al. 2000). Supernatants were assayed for the presence of cytokines using commercial ELISA kits according to manufacturer's instruction or as previously described (Kap et al. 2010).

Neuropathological examination

Brains collected at necropsy were routinely separated into symmetrical halves in anterior-posterior direction, taking care that both brain halves contained one hemisphere and half of the cerebellum and brain stem. One brain half was fixed in 4 % buffered formalin for at least 2 weeks to stabilize magnetic resonance (MR) relaxation time characteristics (Blezer et al. 2007). The other brain half was snap-frozen in liquid nitrogen. Spinal cord was also collected at necropsy and cut into three parts, cervical, thoracic and lumbar. Each part was subsequently cut in two pieces, one piece stored in 4 % buffered saline and the other in liquid nitrogen. As the spinal cord is too small to obtain meaningful data with current MRI technology, it was only included in the histological analysis.

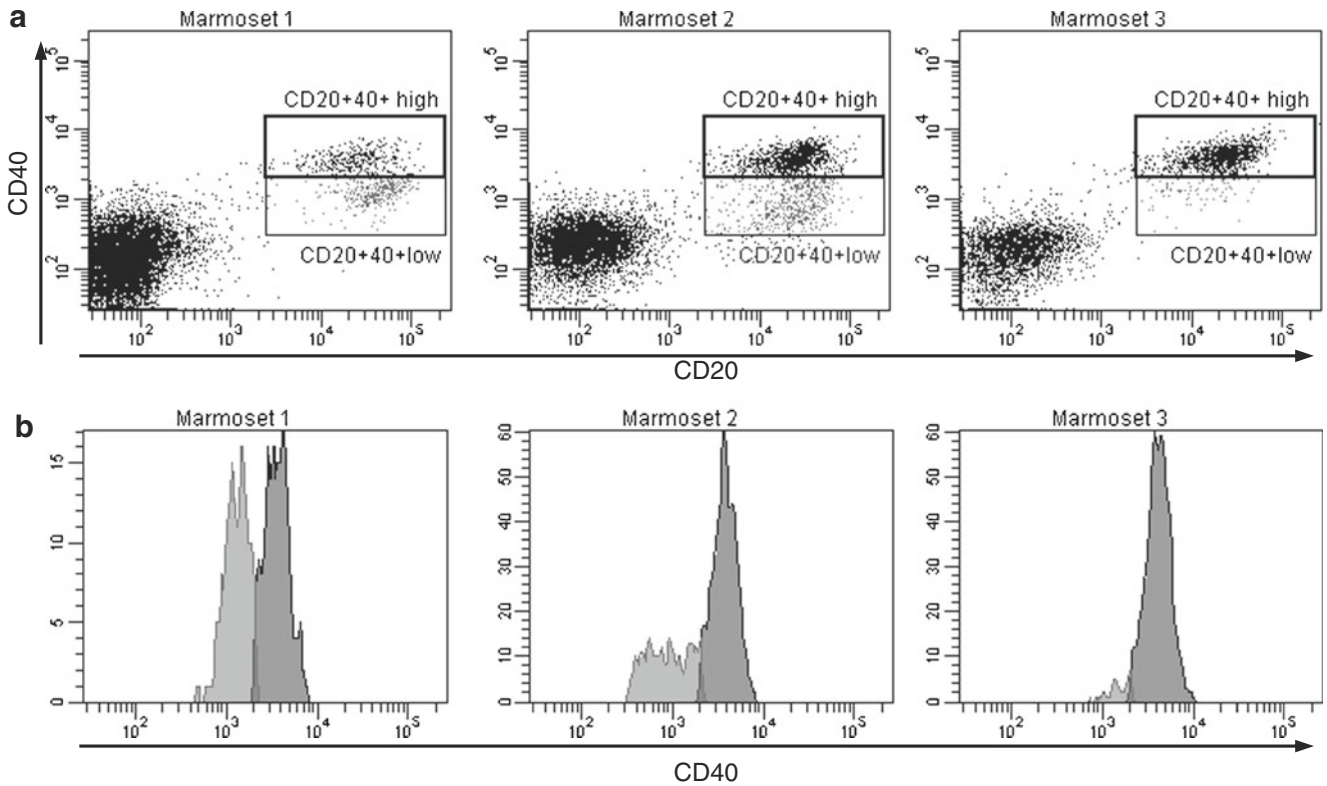

Fig. 2 Expression of CD40 and CD20 on marmoset MNC. Freshly isolated MNC from the venous blood of three different naive marmosets were stained with mAb against human CD20 and CD40. The results are presented as *dot-blots* (**a**) and single histogram (**b**). The results illustrate that CD40 is expressed with variable intensity by marmoset CD20+ B cells

The currently reported data were obtained from the analysis of formalin-fixed brain samples. Formalin-fixed samples were stored in buffered saline containing sodium-azide before scanning for magnetic resonance imaging (MRI) analysis. High contrast post mortem MR images were recorded on a 9.4 T horizontal bore NMR spectrometer (Varian, Palo Alto, CA), equipped with a quadrature coil (RAPID, Biomedical, Rimpar, Germany) as previously described (Kap et al. 2011b).

After completion of the MRI scanning, the fixed brains were processed for (immuno)histological examination of inflammation and demyelination as described (Kap et al. 2011b).

Statistical analysis

Data are presented as mean±SEM. Statistical analysis was performed using the Mann Whitney *U* test. *P* values≤0.05 were considered significant.

Results

The pathogenic fraction of CD20+ B cells is CD40+

Essentially all mature B cells express the costimulatory molecule CD40. However, as shown in Fig. 2 the expression of CD40 on CD20+ cells within freshly isolated marmoset PBMC varies considerably, ranging from high to background level expression. Previous reports document that the different clinical effects of anti-BLyS/APRIL (Jagessar et al. 2012b) and anti-CD20 mAb (Kap et al. 2010) on the rhMOG/CFA induced EAE model might be attributable to a different extent of depletion of $CD40^{high}$ B cells. The conclusion that complete abrogation of EAE development depends on the depletion of CD20 + $CD40^{high}$ cells is in conformity with our previous finding that blockade of CD40+ cells prior to immunization of marmosets with rhMOG/CFA abrogates EAE development (Boon et al. 2001).

These findings prompted us to further examine the pathogenic significance of CD20 + $CD40^{high}$ B cells, in particular focusing on the question whether the CD20 + $CD40^{high}$ fraction may represent a subset of B cells with particular pathogenic function. It is of note that marmosets are naturally infected with a γ-herpesvirus (CalHV3), which just like EBV in humans infects and transforms B cells (Cho et al. 2001). Virus-transformed B cells express CD40 at a high level and are capable to present the immunodominant epitope MOG40-48 to a highly pathogenic T cell subset in the marmoset EAE model (Jagessar et al. 2012c). Therefore, we hypothesized that the CD20 + CD40+ fraction might represent transformed B lymphoblastic cells (BLC). The small amount of blood that can be collected from live marmosets precludes positive isolation of the CD20 + $CD40^{high}$ subset. Hence, we chose to test the effect of titrating doses of anti-BLyS and anti-APRIL mAb on the in vitro survival of transformed marmoset B cells. It was observed that the presence of even high concentrations anti-BLyS or anti-APRIL mAb exerted no detectable effect on the survival of the BLC (data not shown). This experiment shows that B cells transformed by γ-herpesvirus are insensitive to the neutralization of BLyS and APRIL in vitro. This may help explain why CD20 + $CD40^{high}$ B cells were not depleted in vivo by mAbs capturing these growth factors.

Prevalence of CalHV3 infection in a captive colony

Spread of EBV in human populations usually occurs via infected secretions, such as saliva. Since marmosets in our captive colony are housed in family groups where they are engaged in frequent social and physical interactions we expected to find a high frequency of monkeys infected with CalHV3. However, in a significant proportion of the marmosets in our colony (47 out of 94), CalHV3 DNA could not be detected in the blood. It is unknown at this stage whether these monkeys are indeed not infected with CalHV3 or whether the virus load in blood is below the detection level of the quantitative PCR assay. Another possibility might be that CalHV3 negative monkeys have cleared the virus. At this moment established and validated serological assays to detect prior CalHV3 exposure based on specific antibody responses are not available.

CalHV3 titers in EAE-affected control and B cell depleted marmosets

To test whether CalHV3 infected B cells are differentially depleted by treatment with anti-CD20 or anti-BLyS/APRIL mAb in vivo, we determined CalHV3 viral DNA level in spleens of marmosets from three EAE protocols. The results are summarized in Table 2. Experiments 1 and 2 were performed in the rhMOG/CFA EAE model. Clinical data are respectively reported in (Jagessar et al. 2012b) and (Kap et al. 2010). Experiment 3 was performed in the $MOG_{34–56}$/IFA model; clinical data are reported in (Jagessar et al. 2012a). Figure 1 gives the clinical data of the individual experiments as survival curves.

Results from the first experiment show that CalHV3 DNA could not be detected in spleens from several of the untreated control monkeys. Hence we also tested the axillary lymph nodes, which gave essentially the same results. The analysis of the antibody-treated monkeys shows that neither the treatment with anti-BLyS, nor the treatment with anti-APRIL mAb induced reduction of CalHV3 titers.

Of the second experiment, only spleens were available for viral DNA isolation. High titers of CalHV3 DNA were

Table 2 Effect of B cell targeting therapies on CalHV3 concentrations in lymphoid organs. Shown are real time PCR copies of CalHV3/μg DNA in spleen and/or ALN

EAE model	Treatment	Organ	Animal							Mean±SEM	% from control
			1	2	3	4	5	6	7		
1. rhMOG/CFA	Control	Spleen	4400	10500	0	0	2510	776	–	3031±1647	100
		ALN	3090	195	0	0	1370	4000	–	1443±707	100
	Anti-BLyS	Spleen	3270	220	0	0	624	1730	–	974±530	32
		ALN	9400	605	1670	1350	2190	1140	–	2726±1352	180
	Anti-APRIL	Spleen	0	56000	0	0	44000	11000	–	18500±10230	610
		ALN	0	95900	0	0	367000	47600	–	85083±58513	5896
2. rhMOG/CFA	Control	Spleen	15400	10800	9360	8260	64100	5000	TF	18820±9162	100
	Anti-CD20	Spleen	3900	4010	108	28	108	26	184	1195±713	6,3
3. $MOG_{34–56}$/IFA	Control	Spleen	0	TF	923	0	TF	na	–	308±218	100
		ALN	0	3700	11800	3	1180	na	–	3337±2027	100
	Anti-CD20	Spleen	0	27	41	0	TF	na	–	17±8	5,5
		ALN	0	120	3	0	564	na	–	137±100	4,1

TF technical failure, not enough DNA; – = (tissue) not available

detected in all control monkeys. By contrast, CalHV3 DNA was substantially reduced in the anti-CD20 treated monkeys.

In Exp. 3 bone marrow chimeric twins were used. In both siblings of two twins we could not detect CalHV3 DNA in spleen or in ALN. We had insufficient DNA from the spleens of untreated monkeys, but the data from the ALN confirm that CalHV3 DNA levels were substantially reduced by the anti-CD20 treatment.

In vivo T cell activation by BLC presenting MOG34-56

The previous experiments showed that a robust clinical effect of B cell depletion was associated with strong reduction of CalHV3 load. These observations hint at a critical involvement of CalHV3 transformed B cells in the activation of autoreactive T activation. Our previous studies showed that B lymphoblastic cells (BLC) transformed by EBV strain B95-8 can process and present $MOG_{34–56}$ ex vivo to CD4+, CD8+ and CD4 + 8+ T cell lines generated from marmosets immunized with $MOG_{34–56}$ in CFA (Kap et al. 2008) or IFA (Jagessar et al. 2010). This raises the question whether infusion of marmosets with BLC presenting $MOG_{34–56}$ in vivo may induce activation of $MOG_{34–56}$ specific T cells together with signs or symptoms of EAE.

For this experiment we randomly selected five chimeric twins from our colony. Autologous BLC were prepared by ex vivo transformation of blood MNC with EBV strain 95–8. To ensure that both siblings of each twin received the identical BLC preparation, BLC from both fraternal siblings were mixed and subsequently split into two equal portions. One portion was incubated with $MOG_{34–56}$ peptide (BLC_{MOG}) and the other portion was incubated with medium (BLC_0). One sibling of each twin received three infusions at 28 days interval with BLC_{MOG} and the other with BLC_0.

EAE signs Clinical signs of EAE were observed in none of the five twins within the observation period of about 4 months. However, bodyweight measurements, being an accepted surrogate disease marker, yielded different mean bodyweight curves between the two groups (Fig. 3).

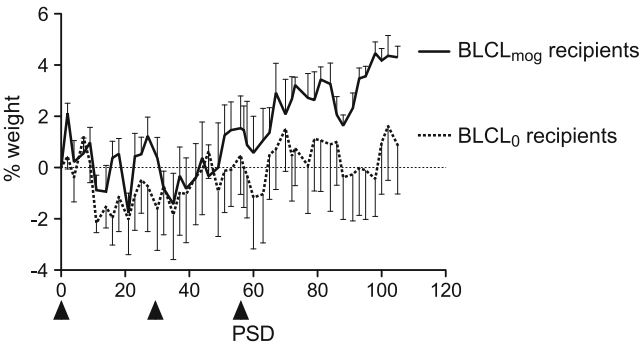

Fig. 3 Mean bodyweight curves. Deviations from the normally ascending growth curves in young adult marmosets can be used as an objective surrogate marker of subclinical disease for the EAE model. The arrowheads point to the time points where non-pulsed B lymphoblastic cells (BLC_0; *dotted lines*) or BLC pulsed with $MOG_{34–56}$ (BLC_{MOG}; *solid lines*) were infused. Note that the only difference between the two cell preparations is the presence or absence of $MOG_{34–56}$. Although differences between the growth curves were not significant the data suggest that the infusion of BLC_{MOG} and BLC_0 exert a different systemic effect on a relevant disease parameter. Data is presented as percentage weight change relative to day 0. *PSD* post sensitization day

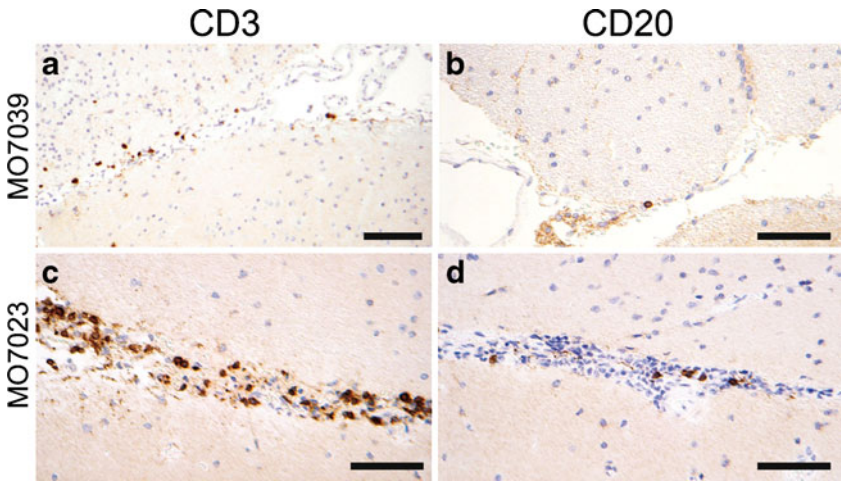

Fig. 4 Infiltrated T and B cells in the meninges of BLC_{MOG} recipients. Histological sections of paraffin-embedded fixed brains were stained with anti-CD3 and anti-CD20 mAb. Data is shown of two recipients of BLC_{MOG} in which signs of meningeal inflammation were detected, namely infiltration of CD3+ (A + C) (100×, *bar*: 200 μm) and CD20+ cells (B + D) (40×, *bar*: 100 μm). Results from the other three recipients of BLC_{MOG} were negative, as were all of the five BLC_0 recipients

Although the bodyweight curves did not differ significantly, they hint at a different systemic effect of BLC_0 and BLC_{MOG}, which differ only in the presentation of $MOG_{34–56}$. All monkeys were humanely killed without clinically evident EAE after >110 days observation.

Postmortem MRI and histology At necropsy brains and spinal cord were removed and processed for examination with high contrast T2W MRI and histology. On highly sensitive T2-weighted MRI scans (Blezer et al. 2007) no abnormalities within the brain parenchyma were visible

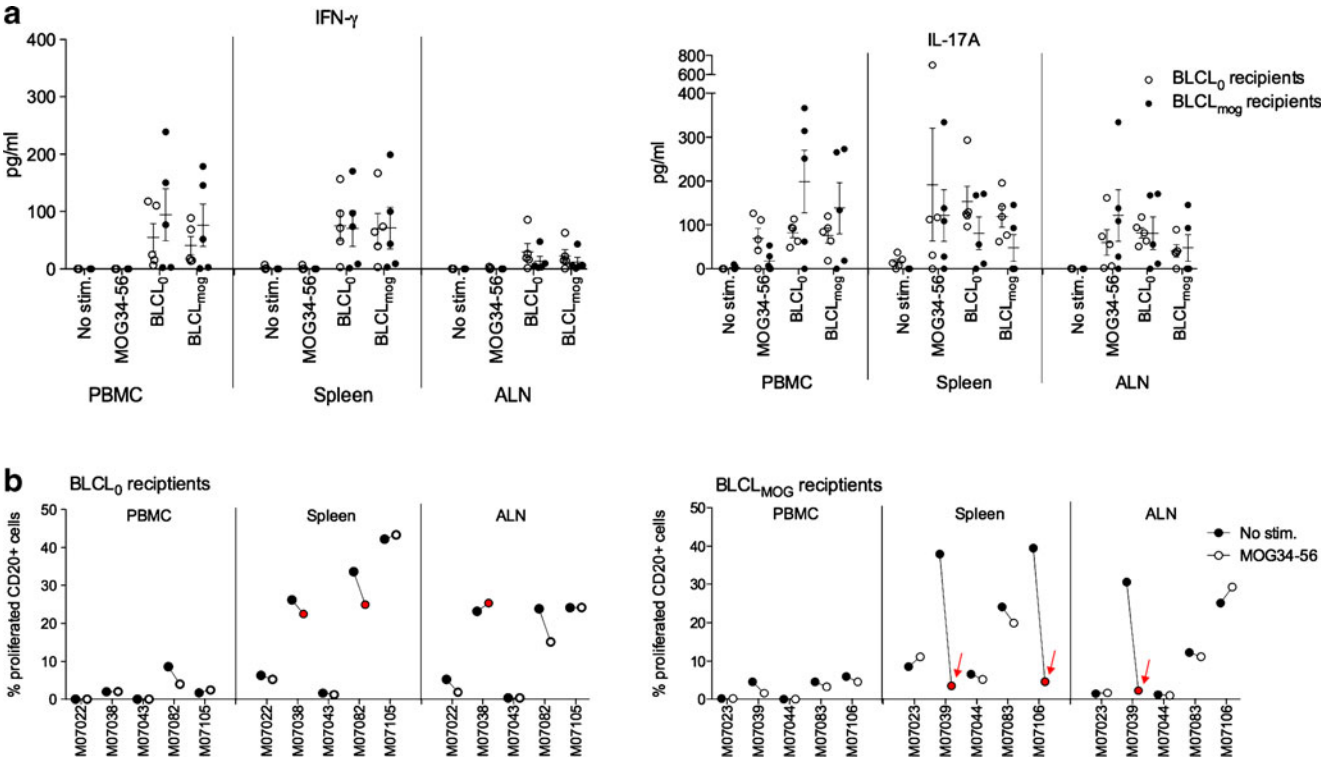

Fig. 5 Ex vivo induction of IL-17A and survival of transformed CD20+ cells. **a** Between psd 112 and 114 all monkeys were humanely killed and spleen cells were collected. MNC from both twin siblings were stimulated in culture with the indicated antigens as soluble proteins or presented by (semi-) autologous BLC. **a** IFNγ and IL-17A concentrations in culture supernatants were determined with ELISA. Group means are shown. **b** MNC suspensions were prepared from blood, spleen or axillary lymph nodes (ALN) of all monkeys. MNC were labeled with the vital dye CFSE and cultured for 7 days with (*open symbols*) or without (*closed symbols*) MOG34-56. After 7 days of culture the cells were harvested and stained with anti-CD20 mAb. Depicted are the percentages of CD20+ cells with dilution of CFSE as a marker of their transformed state. It is shown that in three twins MNC from spleen and ALN contained high percentages of CD20+ cells with CFSE dilution. Addition of MOG34-56 depletes CD20+ cells in MNC cultures from BLC_{MOG} recipients (red arrows), but not in fraternal siblings, which had been infused with BLC_0 and thus had not yet seen $MOG_{34–56}$. Data of individual twins are shown. Mann–Whitney *U* test on group means was performed, but no significant effects were obtained

(data not shown). Histological examination confirmed the absence of perivascular cuffs in CNS parenchyma. However, in two monkeys (M07023 and M07039), which were both recipients of BLC_{MOG}, infiltrated CD3+ and CD20+ lymphocytes were detected in the meninges (Fig. 4). Such infiltrates were not observed in the fraternal twin siblings, which had been infused with BLC_0.

T cell activation The two main immunological activities of in vivo activated T cell subsets in the $MOG_{34–56}$/IFA model were production of IL-17A and in vitro specific cytolysis of BLC_{MOG} target cells (Jagessar et al. 2012c). Hence we chose to analyze both parameters also in the current experiment. MNC from blood, spleen and ALN were stimulated ex vivo with or without $MOG_{34–56}$. In addition, the MNC were stimulated with BLC_0 and BLC_{MOG}. Culture supernatants were tested with ELISA for the presence of IFNγ and IL-17A. Data of individual monkeys depicted in Fig. 5a show that stimulation of MNC from blood, spleen or ALN with $MOG_{34–56}$ induced the release of IL-17A, but not IFNγ. Remarkably, the inducted IL-17A production was lower in the monkeys infused with BLC_{MOG} than in recipients of BLC_0. Stimulation of the cultures with BLC_{MOG} or BLC_0 induced an equal level of IFNγ and IL-17A in recipients of BLC_{MOG} or BLC_0.

We also tested whether the in vivo exposure BLC_{MOG} might have induced $MOG_{34–56}$-specific cytotoxic T cells. MNC collected from blood, spleen and ALN were first stained with the vital dye CFSE and subsequently cultured in the presence or absence of $MOG_{34–56}$. After 7 days in culture the MNC were harvested and stained with anti-CD20 mAb. We subsequently analyzed the stained cells using flow cytometry for the frequency of CD20+ cells with CFSE dilution, defining spontaneously proliferating transformed B cells. The results in Fig. 5b show again data of individual monkeys. In three of the five twins a substantial number of CD20+ cells with CFSE dilution could be detected in spleen and ALN of both siblings. Addition of $MOG_{34–56}$ to the MNC cultures of BLC_0 recipients exerted only a marginal effect. However, the addition of $MOG_{34–56}$ to the MNC cultures of BLC_{MOG} recipients induced complete elimination of CD20+ cells with CFSE dilution in two cases.

Discussion

Previously reported studies in the marmoset EAE model have shown that B cells are intimately involved in the activation of a newly discovered subpopulation of autoreactive NK-CTL cells, which mediate CNS grey matter pathology and neurological deficits (Kap et al. 2010, 2011b; Jagessar et al. 2012a). The current study shows that this activity may be mediated by a subset of virus-transformed B cells.

It is deemed unlikely that the remarkable clinical effect of anti-CD20 mAbs in relapsing-remitting MS can be fully explained by the prevention of autoantibody production, since beneficial effects develop very rapidly. Moreover, as plasma cells do not express CD20 they are most likely not depleted by anti-CD20 mAb (Barun and Bar-Or 2012). As alternative explanation, it has been proposed that B cells may function as antigen presenting cells (APC) for autoreactive T cells that drive MS pathogenesis (Barun and Bar-Or 2012). This assumption is supported by data from EAE models in mouse (Weber et al. 2010) and marmoset (Kap et al. 2010, 2011b).

An intriguing observation in the C57BL/6 mouse EAE model is that the depletion of CD20+ B cells suppresses EAE in recombinant mouse MOG-immunized mice, but exacerbates EAE elicited with $MOG_{35–55}$/CFA (Weber et al. 2010). The different clinical effect was explained by the observation that in the EAE model induced with recombinant mouse MOG (rmMOG) protein B cells are activated and infiltrate the CNS where they can present rmMOG epitopes to infiltrating T cells. Treatment with anti-CD20 mAb was found to remove these intra-CNS APC. B cell activation and CNS infiltration did not occur in the EAE model induced with $MOG_{35–55}$ peptide, precluding action of the anti-CD20 mAb within the CNS. The situation in the marmoset EAE model is remarkably different as the depletion of CD20+ B cells led to suppression of EAE induced with rhMOG/CFA as well as with $MOG_{34–56}$/IFA (Kap et al. 2010; Jagessar et al. 2012a).

How can this discrepancy between essentially equivalent EAE models in marmoset and mouse be explained? We postulate here a critical MOG antibody-independent role of B cells in a recently discovered non-classical EAE pathway in marmosets, which mediates the progression of EAE to clinically evident disease(Kap et al. 2008). This non-classical EAE progression pathway has not been found in rodent EAE models (’t Hart et al. 2011) and seems typical for the immune system of conventionally housed and adult outbred primates, that has been trained by genetic diversity and the lifelong combat against infections. Just like humans, but unlike SPF laboratory mice, Old and New World primates are latently infected with equivalents of human CMV and EBV (Lerche 2005). Both viruses have been implicated in human inflammatory demyelinating disease, namely CMV in ADEM (Wroblewska et al. 1979; Kanzaki and Yabuki 1994; Zaguri et al. 2009) and EBV in MS (Ascherio and Munger 2007; Lunemann and Munz 2009). Moreover, infection with γ-herpesvirus was found associated with MS-like disease in mice (Casiraghi et al. 2012) and in Japanese macaques (Axthelm et al. 2011).

Although EBV infection is recognized as a strong environmental risk factor for MS, or may even be a prerequisite for development of the disease (Pakpoor et al. 2013), a

mechanistic explanation is still lacking. On the basis of the here reported data we like to propose that EBV-infected B cells may be the obligatory APC for the MHC class I (Caja-E) restricted NK-CTL cells that drive the non-classical EAE progression pathway. The notion that the EBV-infected B cell has a critical pathogenic role in the marmoset EAE model raises the question which B cell functions are affected by the virus: 1. Primary B cells are well capable of presenting soluble antigens via MHC class II molecules to CD4+ T cells. However, they have limited capacity to present soluble antigen via MHC class I molecules to cytotoxic T cells, depending on co-stimulatory signals received via Toll-like receptors (Heit et al. 2004; Jiang et al. 2011). Why are EBV-infected B cells of marmosets better equipped for this task, at least in vitro (Jagessar et al. 2012c)? To answer this question we are now working on the unraveling the processing and MHC restricted presentation of rhMOG protein and peptides by primary and EBV-infected B cells. 2. It is becoming increasingly clear that some primary B cells, characterized by TIM-1 and IL-10 expression, exert regulatory functions in the immune response (Ding et al. 2011), including in the (mouse) EAE model (Matsushita et al. 2008). What is the effect of EBV infection on the regulatory functions of B10 cells? To answer this question we are also working on the identification of the marmoset correlate of mouse and human regulatory B10 cells.

The data presented in this publication support intimate involvement of transformed B cells in the pathogenesis of the marmoset EAE model. First, the retrospective analysis of stored lymphoid tissues from earlier preclinical mAb efficacy studies shows that the different clinical effects of B cell depletion via anti-CD20 mAb (Kap et al. 2010) or via neutralization of the essential B cell survival factors BLyS and APRIL (Jagessar et al. 2012b) are associated with a different extent to which CalHV3 is depleted. The data show that the anti-CD20 mAb, which had a robust clinical effect, induced systemic depletion of CalHV3, whereas treatment with anti-BLyS or anti-APRIL mAb, which were only partially effective, did not reduce viral load.

Secondly, we observed that infusion of EBV-infected B cells prepulsed with $MOG_{34–56}$ induced immunological and pathological features of EAE. We observed in two recipients of MOG peptide-pulsed BLC histological signs of meningeal inflammation, together with immune parameters of the $MOG_{34–56}$ induced pathogenic T cells. These features were not observed in monkeys infused with BLC that had not been pulsed with $MOG_{34–56}$. We also observed immunological features reminiscent of the EAE progression pathway, namely production of IL-17A and specific cytotoxicity towards BLC.

Unfortunately, the effects exerted by the infusion of BLC were not uniform and did not reach statistical significance due to high interindividual variation between monkeys. It is pertinent to emphasize here that preclinical research in non-human primates is notoriously complex, as the many genetic, immunological and microbiological differences create high variation between individual monkeys. One of the inevitable consequences is that it is often difficult to obtain robust statistical significance of an experimental treatment, unless high numbers of animals are used (Bacchetti et al. 2011). However, due to the high costs and ethical constraints this is often not possible. It is also possible that effects of a treatment can only be observed in certain susceptible individuals. We believe that this is the case in the BLC infusion experiment.

If the assumption is correct that virus transformed B cells have a central pathogenic role, the question is warranted why clinically evident EAE was not observed. This important question can be answered in several ways. A first important factor may be exposure time to the virus. The interval between infection with EBV and the onset of clinical signs in MS has been estimated at many years. A long period without symptoms between γ-herpesvirus infection and onset of MS-like disease (± 20 years) has also been observed in the macaque colony at the Oregon primate center (Axthelm et al. 2011). In the current study, marmosets were humanely killed at 4 months after the first BLC infusion. It is thus well possible that more severe pathology and clinically evident EAE might have developed when the monkeys would have been monitored for much longer time than 112 days. It is clearly unpractical and extremely expensive, however, to replicate a long EBV incubation period in an experimental setting. A second factor is the heterogeneity of immunopathogenic mechanisms and in clinical or pathological presentation of EAE. A final point of discussion is the discrepancy between the low prevalence of MS (0,1 %) and the high prevalence (>90 %) of EBV infection in the human population. It is conceivable that the low MS prevalence may reflect the low chance that myelin-specific B cells are infected with EBV, being a prerequisite for effective cross-presentation.

In conclusion, our current data point at a central pathogenic role of virus-transformed B cells, being the activation of autoreactive NK-CTL, which have been identified as the core mediators of demyelination in grey matter of the marmoset brain and spinal cord and of neurological dysfunction (Kap et al. 2008). The virus transformation may not be an absolute requirement for B cells to execute a pathogenic role in the EAE model, as we were able to induce EAE in monkeys in which CalHV3 could not be detected with the applied PCR technology. These findings seem to be compatible with the existence of two distinct pathogenic pathways towards EAE in marmosets, respectively characterized by the signature cytokines IFNγ and IL-17A. As explained elsewhere B cells have a different pathogenic contribution in these pathways [see accompanying paper and (’t Hart et al. 2011)].

Acknowledgments We like to thank the veterinarians and biotechnicians of BPRC for excellent care of the monkeys. We are also grateful to Mr. Henk van Westbroek for assistance with the artwork.

Conflict of interest The authors of this manuscript report no conflict of interest with the exception of drs Luke Oh and Thi Migone who were employees of Human Genome Sciences at the time the original efficacy study with anti-BlyS and anti-APRIL were performed.

References

't Hart BA, Jagessar SA, Haanstra K, Verschoor E, Laman JD, Kap YS (2012) The marmoset EAE model proposes (EBV-transformed) B cells as preferential therapy target in multiple sclerosis. J Neuroimmune Pharmacol (submitted)

't Hart BA, Massacesi L (2009) Clinical, pathological, and immunologic aspects of the multiple sclerosis model in common marmosets (Callithrix jacchus). J Neuropathol Exp Neurol 68:341–355

't Hart BA, Bauer J, Muller HJ, Melchers B, Nicolay K, Brok H, Bontrop RE, Lassmann H, Massacesi L (1998) Histopathological characterization of magnetic resonance imaging- detectable brain white matter lesions in a primate model of multiple sclerosis: a correlative study in the experimental autoimmune encephalomyelitis model in common marmosets (Callithrix jacchus). Am J Pathol 153:649–663

't Hart BA, Brok HP, Remarque E, Benson J, Treacy G, Amor S, Hintzen RQ, Laman JD, Bauer J, Blezer EL (2005) Suppression of ongoing disease in a nonhuman primate model of multiple sclerosis by a human-anti-human IL-12p40 antibody. J Immunol 175:4761–4768

't Hart BA, Hintzen RQ, Laman JD (2009) Multiple sclerosis—a response-to-damage model. Trends Mol Med 15:235–244

't Hart BA, Gran B, Weissert R (2011) EAE: imperfect but useful models of multiple sclerosis. Trends Mol Med 17:119–125

Antunes SG, de Groot NG, Brok H, Doxiadis G, Menezes AA, Otting N, Bontrop RE (1998) The common marmoset: a new world primate species with limited Mhc class II variability. Proc Natl Acad Sci U S A 95:11745–11750

Ascherio A, Munger KL (2007) Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol 61:288–299

Axthelm MK, Bourdette DN, Marracci GH, Su W, Mullaney ET, Manoharan M, Kohama SG, Pollaro J, Witkowski E, Wang P, Rooney WD, Sherman LS, Wong SW (2011) Japanese macaque encephalomyelitis: a spontaneous multiple sclerosis-like disease in a nonhuman primate. Ann Neurol 70:362–373

Bacchetti P, Deeks SG, McCune JM (2011) Breaking free of sample size dogma to perform innovative translational research. Sci Transl Med 3:87ps24

Barun B, Bar-Or A (2012) Treatment of multiple sclerosis with Anti-CD20 antibodies. Clin Immunol 142:31–37

Blezer EL, Bauer J, Brok HP, Nicolay K, 't Hart BA (2007) Quantitative MRI-pathology correlations of brain white matter lesions developing in a non-human primate model of multiple sclerosis. NMR Biomed 20:90–103

Boon L, Brok HP, Bauer J, Ortiz-Buijsse A, Schellekens MM, Ramdien-Murli S, Blezer E, van Meurs M, Ceuppens J, de Boer M, 't Hart BA, Laman JD (2001) Prevention of experimental autoimmune encephalomyelitis in the common marmoset (*Callithrix jacchus*) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B cell responses. J Immunol 167:2942–2949

Brok HP, Uccelli A, Kerlero De Rosbo N, Bontrop RE, Roccatagliata L, de Groot NG, Capello E, Laman JD, Nicolay K, Mancardi GL, Ben-Nun A, 't Hart BA (2000) Myelin/oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis in common marmosets: the encephalitogenic T cell epitope pMOG24-36 is presented by a monomorphic MHC class II molecule. J Immunol 165:1093–1101

Brok HP, Hornby RJ, Griffiths GD, Scott LA, 't Hart BA (2001) An extensive monoclonal antibody panel for the phenotyping of leukocyte subsets in the common marmoset and the cotton-top tamarin. Cytometry 45:294–303

Brok HP, Van Meurs M, Blezer E, Schantz A, Peritt D, Treacy G, Laman JD, Bauer J, 't Hart B (2002) Prevention of experimental autoimmune encephalomyelitis in common marmosets using an anti-IL-12p40 monoclonal antibody. J Immunol 169:6554–6563

Casiraghi C, Shanina I, Cho S, Freeman ML, Blackman MA, Horwitz MS (2012) Gammaherpesvirus latency accentuates EAE pathogenesis: relevance to Epstein-Barr virus and multiple sclerosis. PLoS Pathog 8:e1002715

Cho Y, Ramer J, Rivailler P, Quink C, Garber RL, Beier DR, Wang F (2001) An Epstein-Barr-related herpesvirus from marmoset lymphomas. Proc Natl Acad Sci U S A 98:1224–1229

Ding Q, Yeung M, Camirand G, Zeng Q, Akiba H, Yagita H, Chalasani G, Sayegh MH, Najafian N, Rothstein DM (2011) Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. J Clin Invest 121:3645–3656

Genain CP, Nguyen MH, Letvin NL, Pearl R, Davis RL, Adelman M, Lees MB, Linington C, Hauser SL (1995) Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. J Clin Invest 96:2966–2974

Haig D (1999) What is a marmoset? Am J Primatol 49:285–296

Heit A, Huster KM, Schmitz F, Schiemann M, Busch DH, Wagner H (2004) CpG-DNA aided cross-priming by cross-presenting B cells. J Immunol 172:1501–1507

Jagessar SA, Kap YS, Heijmans N, van Driel N, Brok HPM, Blezer ELA, Laman JD, Bauer J, 't Hart BA (2010) Induction of progressive demyelinating autoimmune encephalomyeitis in common marmoset monkeys using MOG34-56 peptide in incomplete Freund's adjuvant. J Neuropathol Exp Neurol 69:372–385

Jagessar SA, Heijmans N, Bauer J, Blezer ELA, Laman JD, Hellings N, 't Hart BA (2012a) B-cell depletion abrogates T-cell mediated demyelination in an antibody nondependent common marmoset EAE model. J Neuropathol Exp Neurol (in press)

Jagessar SA, Heijmans N, Bauer J, Blezer ELA, Laman JD, Delavaraja MN, 't Hart BA (2012b) Antibodies against human BLyS and APRIL mitigate EAE development in marmoset monkeys. J Neuroimmune Pharmacol (in press)

Jagessar SA, Heijmans N, Blezer EL, Bauer J, Blokhuis JH, Wubben JA, Drijfhout JW, van den Elsen PJ, Laman JD, Hart BA (2012c) Unravelling the T-cell-mediated autoimmune attack on CNS myelin in a new primate EAE model induced with MOG34-56 peptide in incomplete adjuvant. Eur J Immunol 42:217–227

Jiang W, Lederman MM, Harding CV, Sieg SF (2011) Presentation of soluble antigens to CD8+ T Cells by CpG Oligodeoxynucleotide-primed human naive B cells. J Immunol 186:2080–2086

Kanzaki A, Yabuki S (1994) Acute disseminated encephalomyelitis (ADEM) associated with cytomegalovirus infection—a case report. Rinsho Shinkeigaku 34:511–513

Kap YS, Smith P, Jagessar SA, Remarque E, Blezer E, Strijkers GJ, Laman JD, Hintzen RQ, Bauer J, Brok HP, 't Hart BA (2008) Fast progression of recombinant human myelin/oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis in marmosets is associated with the activation of MOG34-56-specific cytotoxic T cells. J Immunol 180:1326–1337

Kap YS, van Driel N, Blezer E, Parren PW, Bleeker WK, Laman JD, Craigen JL, 't Hart BA (2010) Late B cell depletion with a human

anti-human CD20 IgG1kappa monoclonal antibody halts the development of experimental autoimmune encephalomyelitis in marmosets. J Immunol 185:3990–4003

Kap YS, Jagessar SA, van Driel N, Blezer E, Bauer J, van Meurs M, Smith P, Laman JD, t Hart BA (2011a) Effects of early IL-17A neutralization on disease induction in a primate model of experimental autoimmune encephalomyelitis. J Neuroimmune Pharmacol 6:341–353

Kap YS, Bauer J, Driel NV, Bleeker WK, Parren PW, Kooi EJ, Geurts JJ, Laman JD, Craigen JL, Blezer E, t Hart BA (2011b) B-cell depletion attenuates white and gray matter pathology in marmoset experimental autoimmune encephalomyelitis. J Neuropathol Exp Neurol 70:992–1005

Lerche NW (2005) Common viral infections of laboratory primates. In: Wolfe-Coote SP (ed) The laboratory primate. Elsevier Science, Amsterdam, pp 75–85

Liu Z, Davidson A (2011) BAFF and selection of autoreactive B cells. Trends Immunol 32:388–394

Lunemann JD, Munz C (2009) EBV in MS: guilty by association? Trends Immunol 30:243–248

Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF (2008) Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest 118:3420–3430

Menge T, von Budingen HC, Lalive PH, Genain CP (2007) Relevant antibody subsets against MOG recognize conformational epitopes exclusively exposed in solid-phase ELISA. Eur J Immunol 37:3229–3239

Merkler D, Boscke R, Schmelting B, Czeh B, Fuchs E, Bruck W, Stadelmann C (2006) Differential macrophage/microglia activation in neocortical EAE lesions in the marmoset monkey. Brain Pathol 16:117–123

Niblack GD, Kateley JR, Gengozian N (1977) T-and B-lymphocyte chimerism in the marmoset. Immunology 32:257–263

Pakpoor J, Disanto G, Gerber JE, Dobson R, Meier UC, Giovannoni G, Ramagopalan SV (2013) The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. Mult Scler 19:162–166

Pomeroy IM, Matthews PM, Frank JA, Jordan EK, Esiri MM (2005) Demyelinated neocortical lesions in marmoset autoimmune encephalomyelitis mimic those in multiple sclerosis. Brain 128:2713–2721

Raab-Traub N, Dambaugh T, Kieff E (1980) DNA of Epstein-Barr virus VIII: B95-8, the previous prototype, is an unusual deletion derivative. Cell 22:257–267

Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-Gort R, Kasper LH (2008) Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol 7:796–804

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM (1996) Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 34:1666–1671

Villoslada P, Abel K, Heald N, Goertsches R, Hauser SL, Genain CP (2001) Frequency, heterogeneity and encephalitogenicity of T cells specific for myelin oligodendrocyte glycoprotein in naive outbred primates. Eur J Immunol 31:2942–2950

Weber MS, Prod'homme T, Patarroyo JC, Molnarfi N, Karnezis T, Lehmann-Horn K, Danilenko DM, Eastham-Anderson J, Slavin AJ, Linington C, Bernard CC, Martin F, Zamvil SS (2010) B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. Ann Neurol 68:369–383

Wroblewska Z, Gilden D, Devlin M, Huang ES, Rorke LB, Hamada T, Furukawa T, Cummins L, Kalter S, Koprowski H (1979) Cytomegalovirus isolation from a chimpanzee with acute demyelinating disease after inoculation of multiple sclerosis brain cells. Infect Immun 25:1008–1015

Zaguia F, Saikali P, Ludwin S, Newcombe J, Beauseigle D, McCrea E, Duquette P, Prat A, Antel JP, Arbour N (2013) Cytotoxic NKG2C+ CD4 T cells target oligodendrocytes in multiple sclerosis. J Immunol 8:2013, published on line Feb

Zaguri R, Shelef I, Ifergan G, Almog Y (2009) Fatal acute disseminated encephalomyelitis associated with cytomegalovirus infection. BMJ Case Rep 2009