



Inhibition of B cell activation following *in vivo* co-engagement of B cell antigen receptor and Fcγ receptor IIb in non-autoimmune-prone and SLE-prone mice

Seung Y. Chu^a, Erik Pong^a, Christine Bonzon^a, Ning Yu^b, Chaim O. Jacob^b, Samantha A. Chalmers^c, Chaim Putterman^c, David E. Szymkowski^{a,1}, William Stohl^{b,*,1}

^a Xencor, Inc., Monrovia, CA, 91016, USA

^b Division of Rheumatology, Department of Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA, 90033, USA

^c Division of Rheumatology and Department of Microbiology and Immunology, Albert Einstein School of Medicine, Bronx, NY, 10461, USA

ARTICLE INFO

Keywords:

B cells
FcγRIIb
Lupus
Murine

ABSTRACT

Engagement of Fcγ receptor IIb (FcγRIIb) suppresses B cell activation and represents a promising target for therapy in autoimmunity. Obixelimab is a non-depleting anti-human CD19 mAb with an Fc region engineered to have high affinity for human FcγRIIb, thereby co-engaging BCR and FcγRIIb. To assess its ability to suppress B cell activation *in vivo*, we generated non-autoimmune-prone C57BL/6 (B6) and SLE-prone NZM 2328 (NZM) mice in which the human FcγRIIb extracellular domain was knocked into the mouse *Fcgr2b* locus (B6.hRIIb and NZM.hRIIb mice, respectively, the latter retaining features of SLE). XENP8206, a mAb which bears the same FcγRIIb-enhanced human Fc domain as does obixelimab but which recognizes murine CD19 rather than human CD19, inhibited *in vitro* BCR-triggered activation of B cells from both B6.hRIIb and NZM.hRIIb mice. Following administration of XENP8206 to B6.hRIIb or NZM.hRIIb mice, B cell numbers in the spleen and lymph nodes remained stable but became hyporesponsive to BCR-triggered activation for at least 14 days. These findings demonstrate proof-of-principle that pharmacologic co-engagement of BCR and human FcγRIIb inhibits B cell activation in non-autoimmune and SLE-prone hosts while preserving B cell numbers. These observations lay a strong foundation for clinical trials in human SLE with agents that co-engage BCR and FcγRIIb. Moreover, B6.hRIIb and NZM.hRIIb should serve as powerful *in vivo* models in the elucidation of the cellular and molecular underpinnings of the changes induced by BCR/FcγRIIb co-engagement.

1. Introduction

The success of B cell-targeting therapies in many systemic rheumatic diseases, including systemic lupus erythematosus (SLE), provides *prima facie* evidence for a pathogenic role for B cells in those disorders. As examples, the anti-CD20 mAb rituximab is FDA-approved for the treatment of rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis, and the anti-BAFF mAb belimumab is FDA-approved for the treatment of SLE. Treatment with rituximab results in profound B cell depletion [1], and treatment with belimumab results in substantial, albeit less dramatic, B cell depletion as well [2].

Given that SLE patients are immunocompromised from their

underlying disease and concurrent immunosuppressive medications, a major concern surrounding B cell depletion in SLE is increased risk of serious infection. Once B cells are physically depleted, reconstitution of B cells to normal levels can take weeks to months, leaving the host B cell-deficient and at increased risk for serious infection for protracted stretches of time.

Indeed, two phase-II/III clinical trials in SLE of atacept, an inhibitor of both BAFF and APRIL [3], could not be completed as originally planned. One was prematurely terminated in its entirety due to serious (including fatal) infections [4], and the other had a treatment arm prematurely terminated due to infection-associated deaths [5]. A phase-III clinical trial in SLE nephritis of the anti-CD20 mAb ocrelizumab was

* Corresponding author. Division of Rheumatology, University of Southern California Keck School of Medicine, 2011 Zonal Ave HMR 711, Los Angeles, CA, 90033, USA.

E-mail address: stohl@usc.edu (W. Stohl).

¹ DS and WS are co-senior authors.

<https://doi.org/10.1016/j.jtauto.2020.100075>

Received 4 December 2020; Accepted 9 December 2020

2589-9090/© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

terminated early due to an unacceptable imbalance in serious infections between ocrelizumab-treated patients and placebo-treated patients [6]. Clinically significant hypogammaglobulinemia, leading to serious infections and requiring IgG replacement therapy, is not rare in SLE patients undergoing B cell-depleting therapy [7], and development of progressive multifocal leukoencephalopathy remains a lingering worry in such patients [8,9].

Since the goal of B cell-targeting approaches is to eliminate the function of pathogenic B cells, *inactivation* of pathogenic B cells, rather than their physical depletion, could achieve the same end. This could be accomplished by recapitulating the homeostatic pathway naturally utilized by the host immune response. In the course of an antigen-driven humoral response, antibody levels rise, form specific immune complexes with their cognate antigens, and co-engage Fc γ receptor IIb (Fc γ RIIb) and B cell antigen receptor (BCR) with high avidity. This results in the suppression of those B cells that recognize cognate antigen [10].

Obixelimab (previously known as XmAb5871) is an anti-human CD19 mAb whose Fc region has been engineered to have ~430-fold greater affinity for human Fc γ RIIb than that of the corresponding unmodified anti-CD19 mAb [11]. Given the physical association between CD19 and BCR during antigen-triggered activation of human B cells [12], obixelimab co-engages BCR and Fc γ RIIb on human B cells and strongly inhibits BCR-induced activation of normal human B cells *in vitro* through an SH2-containing inositol polyphosphate 5-phosphatase (SHIP)-mediated pathway [11,13]. Of note, obixelimab does not deplete human peripheral blood mononuclear cell (PBMC) cultures of B cells, in contrast to the considerable B cell reduction in parallel cultures containing either rituximab or tafasitamab (an anti-human CD19 mAb identical to obixelimab but with an Fc domain engineered to have enhanced affinity for stimulatory Fc γ RIIA and Fc γ RIIIa) [14]. The decreased expression of Fc γ RIIb by memory B cells in human SLE notwithstanding [15,16], obixelimab-driven *in vitro* suppression of activation and proliferation of B cells from SLE patients is as strong as the corresponding *in vitro* suppression of activation and proliferation of B cells from healthy controls [14]. Moreover, obixelimab inhibits *in vivo* anti-tetanus antibody responses generated in immunodeficient SCID mice engrafted with human PBMC, regardless of whether the PBMC were donated from a healthy control donor or a SLE patient [14]. Importantly, no drug-related serious adverse events were reported in a phase-I bioavailability study of intravenous or subcutaneous obixelimab in healthy subjects (NCT02867098), and a phase-II study in SLE (NCT02725515) demonstrated a lower-than-expected infection rate among obixelimab-treated patients as well as a trend to greater improvement in disease activity among obixelimab-treated patients than among placebo-treated patients. Similar encouraging results were noted in a phase-II trial of obixelimab in IgG4-related disease (NCT02725476) [17]. Whereas pre-clinical and early clinical studies have been promising, such studies are inherently incapable of fully elucidating the cellular and molecular underpinnings of the changes induced *in vivo* by BCR/Fc γ RIIb co-engagement. To achieve such understanding, a suitable *in vivo* model becomes indispensable. Accordingly, we turned to C57BL/6 (B6) and NZM 2328 (NZM) mice as models of non-autoimmune-prone and SLE-prone mice, respectively.

Wild-type (WT) mice are not suitable for the study of the *in vivo* effects of obixelimab, since its modified Fc region binds with high affinity to human Fc γ RIIb but not to murine Fc γ RIIb. Accordingly, we first generated B6 mice in which 68 amino acids of the human Fc γ RIIb extracellular domain was knocked into the mouse *Fc γ r2b* locus (B6.hRIIb mice). The resulting chimeric receptor retains the mouse Fc γ RIIb intracellular domain as well as the mouse chromosomal locus and promoter. Accordingly, Fc γ RIIb expression levels, distribution, and signaling mechanisms are identical in B6.hRIIb and B6 WT mice. We then introgressed the *Fc γ r2b* chimeric genotype into SLE-prone NZM mice to yield NZM.hRIIb mice.

Since obixelimab recognizes human CD19 but not murine CD19, we had to modify obixelimab to effect co-engagement of murine BCR and human chimeric Fc γ RIIb in B6.hRIIb and NZM.hRIIb mice. To that end,

we generated XENP8206, a mAb which bears the same Fc γ RIIb-enhanced human Fc domain as does obixelimab but which recognizes murine CD19 rather than human CD19.

In this report, we demonstrate that BCR/Fc γ RIIb co-engagement with XENP8206 inhibits BCR-triggered activation of B cells from B6.hRIIb mice while having no effect on the number of B cells in lymph nodes or spleen. The inhibitory effect must be due to engagement of the human chimeric Fc γ RIIb, since XENP8206 has no effect in B6 WT mice which do not express the human chimeric Fc γ RIIb.

We also demonstrate that introgression of the hRIIb-knockin construct onto the NZM genetic background generated NZM.hRIIb mice that retain serologic and immunopathological features of SLE. As with B6.hRIIb mice, XENP8206 inhibits BCR-triggered activation of B cells from NZM.hRIIb mice. Thus, B6.hRIIb and NZM.hRIIb mice should serve as suitable models to investigate the *in vivo* consequences of BCR/Fc γ RIIb co-engagement in non-autoimmune-prone and SLE-prone hosts. Results in NZM.hRIIb mice should well model and inform the effects that obixelimab will have in human SLE subjects.

2. Materials and methods

General. The reported studies were approved by the Xencor and University of Southern California Institutional Animal Care and Use Committees.

Generation of B6.hRIIb mice. B6.hRIIb mice were generated by knocking in the construct illustrated in Supplemental Figure 1 into B6 ES cells. The resulting primary structure of the chimeric human extracellular domain and mouse intracellular Fc γ RIIb is shown in Supplemental Figure 2. B6.hRIIb mice bore two copies of the human/mouse chimeric *Fc γ r2b* gene. In these mice, the human extracellular domain can engage a mAb that bears the engineered human Fc domain that binds to human Fc γ RIIb with high affinity. The resulting chimeric receptor retains the mouse Fc γ RIIb intracellular domain as well as the mouse chromosomal locus and promoter. Thus, Fc γ RIIb expression levels, distribution, and signaling mechanisms are identical in B6.hRIIb and B6 WT mice.

Generation of NZM.hRIIb mice. NZM.hRIIb mice were generated by introgressing the *Fc γ r2b* chimeric genotype from B6.hRIIb mice into NZM WT mice. The *Fc γ r2b* chimeric genotype was monitored by PCR, using the primers 5'-GTG GAA CCA CTG AAT GGA GTC GCT C and 5'-AGT GTG CTT TAT CCA GGA AGT CCC AG. The chimeric genotype yielded a band of 396 bp, whereas the native murine *Fc γ r2b* genotype yielded no band. Replacement of the B6 genetic background with the NZM genetic background was accomplished by the marker-assisted selection protocol [18, 19] using 47 microsatellite markers across the genome chosen to include those regions identified as susceptibility loci in NZM mice [20]. As assessed by these microsatellite markers, the N7 backcross generation of NZM.hRIIb mice was congenic with NZM WT mice.

Since the *Fc γ r2b* gene lies within the *Sle1* region (the region which is indispensable to development of autoimmunity in NZM mice), it is likely that DNA sequences near the human/mouse chimeric *Fc γ r2b* gene are derived from B6, rather than from NZM, mice. Studies in B6 congenic mice documented that mice that do not bear at least one intact *Sle1* region do not develop serologic autoimmunity [21,22]. Thus, to ensure that all NZM.hRIIb mice bore at least one copy of the intact NZM *Sle1* region, NZM.hRIIb mice were mated to NZM WT mice, and the pups were genotyped for the chimeric *Fc γ r2b* gene by PCR as above. Through this approach, our NZM.hRIIb mice bore one copy of the native (murine) *Fc γ r2b* gene (with a fully intact *Sle1* region) and one copy of the human/mouse chimeric *Fc γ r2b* gene (with a *Sle1* region that likely is partially disrupted).

Expression of murine and human chimeric Fc γ RIIb on B cells. Whole blood or splenocytes from mice were stained for murine CD3 (145-2C11, Life Technologies), CD32b (AT130-2, Invitrogen), B220 (RA3-6B2, BioLegend), CD335 (29A1.4, BioLegend), and CD116/32 (93, BioLegend) and human CD32b (9B5, Xencor, Inc.). The cells were analyzed using a Fortessa flow cytometer (BD).

Chimeric mAb. The human IgG1-Fc domain was engineered to have enhanced affinity to human FcγRIIb, through mutations in amino acid residues S267 to E and L328 to F (S267E/L328F or IIbE), or to ablate binding to any FcγR, through mutations in amino acid residues G236 to R and L328 to R (G236R/L328R or Fc-KO) [11,23]. Obixelimab (XmAb5871) is an anti-human CD19 mAb with IIbE-Fc. XENP8206 is the obixelimab mouse surrogate mAb, with anti-mouse CD19 (clone 1D3) [24] and IIbE-Fc. To control for the engagement of FcγRIIb, XENP8207 was generated with anti-mouse CD19 (1D3) with Fc-KO.

Serum total IgG and IgG autoantibody levels. Serum levels of total IgG and IgG autoantibodies were determined by ELISA [25]. Autoantibody OD values were normalized to the mean OD of serum from 5-month-old *MRL-*lpr** mice, the latter arbitrarily assigned a value of 100 U/ml.

Kidney histology. Sections of formalin-fixed kidneys were stained with hematoxylin and eosin and assessed by light microscopy [26].

Kidney immunofluorescence. Sections of snap-frozen kidneys were stained for IgG or C3 deposition using FITC-conjugated goat F(ab')₂ fragment anti-mouse IgG or C3 antibodies (MP Biomedicals) [25].

Assessment of proteinuria. Reagent strips for urinary protein (Albustix, Bayer) were dipped in mouse urine and were assigned a score (0–4) by visual color comparison to the supplied standard color key.

Calcium mobilization assay. Splenocytes (1×10^7 cells/ml) were pre-incubated with mAbs XENP8206 or XENP8207 (10 μg/ml) with calcium dye (Fluo-4 NW Calcium assay kit, Molecular Probes, Invitrogen) in HBSS. Anti-mouse CD3-APC (clone 145-2C11, eBiosciences) and CD49b-APC (clone DX5, BioLegend) were used (1 μg/ml) to gate out T and NK cells. The pre-loaded cells were diluted 10x in HBSS, and calcium flux (a well-established surrogate for BCR-triggered activation) was induced with F(ab')₂ fragment of goat anti-mouse pentameric IgM antibody (Jackson ImmunoResearch Labs.) (10 μg/ml). Intracellular calcium release was recorded on APC-negative lymphocytes using a FACS Canto 2 flow cytometer, and area under curve (AUC) values were obtained through 30-s running averages of the scatter plots.

Western blot analysis. Four spleens from B6 WT or B6.hRIIb mice were pooled, and B cells were isolated using a negative-selection kit (StemCell technology) and resuspended in 1×10^7 cell/ml in RPMI 8226 with 10% FBS. Isolated B cells (1×10^7 cells/ml in RPMI 8226 with 10% FBS) were incubated with PBS, XENP8206, or XENP8207 (10 μg/ml) for 3 min and lysed with 1% NP-40. Lysates were pre-cleared, fractionated in SDS-PAGE, and transferred to nitrocellulose membrane. The blot was probed with anti-p-Tyr292-CD32b antibody (EP926Y, AbCam) and quantified using chemiluminescence.

Statistical analysis. Parametric testing between two groups was performed by the unpaired t-test and among three or more groups by one-way ANOVA. When the data were not normally distributed or the equal variance test was not satisfied, non-parametric testing was performed by the Mann-Whitney rank sum test between two groups and by Kruskal-Wallis one-way ANOVA on ranks among three or more groups.

3. Results

Expression of murine and human chimeric FcγRIIb in B6 WT and B6.hRIIb mice. B cell expression of murine and human chimeric FcγRIIb in B6 WT and B6.hRIIb mice was consistent with their genotypes, with B6 WT B cells expressing high levels of murine, but not human chimeric, FcγRIIb, and B6.hRIIb B cells expressing high levels of human chimeric, but not murine, FcγRIIb (Fig. 1A). FcγRIIb was not appreciably expressed on T cells in either B6 WT or B6.hRIIb mice, whereas low levels of murine and human chimeric FcγRIIb were expressed on NK cells from B6 WT and B6.hRIIb mice, respectively (Fig. 1B), consistent with the low-level expression of FcγRIIb by NK cells previously reported in mice and humans [27,28].

Inhibition of *in vitro* BCR-induced intracellular calcium release and phosphorylation of FcγRIIb by XENP8206 in B6.hRIIb B cells. Incubation of B cells from B6.hRIIb mice with anti-mouse IgM triggered vigorous release of intracellular calcium (Fig. 2A). Treatment of these cells with

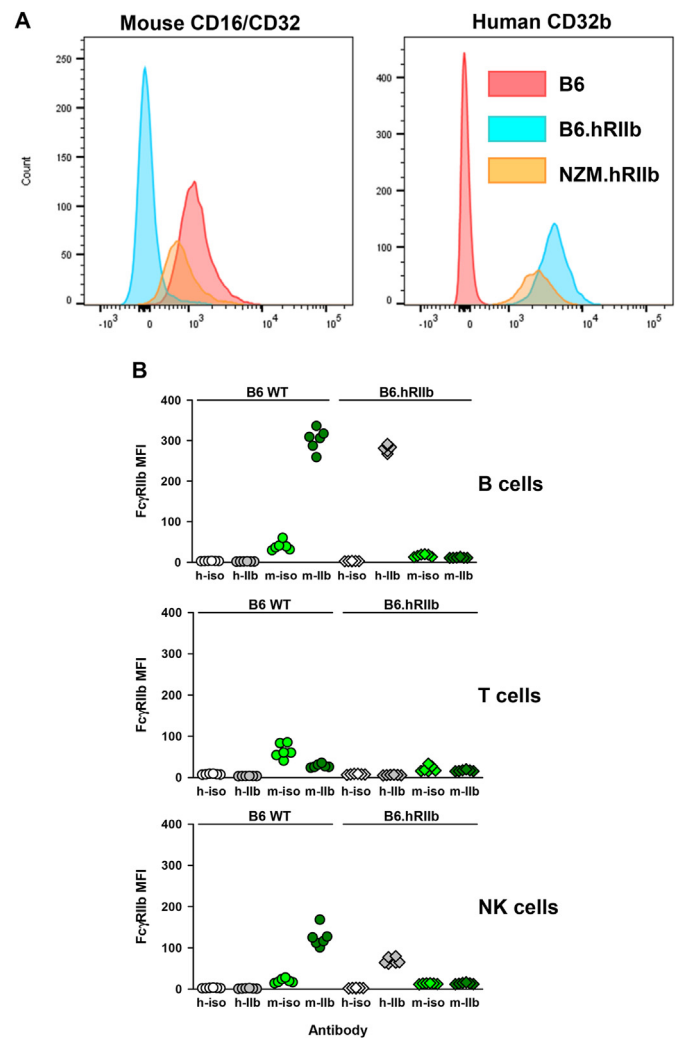


Fig. 1. Expression of murine and human chimeric FcγRIIb on B cells from B6, B6.hRIIb, and NZM.hRIIb mice. Panel A: Spleen cells from B6 (red), B6.hRIIb (blue), and NZM.hRIIb (orange) mice were isolated and stained with combinations of mAb against murine B220 and murine or human FcγRIIb. Whereas the anti-mouse FcγRIIb antibody cross-reacts with FcγRIII, since mouse B cells do not express FcγRIII, only FcγRIIb on the mouse B cells was being detected. The cells were gated on B220⁺ B cells, and histograms were generated to illustrate murine and human chimeric FcγRIIb staining. Panel B: B220⁺ B cells (top), CD3⁺ T cells (center) and CD335⁺ NK cells (bottom) from B6 WT (circles) and B6.hRIIb mice (diamonds) were stained with anti-murine FcγRIIb (m-Iib), corresponding isotype control (m-Iso), anti-human FcγRIIb (h-Iib) or corresponding isotype control (h-Iso), and mean fluorescence intensity (MFI) was recorded. Each symbol represents an individual mouse (N = 6).

XENP8206, which co-engages BCR and FcγRIIb on B cells from these mice, blunted the calcium response. Treatment with XENP8207, which bears the same anti-CD19 moiety as XENP8206 but is incapable of binding to FcγRIIb, failed to blunt the calcium response in B6.hRIIb splenocytes. In addition, XENP8206 did not blunt the calcium response in splenocytes from B6 WT mice (Fig. 2B), indicating that BCR/FcγRIIb co-engagement was required.

We have previously demonstrated that incubation of human PBMC with obixelimab induces phosphorylation of FcγRIIb at tyrosine 292 (Y292) in the ITIM motif, a step critical to stimulation of downstream inhibitory signaling pathways [14]. Importantly, XENP8206, which bears the same FcγRIIb-enhanced human Fc domain as obixelimab, triggered phosphorylation of FcγRIIb in B cells from B6.hRIIb mice at Y317 (the equivalent amino acid residue in the human chimeric receptor to Y292 in the native human receptor; Supplemental Fig. 2 and Fig. 2C).

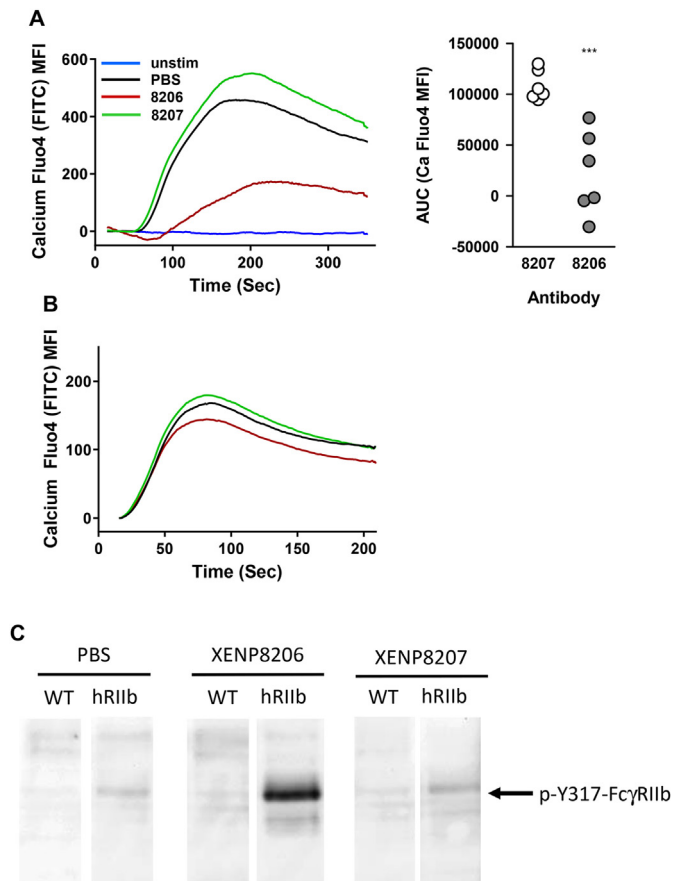


Fig. 2. Inhibition of BCR-induced intracellular calcium release and phosphorylation of Fc γ RIIb by XENP8206. Panel A: Fluo-4-loaded B cells from B6.hRIIb mice were unstimulated (blue) or stimulated with anti-IgM in the presence of PBS (black), XENP8206 (red), or XENP8207 (green), and intracellular calcium release was recorded over time. A representative experiment is illustrated on the left, and the aggregate results of six independent experiments are plotted as area under curve (AUC) on the right. Each symbol represents an individual mouse. “****” denotes $p = 0.002$. Panel B: Fluo-4-loaded B cells from B6 WT mice were stimulated with anti-IgM in the presence of PBS (black), XENP8206 (red), or XENP8207 (green), and intracellular calcium release was recorded over time. Panel C: B cells from B6 WT (WT) or B6.hRIIb (hRIIb) mice were incubated with PBS, XENP8206, or XENP8207 (10 μ g/ml) for 3 min and lysed with 1% NP-40. Lysates were pre-cleared, fractionated in SDS-PAGE, and transferred to nitrocellulose membrane. The blot was probed with anti-p-Tyr317-CD32b antibody and developed using chemiluminescence.

No such phosphorylation occurred in B6 WT cells which do not express the human chimeric Fc γ RIIb, and XENP8207, which does not engage human Fc γ RIIb, did not induce phosphorylation of Y317.

Preservation and inactivation of B cells in B6.hRIIb mice following *in vivo* treatment with XENP8206. We previously reported that obixelimab does not deplete B cells in human PBMC cultures [14]. To assess the *in vivo* effects of the obixelimab surrogate, XENP8206, on B cell numbers, B6.hRIIb mice were treated with PBS or XENP8206 (10 mg/kg) on days 0 and 7 and were assessed for B cells on day 10. No differences in lymph node B cells or in spleen B cells between the two were observed, although B cells in the blood were lower in XENP8206-treated mice than in PBS-treated controls (Fig. 3A).

The preservation of B cells in the spleens and lymph nodes of the XENP8206-treated B6.hRIIb mice is not due to an inability of XENP8206 to penetrate the lymph nodes or spleen. In mice treated with XENP8206, detection of CD19 and Fc γ RIIb on B cells from lymph nodes and spleen (i.e., receptor occupancy) was decreased to the same degree as on blood B cells (Fig. 3B). Importantly, B cells from lymph nodes and spleens of the

XENP8206-treated B6.hRIIb mice were less responsive to subsequent BCR-triggered activation than were corresponding B cells from PBS-treated B6.hRIIb mice (Fig. 3C), thereby confirming that the administered XENP8206 effectively reached B cells in the secondary lymphoid tissues and was functionally active. The reduction in blood B cells in XENP8206-treated B6.hRIIb mice may be a reflection of non-depletive events, such as homing to extravascular sites or margination.

Expression of murine and human chimeric Fc γ RIIb in NZM.hRIIb mice. As was the case for B6 mice, B cell expression of murine and human chimeric Fc γ RIIb in NZM.hRIIb mice was consistent with its genotype. NZM.hRIIb mice genetically express one copy each of the murine and human chimeric Fc γ RIIb genes, and expression of both murine and human chimeric Fc γ RIIb were intermediate between the levels expressed by B6 WT and B6.hRIIb mice (Fig. 1A).

Development of SLE features in NZM.hRIIb mice. Since NZM.hRIIb mice bear only one copy of the native (murine) Fc γ RIIb gene (with a fully intact *Sle1* region) and one copy of the human/mouse chimeric Fc γ RIIb gene (with a partially-disrupted *Sle1* region), we needed to establish that NZM.hRIIb mice do, in fact, develop features of SLE. To that end, a cohort of 11 NZM.hRIIb female mice were bled monthly from 4 months of age through 12 months of age. Serum IgG anti-dsDNA and anti-chromatin antibody levels steadily increased over time, with these autoantibody levels being significantly greater by 6 and 7 months of age, respectively, relative to their respective baseline (4 months) levels (Fig. 4A). This development of serologic autoimmunity in NZM.hRIIb mice is consistent with the development of serologic autoimmunity in B6 congenic mice that bear a single copy of the *Sle1* region [21,22]. Of note, serum IgG autoantibody levels in 6- to 7-month-old NZM.hRIIb mice were similar to those previously observed in 6- to 7-month-old NZM WT mice [29].

In addition to serologic autoimmunity, NZM.hRIIb mice developed substantial renal immunopathology. H&E evaluation demonstrated swollen and hypercellular glomeruli with mesangial deposition along with interstitial and perivascular infiltrates (Fig. 4Ba-c). Immunofluorescence analysis consistently revealed glomerular deposition of IgG of variable intensity (Fig. 4Bd), although C3 deposition was routinely minimal (not shown).

By 6 months of age, NZM WT mice routinely begin developing severe ($\geq 3+$ by dipstick) proteinuria, and $>80\%$ develop severe proteinuria by 10 months of age [26,29–33]. Variable degrees of proteinuria also developed in NZM.hRIIb mice. Of the 17 NZM.hRIIb mice tested, 2+ proteinuria (measured by dipstick) developed in 6 mice, 1+ proteinuria developed in 6 mice, and trace proteinuria developed in 5 mice. No mouse developed $\geq 3+$ proteinuria, so clinical disease did not develop in NZM.hRIIb mice but was not as severe as in NZM WT mice. The degree of proteinuria correlated neither with serum levels of IgG anti-dsDNA nor with serum levels of IgG anti-chromatin (not shown).

Inhibition of B cell activation without B cell depletion following administration of XENP8206 to NZM.hRIIb mice. *In vivo* treatment with XENP8206 did not lead to a reduction either in blood B cells or in spleen B cells (Fig. 5A). In 8 of 11 XENP8206-treated mice, receptor occupancy on B cells was very high, as evidenced by the inability of exogenous labeled XENP8206 to bind to B cells from XENP8206-treated mice. This is in striking contrast to the copious binding of exogenous labeled XENP8206 to the 14 untreated day-0 mice (Fig. 5B). This high *in vivo* receptor occupancy by XENP8206 on both blood and splenic B cells persisted through day 14 post-treatment.

Surprisingly, receptor occupancy was nil (i.e., exogenous labeled XENP8206 bound to blood and splenic B cells from these mice to the same extent it bound to B cells from untreated mice) in 3 of the 11 mice treated with XENP8206. Whether this reflected a technical issue with the administration of XENP8206 or an exceptionally rapid turnover of CD19 and Fc γ RIIb in these mice is not known. Of note, however, is that *in vitro* spiking of splenic B cells isolated from those eight NZM.hRIIb mice with high receptor occupancy did not further suppress the existing low anti-IgM-induced B cell activation, even when the XENP8206 had been administered as long as 14 days earlier ($p = 0.787$; Fig. 5C). In stark

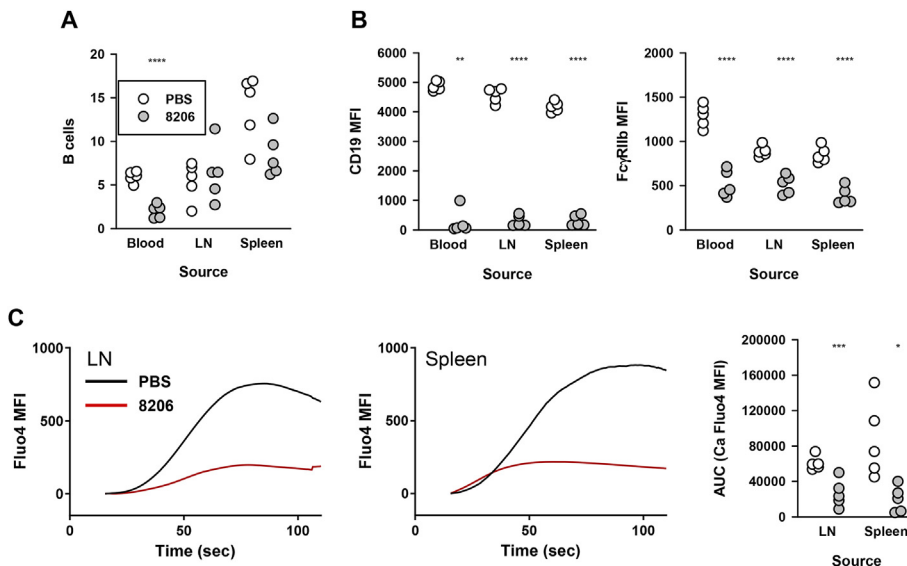


Fig. 3. Preservation and inactivation of B cells following *in vivo* treatment with XENP8206. Panel A: B6.hRIIb mice (N = 5) were treated with PBS or XENP8206 (10 mg/kg) on days 0 and 7 and assessed for B cells in the blood, lymph nodes (LN), and spleen on day 10. Results are expressed as B cells ($\times 10^{-2}$) per μ l blood, B cells ($\times 10^{-5}$) per lymph node, and B cells ($\times 10^{-6}$) per spleen. Panel B: CD19 mean fluorescence intensity (MFI; left) and Fc γ RIIb MFI (right) of the B cells harvested on day 10 from the mice in panel A are plotted. Panel C: B cells harvested on day 10 from the mice in panel A treated with PBS (black) or XENP8206 (red) were loaded with Fluo-4, stimulated with anti-IgM, and assessed for intracellular calcium release as in Fig. 2. Representative experiments are illustrated for LN (left) and spleen (center), and the aggregate results of five independent experiments are illustrated on the right. Each symbol represents an individual mouse. “*” indicates $p \leq 0.05$; “**” indicates $p \leq 0.01$; “***” indicates $p \leq 0.005$; “****” indicates $p \leq 0.001$ compared to the corresponding PBS values.

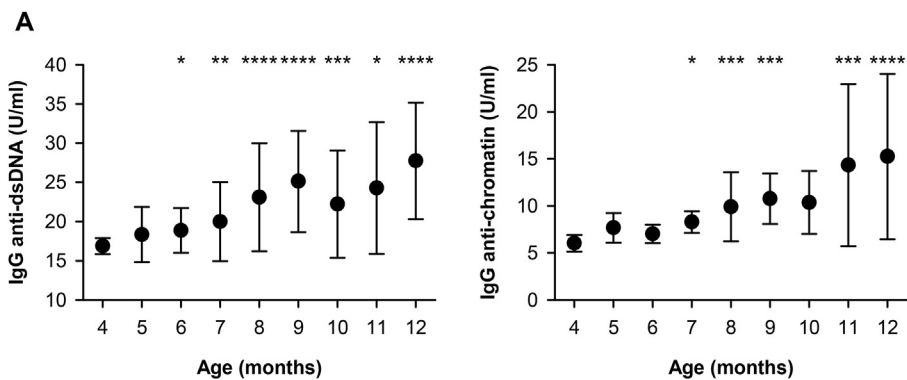
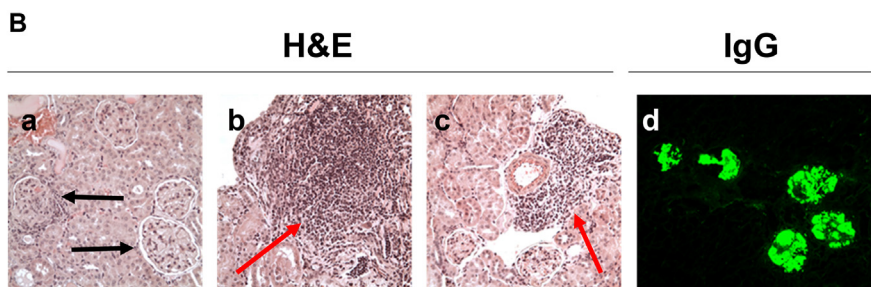


Fig. 4. Serum autoantibody levels and renal immunopathology in NZM.hRIIb mice. Panel A: Mean serum levels (± 1 SD) of IgG anti-dsDNA (left) and IgG anti-chromatin (right) are illustrated for the 11 NZM.hRIIb mice serially bled at the indicated ages. “*” indicates $p \leq 0.05$; “**” indicates $p \leq 0.01$; “***” indicates $p \leq 0.005$; “****” indicates $p \leq 0.001$. Panel B: Kidney sections from 12-month-old NZM.hRIIb mice were stained with hematoxylin and eosin (H&E, a-c) or for IgG immunofluorescence (d). The black arrows point to swollen/hypercellular glomeruli, and the red arrows point to interstitial/perivascular infiltrates. Original magnification is 200X.



contrast, *in vitro* activation of B cells from the three mice with negligible *in vivo* receptor occupancy could be suppressed by an additional spike of 10 μ g/mL XENP8206 ($p < 0.001$). *In vitro* suppression of B cells from the three mice not showing *in vivo* receptor occupancy was similar to *in vitro* suppression of B cells from the three mice that had never been injected with XENP8206 ($p = 0.046$). Taken together, these observations strongly suggest that blood and splenic B cells that had been engaged by XENP8206 and retained XENP8206 on their surfaces remained hypo-responsive to BCR-triggered activation for at least 2 weeks post-injection. This, in turn, points to XENP8206 (and, by inference, to obixelimab) being an active signaling agent, rather than a blocking agent, that turns on a natural signal (BCR/Fc γ RIIb co-engagement) that shuts off B cells.

4. Discussion

Of the published late-stage clinical trials in SLE of B cell-targeting agents, only six have met their primary endpoints [34–39]. Even in the successful trials, 38–61% of the SLE patients treated with drug were deemed non-responders, and, in the case of one of these agents (tabalumab), the less-than-resounding results prompted the sponsor to discontinue development of the drug for SLE.

The therapeutic agent in each of the six successful trials was a BAFF antagonist (belimumab in five trials; tabalumab in one trial), so the salutary clinical effects arose through the neutralization of the vital B cell survival factor, BAFF, which, in turn, likely led to death of pathogenic B cells. However, neither BAFF antagonists nor other extant B cell-targeting agents are specific for pathogenic B cells. In fact, large populations of B

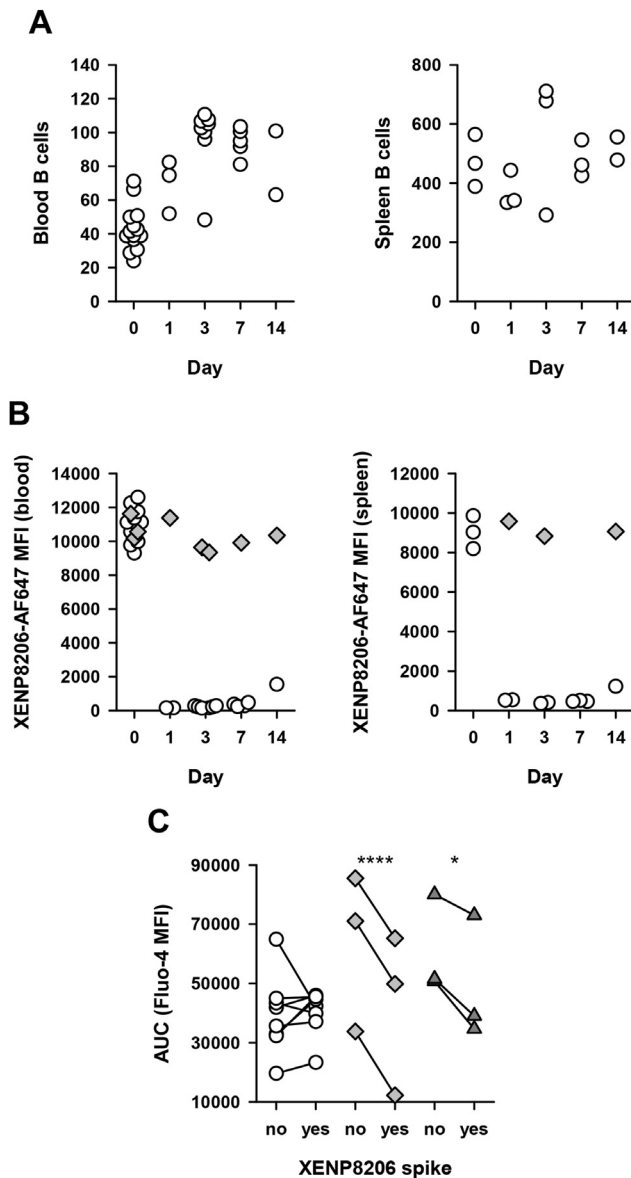


Fig. 5. Inhibition of B cell activation without B cell depletion following administration of XENP8206 to B6.hRIIb mice. Blood samples from 14 NZM.hRIIb mice were obtained on day 0 before treatment with XENP8206. Three mice were sacrificed before treatment to quantify baseline splenic B cells, and the remaining 11 mice were injected with XENP8206 (10 mg/kg). Three additional mice each were sacrificed on days 1, 3, and 7, and the remaining two mice were sacrificed on day 14 to quantify splenic B cells. Blood B cells were also analyzed from all remaining treated mice, except on day 1 when blood was drawn only from the 3 mice slated for sacrifice. Panel A: B cells/ μ l blood (left) and B cells/spleen ($\times 10^{-3}$, right) were enumerated on days 0 (pre-injection), 1, 3, 7, or 14 post-injection. Symbols are as described in Panels B and C. Panel B: Blood (left) and spleen (right) B cells were stained with Alexa Fluor-647 (AF647)-labeled XENP8206 at the indicated days to assess *in vivo* receptor occupancy (RO) by unlabelled XENP8206. Reduction in XENP8206-AF647 MFI is evidence of RO by XENP8206 due to its *in vivo* administration after Day 0 blood draws. Eight of the 11 treated mice showed extensive and prolonged receptor occupancy on B cells in both blood and spleen (open circles) while three mice showed no receptor occupancy (light gray diamonds). Panel C: Spleen B cells from the 8 mice that displayed receptor occupancy by XENP8206 (white circles), from the 3 mice that displayed no receptor occupancy (light gray diamonds) after dosing, and from the 3 untreated day 0 mice (dark gray triangles) were assessed for intracellular calcium release after stimulation with anti-IgM as described in Fig. 2 in the absence (no) or presence (yes) of 10 μ g/ml XENP8206. The lines connect the respective results for each individual mouse. * denotes $p < 0.05$; **** denotes $p < 0.001$.

cells, including protective B cells, are targeted by these agents. Once protective B cells are physically depleted, their reconstitution to normal levels can take several weeks to months following discontinuation of the B cell-targeting agent, leaving the host immunocompromised and at increased risk for serious infection for protracted stretches of time.

Rather than target B cells for death, an alternate and safer approach entails the inactivation of B cells without killing them. Should the clinical need to discontinue a B cell-inactivating agent arise, B cells would likely be restored to their baseline functional states in a time frame much quicker than that required to reconstitute physically depleted B cells.

With that in mind, we asked whether *in vivo* administration of a pharmacologic agent which strongly co-engages BCR and human Fc γ RIIb could inactivate B cells. Since the Fc domain of XENP8206 is identical to that of obixelimab and the extracellular domain of Fc γ RIIb in B6.hRIIb and NZM.hRIIb mice is identical to that in human subjects, XENP8206 will co-engage BCR and Fc γ RIIb on B cells in B6.hRIIb and NZM.hRIIb mice in a manner very similar to that in which obixelimab co-engages BCR and Fc γ RIIb on human B cells.

Administration of XENP8206 did not reduce B cell numbers in secondary lymphoid tissues (i.e., lymph node or spleen), consistent with XENP8206 being a non-depleting drug. This is in stark contrast to the marked reduction in lymph node and spleen B cells following treatment with B cell-depleting anti-CD20 mAb or BAFF-neutralizing agents [40–42]. Nevertheless, B cell numbers on the blood of XENP8206-treated B6 mice did decline. However, given that liver sinusoidal endothelial cells (LSECs) abundantly express Fc γ RIIb and mediate clearance of circulating immune complexes [43], some of the administered XENP8206 may have co-engaged CD19 on B cells in the blood with Fc γ RIIb on LSECs, leading to some sequestration of B cells to the liver from the circulation. Regardless and importantly, XENP8206 substantially inhibited activation of B cells from both non-autoimmune-prone B6.hRIIb mice and from SLE-prone NZM.hRIIb mice. Thus, the normal physiological homeostatic process, in which the Fc portion of an antibody engages with Fc γ RIIb, is replicated/amplified by XENP8206.

The inhibition of NZM.hRIIb B cells by XENP8206 is especially noteworthy on two fronts. First, since NZM.hRIIb mice bear only one copy of the human/mouse chimeric *Fcgr2b* gene (whereas B6.hRIIb mice have two copies of the chimeric gene), expression of human chimeric Fc γ RIIb on NZM.hRIIb B cells is lower than that on B6.hRIIb B cells. Conversely, since NZM.hRIIb mice bear a copy of the native mouse *Fcgr2b* gene (whereas B6.hRIIb mice bear no copies), expression of murine Fc γ RIIb (which cannot be engaged by XENP8206) on NZM.hRIIb B cells is substantial. Nevertheless, XENP8206-mediated suppression of B cell activation was similar in both mice, suggesting that effective suppression can be achieved even when a considerable fraction (half) of the Fc γ RIIb on the B cell surface is not therapeutically co-engaged with BCR. If mimicked in humans, this suggests that only a fraction (perhaps a small fraction) of Fc γ RIIb would need to be co-engaged with BCR to promote a clinically meaningful suppression of (pathogenic) B cells.

Second, the success in co-targeting Fc γ RIIb in murine SLE raises hope that it will also be successful in human SLE, and provisional results support that hope [17]. It is well established that altered Fc γ RIIb-based inhibitory function assumes great importance in the context of SLE. This is highlighted by: 1) the development of SLE-like disease in mice genetically deficient in Fc γ RIIb [44]; 2) the reduction in serologic, immunopathological, and clinical disease in several strains of SLE-prone mice following irradiation and reconstitution with bone marrow cells transduced with a Fc γ RIIb-expressing retrovirus [45]; 3) the amelioration of serologic and clinical disease in SLE-prone MRL.*lpr* mice that over-express Fc γ RIIb specifically on their B cells [46]; 4) the decreased expression of Fc γ RIIb by human memory B cells in human SLE [15,47]; and 5) the impaired function of an SLE-associated Fc γ RIIb polymorphism [48]. Thus, the ability of XENP8206 to inhibit B cell activation, not just in non-autoimmune-prone B6.hRIIb mice but in SLE-prone NZM.hRIIb mice as well, suggests that BCR/Fc γ RIIb co-engagement in human SLE may be effective.

Therapeutic BCR/FcγRIIb co-engagement may be practicable not just for SLE but for other diseases in which B cell-depletion therapy has heretofore been considered. For example, BCR/FcγRIIb co-engagement with obexelimab inhibits *in vitro* activation of B cells from rheumatoid arthritis (RA) patients at least as well as it inhibits *in vitro* activation of B cells from healthy control subjects [49]. Importantly, whole-blood assays documented that obexelimab-mediated inhibition is independent of rheumatoid factor (RF) levels in the blood, strongly suggesting that circulating RF in patients with RA would not inhibit obexelimab activity. A phase-Ib/IIa clinical trial of obexelimab in RA has been completed, and the results are encouraging. In addition, a phase-II trial of obexelimab in IgG4-related disease (NCT02725476) has been completed, and initial results have also been encouraging [17].

In summary, we have generated non-autoimmune-prone B6.hRIIb mice and SLE-prone NZM.hRIIb mice through knockin technology that express a human chimeric FcγRIIb. Our studies collectively demonstrate proof-of-principle that pharmacologic co-engagement of BCR and human chimeric FcγRIIb inhibits B cell activation in both B6.hRIIb mice and NZM.hRIIb mice without concomitant B cell depletion. Our studies not only lay a strong foundation for continued clinical trials in human SLE with agents that co-engage BCR and FcγRIIb, such as obexelimab, but also introduce B6.hRIIb and NZM.hRIIb mice as powerful *in vivo* models to aid in the elucidation of the cellular and molecular changes induced by BCR/FcγRIIb co-engagement under normal and disease-prone conditions.

Funding

This work was supported in part by a grant from the Alliance for Lupus Research (WS). Authors SYC, EP, CB, and DES are employees of Xencor, Inc, and hold stock and stock options in the company. NY, SAC, CP, COJ, and WS have no financial support or other benefits from commercial sources to declare for the work reported in this manuscript

Declaration of competing interest

This work was supported in part by a grant from the Alliance for Lupus Research (WS). Authors SYC, EP, CB, and DES are employees of Xencor, Inc, and hold stock and stock options in the company. NY, SAC, CP, COJ, and WS have no financial support or other benefits from commercial sources to declare for the work reported in this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtauto.2020.100075>.

References

- J.C.W. Edwards, L. Szczepanski, J. Szechinski, A. Filipowicz-Sosnowska, P. Emery, D.R. Close, et al., Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis, *N. Engl. J. Med.* 350 (2004) 2572–2581.
- W. Stohl, F. Hiepe, K.M. Latinis, M. Thomas, M.A. Scheinberg, A. Clarke, et al., Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus, *Arthritis Rheum.* 64 (2012) 2328–2337.
- M. Dall'Era, E. Chakravarty, D. Wallace, M. Genovese, M. Weisman, A. Kavanaugh, et al., Reduced B lymphocyte and immunoglobulin levels after ataccept treatment in patients with systemic lupus erythematosus, *Arthritis Rheum.* 56 (2007) 4142–4150.
- E.M. Ginzler, S. Wax, A. Rajeswaran, S. Copt, J. Hillson, E. Ramos, et al., Ataccept in Combination with MMF and Corticosteroids in Lupus Nephritis: Results of a Prematurely Terminated Trial, *Arthritis Res Ther* 14 (2012) R33.
- D. Isenberg, C. Gordon, D. Licu, S. Copt, C.P. Rossi, D. Wofsy, Efficacy and safety of ataccept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial), *Ann. Rheum. Dis.* 74 (2015) 2006–2015.
- E.F. Mysler, A.J. Spindler, R. Guzman, M. Bijl, D. Jayne, R.A. Furie, et al., Efficacy and safety of ocrelizumab in early proliferative lupus nephritis: results from a randomized, double-blind, phase III study, *Arthritis Rheum.* 65 (2013) 2368–2379.
- D.M. Roberts, R.B. Jones, R.M. Smith, F. Alberici, D.S. Kumaratne, S. Burns, et al., Immunoglobulin G replacement for the treatment of infective complications of rituximab-associated hypogammaglobulinemia in autoimmune disease: a case series, *J. Autoimmun.* 57 (2015) 24–29.
- K.R. Carson, A.M. Evens, E.A. Richey, T.M. Habermann, D. Focosi, J.F. Seymour, et al., Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the Research on Adverse Drug Events and Reports project, *Blood* 113 (2009) 4834–4840.
- C.A. Fredericks, K.A. Kvam, J. Bear, G.S. Crabtree, S.A. Josephson, A case of progressive multifocal leukoencephalopathy in a lupus patient treated with belimumab, *Lupus* 23 (2014) 711–713.
- F. Nimmerjahn, J.V. Ravetch, Fcγ receptors as regulators of immune responses, *Nat. Rev. Immunol.* 8 (2008) 34–47.
- S.Y. Chu, I. Vostiar, S. Karki, G.L. Moore, G.A. Lazar, E. Pong, et al., Inhibition of B cell receptor-mediated activation of primary human B cells by coengagement of CD19 and FcγRIIb with Fc-engineered antibodies, *Mol. Immunol.* 45 (2008) 3926–3933.
- D. Depoil, S. Fleire, B.L. Treanor, M. Weber, N.E. Harwood, K.L. Marchbank, et al., CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand, *Nat. Immunol.* 9 (2008) 63–72.
- D. Szili, M. Cserhalmi, Z. Bankó, G. Nagy, D.E. Szymkowski, Sármy Suppression of innate and adaptive B cell activation pathways by antibody coengagement of FcγRIIb and CD19, *mAbs* 6 (2014) 991–999.
- H.M. Horton, S.Y. Chu, E.C. Ortiz, E. Pong, S. Cemerski, I.W.L. Leung, et al., Antibody-mediated coengagement of FcγRIIb and B cell receptor complex suppresses humoral immunity in, systemic lupus erythematosus *Journal of Immunology* 186 (2011) 4223–4233.
- M. Mackay, A. Stanevsky, T. Wang, C. Aranow, M. Li, S. Koenig, et al., Selective dysregulation of the FcγRIIb receptor on memory B cells in SLE, *J. Exp. Med.* 203 (2006) 2157–2164.
- K.H. Su, H.X. Yang, X.R. Li, X.L. Li, A.W. Gibson, J.M. Cafardi, et al., Expression profile of FcγRIIb on leukocytes and its dysregulation in systemic lupus erythematosus, *J. Immunol.* 178 (2007) 3272–3280.
- Q. Zhao, Bispecific antibodies for autoimmune and inflammatory diseases: clinical progress to date, *BioDrugs* 34 (2020) 111–119.
- L. Morel, Y. Yu, K.R. Blenman, R.A. Caldwell, E.K. Wakeland, Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain, *Mamm. Genome* 7 (1996) 335–339.
- P. Markel, P. Shu, C. Ebeling, G.A. Carlson, D.L. Nagle, J.S. Smutko, et al., Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains, *Nat. Genet.* 17 (1997) 280–284.
- S.T. Waters, S.M. Fu, F. Gaskin, U.S. Deshmukh, S.S.J. Sung, C.C. Kannappell, et al., NZM2328: a new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci, *Clin. Immunol.* 100 (2001) 372–383.
- L. Morel, C. Mohan, Y. Yu, B.P. Croker, N. Tian, A. Deng, et al., Functional dissection of systemic lupus erythematosus using congenic mouse strains, *J. Immunol.* 158 (1997) 6019–6028.
- L. Morel, K.R. Blenman, B.P. Croker, E.K. Wakeland, The major murine systemic lupus erythematosus susceptibility locus, *Sle1*, is a cluster of functionally related genes, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 1787–1792.
- G.L. Moore, C. Bautista, E. Pong, D.H.T. Nguyen, J. Jacinto, A. Eivazi, et al., A novel bispecific antibody format enables simultaneous bivalent and monovalent co-engagement of distinct target antigens, *mAbs* 3 (2011) 546–557.
- A. Rolink, E. tenBoekel, F. Melchers, D.T. Fearon, I. Krop, J. Andersson, A subpopulation of B220⁺ cells in murine bone marrow does not express CD19 and contains natural killer cell progenitors, *J. Exp. Med.* 183 (1996) 187–194.
- N. Jacob, S.H. Guo, A. Mathian, M.N. Koss, S. Gindea, C. Putterman, et al., B cell and BAFF dependence of IFN-α-exaggerated disease in systemic lupus erythematosus-prone NZM 2328 mice, *J. Immunol.* 186 (2011) 4984–4993.
- C.O. Jacob, S.H. Guo, N. Jacob, R.D. Pawar, C. Putterman, W.J. Quinn, et al., Dispensability of APRIL to the development of systemic lupus erythematosus in NZM 2328 mice, *Arthritis Rheum.* 64 (2012) 1610–1619.
- A.L. Tutt, S. James, S.A. Laversin, T.R.W. Tipton, M. Ashton-Key, R.R. French, et al., Development and characterization of monoclonal antibodies specific for mouse and human Fcγ receptors, *J. Immunol.* 195 (2015) 5503–5516.
- C.A. Dutertre, E. Bonnin-Gelize, K. Pulford, D. Bourel, W.H. Fridman, J.L. Teillaud, A novel subset of NK cells expressing high levels of inhibitory FcγRIIb modulating antibody-dependent function, *J. Leukoc. Biol.* 84 (2008) 1511–1520.
- C.O. Jacob, N. Yu, V. Sindhava, M.P. Cancro, R.D. Pawar, C. Putterman, et al., Differential development of systemic lupus erythematosus in NZM 2328 mice deficient in discrete pairs of BAFF receptors, *Arthritis & Rheumatology* 67 (2015) 2523–2535.
- C.O. Jacob, L. Pricop, C. Putterman, M.N. Koss, Y. Liu, M. Kollaros, et al., Paucity of clinical disease despite serological autoimmunity and kidney pathology in lupus-prone New Zealand Mixed 2328 mice deficient in BAFF, *J. Immunol.* 177 (2006) 2671–2680.
- H. Agrawal, N. Jacob, E. Carreras, S. Bajana, C. Putterman, S. Turner, et al., Deficiency of type I IFN receptor in lupus-prone New Zealand Mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease, *J. Immunol.* 183 (2009) 6021–6029.
- N. Jacob, H.T. Yang, L. Pricop, Y. Liu, X.N. Gao, S.G. Zheng, et al., Accelerated pathological and clinical nephritis in systemic lupus erythematosus-prone New Zealand Mixed 2328 mice doubly deficient in TNF receptor 1 and TNF receptor 2 via a Th17-associated pathway, *J. Immunol.* 182 (2009) 2532–2541.

- [33] C.O. Jacob, N. Yu, S.H. Guo, N. Jacob, W.J. Quinn, V. Sindhava, et al., Development of systemic lupus erythematosus in NZM 2328 mice in the absence of any single BAFF receptor, *Arthritis Rheum.* 65 (2013) 1043–1054.
- [34] S.V. Navarra, R.M. Guzman, A.E. Gallacher, S. Hall, R.A. Levy, R.E. Jimenez, et al., Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial, *Lancet* 377 (2011) 721–731.
- [35] R. Furie, M. Petri, O. Zamani, R. Cervera, D.J. Wallace, D. Tegzova, et al., A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus, *Arthritis Rheum.* 63 (2011) 3918–3930.
- [36] J.T. Merrill, R.F. van Vollenhoven, J.P. Buyon, R.A. Furie, W. Stohl, M. Morgan-Cox, et al., Efficacy and safety of subcutaneous tabalumab, a monoclonal antibody to B-cell activating factor, in patients with systemic lupus erythematosus: results from ILLUMINATE-2, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study, *Ann. Rheum. Dis.* 75 (2016) 332–340.
- [37] W. Stohl, A. Schwarting, M. Okada, M. Scheinberg, A. Doria, A. Hammer, et al., Efficacy and Safety of Subcutaneous Belimumab in Systemic Lupus Erythematosus: A Randomized, Double-Blind, Placebo-Controlled, 52-week Study, *Arthritis Rheumatol.* 69 (5) (2017) 1016–1027.
- [38] F.C. Zhang, S.C. Bae, D. Bass, M. Chu, S. Egginton, D. Gordon, et al., A Pivotal Phase III, Randomised, Placebo-Controlled Study of Belimumab in Patients with Systemic Lupus Erythematosus Located in China, Japan and South Korea, *Ann. Rheum. Dis.* 77 (3) (2018) 355–363.
- [39] R. Furie, B.H. Rovin, F. Houssiau, A. Malvar, Y.K. Onno Teng, G. Contreras, et al., Two-year, randomized, controlled trial of belimumab in lupus nephritis, *N. Engl. J. Med.* 383 (2020) 1117–1128.
- [40] Q. Gong, Q.L. Ou, S.M. Ye, W.P. Lee, J. Cornelius, L. Diehl, et al., Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy, *J. Immunol.* 174 (2005) 817–826.
- [41] J.S. Thompson, P. Schneider, S.L. Kalled, L. Wang, E.A. Lefevre, T.G. Cachero, et al., BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population, *J. Exp. Med.* 192 (2000) 129–135.
- [42] J.A. Gross, S.R. Dillon, S. Mudri, J. Johnston, A. Littau, R. Roque, et al., TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLyS, *Immunity* 15 (2001) 289–302.
- [43] L.P. Ganesan, J. Kim, Y. Wu, S. Mohanty, G.S. Phillips, D.J. Birmingham, et al., FcγRIIb on liver sinusoidal endothelium clears small immune complexes, *J. Immunol.* 189 (2012) 4981–4988.
- [44] S. Bolland, J.V. Ravetch, Spontaneous autoimmune disease in FcγRIIb-deficient mice results from strain-specific epistasis, *Immunity* 13 (2000) 277–285.
- [45] T.L. McGaha, B. Sorrentino, J.V. Ravetch, Restoration of tolerance in lupus by targeted inhibitory receptor expression, *Science* 307 (2005) 590–593.
- [46] R.J. Brownlie, K.E. Lawlor, H.A. Niederer, A.J. Cutler, Z. Xiang, M.R. Clatworthy, et al., Distinct cell-specific control of autoimmunity and infection by FcγRIIb, *J. Exp. Med.* 205 (2008) 883–895.
- [47] K.H. Su, H.X. Yang, X.R. Li, X.L. Li, A.W. Gibson, J.M. Cafardi, et al., Expression profile of FcγRIIb on leucocytes and its dysregulation in systemic lupus erythematosus, *J. Immunol.* 178 (2007) 3272–3280.
- [48] R.A. Floto, M.R. Clatworthy, K.R. Heilbronn, D.R. Rosner, P.A. MacAry, A. Rankin, et al., Loss of function of a lupus-associated FcγRIIb polymorphism through exclusion from lipid rafts, *Nat. Med.* 11 (2005) 1056–1058.
- [49] S.Y. Chu, K. Yeter, R. Kotha, E. Pong, Y. Miranda, S. Phung, et al., Suppression of rheumatoid arthritis B Cells by XmAb5871, an anti-CD19 antibody that coengages B cell antigen receptor complex and Fcγ receptor I/II inhibitory receptor, *Arthritis & Rheumatology* 66 (2014) 1153–1164.