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Antibody-Mediated Coengagement of FcγRIIb and B Cell Receptor Complex Suppresses Humoral Immunity in Systemic Lupus Erythematosus

Holly M. Horton,*[,1] Seung Y. Chu,*[,1] Elizabeth C. Ortiz,[†,‡] Erik Pong,* Saso Cemerski,* Irene W. L. Leung,* Noam Jacob,[‡] Jonathan Zalevsky,* John R. Desjarlais,* William Stohl,[†,‡] and David E. Szymkowski*

Engagement of the low-affinity Ab receptor FcγRIIb downregulates B cell activation, and its dysfunction is associated with autoimmunity in mice and humans. We engineered the Fc domain of an anti-human CD19 Ab to bind FcγRIIb with high affinity, promoting the coengagement of FcγRIIb with the BCR complex. This Ab (XmAb5871) stimulated phosphorylation of the ITIM of FcγRIIb and suppressed BCR-induced calcium mobilization, proliferation, and costimulatory molecule expression of human B cells from healthy volunteers and systemic lupus erythematosus (SLE) patients, as well as B cell proliferation induced by LPS, IL-4, or BAFF. XmAb5871 suppressed humoral immunity against tetanus toxoid and reduced serum IgM, IgG, and IgE levels in SCID mice engrafted with SLE or healthy human PBMC. XmAb5871 treatment also increased survival of mice engrafted with PBMC from a unique SLE patient. Unlike anti-CD20 Ab, coengagement of FcγRIIb and BCR complex did not promote B cell depletion in human PBMC cultures or in mice. Thus, amplification of the FcγRIIb inhibitory pathway in activated B cells may represent a novel B cell-targeted immunosuppressive therapeutic approach for SLE and other autoimmune diseases that should avoid the complications associated with B cell depletion. ***The Journal of Immunology*, 2011, 186: 4223–4233.**

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As the only FcR on B cells, FcγRIIb serves as an Ab-sensing downregulator of humoral immunity that is naturally engaged by immune complexes (ICs). When sufficient Ab is raised against a given Ag, specific IC form and coengage FcγRIIb and BCR with high avidity, selectively suppressing only B cells recognizing cognate Ag (1). In addition, FcγRIIb regulates activity of other B cell stimulators including IL-4, LPS, and BAFF (2) that amplify BCR-driven proliferation and differentiation, although the signaling mechanisms are currently poorly understood. By suppressing expression of costimulatory molecules, FcγRIIb also downregulates the APC function of B cells (3).

FcγRIIb plays a crucial role in suppressing autoimmunity. For example, autoimmune disease is exacerbated in mice lacking FcγRIIb (4, 5), and its restoration rescues mice in systemic lupus erythematosus (SLE), arthritis, and asthma models (6–8). Moreover, FcγRIIb polymorphisms affecting activity or expression are associated with human autoimmunity (9–11), and B cell expression of FcγRIIb is abnormally low in SLE, leading to inadequate suppression of autoantigen-mediated BCR activation (12–14). In addition, this receptor has recently been demonstrated to be a tractable drug target, with a dual-affinity diabody against CD79b and FcγRIIb showing efficacy in a mouse collagen-induced arthritis model (15).

To develop new therapies that exploit FcγRIIb signaling, we reasoned that it should be possible to induce B cell suppression via pharmacologic coengagement of FcγRIIb and the BCR complex using a recombinant Ab. We therefore engineered an Fc domain with >400-fold increased FcγRIIb affinity relative to native IgG1 Fc. By combining this Fc with a humanized Fv domain that recognizes human CD19 (16), we generated an Ab that coengages FcγRIIb with the BCR complex on all human CD19^{+} B cells. We previously demonstrated that this Ab, XmAb5871, suppressed BCR-mediated activation of normal human B cells through a SHIP-mediated inhibitory pathway (17). We now show that XmAb5871 also overcame FcγRIIb dysregulation to suppress activation of SLE B cells and inhibited proliferation induced by multiple B cell activation signals including BCR cross-linking, IL-4, BAFF, and LPS. In agreement with the known initial signaling event induced by IC, phosphorylation of the FcγRIIb ITIM in normal and SLE human B cells was stimulated only when the receptor was coengaged with BCR complex by XmAb5871. XmAb5871 also suppressed induction of costimulatory molecules CD80 and CD86, suggesting that coengagement of CD19 and FcγRIIb can inhibit B cell APC function and T cell stimulation. In immunodeficient SCID mice engrafted with PBMC from normal or SLE donors, XmAb5871 potently suppressed the human humoral immune response to tetanus toxoid (TTd), a T cell-dependent Ag. In SLE PBMC-engrafted mice, XmAb5871 also increased survival and reduced human Ab production. In contrast with anti-CD19 or anti-CD20 Abs that deplete B cells by immune effector functions (18–20), FcγRIIb-mediated immunosuppression neither required nor caused global B cell depletion in human PBMC cultures or in human FcγRIIb-transgenic mice. Our results

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Abbreviations used in this article: AUC, area under the curve; IC, immune complex; SLE, systemic lupus erythematosus; TTd, tetanus toxoid.

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validate the critical role of FcγRIIb in regulating human humoral immunity and suggest that replication of IC-mediated immunosuppression by an Fc-engineered Ab has potential as a novel therapeutic mechanism to suppress autoimmunity in SLE and other diseases.

Materials and Methods

Abs and reagents

Human and murine surrogate anti-CD19 and control Abs are described in Supplemental Table I (66) and elsewhere (17, 18, 21). Anti-FcγRIIb Ab 2B6 (22), anti-CD79b (BCR β subunit) Ab SN8 (23), and anti-CD40 Ab G28.5 (24) were constructed by gene synthesis and subcloned into pcDNA3.1Zeo as full-length chimeric Abs. Mouse surrogate anti-CD19 Ab XENP8206 was generated from the rat IgG2a κ Ab 1D3 sequence (ATCC HB-305 hybridoma) (25) as a chimera with the human FcγRIIb-enhanced IgG1 Fc domain containing S267E and L328F mutations. Mouse anti-CD20 Ab XENP8243 (a surrogate for rituximab) was derived from the rat IgG2a κ Ab 18B12 sequence (26). Abs were expressed in HEK293 cells and purified using protein A chromatography. Full-length 2B6 was labeled with PerCp (Prozyme, Hayward, CA) according to the manufacturer's instructions. Goat anti-human IgG Fcγ-specific, goat anti-human IgG Fcγ-specific $F(ab')_2$, and IgM Fc5μ fragment-specific cross-linking Abs were from Jackson ImmunoResearch (West Grove, PA). Rituximab was purchased from RxUSA (Port Washington, NY). Two anti-human CD79b Abs were used; XENP6293 contains L328R and G236R mutations to abolish FcγRIIb binding (17), whereas SN8 is mouse IgG1 κ (Santa Cruz Biotechnology, Santa Cruz, CA). Fluorescently labeled Abs to CD19, CD27, CD80, and CD86 were from BD Biosciences (San Jose, CA). BAFF was from R&D Systems (Minneapolis, MN), IL-4 was from PeproTech (Rocky Hill, NJ), and activating anti-CD40 Ab 82111 was from R&D Systems. LPS (serotype O127:B8) was from Sigma-Aldrich (St. Louis, MO). Rabbit anti-FcγRIIb phosphotyrosine 292 (Epitomics, Burlingame, CA), anti-Erk1/2 (Santa Cruz Biotechnology) and HRP-conjugated goat anti-rabbit and goat anti-mouse Abs (BioRad, Hercules, CA) were used for Western blot analysis. For human IgG2 determination in SCID mice, mouse anti-human IgG2 Ab (BD Biosciences, San Jose, CA) and HRP-conjugated donkey anti-human IgG (Jackson ImmunoResearch) was used. Serum samples were compared with a human IgG2 standard curve (Calbiochem/EMD, San Diego, CA). Reagents for other ELISAs were for human total IgM (Alpha Diagnostics, San Antonio, TX), IgG (Zeptometrix), IgG2 (BD Biosciences), IgE (Mabtech, Mariemont, OH), and anti-tetanus IgG (IBL, Minneapolis, MN).

Human PBMC and B cells

Human PBMC were obtained from whole blood or from leukapheresis with informed consent (HemaCare, Van Nuys, CA) for healthy donors, and from SLE patients seen at Los Angeles County + University of Southern California Medical Center Rheumatology Clinics with University of Southern California Institutional Review Board approval. SLE disease activity was assessed by the Safety of Estrogens in Lupus Erythematosus National Assessment SLE disease activity index (27, 28). PBMC were purified by density gradient isolation using Ficoll–Paque (GE Healthcare, Waukesha, WI). For some experiments, B cells were purified from frozen or fresh PBMC using the EasySep negative B cell enrichment kit (StemCell Technology, Vancouver, BC, Canada).

Surface marker expression

To examine B cell CD80 and CD86 surface expression, purified B cells were activated with polyclonal anti-IgM in the presence of IL-4 and anti-CD40 Ab (G28.5). To minimize potential Fc contribution to activity, the anti-CD40 Ab (G28.5) contained two Fc mutations (G236R and L238R) that abolish FcγRIIb binding. At each time point, cells were collected and stained with anti-CD80 and anti-CD86 Abs, examined using a FACSCanto II flow cytometer (BD Biosciences), and plotted using the FlowJo analysis package (Ashland, OR).

Ex vivo B cell depletion

PBMC were incubated in RPMI 1640 with 10% FBS for 2 d in varying concentrations of Abs. Lymphocytes were identified based on side and forward scatter, from which the $CD40^+$ cell fraction was identified as B cells using a FACSCanto II flow cytometer.

B cell proliferation assays

For proliferation assays, we used ATP-dependent luminescence (CellTiter-Glo; Promega, Madison, WI) as a marker of total cell viability after 3–4 d incubation at 37°C. B cells were purified from PBMC obtained from healthy and SLE donors using the EasySep negative B cell enrichment kit.

Normal and SLE donor B cell analysis

PBMC were isolated from ~30 ml peripheral blood from normal and SLE donors using a density gradient of Ficoll–Hypaque. B cells were analyzed for expression of CD19 and FcγRIIb, using CD27 to distinguish memory versus naive B cells. The B cell fraction from SLE donor 14 (Supplemental Table II) was insufficient for marker analysis. In addition, $CD40^+$ B cells in PBMC were assayed for calcium mobilization as described previously (17). In brief, PBMC were loaded with Fluo-4 NW calcium dye (Molecular Probes, Eugene, OR) and incubated at 37°C for 30–40 min in 1× HBSS with calcium and magnesium (Invitrogen, Carlsbad, CA) containing 0 or 10 μg/ml XmAb5871 and anti–CD40-allophycocyanin (for B cell identification). The cells were diluted 10-fold in HBSS, and baseline calcium response was recorded for 90 s, followed by 10 min of recording after BCR activation induced by 10 μg/ml anti-CD79b Ab XENP6293 premixed with 10 μg/ml cross-linking goat anti-human IgG Fcγ-specific Ab (Jackson ImmunoResearch Lab). Calcium response was detected using a FACSCanto II flow cytometer and quantified by determining the baseline-adjusted area under the curve (AUC). B cell fractions from SLE donors 4 and 14 were insufficient in number to perform calcium mobilization studies.

Western blotting

Purified B cells or PBMC (1.5×10^6 cells) were incubated with or without 10 μg/ml engineered Abs (as indicated) for 45 min at 37°C. On incubation, cells were stimulated for 2 min with 10 μg/ml anti-CD79b Ab XENP6293 premixed with 5 μg/ml goat anti-human IgG Fcγ-specific $F(ab')_2$, centrifuged, and lysed for 20 min in 1% NP-40 lysis buffer (0.15 M NaCl, 0.05 M Tris pH 8.0, 1 mM EDTA) in the presence of 0.2 M Na_3VO_4, 0.1 M NaF, 0.1 M PMSF (all from Sigma-Aldrich, St. Louis, MO), and a mixture of protease inhibitors (Roche Applied Sciences, Mannheim, Germany). Lysates were centrifuged 10 min at 12,000 rpm; then postnuclear lysates were resolved by SDS-PAGE, and proteins were transferred to polyvinylidene difluoride membranes by Western blotting. Membranes were probed using anti-phosphotyrosine 292 FcγRIIb and anti-Erk1/2 primary Abs plus HRP-conjugated secondary Abs.

B cell depletion studies in FcγRIIb transgenic mice and calcium activation from splenocytes

C57BL/6 mice transgenic for human FcγRIIb and null for mouse FcγRIIb were provided by Jeffrey Ravetch of the Rockefeller University. Mice were treated with Abs by i.v. or i.p. injection. Blood and spleens were collected according to Xencor's Institutional Animal Care and Use Committee-approved protocols. For B cell staining, 50 μl mouse whole blood or splenocytes was prepared by standard methods and stained with anti-mouse CD45R/B220 Ab (clone RA3-6B2; BD Biosciences), and the fraction of lymphocytes (based on forward and side scattering) was determined using a FACSCanto II flow cytometer. For calcium assays of splenocytes, prepared cells were loaded with Fluo-4 NW calcium dye (Molecular Probes, Eugene, OR) with anti-mouse CD3-allophycocyanin Ab (used to exclude T cells from the analysis) and 10 μg/ml anti-mouse CD19 Ab in 1× HBSS. Cells were induced using goat anti-mouse IgM (Jackson ImmunoResearch), and calcium flux of $CD3^-$ lymphocytes was measured using a FACSCanto II cytometer and analyzed with FlowJo software.

Human PBMC engraftment of SCID mice

Female C.B-17 SCID mice (Taconic, Hudson, NY) were maintained in microisolator cages (Innovive, San Diego, CA) and used at 6–12 wk of age. All experiments were approved by Xencor's Institutional Animal Care and Use Committee. The human PBMC–SCID model was established as previously described (29, 30). Mice were depleted of NK cells on day −1 by i.p. injection of rabbit anti–asialo-GM_1 Ab (Wako Chemical, Richmond, VA). On day 0, mice were engrafted by i.p. injection with 3×10^7 human PBMC (Figs. 4, 9*A*); 1×10^7 PBMC were used for Fig. 9*B* and 9*C* because of limited apheresis yield for SLE donor 28. On day 7, blood was collected and serum human IgG level was assayed (ZeptoMetrix, Buffalo, NY) to assess engraftment. Mice were then injected i.p. with 10 mg/kg Abs or PBS on day 9 post-PBMC injection (for normal PBMC, Abs were dosed once; for SLE PBMC, Abs were dosed twice weekly). On day 11, mice were injected i.p. with 15 μg TTd (List Biologicals, Campbell, CA) in PBS. On day 21 or as indicated, blood was collected and assayed by ELISA for human total IgM, IgG, IgG2, IgE, and anti-tetanus IgG (IBL).

Statistical analysis

Statistical significance between two groups was calculated using the two-tailed Student *t* test using Prism software (GraphPad Software, La Jolla,

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CA), including Welch's correction if the variances as determined by F-test were significantly different. Kaplan–Meier survival curves were compared using a log-rank test.

Results

FcγRIIb inhibits human B cell proliferation induced by diverse activation factors

We previously showed that coengagement of FcγRIIb with BCR complex by XmAb5871 inhibits BCR-mediated B cell calcium mobilization and proliferation (17). Because factors such as LPS (via TLR4 receptor), BAFF, and IL-4 influence B cell activation and differentiation, we tested whether these signals, alone or in combination with BCR activation, could be inhibited by XmAb5871. As shown in Fig. 1, these activation factors promoted proliferation of normal human B cells, which was further amplified by BCR cross-linking using anti-CD79b. Under all treatment conditions, XmAb5871 was inhibitory; in contrast, two anti-CD19 control Abs with reduced binding to FcγRIIb (17) showed only modest or no inhibitory effects against all stimuli (see Supplemental Table I for a description of all Abs used). Although the mechanisms for cross talk between FcγRIIb and non-BCR B cell activation signals remain unclear, these data indicate that XmAb5871 efficiently inhibits multiple discrete immune activators, with or without concomitant BCR signaling. The lack of inhibition by native IgG1 and FcγR-knockout Abs demonstrates that activity requires high-affinity coengagement of FcγRIIb and BCR complex, mimicking the high-avidity effects of IC.

XmAb5871 inhibits B cell expression of T cell costimulatory molecules

On activation, B cells upregulate T cell costimulatory molecules CD80 (B7.1) and CD86 (B7.2) that bind to CD28 expressed on the surface of T cells, thereby promoting TCR-induced $CD4^+$ T cell activation. Engagement of CD28 with CD80 and/or CD86 is critical for an immune response, because TCR signaling in the absence of such interactions results in T cell anergy (31). Therefore, we asked whether XmAb5871 could inhibit CD80 and CD86 induction on B cell activation with IL-4 and CD40 costimulation. As shown in Fig. 2*A*, CD80 and CD86 surface expression was upregulated on human B cells stimulated with IL-4 and anti-CD40 Ab, with CD86 detected within 1 d, and CD80 developing more slowly over 3 d. As with B cell proliferation, XmAb5871 suppressed induction of both CD80 and CD86, whereas three Fc and isotype control Abs did not.

Murine surrogate of XmAb5871 suppresses B cell activation in human FcγRIIb-transgenic mice

XmAb5871 does not cross-react with murine CD19. Therefore, to extend human ex vivo results into in vivo mouse models, we developed XENP8206, an anti-murine CD19 Ab containing the identical FcγRIIb-enhanced human Fc domain. To verify the immunosuppressive potency of XENP8206, we assessed its ability to suppress activation of mouse B cells as measured by BCR-induced calcium mobilization. Because the engineered human Fc domain does not have high affinity for the mouse FcγRIIb inhibitory receptor, it was necessary to use C57BL/6 mice transgenic for human FcγRIIb. In these transgenic mice, XENP8206, but not two anti-murine CD19 control Abs that bind with low affinity to human FcγRIIb, suppressed B cell activation induced by anti-IgM–mediated BCR cross-linking (Fig. 2*B*). In contrast, XENP8206 was not effective in littermate control mice lacking human and mouse FcγRIIb (Fig. 2*C*), further demonstrating the critical role of the Fc/FcγRIIb interaction in B cell suppression. Notably, inhibitory activity required coengagement of both FcγRIIb

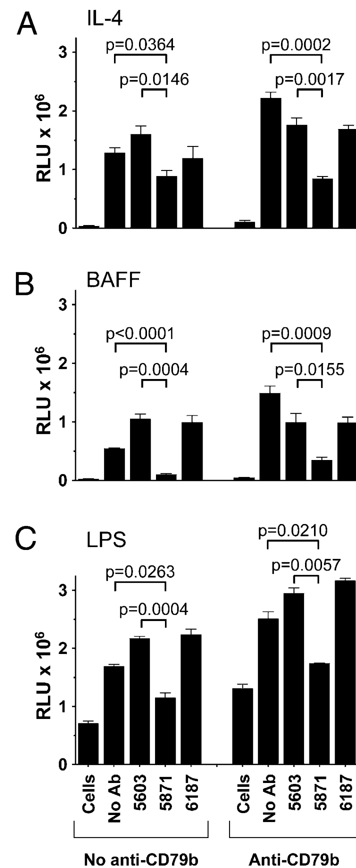

FIGURE 1. XmAb5871 inhibits B cell proliferation signals in normal human B cells. Purified B cells (5×10^4) from healthy donors were activated with 2 ng/ml IL-4 (*A*), 2 ng/ml BAFF (*B*), or 1 μg/ml LPS (*C*) with or without 1 μg/ml anti-CD79b Ab XENP6293. After 4 d, proliferation was quantified using an ATP-dependent luminescence assay. XmAb5871 (anti-CD19 with FcγRIIb-enhanced Fc domain), XENP5603 (anti-CD19 with native IgG1 Fc), and XENP6187 (anti-CD19 lacking FcγR binding) were used at 0.5 μg/ml. XENP6293 contains the same knockout Fc domain as XENP6187 to preclude FcR-mediated effects on BCR activation. Data shown are representative of two experiments. The *p* values are shown for two-tailed Student *t* test; error bars represent SEM. Cells, untreated controls; No Ab, stimulated controls without Ab treatment; RLU, relative luciferase units.

and CD19 by Fc and Fv domains, respectively, on the same Ab as opposed to simultaneous engagement by different Abs, because the combination of isotype control and Fc control Abs was ineffective in suppressing B cell activation (Fig. 2*D*). This requirement for coengagement of FcγRIIb and BCR complex agrees with earlier results demonstrating that specific cross-linking of FcγRIIb by a selective Ab has no in vitro or in vivo effects in the absence of immune cell-dependent effector functions (32).

FcγRIIb-enhanced anti-CD19 Abs do not induce effector cell-dependent B cell depletion, yet suppress B cell activation in vivo

By optimizing engagement of stimulatory FcγRs (rather than inhibitory FcγRIIb) on immune effectors including NK cells, Fc-engineered Abs can be created that effectively deplete opsonized target cells (21). In contrast, because XmAb5871 was engineered to selectively enhance Fc binding to FcγRIIb relative to activating receptors FcγRI, FcγRIIa, and FcγRIIIa (17), we hypothesized that its capacity to stimulate such effector cell-dependent cytotoxicity would be minimized. As shown in Fig. 3*A*, B cells were not depleted from human PBMC cultures even after 2 d of ex-

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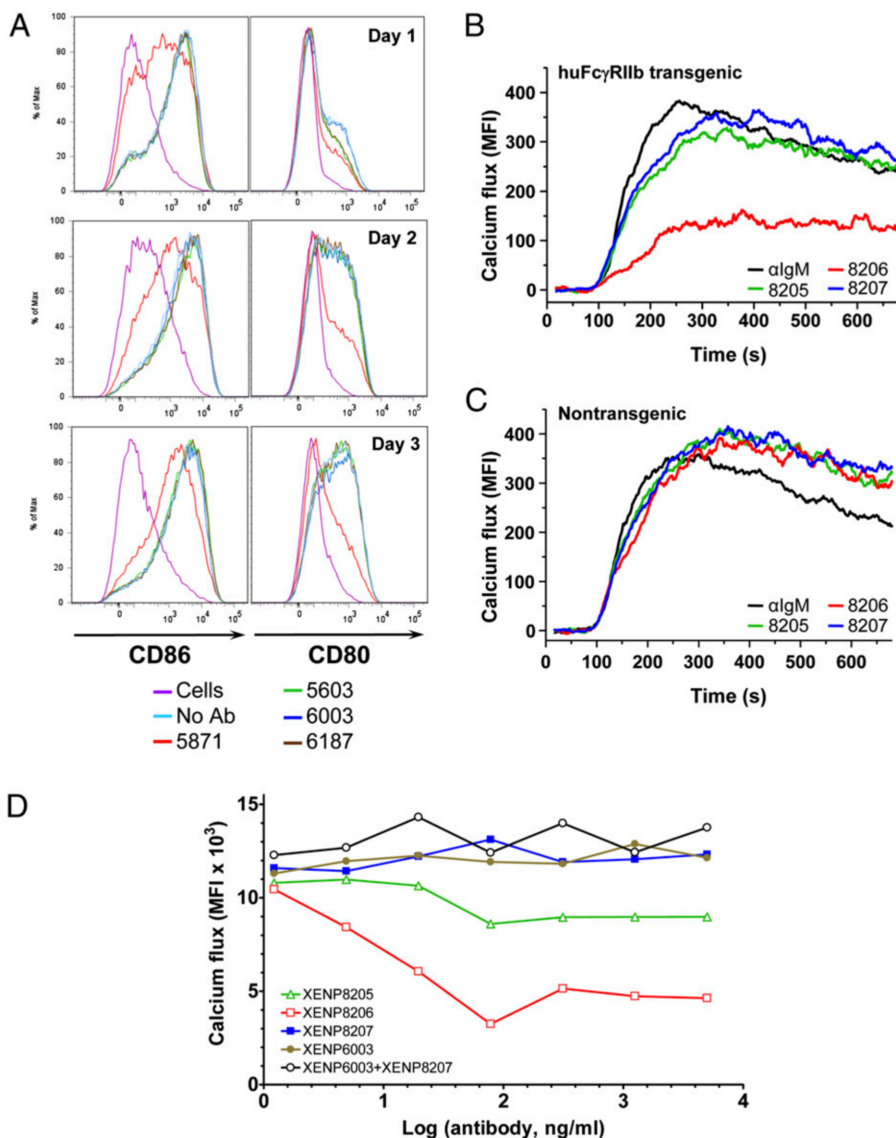

FIGURE 2. XmAb5871 suppresses T cell costimulatory molecules and BCR activation in human and mouse B cells. *A*, Purified human B cells (1×10^6) were activated using 2 μg/ml anti-IgM to cross-link BCR in the presence of 5 ng/ml IL-4 and 0.1 μg/ml anti-CD40. CD80 and CD86 expression was measured on days 1, 2, and 3. XmAb5871 (red), XENP5603 (green), XENP6187 (brown), and XENP6003 (anti-respiratory syncytial virus with FcγRIIb-enhanced Fc domain, an isotype control; blue), were used at 1 μg/ml. Untreated cells (purple) and cells treated with IL-4, anti-CD40, and anti-IgM without anti-CD19 Ab (No Ab, cyan) were used as baseline and stimulated controls, respectively. *B* and *C*, B cell activation measured by calcium mobilization in splenic B cells of human FcγRIIb transgenic mice (*B*) and littermate controls (*C*) induced by 5 μg/ml anti-murine IgM Ab (black line). XENP8206 (red), a surrogate for XmAb5871 containing the identical human Fc domain with an anti-mouse CD19 Fv domain, suppresses activation in transgenic mice but not littermate controls. Native IgG1 control XENP8205 (green) and FcγR knockout control XENP8207 (blue) do not inhibit calcium flux in transgenic mice or littermates (all Abs at 10 μg/ml). *D*, Effect of coengagement versus simultaneous engagement of FcγRIIb and CD19 on suppression of calcium mobilization of B cells from FcγRIIb transgenic mice. XENP8206 (open squares, red) suppresses activation in a dose-dependent fashion, whereas controls XENP6003 (filled circles, brown) and XENP8207 (filled squares, blue) do not, even when combined (open circles, black). *A*, Data are representative of two duplicate experiments; *B* and *C* each show one representative mouse of two examined, and *D* was done once in the exact format shown but has been repeated more than five times with related Abs.

posure to XmAb5871. In contrast, rituximab (anti-CD20) (20) and XmAb5574 (anti-CD19) (18, 19), known to be potent depleting Abs, eliminated B cells in a dose-dependent manner, presumably through FcγRIIIa-mediated lysis by NK cells. Notably, XmAb5574 and XmAb5871 have identical anti-CD19 Fv domains, differing only in the two mutations made to their respective Fc domains to modulate FcγR interactions (Supplemental Table I). Like XmAb5871, Fc and isotype control Abs did not deplete B cells, indicating that affinity for activating FcγRs is critical to effector cell-mediated depletion.

We next asked whether this ex vivo result using human PBMC could be extended into an in vivo setting, again using XENP8206 as the murine anti-CD19 surrogate for XmAb5871. Peripheral blood B cell numbers in XENP8206-treated human FcγRIIb-transgenic mice 2 d posttreatment were identical to those in vehicle-treated mice (Fig. 3*B*). In contrast, peripheral blood B cells of mice treated with XENP8243, an anti-murine CD20 surrogate for rituximab (26), were profoundly depleted.

It was important to understand whether the nondepleted B cell population as shown in Fig. 3*B* was functionally resistant to activation due to Ab coengagement of FcγRIIb and CD19. Therefore, we examined effects of XENP8206 administration on B cell function in FcγRIIb transgenic mice. Fig. 3*C* shows that 24 h after mice were treated with XENP8206, splenic B cells remained refractory to BCR activation, whereas (as in Fig. 3*B*) splenic B cell numbers in these mice again matched vehicle controls (Fig. 3*D*), demonstrating that suppression of B cell function in vivo does not require B cell depletion.

XmAb5871 inhibits B cell activation in SCID mice engrafted with normal human PBMC

To assess the effects of XmAb5871 on human B cell activation in vivo, we exploited an established model in which the human immune system is partially reconstituted in SCID mice through engraftment of human PBMC (29, 30). Because it is generally accepted that the generation of pathogenic autoantibodies in SLE is highly T cell dependent, we focused our attention on T cell-dependent Ab responses. To that end, we immunized PBMC-engrafted mice with a prototypic T cell-dependent Ag, TTd, and measured human anti-tetanus titers. Because PBMC donors had been previously immunized with TTd, this model recapitulates a T cell-dependent recall response requiring differentiation of memory B cells to plasma cells. Immunization of engrafted mice stimulated a strong human anti-tetanus response in vehicle-treated

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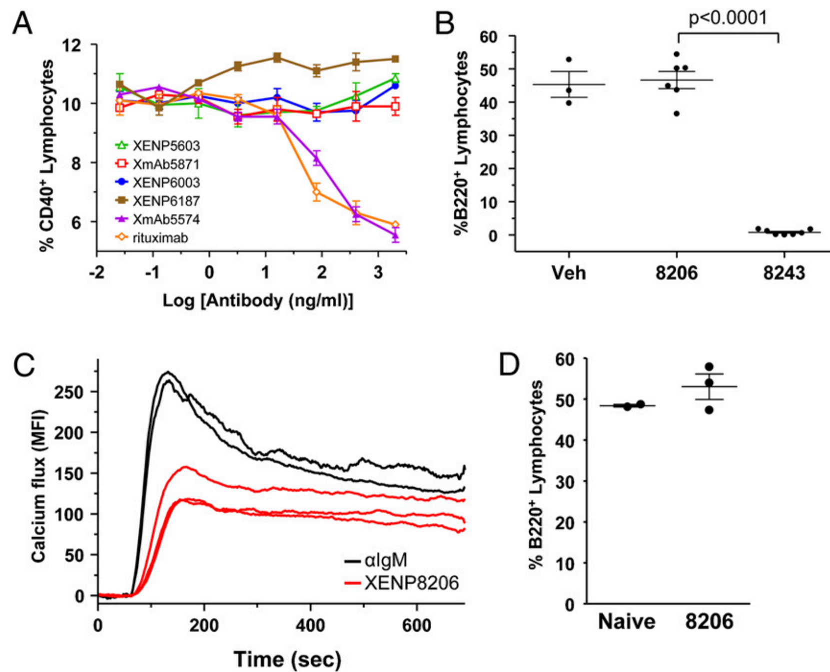

FIGURE 3. XmAb5871 and murine surrogate XENP8206 do not deplete B cells in human PBMC culture or in human FcγRIIb transgenic mice. *A*, PBMC (5×10^5) from healthy human donors were incubated with varying concentrations of Abs for 2 d, and CD40$^+$ B cell depletion was quantified by flow cytometry. XmAb5871 (open squares, red) was compared with Fv and isotype control Abs XENP5603 (open triangles, green), XENP6187 (filled squares, brown), and XENP6003 (filled circles, blue). The depleting Abs rituximab (anti-CD20; open diamonds, orange) and XmAb5574 (anti-CD19; filled triangles, purple) were included as positive controls. Data shown are from one donor and are representative of results from three donors. *B*, XENP8206, a murine surrogate for XmAb5871, was dosed at 10 mg/kg in C57BL/6 mice transgenic for human FcγRIIb and lacking mouse FcγRIIb, and the blood B cell fraction was determined after 2 d. XENP8243, an anti-mouse CD20 Ab with native mouse IgG2a Fc domain, was included as a known depleting control and surrogate for rituximab (26). The *p* values are shown for two-tailed Student *t* test; error bars represent SEM. *C*, Splenic B cells are refractory to BCR activation by 5 μg/ml anti-murine IgM (black) after treatment of FcγRIIb-transgenic mice with XENP8206 (red). Each line represents one mouse. *D*, Splenic B cell number in mice from *C*. B cell function is suppressed by XENP8206 without B cell depletion at 24 h after drug treatment. *B*, Data are representative of four similar experiments; experiments in *C* and *D* were repeated twice.

mice that was potently suppressed by XmAb5871 (Fig. 4*A*). Such inhibition was critically dependent on coengagement of FcγRIIb with CD19, because the FcγR knockout control XENP6187 was inactive. Of note, rituximab also partially suppressed anti-tetanus titer, presumably through depletion of peripheral human B cells (20). Because the number of human B cells in PBMC-engrafted SCID mice is routinely below the limit of detection (29), the nondepleting aspect of FcγRIIb engagement could not be assessed in this model.

To explore the specific Ig pools that are affected by XmAb5871, we assessed total serum levels of IgM, IgG, and IgE in a subsequent SCID mouse study using a different human PBMC donor. Notably, XmAb5871 suppressed production of these three human isotypes in PBMC-engrafted mice (Fig. 4*B*). Because XmAb5871 coengages FcγRIIb with CD19 targets found on both naive and memory B cells of all BCR isotypes, it is not surprising that coengagement suppresses Ig production in the acute humoral response of the PBMC–SCID model. This result also suggests that through careful choice of Ab targets, selective immunosuppression of particular B cell subpopulations involved in allergic and autoimmune diseases may be possible through amplified FcγRIIb engagement.

Activation markers and XmAb5871 targets are expressed on human B cells from normal and SLE donors

Because dual expression of CD19 and FcγRIIb on B cells is essential for XmAb5871 activity, and CD80 and CD86 are essential for T cell activation, we examined levels of these markers on B cells from 30 healthy donors and 29 SLE patients with varying degrees of disease activity (SLE disease activity index scores 0–20; Supplemental Table II). Expression of CD19 was reduced on both CD27$^-$ (naive) and CD27$^+$ (memory) B cells in SLE relative to normal donors (Fig. 5*A*). FcγRIIb expression on CD27$^+$ B cells trended lower in SLE patients but was not significantly different from normal donors, and it increased slightly in CD27$^-$ B cells (Fig. 5*B*). In agreement with previous reports (33, 34), in SLE patients, CD86 expression was upregulated on both CD27$^-$ and CD27$^+$ B cells (Fig. 5*C*), whereas only CD27$^-$ B cells showed significantly higher expression of CD80 (Fig. 5*D*). These data also demonstrate that in all SLE patients examined, CD19 and FcγRIIb were readily detectable, indicating that both targets of XmAb5871 are accessible to facilitate coengagement and subsequent B cell suppression.

XmAb5871 suppresses B cell activation in PBMC from SLE patients

Given many reports that FcγRIIb signaling is perturbed in SLE (4, 9–14, 35), it was essential to determine whether XmAb5871 retained activity against SLE B cells. As measured by calcium mobilization, BCR cross-linking robustly activated B cells from normal and SLE donors, and XmAb5871 efficiently inhibited this activation (Fig. 6*A*, 6*B*). The AUC for calcium responses of 30 normal and 28 SLE donors is plotted in Fig. 6*C* (B cell recovery from 2 SLE donors was insufficient to assay). Calcium mobilization was induced in B cells from all patients, but the relative increase in SLE was generally lower than in healthy donors because of a higher unstimulated baseline in SLE. SLE B cell activation was inhibited in every case by XmAb5871, with average percent inhibition not significantly different from normal donors

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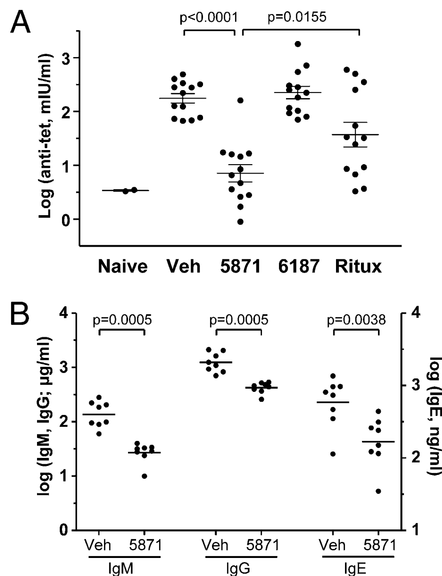

FIGURE 4. XmAb5871 and surrogate XENP8206 inhibit human immune response in PBMC-engrafted SCID mice. *A*, C.B-17 SCID recipient mice were engrafted with 3×10^7 human PBMC on day 0 and injected with 10 mg/kg XmAb5871, XENP6187, rituximab, or PBS on day 9. On day 11, mice were immunized with TTd, and on day 21, blood was assayed for human antitetanus IgG. Two mice were engrafted but not vaccinated, to establish the baseline tetanus titer. Data shown represent one of three similar experiments. *B*, Mice were engrafted as in *A* and treated with a single 10-mg/kg dose of Abs on day 8. Serum levels of IgM, IgG, and IgE were determined at day 22 after engraftment. XmAb5871 suppresses total human IgM, IgG, and IgE levels. The *p* values are shown for two-tailed Student *t* test; error bars represent SEM for 13 (*A*) or 8 (*B*) mice per group. *A*, Data are representative of four studies; (*B*) data are representative of three studies.

(Fig. 6*D*). These data indicate that despite differences in CD19 and FcγRIIb expression on SLE and normal B cells, XmAb5871 efficiently suppressed BCR activation, suggesting that FcγRIIb signaling remains functional in SLE.

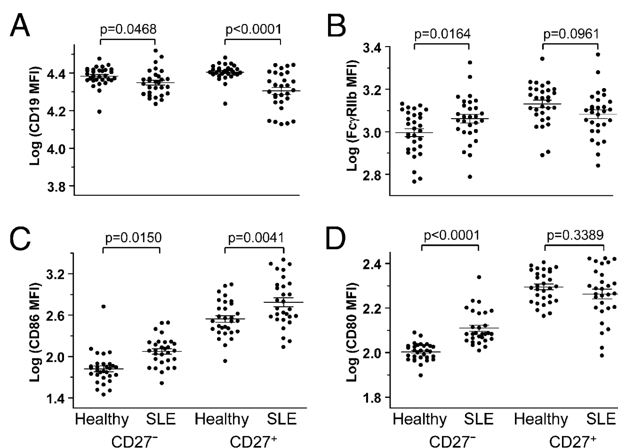

FIGURE 5. XmAb5871 Fc and Fv targets, and CD80 and CD86 are expressed on B cells from 30 healthy donors and 29 SLE patients. PBMC were analyzed for CD19 (*A*), FcγRIIb (*B*), CD86 (*C*), and CD80 (*D*) expression on the surface of $CD27^-$ (naive) and $CD27^+$ (memory) B cells. Expression is shown as relative mean fluorescence intensity (MFI). The *p* values are shown for two-tailed Student *t* test, with error bars representing SEM. PBMC from each donor was assayed once.

XmAb5871 inhibits multiple stimulatory pathways in SLE B cells

Many stimulatory molecules can supplement BCR-mediated B cell activation in SLE. IL-4 and BAFF stimulate autoreactive B cell expansion, and circulating BAFF levels are increased in SLE patients (36, 37). In addition, development of SLE-like features in mice requires MyD88 signaling (38), suggesting that TLRs and innate immunity play an important role in the disease. Therefore, we examined the effects of XmAb5871 on SLE B cell proliferation induced by IL-4, BAFF, and LPS. As shown in Fig. 7, each factor induced proliferation that was further amplified by BCR activation. In the presence or absence of BCR cross-linking, XmAb5871 inhibited proliferation approximately to baseline level. Suppression was dependent on Fc interaction with FcγRIIb, because anti-CD19 control Abs were ineffective. Thus, XmAb5871 inhibits multiple proliferative signals that contribute to SLE B cell expansion in the absence and presence of direct BCR activation.

XmAb5871 stimulates the known FcγRIIb-mediated inhibitory signaling pathway

The inhibitory effects of IC coengagement of BCR and FcγRIIb are initiated through phosphorylation of the ITIM motif of FcγRIIb, thereby stimulating downstream inhibitory signaling pathways (39). We therefore asked whether B cell inhibition by XmAb5871 was accompanied by phosphorylation of tyrosine 292 of FcγRIIb, a known result of IC binding. Using an Ab recognizing the phosphotyrosine 292 epitope on Western blots, we analyzed FcγRIIb ITIM phosphorylation in human B cells treated with XmAb5871 or multiple Fv and isotype controls. Purified B cells pretreated with XmAb5871 showed strong phosphorylation at tyrosine 292 (Fig. 8*A*) with or without BCR cross-linking by anti-CD79b Ab. Controls including native IgG1 anti-CD19, Fc knockout anti-CD19, and the FcγRIIb-enhanced anti-respiratory syncytial virus isotype control (66) did not induce FcγRIIb phosphorylation, demonstrating again that engagement of FcγRIIb or CD19 alone is insufficient to stimulate signaling. Notably, as in Fig. 2*A*, the combination of Fv and isotype control Abs (XENP6187, binding CD19, and XENP6003, with Fc enhanced for FcγRIIb) was also inactive. This result further demonstrates that coengagement of FcγRIIb and the BCR coreceptor complex by the Fc and Fv domains of a single Ab (not simultaneous engagement by the Fc and Fv domains of different Abs) is necessary for inhibitory activity of XmAb5871.

We next tested the ability of XmAb5871 to trigger phosphorylation of tyrosine 292 in human PBMC (which includes non-B cells that potentially could affect signaling events in B cells). As shown in Fig. 8*B*, some FcγRIIb phosphorylation was detected in XmAb5871-treated PBMC in the absence of BCR cross-linking. However, anti-CD79b treatment caused an additional increase in FcγRIIb phosphorylation by XmAb5871, suggesting that in the complex environment of PBMC, FcγRIIb-mediated suppression may be more effective against activated B cells. Isotype, Fv, and combination controls were again negative. Of note, rituximab, as expected from its unrelated target and mechanism, was unable to activate FcγRIIb, presumably because CD20 is not a component of the BCR complex.

Based on the similar suppression of calcium mobilization previously shown for normal and SLE B cells (Fig. 6), we next asked whether phosphorylation of FcγRIIb by XmAb5871 occurred in SLE PBMC, as well as in normal PBMC. Fig. 8*C* shows that XmAb5871 does stimulate FcγRIIb phosphorylation in SLE PBMC relative to untreated control. Taken together, these results show that the immunosuppressive effects of XmAb5871 on human

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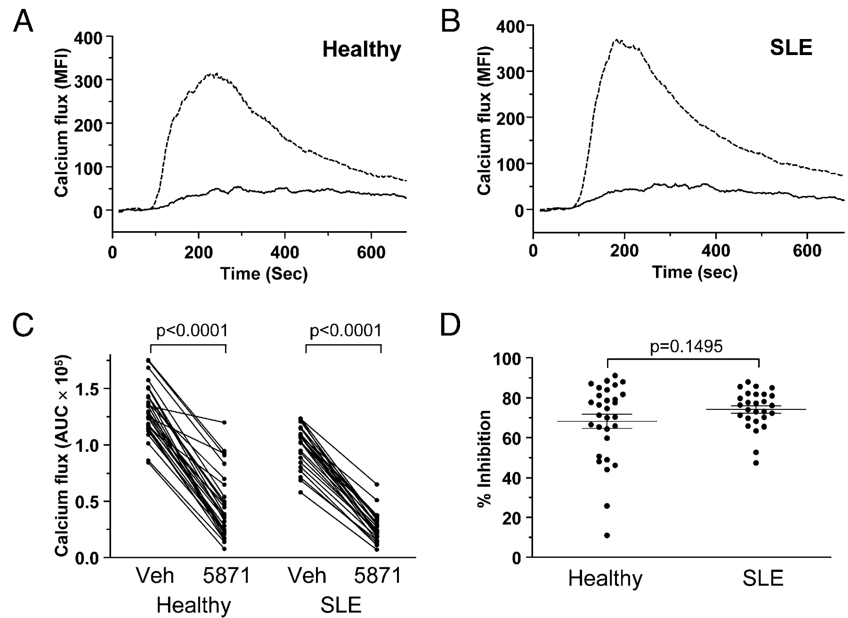

FIGURE 6. XmAb5871 suppresses BCR activation-mediated calcium flux in human B cells sampled from a population of 30 healthy and 28 SLE donors. *A* and *B*, Representative calcium mobilization traces induced by anti-CD79b Ab XENP6293 cross-linked with anti-human IgG Fcγ-specific polyclonal Ab (dotted line) and its inhibition by XmAb5871 (solid line) in B cells from healthy and SLE donors (Abs at 10 μg/ml). The AUC from each response curve as in *A* and *B* was determined for healthy and SLE cohorts and plotted in *C*, to quantify calcium mobilization for each sample. Percent inhibition for each healthy and SLE donor (*D*) was determined by normalizing the AUC of XmAb5871 to vehicle. The *p* values are shown for two-tailed Student *t* test; error bars represent SEM. PBMC from each donor was assayed once.

B cell responses in vitro and in vivo are associated with the recognized first step in inhibitory signaling by IC: phosphorylation of the tyrosine 292 residue in the ITIM of FcγRIIb.

XmAb5871 inhibits human B cell activation in SCID mice engrafted with SLE PBMC

Given our results demonstrating that FcγRIIb signaling was stimulated by XmAb5871 in B cells from SLE patients, suppressing BCR-mediated calcium activation and proliferation, we next asked whether this Ab was immunosuppressive in the more physiologically relevant setting of SCID mice engrafted with PBMC from SLE patients. Similar to results for SCID mice reconstituted with normal human PBMC (Fig. 4), mice reconstituted with SLE PBMC generated a robust human anti-tetanus titer (Fig. 9*A*). XmAb5871 treatment beginning 8 d after engraftment strongly suppressed the anti-tetanus Ab response to a degree similar to that shown by the B cell-depleting agent rituximab.

We next assessed activity in a more stringent SCID mouse model using PBMC from another SLE donor (donor 28, as noted in Supplemental Table I). In pilot studies (not shown), we discovered that PBMC from this particular patient stimulated an unusually severe illness when engrafted into SCID mice, resulting in fatal hemorrhage, often within 2 wk. To determine whether XmAb5871 could ameliorate this rapid and virulent illness (which may or may not be related to either the SLE or antiphospholipid syndrome noted in this patient; Supplemental Table II), we engrafted mice with PBMC from this donor after dosing prophylactically with XmAb5871 or vehicle. Pretreatment of mice with XmAb5871 significantly increased survival (Fig. 9*B*) and also suppressed total human IgG2 levels (Fig. 9*C*) over time. Because human autoantibodies such as anti-dsDNA have been reported to develop in a fraction of SCID mice engrafted with SLE PBMC (30, 40), we also assessed levels of human anti-dsDNA in mouse serum; however, none could be detected in either treatment group (not shown). Although more studies need to be done to characterize the disease pathology of mice engrafted with PBMC from this unique SLE donor, Fig. 9*C* suggests that the engrafted B cells generated an unrestrained human Ab response that could be suppressed by activation of the FcγRIIb inhibitory pathway. Taken together with Figs. 6–8, these results demonstrate that XmAb5871 is capable of suppressing SLE B cell activation and differentiation under in vivo conditions, and can prolong survival and suppress Ab production in mice engrafted with PBMC from SLE patients.

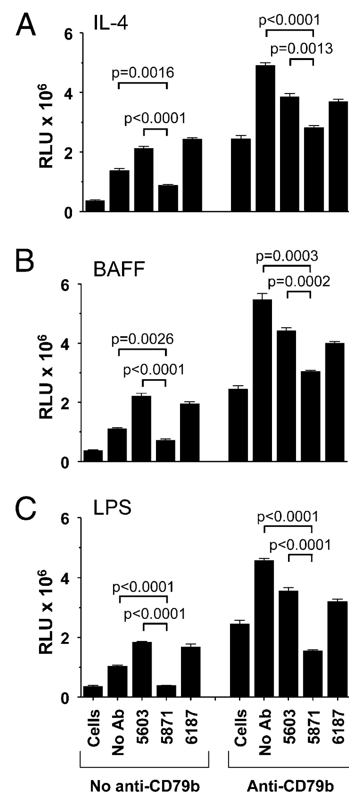

FIGURE 7. XmAb5871 inhibits multiple SLE B cell proliferation signals. B cells (5×10^4) purified from PBMC of SLE donor 18 (Supplemental Table II) were activated with 2 ng/ml IL-4 (*A*), 2 ng/ml BAFF (*B*), or 1 μg/ml LPS (*C*) in the presence or absence of 1 μg/ml anti-CD79b Ab XENP6293, and proliferation was quantified after 4 d by ATP assay. XmAb5871 and controls XENP5603 (native IgG1 Fc) and XENP6187 (FcγR knockout) were used at 0.5 μg/ml. The *p* values are shown for two-tailed Student *t* test; error bars represent SEM for three replicates of a single experiment. Cells, untreated B cells; No Ab, stimulated cells without Ab treatment.

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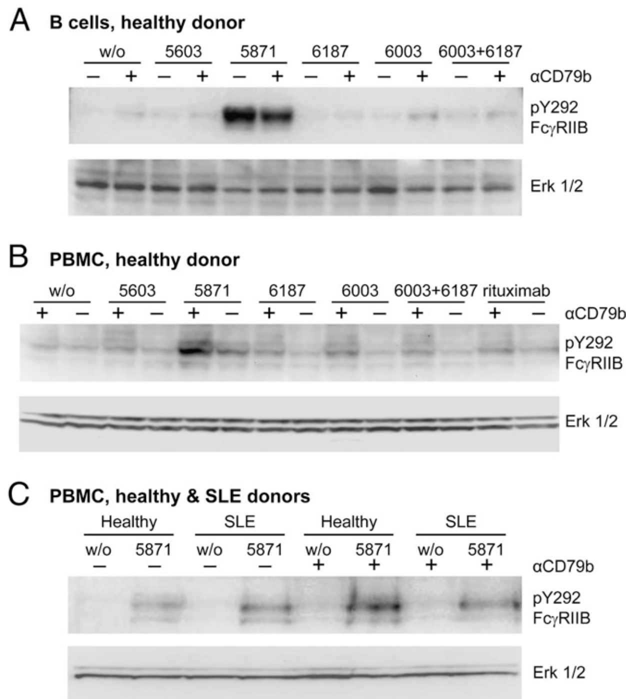

FIGURE 8. XmAb5871 induces phosphorylation of the FcγRIIb ITIM in B cells and PBMC from normal and SLE donors. Purified B cells (*A*) and PBMC (*B*, *C*) from healthy donors, and PBMC from SLE donor 18 (*C*) (each 1.5×10^6 cells) were incubated for 45 min with indicated Abs (10 μg/ml). After incubation, cells were stimulated (+) or not (−) for 2 min with 10 μg/ml anti-CD79b Ab XENP6293 and 5 μg/ml goat anti-human IgG Fcγ-specific $F(ab')_2$. Western blots were probed with anti-phosphotyrosine 292 Ab (specifically recognizing the phosphorylated ITIM of FcγRIIb) and reprobed with an anti-Erk1/2 Ab as a loading control. Results shown are representative of at least two independent experiments. w/o, no Ab treatment.

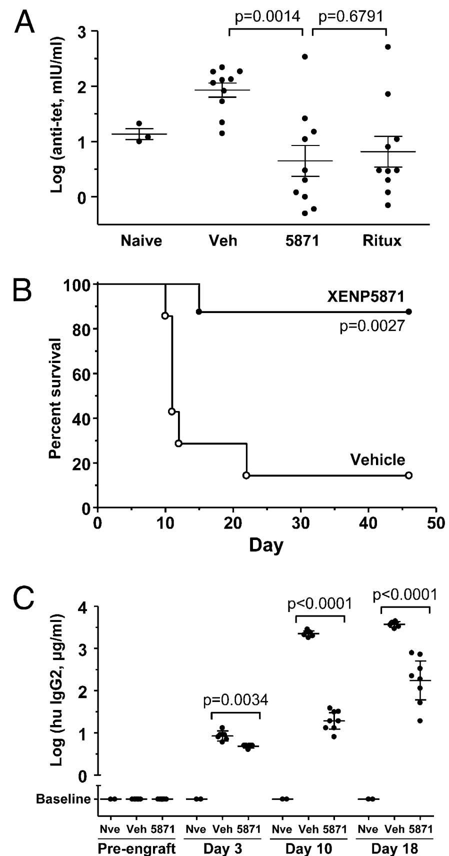

FIGURE 9. XmAb5871 inhibits immune response in SCID mice engrafted with SLE PBMC. *A*, PBMC obtained by leukapheresis were engrafted into SCID mice, and therapeutic and control Ab effects on human antitetanus titer after TTd immunization were assessed as described in Fig. 4, with Abs dosed at 10 mg/kg twice weekly starting at day 8 after engraftment. XmAb5871 and rituximab show similar suppression of humoral immunity. The *p* values are shown for two-tailed Student *t* test; error bars represent SEM for 10 mice per treatment group (3 for naive group). *B* and *C*, Mice were dosed with 10 mg/kg XmAb5871 (n = 8) or vehicle (n = 7) twice weekly starting on day −2, then engrafted with 1×10^7 human PBMC from a second SLE donor on day 0. *B*, Survival is prolonged by XmAb5871 (closed circles) over vehicle (open circles). *C*, Serum levels of human IgG2 from mice in *B*. Day 18 includes all surviving mice plus those dying between days 10 and 18. Total human serum IgG2 is suppressed by XmAb5871. The *p* values are shown for two-tailed Student *t* test; bars represent mean ± 95% confidence interval. Results represent independent studies done with two SLE PBMC donors [donors 18 (*A*) and 28 (*B*, *C*)].

Discussion

Given their central role in SLE pathogenesis, it is not surprising that targeting B cells has emerged as an attractive therapeutic approach. Early results with rituximab, a B cell-depleting Ab, demonstrated meaningful efficacy in SLE (41). Although two recent phase II/III SLE studies failed to meet clinical end points (42, 43), this may be because of poor trial design rather than inherent inadequacy of rituximab (44). A disconcerting issue, however, is the long-term safety of B cell-depleting agents. Fatal progressive multifocal leukoencephalopathy after JC virus reactivation is associated with suppression of T cell-mediated immunity (45, 46) and has been reported in five rituximab-treated autoimmune disease patients (including two with SLE) (47), and clinical trials of ocrelizumab, a next-generation version of rituximab, were recently halted because of unacceptable serious and fatal infections.

Such adverse events associated with CD20-based depletion therapy do not mean that unacceptable toxicity invariably accompanies all B cell-directed approaches. For example, belimumab, an anti-BAFF Ab that generates only a modest reduction in B cell number, has a highly favorable safety profile (48, 49). Unfortunately, clinical efficacy of belimumab is rather modest, perhaps because of other B cell survival factors that affect persistence of autoreactive B cells. Indeed, experience with rituximab suggests that clinical response and B cell depletion are linked; for example, reappearance of B cells in the peripheral blood of rituximab-treated patients can foretell disease flare (50, 51). Thus, clinical efficacy of B cell-targeted therapies may require functional suppression of autoreactive B cells, whereas clinical safety may require survival of protective nonautoreactive B cells. Therefore, a preferred approach in SLE may be one that inhibits, rather than depletes, B cells.

This strategy of inhibition rather than depletion is naturally used by the immune system. FcγRIIb tightly regulates pleiotropic effects of BCR activation, including proliferation, class switching, Ag presentation, and differentiation to plasma cells. As the only FcR present on B cells, engagement of FcγRIIb triggers an essential homeostatic downregulation of these processes to guarantee that B cell responses to foreign Ags (pathogens) are sufficient but not excessive. In states of inadequate FcγRIIb function, the ensuing excessive B cell responses could facilitate autoimmunity. Indeed, the importance of FcγRIIb in SLE is highlighted by development of SLE-like disease in mice knocked out for the gene (52), by amelioration of serological and clinical disease in SLE-prone MRL-*lpr/lpr* mice that selectively overexpress FcγRIIb on B cells (6), by decreased FcγRIIb expression on memory B cells

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in human SLE (12, 13), and by impaired function of an FcγRIIb polymorphism associated with human SLE (9). In support of our focused approach of activating this inhibitory pathway specifically on B cells, Brownlie et al. (6) showed that FcγRIIb overexpression on macrophages sensitizes mice to *Streptococcus pneumoniae* infection; in marked contrast, its overexpression on B cells does not reduce survival.

Of great importance, notwithstanding the potential differences in FcγRIIb expression or function in SLE, our data demonstrate that this inhibitory pathway in human SLE cells can be activated by high-affinity coengagement of FcγRIIb and BCR complex under in vivo conditions.

Although the role of FcγRIIb as a rheostat for the immune system has been recognized for many years (1, 5, 6, 53), to our knowledge, our report is the first to amplify this signaling pathway by optimizing the natural interaction of Fc with FcγRIIb in the context of IC. In principle, it should be possible to trigger the inhibitory FcγRIIb pathway with other biologic agents. Indeed, it has been suggested that the efficacy of high-dose i.v. Ig (IVIG) in autoimmunity results from Fc engagement of FcγRIIb (54). However, IVIG therapy has drawbacks not associated with recombinant biologics, including the need for daily infusions over several days, each requiring several hours, and the significant production, economic, and safety issues related to therapeutic use of a pooled human blood product. We have also tested activity of the FcγRIIb-enhanced Fc domain incorporated into Abs targeting the BCR itself (including IgM, IgE, CD79a, and CD79b) and other BCR coreceptors including CD22. In agreement with a report using a bispecific diabody targeting CD79b and FcγRIIb (15), FcγRIIb-enhanced anti-CD79b Abs could also suppress B cell activation (not shown); however, we found that the most effective pan-B cell target for this inhibitory mechanism was CD19, which is reported to be an essential coreceptor required for BCR activation (55).

It must be stressed that factors independent of Ag interaction with BCR also stimulate B cells, with potential important consequences for autoimmune disease (56). For example, IL-4 induces B cell proliferation, differentiation, and class switching, and initiates T cell-mediated adaptive immunity (57). BAFF produced by myeloid cells stimulates B cell survival and differentiation (58), its serum levels are increased in human SLE and in mouse SLE models (37, 59), and its overexpression in transgenic mice results in SLE-like manifestations (60, 61). Pathogen-associated molecular patterns such as LPS can potently induce B cell proliferation and differentiation via binding to TLRs and other pattern-recognition receptors (38, 62). The work presented in this article demonstrates that such Ag- and BCR-independent activation signals can be inhibited by enhanced coengagement of FcγRIIb with BCR complex, suggesting that this approach has potential to suppress multiple B cell activation signals in autoimmune diseases.

Coengagement of BCR complex and FcγRIIb may not only represent a therapeutic modality for autoantibody-associated diseases such as SLE, idiopathic thrombocytopenic purpura, and myasthenia gravis, but it may be more widely applicable across T cell-driven autoimmune diseases. The ability of BCR complex-FcγRIIb coengagement to inhibit CD80 and CD86 expression by B cells points to inhibition of their APC capacity. In addition, activity of XmAb5871 in PBMC-engrafted SCID mice against a human antitetanus response, a T cell-mediated process, also suggests potential clinical applications beyond SLE. Given that B cell depletion is effective in murine models of classical T cell-mediated diseases, and also in human multiple sclerosis, rheumatoid arthritis, and type I diabetes (63–65), it stands to reason that inhibition of B cell functions such as Ag presentation will attenuate development of autoreactive pathogenic T cells.

Of great potential importance from a safety standpoint, our results demonstrate that suppression of B cell activation can be achieved without global B cell depletion. This markedly contrasts with data presented in this article and elsewhere showing that XmAb5574, rituximab, and a murine surrogate for rituximab act via potent B cell depletion (18, 20, 26). Notably, XmAb5574 has an identical anti-CD19 Fv domain to XmAb5871 but contains an Fc domain engineered for high affinity to activating receptors FcγRIIa and FcγRIIIa (Supplemental Table I) (18, 21). In marked contrast with XmAb5871, XmAb5574 depletes B cells in human PBMC and in cynomolgus monkeys, and is intended as therapy for B cell leukemias and lymphomas, where potent B cell depletion is desirable (19). The dramatically different properties of these two therapeutic Abs, generated solely by manipulating Fc affinity for inhibitory versus activating FcγRs, demonstrates the central role of IgG Fc and its receptors in modulating diverse immune responses to specific Ags.

In conclusion, our results demonstrate an effective method to suppress activated B cells via the natural negative feedback pathway normally stimulated by IC. We show that a recombinant Ab engineered to engage FcγRIIb via its Fc domain is a potent suppressor of B cell activation ex vivo and of humoral immune responses in humanized mouse models. The inhibitory effects of XmAb5871 on costimulatory molecules, cytokine- and LPS-mediated proliferation, and BCR activation indicate that high-affinity coengagement of FcγRIIb and BCR complex may be effectively immunosuppressive in multiple in vivo settings. This FcγRIIb-optimized Fc domain may also be incorporated into Abs against other targets on B cells and on other FcγRIIb-expressing cells of the immune system; for example, its combination with an anti-IgE Fv domain may facilitate suppression of only IgE^+ allergic B cells. The therapeutic mechanism described in this article can globally suppress activated B cells without depletion and also has the potential to selectively suppress specific B cell populations in allergic and autoimmune diseases.

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Disclosures

H.M.H., S.Y.C., E.P., S.C., I.W.L.L., J.Z., J.R.D., and D.E.S. are employees of Xencor and hold stock options in the company. All other authors have no financial conficts of interest.

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Supplemental Table I. Therapeutic and control antibodies used.

Antibody	Antigen specificity[a]	Fc domain & species	Fc mutations	FcγR binding properties	Purpose
XmAb5871	Human CD19	Human IgG1	S267E L328F	↑FcγRIIb	Therapeutic candidate for autoimmune disease (17)
XmAb5574	Human CD19	Human IgG1	S239D I332E	↑FcγRIIa ↑FcγRIIIa	Control B cell depleter with enhanced binding to activating Fcγ receptors (FcγR) (18, 19)
XENP6187	Human CD19	Human IgG1	L328R G236R	↓FcγRI, ↓FcγRIIa ↓FcγRIIb, ↓FcγRIIIa	Fv control lacking FcγR interactions (17)
XENP5603	Human CD19	Human IgG1	None	Native	Fv control possessing native FcγR interactions (17)
Rituximab	Human CD20	Human IgG1	None	Native	Control; known B cell depleter (20)
XENP6003	RSV[b] F protein	Human IgG1	S267E L328F	↑FcγRIIb	Isotype control for XmAb5871 and XENP8206 (17, 66)
XENP8206	Mouse CD19	Human IgG1	S267E L328F	↑FcγRIIb	Surrogate of XmAb5871 for human FcγRIIb transgenic mouse models (25)
XENP8205	Mouse CD19	Human IgG1	None	Native	Surrogate of XENP5603; control possessing native FcγR interactions (25)
XENP8207	Mouse CD19	Human IgG1	L328R G236R	↓FcγRI, ↓FcγRIIa ↓FcγRIIb, ↓FcγRIIIa	Surrogate of XENP6187; control lacking FcγR interactions (25)
XENP8243	Mouse CD20	Mouse IgG2a	None	Native	Surrogate of rituximab for mouse models (26)
XENP6293	Human CD79b	Human IgG1	L328R G236R	↓FcγRI, ↓FcγRIIa ↓FcγRIIb, ↓FcγRIIIa	Tool to crosslink and activate BCR without interference by FcγR interactions (17)

[a]All antibodies to individual antigens target same epitope and have identical Fv domains
[b]RSV, Respiratory Syncytial Virus

Supplemental Table II. Clinical data on SLE donors.

Donor	Age (yr)	Gender	Race	SLEDAI	Daily prednisone equivalent (mg)	Other immunomodulatory medications	Other clinical conditions	ANA titer	dsDNA titer	Suppression by XmAb5871
1	20	F	H	6	17.5	MMF, HCQ		160	<10	Yes
2	23	F	H	NA	17.5	MMF, HCQ		1280	<10	Yes
3	34	F	A	12	40	AZA		1280	160	Yes
4	32	F	H	6	20	AZA, HCQ		640	<10	ND
5	31	F	A	4	20	MMF, HCQ		NA	10	Yes
6	36	F	H	NA	10	AZA, HCQ	Hemodialysis	320	160	Yes
7	24	F	A	8	5	MMF, HCQ		NA	160	Yes
8	49	F	H	20	20	MTX, HCQ		1280	2560	Yes
9	49	F	A	0	0	AZA, HCQ		320	<10	Yes
10	33	F	H	8	20	CYC		640	<10	Yes
11	45	F	H	2	40	MMF, HCQ		80	<10	Yes
12	46	F	H	16	40	CYC, HCQ		640	40	Yes
13	22	F	H	10	3	AZA, HCQ		1280	640	Yes
14	21	F	H	18	70	RTX, CTX, HCQ		1280	<10	ND
15	41	F	H	14	5	MMF, HCQ		640	160	Yes
16	31	F	H	10	10	MMF, HCQ		1280	<10	Yes
17	43	F	H	10	0	MMF, HCQ	Anti-phospholipid syndrome	80	<10	Yes
18[*]	19	F	H	12	20	MMF, HCQ		1280	640	Yes
19	53	F	H	NA	3	LEF, HCQ	Concurrent rheumatoid arthritis	160	10	Yes
20	28	F	H	4	0	HCQ		80	<10	Yes
21	46	F	H	6	0	None	Hemodialysis	<40	160	Yes
22	23	F	H	10	25	MMF, HCQ		320	20	Yes
23	48	F	H	4	10	AZA, HCQ		320	20	Yes
24	34	F	H	4	5	MMF, HCQ		NA	160	Yes
25	32	M	H	4	20	AZA, HCQ		320	<10	Yes
26	61	F	AA	NA	0	None		320	<10	Yes
27	53	M	H	2	10	MTX, HCQ		1280	<10	Yes
28[*]	39	F	H	12	4	AZA, HCQ	Anti-phospholipid syndrome	160	160	Yes
29	32	F	H	0	30	MMF, HCQ		640	<10	Yes
30	21	F	C	8	60	MMF, HCQ		1280	10240	Yes

Abbreviations: F, Female; M, Male; H, Hispanic; A, Asian; AA, African-American; C, Caucasian; NA, not available (1 or more elements of the SLEDAI were not obtained); ND, not done (insufficient numbers of B cells to perform calcium mobilization assay); MMF, mycophenolate mofetil; HCQ, hydroxychloroquine; AZA, azathioprine; CYC, cyclophosphamide; RTX, rituximab; LEF, leflunomide.

[*]Donors 18 and 28 underwent leukapheresis for PBMC-SCID mouse studies in Fig. 9*A* and 9*B* and *C*; respectively; donor 18 B cells were used in Fig. 7.