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B cell delivered gene therapy for tolerance induction: role of autoantigen-specific B cells

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Abstract

Antigen-specific tolerance induction using autologous B-cell gene therapy is a potential treatment to eliminate undesirable immune responses. For example, we have shown that experimental autoimmune encephalomyelitis (EAE) and type 1 diabetes in NOD mice can be ameliorated using antigen-Ig fusion protein transduced B cells. However, it is well established that autoreactive antigen-specific B cells are activated in many autoimmune diseases and can contribute to pathogenesis. While syngeneic B cells from immunized or autoimmune mice can serve as tolerogenic antigen-presenting cells (APC), this observation begs the question of whether the antigen-specific B cells *per se* can be transduced as tolerogenic APC. To test this, we employed two model systems employing B cell receptor (BCR) transgenic or wild type (wt) mice as B cell donors. While adoptively transferred MOG-Ig transduced wt C57Bl/6 B cells were highly tolerogenic and ameliorated EAE, MOG-Ig transduced anti-MOG B cells from BCR transgenic mice were not. This phenomenon was reproduced in the NOD diabetes model in which pro-insulin-Ig transduced polyclonal wt NOD B cells were protective, whereas similarly transduced anti-insulin BCR B cells were not. Since the frequency of antigen-specific B cells in an immunized animal is quite low, we wished to determine the threshold numbers of BCR transgenic B cells that could be present in an effective transduced population. Therefore, we “spiked” polyclonal wt C57Bl/6 B cells with different numbers of anti-MOG BCR transgenic B cells. In the EAE model, we found protection when BCR B cells were present at 1%, but they prevented tolerance induction at 10%. Antigen-specific B cells expressed normal levels of co-stimulatory molecules and were tolerogenic when transduced with an irrelevant antigen (OVA). Thus, the presence of a BCR specific for the target autoantigen may interfere with the tolerogenic process to that antigen, but BCR-specific B cells are not intrinsically defective as tolerogenic APC. Taken together, these data suggest that antigen-specific tolerance induction can be achieved in the presence of a limited number of antigen-specific B cells, but higher numbers of pathogenic B cells

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may mask this induction. This observation should guide future development of therapies using autologous B cells to treat patients with autoimmune diseases.

Keywords

EAE; Tolerance; B cells; Gene Therapy

1. Introduction

Multiple sclerosis (MS) is a common inflammatory disease of the central nervous system (CNS), characterized by leukocyte infiltration and white matter demyelination [1]. Much of our current understanding of MS comes from the study of experimental autoimmune encephalomyelitis (EAE), a model which recapitulates human disease in both the clinical course and pathogenesis [2]. T cells have long been considered the major player in the pathogenesis of MS. This is mainly based on the presence of activated T cells in CNS lesions, alterations in T-cell subsets in the blood of MS patients, and the fact that EAE could be adoptively transferred using myelin specific CD4⁺ T cells [3,4]. However, the importance of B cells and myelin-specific antibody in the pathogenesis of MS has been re-emphasized recently [5–8]. To date, an effective specific therapy for MS is still lacking. While there are several approved immune modulation treatments available for MS [9–12], the efficacy of these treatments is limited, and a majority of patients eventually will become disabled [13]. Thus, it is important to have new and more effective therapy developed for treating MS patients.

We have developed a B cell gene therapy approach for antigen-specific tolerance induction which is based on the tolerogenicity of IgG carrier and B cells as “non-professional” APC [14]. B cells, transduced with a retroviral vector encoding a target peptide antigen fused with murine IgG1 heavy chain carrier, are highly tolerogenic *in vivo*. This approach has been successful in multiple animal models of human autoimmune disease, including multiple sclerosis (MS), diabetes, rheumatoid arthritis and uveitis [15–23], and has been confirmed and extended by other groups using endosomal targeting sequences [24,25]. Our mechanistic studies have shown that transduced B cells must express MHC class II and B7.2 co-stimulatory molecules for successful tolerance induction [26,27]. In addition, transduced B cells present endogenously expressed peptide-Ig with MHC class II molecules via a gamma interferon lysosomal thiol reductase-dependent pathway [28], and this leads to the activation or recruitment of CD25⁺, FoxP3⁺ regulatory T cells (Skupsky et al., Molecular Therapy, in press) [19,21,29]. Interestingly, Ahangarani *et al.* reported recently that treatment of mice with B cells transduced with a retroviral vector encoding a major T cell epitope from a common allergen led to induction of type 1-like regulatory T cells, and adoptive transfer of such T cells prevented the development of asthma induced by the same allergen [25].

The long-term goal for antigen-Ig therapy would be to transduce a patient’s own B cells with the tolerogenic construct appropriate for the disease. However, one possible hurdle for antigen-specific based therapy is pre-existent pathogenic autoantigen-specific B cells in autoimmune patients. While B cells from immunized or autoimmune mice can serve as tolerogenic APC [16,21], it is not known whether antigen-specific B cells *per se* could be transduced to be tolerogenic APC. In this study, we sought to address this question by using anti-MOG (8.18C5) and anti-insulin (125Tg) specific B cells from BCR transgenic mice. The former B cells are able to produce demyelinating antibodies and exacerbate EAE in MOG₃₅₋₅₅ immunized mice [30]. The anti-insulin BCR transgene fully supports the development of diabetes in NOD mice [31]. We found that antigen-specific B cells can be transduced as tolerogenic APC, but that a high frequency of pathogenic antigen-specific B

cells can mask this tolerance outcome. In order to treat autoimmune patients with autologous B cells, it is necessary that the therapy be effective in the presence of the physiologically relevant level of pathogenic antigen-specific B cells. Nonetheless, antigen-specific tolerance induction can be achieved in the presence of low numbers of pathogenic antigen-specific B cells, which is necessary in developing methods to overcome clinical limitations in the use of autologous B cells to treat patients with autoimmune diseases.

2. Materials and Methods

2.1. Mice and Reagents

Six-week-old C57BL/6 (B6) and NOD mice were purchased from Jackson Laboratory (Bar Harbor, ME). Anti-MOG-specific BCR transgenic mice, originally made by Litzenburg *et al* [30], were kindly provided by Drs. Vijay Kuchroo and Denise Chung (Brigham and Women's Hospital, Harvard Medical School, Boston). Spleen cells from mice expressing anti-insulin transgenes (125Tg), which harbor anti-insulin BCR (H + L chains), were from Dr. James W. Thomas (Vanderbilt University Medical Center, Nashville, TN) [31]. All mice were housed in pathogen-free condition, and the animal experiments were approved by the University of Maryland Animal Care and Use Committee. The MOG₃₅₋₅₅ peptide (MEVGWYRSPFSRVVHLYRNGK) was synthesized by New England Peptide (Fitchburg, MA). *Escherichia coli* expressing murine His₆-tagged recombinant mouse MOG (rMOG) was kindly provided by Dr. Joan Goverman (University of Washington, Seattle, WA). Preparation of non-glycosylated His-tagged rMOG was as described [32]. Biotin anti-mouse IgM^a, FITC anti-mouse B7.2, FITC anti-mouse I-A^b, and PE anti-mouse CD19 were from BD Pharmingen (San Jose, CA).

2.2. EAE induction

EAE was actively induced in 6–8 week old female B6 mice by subcutaneous immunization with 100–200 µg of MOG₃₅₋₅₅ peptide emulsified in CFA containing 4 mg/ml of *Mycobacterium tuberculosis* H37Ra (DIFCO, Detroit, MI). On the day of immunization (day 0) and 48 h later, mice also received 200 ng of pertussis toxin (Sigma-Aldrich) in 0.5 ml PBS intraperitoneally. Clinical signs of EAE were assessed daily with a 0 to 5 scoring system [33]: 0, normal; 0.5, partially limp tail; 1, paralyzed tail; 2, loss in coordinated movement; 2.5, one hind limb paralyzed; 3, hind limbs paralyzed; 3.5, hind limbs paralyzed and forelimbs weakness; 4, forelimbs paralyzed; 5, moribund.

2.3. Diabetes in NOD mice

In female NOD mice, spontaneous diabetes begins to appear by 12 weeks of age (increasing to an 80–90% incidence at 20–25 weeks of age). Glucose levels in blood taken from tail snips were measured once a week after 8 weeks of age by standard methods using OneTouch Ultra Test strips and monitoring system (LifeScan Technologies, Milpitas, CA). Mice were considered diabetic when glucose levels reached >250 mg/dl. Animals were also evaluated daily for clinical signs of diabetes (e.g., weight loss, cage wetting, morbidity and mortality).

2.4. Retroviral constructs and packaging cell lines

The retroviral vector MSCV-IRES-GFP was kindly provided by Dr. Kevin Bunting (Case Western Reserve University, Cleveland, OH). Generation of the retrovirus vector, MSCV-MOG₃₅₋₅₅-Ig-IRES-GFP (MOG-Ig), MSCV-Pro-Insulin-Ig-IRES-GFP (pro-insulin-Ig) and control vector MSCV-OVA₃₂₃₋₃₃₉-Ig-IRES-GFP (OVA-Ig) were as previously described [16]. Briefly, as illustrated in Fig. 1A, PCR amplified major T-cell epitopes from different antigens were inserted into the N-terminal of murine IgG1 H chain using Not I and Xho I

restriction sites. The resulting peptide-Ig fragments were further cloned into MSCV-IRES-GFP vector using a Sal I restriction site.

For generating packaging cell lines, retroviral vectors generated as described above were first transiently transfected into 293T cells, and then transduced to GPE-86 packaging cell line using the supernatants from the 293 T cells. The stably transduced GPE-86 packaging cell lines were established by FACS sorting of GFP positive cells. The viral titers of GPE-86 packaging cell lines were tested using NIH3T3 cells; titers $>1 \times 10^6$ CFU/ml were achieved before use of cells for co-culture.

2.5. B-cell purification and retroviral transduction

The day before co-culture, spleen B cells were purified to ~95% homogeneity by depleting T cells using anti-T cell antibody mixture (anti-Thy1, anti-CD4, and anti-CD8) plus rabbit complement (LowTox-M, Cedarlane, Burlington, NC). Purified B cells were stimulated overnight with 2 μ g/ml LPS (Sigma-Aldrich, St. Louis, MO) in RPMI1640 medium (InVitrogen, Carlsbad, CA) supplemented with 5% FBS, 2 mM L-glutamine and 2-Mercaptoethanol. The day before co-culture, packaging cells were irradiated at 1500 rads and plated on 0.1% gelatin (Fisher Scientific, Pittsburgh, PA) treated 150mm dishes (Corning, Lowell, MA) at 2×10^7 cells/dish. The next day, LPS-stimulated B cells were added onto packaging cells and co-cultured for 24 h in the presence of 6 μ g/ml of polybrene and 2 μ g/ml LPS (Sigma, St. Louis, MO).

2.6. ELISA

MOG-specific antibody production was measured by ELISA using plates coated with 10 μ g/ml of rMOG protein. Plates were incubated with diluted serum sample, and bound IgG antibodies detected using HRP-conjugated rabbit anti-mouse IgG (H+L) and IgG1 (Zymed-InVitrogen, San Francisco, CA), respectively.

2.7. FACS analysis

B cells were initially incubated with 2.4G2 monoclonal antibody (BD Pharmingen, San Diego, CA) for 15 min to block FcR, and then stained with PE-CD19, FITC- I-A^b or FITC-B7.2 antibodies for 20 min at 4°C. Propidium iodide (PI) was used to gate live cells. Data acquisition was performed on FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) using FlowJo software (Tree Star Inc. San Carlos, CA).

2.8. Statistics

Data are shown as mean $\pm$ SEM unless otherwise indicated. Student's *t* test was used to determine significance between groups. A *p* value of less than 0.05 was considered significant.

3. Results

3.1. Delayed onset and decreased severity of EAE by B cell gene therapy s

We previously reported that B cell delivery of MOG-Ig or MBP-Ig could ameliorate EAE in both pretreatment and therapeutic protocols [15,16]. To confirm the efficacy of tolerance induction using B cell gene therapy, we treated wt B6 mice with MOG-Ig transduced B cells 7 days before actively inducing EAE by MOG₃₅₋₅₅/CFA immunization. Control mice were treated with OVA-Ig transduced wt B6 B cells. The general protocol is shown as in the flow chart of Fig. 1B. Whereas control mice developed a typically severe and chronic relapsing EAE in 100% of the mice (Fig 2, left panel), MOG-Ig transduced B6 B cells treatment protected mice from EAE, significantly delaying day of onset (13.6 ± 0.5 for MOG-Ig, 11.6

± 0.4 for OVA-Ig, $p < 0.05$) and disease severity. That is, both the maximum disease score and cumulative scores were significantly lower in the MOG-Ig group than in the OVA-Ig group (2.6 ± 0.2 and 59.2 ± 9.2 for MOG-Ig, 3.4 ± 0.4 and 96.8 ± 13.3 for OVA-Ig, respectively, $p < 0.05$). Furthermore, our data suggested that tolerance was achieved at both T_H1 and T_H2 compartments (Supplemental Figure 1). H&E staining of lumbar spinal section showed greatly decreased CNS mononuclear cell infiltration in the MOG-Ig group of mice compared to mice receiving OVA-Ig B cells. MOG specific total IgG and IgG1 antibodies levels were also significantly decreased by treatment of MOG-Ig transduced B cells from wt B6 donors. These data agree with previous work from our lab with B cell presentation of MOG-Ig and MBP-Ig [16].

3.2. Transduced autoantigen-specific B cells failed to protect mice from EAE in B6 mice and autoimmune diabetes in NOD mice

To test whether EAE could be prevented by transduced *antigen-specific* B cells, we used anti-MOG-specific B cells (8.18C5) from mice in which the germ line J_H locus had been replaced with the rearranged immunoglobulin (Ig) H chain variable (V) region of a pathogenic MOG-specific monoclonal antibody [30]. B cells from homozygous transgenic mice exclusively express rearranged transgenic H chain as determined by anti-allotype (IgM^a) staining (data not shown). LPS activated 8.18C5 B cells were transduced with either MOG-Ig or control vector OVA-Ig, and 1×10^7 transduced B cells were adoptively transferred into wt B6 recipient mice 7 days before MOG₃₅₋₅₅/CFA immunization. As shown in Figure 2, when MOG antigen-specific 8.18C5 B cells were used, the disease course and severity of MOG-Ig and control OVA-Ig groups were similar. The levels of anti-MOG Ig antibodies were also similar between the two groups (data not shown).

To determine whether this phenomenon was unique to MOG-induced EAE and anti-MOG B cells, we tested antigen-specific B cells in another autoimmune disease model, the NOD mouse. We previously demonstrated that B cells from NOD donors (including those from diabetic females) could be transduced with an insulin B₉₋₂₃-Ig construct to significantly delay the onset and reduce the severity of diabetes [15]. To test whether insulin-specific B cells could function as tolerogenic APC, we used B cells from anti-insulin BCR (125Tg) transgenic NOD mice *versus* wild type NOD donors. In studies published to date, the 125Tg is the only BCR that fully supports the development of diabetes in NOD. We transduced B cells from NOD *female* donors or from 125Tg BCR transgenic donors with pro-insulin-Ig. The results in Figure 3 confirm that B cells from NOD donors can be transduced with a pro-insulin-Ig to provide protection from diabetes in terms of disease incidence and mean blood glucose. However, the transduced 125Tg transgenic B cells failed to protect NOD recipients from diabetes, in agreement with the results in the EAE model.

3.3. OVA-Ig transduced 8.18C5 B cells induced tolerance to OVA/CFA immune challenge in wt B6 recipient mice

Tolerogenicity of antigen-specific B cells in the above autoimmune disease models could be masked by the pathogenic nature of these B cells. Alternatively, these cells may be functionally deficient as tolerogenic APC independent of their receptor specificity. To test the latter hypothesis, we transduced 8.18C5 B cells with an OVA-Ig construct, which encodes a major T-cell epitope from a non-relevant chicken ovalbumin antigen, OVA₃₂₃₋₃₃₉. Wt B6 mice were received 1×10^7 transduced B cells from either anti-MOG transgenic or wt B6 donors, and were challenged with OVA/CFA one week after adoptive transfer of B cells. Two weeks after immunization, the T-cell proliferation recall response to OVA peptide was analyzed. As shown in Figure 4, both 8.18C5 and wt B6 B cells were equally efficient in inducing tolerance to OVA, compared with the corresponding B cells that were

transduced with control vectors ($p < 0.01$). This indicates that the BCR transgenic B cells are fully functional for tolerance induction when a non-relevant antigen is targeted.

3.4. BCR transgenic B cells are equally transduced and express similar levels of MHC-II and the B7.2 co-stimulatory molecule

As reported for other BCR transgenic B cells [34], we found that 8.18C5 B cells have an enlarged marginal zone compartment ($CD21^{\text{hi}}CD23^{\text{int}}$). Both resting and LPS activated 8.18C5 B cells had about 2–3 fold more marginal zone B cells (Fig. 5A). To determine whether this phenotypic change affects the transduction efficiency of 8.18C5 B cells, we looked at GFP expression in transduced B cells. FACS analysis (figure 5B) showed that the transduction efficiency was similar in the BCR transgenic B cells and wt B6 B cells. In addition, we also found that LPS activated 8.18C5 B cells and wt B6 B cells expressed similar levels of MHC class II molecule I-A^b and co-stimulatory molecule B7.2 (Fig. 5C), both of which are required for tolerance [26,27].

3.5. Amelioration of EAE achieved in the presence of low number of antigen-specific B cells

Autoantigen-specific B cells are likely to be a rare population even in autoimmune disease patients due to the broad repertoire of B cells in any given individual. To determine the threshold of antigen-specific B cells that could be used in our transduction protocol, we diluted the MOG-Ig transduced 8.18C5 B cells with MOG-Ig transduced wt B6 B cells and tested their efficacy in the EAE model. While no protection was observed in mice that received B cells containing at least 10% 8.18C5 B cells (Fig. 5A), a protective effect in the MOG-Ig group was found when the number of 8.18C5 B cells was reduced to 1%, a number still in excess of that expected in autoimmune individuals (Fig. 5B). A significant difference was reached at earlier time points as indicated in Figure 5B.

Furthermore, when 8.18C5 B cells were maintained at 10% ratio and FACS sorted to enrich only the GFP⁺ cells, the EAE disease course showed a trend of lower disease severity as compared with that without FACS sorting (Supplement Figure 2).

4. Discussion

Autoimmune diseases remain incurable. It is important to continue developing new treatment methods. Antigen-specific tolerance induction holds great potential in eliminating undesirable immune responses. We previously developed a B cell gene therapy method to achieve efficient antigen-specific tolerance induction in multiple autoimmune disease models [15,16,18,20–23]. However, it is well known that autoantigen-specific B cells may contribute to pathogenicity in both autoimmune disease models and in patients. Indeed, primed and clonally expanded antigen-specific B cells may cross the blood brain barrier and efficiently present autoantigen to specific CD4⁺ T cells, thus enhancing T cell-initiated CNS inflammation. Therefore, before autologous B cell-delivered gene therapy could possibly be translated to the clinic, we need to address the question of whether auto-reactive antigen-specific B cells *per se* could be transduced as tolerogenic APC using our B cell gene therapy approach. By employing two types of BCR transgenic mice as donors of transduced B cells and using two relevant autoimmune disease models, we found that antigen-specific B cells themselves can be transduced as tolerogenic APC. However, we found that antigen-specific B cells interfered with tolerance induction when present in large numbers. Importantly, we demonstrated that significant protection of mice against EAE could be achieved in the presence of a low number of pathogenic antigen-specific B cells, the physiologically relevant condition.

In previous experiments, we successfully transduced B cells from immunized or overtly autoimmune donors to mimic the situation in patients with ongoing autoimmune disease [16,21]. However, using antigen-specific BCR transgenic B cells more directly addresses the central question and allows us to quantitate their effect. The anti-MOG 8.18C5 B cells used in this study were from BCR transgenic mice in which the germ line J_H locus was replaced with the rearranged Ig H chain variable (V) gene. This V gene was cloned from a hybridoma that produces pathogenic demyelinating anti-MOG monoclonal antibody [30]. More than 98% of B cells from homozygous BCR transgenic mice express the transgenic IgH chain (data not shown). The transgenic H chain pairs with endogenous L chain. As a result, more than 50 % of the B cells from homozygous anti-MOG transgenic mice could directly bind rMOG protein [30]. In addition to using 8.18C5 B cells in the mouse EAE model, we employed anti-insulin 125Tg BCR B cells in the autoimmune diabetes model to confirm the results of the EAE experiments. Both types of antigen-specific BCR B cells contribute to pathogenesis in their respective disease model [30,31].

We found that transduced 8.18C5 and 125Tg B cells failed to protect against MOG₃₅₋₅₅-induced EAE in B6 mice or autoimmune diabetes in NOD mice, respectively (Fig. 1C and Fig. 2). This may be because antigen-specific B cells are unable to serve as tolerogenic APC, or it may be that antigen-specific B cells' pathogenic nature interferes with the tolerance effect. Our results suggest that the latter is the case. First, we found the antigen-specific B cells expressed similar levels of MHC class II molecule I-A^b and co-stimulatory molecule B7.2 (Fig. 4B and C), both of which are required for tolerance induction as shown by previous mechanistic studies [26,27]. Second, when a non-relevant antigen, OVA, was targeted in a non-disease model, an equal tolerogenic efficacy could be achieved in terms of *in vitro* T-cell recall response by treatment with either transduced 8.18C5 or wt B6 B cells. Third, in the experiment where transduced wt B cells were "spiked" with fractional (10%) transduced 8.18C5 B cells, we saw a trend of decreased disease severity of EAE simply by increasing transduction efficiency through FACS sorting to enrich GFP⁺ cells (Supplemental Fig. 2).

How non-transduced antigen-specific B cells interfere with tolerance is currently not known. In addition to the production of pathogenic antibodies, another way that antigen-specific B cells could contribute to pathogenesis might be through efficient autoantigen presentation in the context of autoimmune tissue damage and chronic inflammation. Using two-photon microscopy, we found that transduced tolerogenic wt B cells actively engage antigen-specific T cells in the local lymph node, and the immune cells remain paired for a prolonged time (Su, Matheu et al., manuscript in preparation). It would be very interesting to determine if pathogenic antigen-specific B cells could do the same, and whether tolerogenic presentation by antigen-specific B cells could directly overcome pathogenic antigen presentation in the autoimmune disease condition.

Overall, our results in the EAE "spiking" experiment indicate that significant tolerance induction could be achieved in the presence of a low, but significant, number of pathogenic antigen-specific B cells (Fig. 5). This is important, since the absolute number of antigen-specific B cells in autoimmune diseases patients should still be rare considering the huge diversity of antigen specificity in the BCR reservoir. The results obtained in this study encourage us continue to develop methods to overcome clinical limitations in the use of autologous B cells to treat patients with autoimmune diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic depiction of retroviral vectors and the general experimental protocol. *A*. Schematic depiction of retroviral vectors. MSCV-MOG₃₅₋₅₅-Ig-IRES-GFP (MOG-Ig) and MSCV-OVA₃₂₃₋₃₃₉-Ig-IRES-GFP (OVA-Ig) were constructed by inserting the major T-cell epitope MOG₃₅₋₅₅ or OVA₃₂₃₋₃₃₉ at the N-terminus of the murine IgG1 H chain, respectively. *B*. The flow chart of the general experimental protocol. B cells from wild type or BCR transgenic donors were transduced with different retroviral vectors and transferred to recipients who were challenged for clinical disease read out as described in subsequent figures.

Fig. 2.

Specific tolerance was induced in mice EAE with transduced B cells from wild type (wt), but not anti-MOG BCR transgenic (8.18C5 Tg) donors. B6 recipients (n=4) were adoptively transferred with 1×10^7 retrovirally transduced B cells from wt B6 donors or 8.18C5 Tg donors intraperitoneally. One week later, the mice were actively immunized for EAE using MOG₃₅₋₅₅ (100 μ g)/CFA. The clinical score was monitored daily after immunization. Delayed onset and decreased severity of EAE were achieved by pretreatment with MOG-Ig transduced wt B cells, but not by MOG-Ig transduced 8.18C5 B cells. Significant differences between the MOG-Ig group and OVA-Ig control group are indicated: *, $p < 0.05$; **, $p < 0.01$. Representative data from one of two independent experiments with similar results are shown.

Fig. 3.

Protection of autoimmune diabetes by transduced B cells from wt NOD donors, but not anti-insulin BCR transgenic (125Tg) NOD donors. At the age of 8 weeks, female NOD mice (n=8) were injected intraperitoneally with 5×10^6 of either pro-insulin-Ig expressing B cells or control B cells, followed by weekly monitoring of blood glucose levels. The mice with blood glucose levels equal or higher than 250 mg/dl for two consecutive tests were considered as diabetic. One representative experiment from three independent studies is shown. *A*, Incidence of diabetes in the mice that received transduced B cells from either wt NOD donors or 125Tg donors, respectively. *B*, Mean blood glucose levels in the mice that received transduced B cells from different donors as indicated.

Fig. 4.

Antigen-specific B cells are tolerogenic when a non-relevant antigen, OVA, was targeted. Wt B6 recipient mice (n=4) were injected intraperitoneally with 1×10^7 of transduced B6 B cells or 8.18C5 Tg B cells. One week later, the mice were immunized in one foot pad with OVA (2μg)/CFA. Two weeks after immunization, *in vitro* T-cell recall response to OVA₃₂₃₋₃₃₉ peptide antigen was set up using draining lymph node cells. Data are expressed as mean counts $\pm$ SEM from incorporated ^3H -thymidine. No matter what type of donors for the transduced B cells were used, the *in vitro* T-cell proliferation response to OVA₃₂₃₋₃₃₉ antigen in OVA-Ig groups was significantly lower than that in MOG-Ig groups. *, $p < 0.05$; **, $p < 0.01$. Representative data from one of two independent experiments is shown.

Fig. 5. Antigen-specific B cells expressed similar levels of MHC class II and co-stimulatory molecule B7.2, and were equally transducible as wt B cells. **A**, The 8.18C5 Tg mice had an enlarged marginal zone compartment in their spleens. Resting or LPS activated splenic B cells from either wt B6 or 8.18C5 Tg mice were analyzed for surface expression of CD23 and CD21 by FACS. Cells were gated on CD19⁺ B cells. Gates shown are CD23^{hi}CD21^{int} follicular B cells and CD23^{low}CD21^{hi} marginal zone B cells. **B**, FACS analysis of surface I-A^b and B7.2 expression on resting and LPS-activated splenic B cells. MHC class II molecule I-A^b and co-stimulatory molecule B7.2 expressions were both increased to a similar extent on wt B6 and 8.18C5 Tg cells after LPS stimulation for 24 hrs. **C**, Retroviral transduction of LPS-activated splenic B cells measured by FACS analysis of GFP expression. The transduction efficiency was similar for both wt B6 and 8.18C5 Tg B cells.

Fig. 6.

Decreased EAE severity achieved in the presence of physiologically significant numbers (1%) of autoimmune, transduced 8.18C5 Tg B cells. Transduced 8.18C5 Tg and wt B6 B cells were mixed at different ratios as indicated. B-cell pretreatment and EAE induction ($n=4$) were performed as described in Fig. 2, A, EAE disease courses. While the presence of super-physiologic (10%) transduced 8.18C5 Tg B cells prevented tolerance induction (left panel), significant reduction of the disease severity was achieved in the presence of 1% transduced 8.18C5 Tg B cells (right panel). Data are expressed as mean clinical score $\pm$ SEM; representative data from one of two independent experiments with similar results is shown. **B**, Overlay of the disease courses. The disease onset was delayed and the disease severity was significantly reduced in the presence of 1% MOG-Ig transduced 8.18C5 B cells, as compared with 10% of MOG-Ig transduced 8.18C5 B cells. *, $p<0.05$.