

TABLE I. IgE and FcγRIIb binding affinities of therapeutic and control antibodies

Antibody	Anti-IgE Fv	Binding to human IgE				Binding to human FcγRIIb						Purpose
		K _D (nM)*	Fold affinity vs parent mAb	k _a (1/Ms) (× 10 ⁺⁵)	k _d (1/s) (× 10 ⁻⁵)	Fc mutations†	FcγR binding properties	K _D (nM)	Fold affinity vs parent mAb	k _a (1/Ms) (× 10 ⁺⁵)	k _d (1/s) (× 10 ⁻²)	
XENP6727	Chimeric MaE11	0.112	1.0	4.22	4.71	None	Native	3100	1.0	2.33	72.0	Original mouse antihuman IgE chimeric mAb
XENP6728	Humanized‡ MaE11	0.073	1.5	3.76	2.73	None	Native	3430	0.90	1.47	50.3	Fc control possessing native IgG1 FcγR interactions
XmAb7195	Humanized MaE11	0.075	1.5	3.83	2.86	S267E L328F	↑FcγRIIb	7.74	400	4.40	3.41	Therapeutic candidate
XENP7196	Humanized MaE11	0.109	1.0	3.72	4.07	G236R L328R	↓FcγRI ↓FcγRIIa ↓FcγRIIb ↓FcγRIIIa	n.b.	n.b.	n.b.	n.b.	Fc control lacking FcγR interactions
Omalizumab (XENP6402)	Humanized§ MaE11	0.393	0.3	2.15	8.44	None	Native	6370	0.49	0.72	45.9	Approved drug; known to sequester free IgE, increase total IgE

n.b., No detectable binding.

*K_D, Dissociation constant; k_a, on-rate; k_d, off-rate.

†All Fc mutations are in human IgG1 backbone.

‡Humanized by human string content optimization.²¹§Humanized by CDR grafting.⁴

mediated by immune complex coengagement of FcγRIIb and antigen-specific B-cell receptor (BCR) suppresses B-cell proliferation and differentiation into plasma cells.^{13,15} The role of FcγRIIb in modulating IgE immune complex-mediated B-cell responses is less well understood than it is for IgG. However, it is notable that FcγRIIb binds to IgE Fc with a similar low affinity as to IgG1 Fc and may therefore regulate IgE in addition to IgG production.^{16,17} We therefore reasoned that FcγRIIb-mediated B-cell regulation could be exploited for therapeutic intervention in IgE-mediated allergic diseases. Previously, we engineered the Fc domain of human IgG1 to bind selectively and with greater than 400-fold affinity to FcγRIIb.¹⁸ We established that this novel Fc domain, when integrated into a humanized antibody targeting all CD19⁺ B cells, could block BCR-induced activation and suppress an immunization response in human PBMC-engrafted severe combined immunodeficiency (huSCID) mice.¹⁹ In this report, we now refine this approach by using a new therapeutic antibody that suppresses only IgE⁺ B cells. Through humanization, affinity maturation, and Fc engineering of the murine parental antibody of omalizumab, we have created an antibody that sequesters free serum IgE and also coengages FcγRIIb and IgE BCR to selectively suppress the activation and differentiation of allergic B cells without affecting other B-cell isotypes. We show that this antibody, XmAb7195, in contrast to omalizumab, reduces both free and total human IgE levels in huSCID mice without altering the normal production of human IgM and IgG. Likewise, we show that a murine surrogate of XmAb7195 also reduces free and total murine IgE levels in immunocompetent mice expressing human FcγRIIb. Our results suggest that a therapeutic antibody possessing 2 complementary mechanisms of action,

IgE sequestration and IgE⁺ B-cell suppression, may have advantages in the treatment of allergic diseases as compared with antibodies possessing either mechanism alone.

METHODS

Cells and antibodies

For *in vitro* experiments, human PBMCs were purified from the blood of healthy donors obtained under an institutional review board-approved protocol (HemaCare, Van Nuys, Calif) by density gradient using Ficoll-Paque Plus (GE Healthcare, South Plainfield, NJ). The mouse antihuman IgE antibody MaE11,^{4,20} the parental antibody of omalizumab (rhuMab-25), was humanized by using string optimization.²¹ The humanized variable region heavy and light domains were generated by gene synthesis (Blue Heron Biotechnology, Bothell, Wash) and subcloned into the vector pTT5²² encoding human heavy IgG1 and light Cκ constant regions. Fc substitutions (S267E/L328F FcγRIIb binding-enhanced^{18,23} or G236R/L328R Fcγ receptor binding-ablated¹⁹) were introduced by site-directed mutagenesis. Antibodies were produced by using the HEK293E transient expression system²² and purified by using protein A affinity chromatography (GE Healthcare). See Table I for a description of anti-IgE antibodies used.

IgE and FcγRIIb binding affinities

Surface plasmon resonance measurements for calculating binding affinities were performed by using a Biacore 3000 instrument (GE Healthcare). Antibody affinity for FcγRIIb was determined as described²⁴ by immobilizing antibodies on a protein A CM5 biosensor chip (GE Healthcare) and injecting soluble FcγRIIb over antibody-bound surface. To determine the antibody affinity for human IgE, a protein A CM5 biosensor chip was generated by using a standard primary amine coupling protocol. All measurements were performed in buffer containing 10 mM HEPES pH 7.4, 0.15 mol/L NaCl, 3 mM EDTA,

and 0.005% v/v surfactant P20 (GE Healthcare). Antibodies at 25 nM were immobilized on the protein A surface for 48 seconds at 10 μ L/min, giving an approximate gain of 1000 response units. Human IgE in 2-fold serial dilutions (4 concentrations starting at 25 nM) was injected over antibody-bound surface for 2 minutes at 20 μ L/min followed by a 4-minute dissociation phase. After each cycle, the surface was regenerated by injecting glycine buffer (10 mM, pH 1.5). Data were processed by zeroing time and response before the injection of analyte and by subtracting appropriate non-specific signals (response of reference channel and injection of running buffer). Kinetic analyses were performed by global fitting of binding data with a 1:1 Langmuir binding model by using BIAevaluation software (GE Healthcare).

IgE, IgG, and IgM production from human PBMCs

Human PBMCs (3×10^6 cells) were cultured with antibodies at varying concentrations for 14 days at 37°C in RPMI-1640 containing 10% FBS, 5 ng/mL recombinant human IL-4 (Peprotec, Rocky Hill, NJ), and 250 ng/mL agonistic antihuman CD40 antibody (R&D Systems, Minneapolis, Minn). IgE production in culture medium was assessed by ELISA by using a pool of 3 anti-IgE antibodies (clones 101, 107, and 182; MabTech, Cincinnati, Ohio) for capture, with horseradish peroxidase (HRP)-conjugated antihuman IgE antibody for detection (Bethyl Laboratories, Montgomery, Tex). To eliminate IgG assay interference arising from therapeutic antibodies and controls (which were all human IgG1), specific levels of IgG2 were assessed by ELISA by using mouse antihuman IgG2 antibody (clone G18-21, BD Pharmingen, San Jose, Calif) for capture, with HRP-conjugated donkey antihuman IgG (H+L)-specific antibody for detection (Jackson ImmunoResearch Laboratories, West Grove, Pa). For IgM quantitation, polyclonal goat antihuman IgM F(ab')₂ fragment was used for capture and HRP-conjugated polyclonal goat anti-IgM antibody was used for detection. Standard curves were generated by using human IgE (Bethyl Laboratories), human IgG2 (Novabiochem, San Diego, Calif), and human IgM (Jackson ImmunoResearch). For quantification, an HRP substrate system (KPL, Gaithersburg, Md) was used according to the manufacturer's protocol.

Human PBMC engraftment of SCID mice

All animal experiments were performed according to Institutional Animal Care and Use Committee-approved protocols. For huSCID mouse models, immunodeficient SCID mice (Taconic, Cambridge City, Ind) were treated on day 1 with 100 μ L of anti-asialo GM antibody (Wako, Richmond, Va) to deplete natural killer cells and on day 0 with an intraperitoneal injection of 3.0×10^7 human PBMCs obtained by leukapheresis under an institutional review board-approved protocol (HemaCare). Mice were dosed intraperitoneally with antibodies on day 8 or 9 or as described. Blood was collected by retro-orbital sinus puncture for the determination of total human IgM, IgG, and IgE, free human IgE, and therapeutic antibody levels by using the methods described below. In the first huSCID study, mice were also injected on days 11 and 26 with 15 μ g each of dust mite antigens Der p 1 and Der p 2 (LoTox Natural Der p 1 and Der p 2, Indoor Biotechnologies, Charlottesville, Va), although no antigen-specific IgE could be detected in vehicle-treated or any other group (data not shown).

Quantitation of human immunoglobulin and therapeutic antibody levels in PBMC-engrafted SCID mice

Human IgE, IgG, and IgM concentrations in mouse sera were measured by using immunoassays. For total IgE, plates were coated with antihuman IgE antibodies (clones 101, 107, and 182; MabTech). For free IgE, the coating protein was human Fc ϵ RI (produced in-house). The detection system for both assays used a biotin-labeled goat antihuman IgE antibody (KPL, Gaithersburg, Md) with europium-labeled streptavidin (PerkinElmer, Waltham, Mass). The IgG level was determined by using a dissociation-enhanced lanthanide fluorescent immunoassay-based sandwich immunoassay in which a goat

antihuman IgG Fc-specific antibody (Jackson ImmunoResearch) was used for capture and a goat antihuman kappa chain-specific antibody (Invitrogen, Grand Island, NY) was used for detection in the first and third huSCID studies. Signals of all dissociation-enhanced lanthanide fluorescent immunoassays were read by using the Wallac Envision reader (PerkinElmer) with time-resolved fluorescence settings. ELISA kits were used to determine IgG (Zeptometrix, Buffalo, NY) and IgM (Alpha Diagnostic, San Antonio, Tex) levels in the fourth huSCID study, following the manufacturers' protocols.

To quantify the serum concentrations of free omalizumab and XmAb7195, plates were coated with human IgE (Fitzgerald Industries, Acton, Mass). Antibodies were detected by using europium-labeled antihuman IgG (PerkinElmer), and signals were read by using the Wallac Envision reader (PerkinElmer).

Activation of human B cells in huSCID mice by polyclonal anti-IgE

SCID mice were engrafted with human PBMCs as described above. Seven days after engraftment, mice were treated with antibodies XmAb7195 or XENP7196 (the Fc γ R knockout control for XmAb7195) at 10 mg/kg twice weekly throughout the study. After 2 days, mice were injected intraperitoneally with PBS or chicken polyclonal anti-IgE IgY (Abcam, Cambridge, Mass) at doses indicated. Twenty days after engraftment, blood was collected for the determination of total human IgE levels by using ELISA.

Induction of IgE in C57BL/6 mice transgenic for human Fc γ RIIb

C57BL/6 mice transgenic for human Fc γ RIIb (*Fcgr2b*^{-/-} *hFCGR2B*⁺)^{25,26} were obtained from Jeffrey Ravetch (Rockefeller University). Mice were injected intraperitoneally with antibodies XENP8252 or XENP8253 at 10 mg/kg twice weekly throughout the study, starting on day 0. Both antibodies are derived from R1E4, a rat antimouse IgE used as a surrogate for omalizumab^{27,28} and contain identical rat antimouse IgE Fv domains. XENP8252 is a chimeric murine surrogate for omalizumab containing a native human IgG1 Fc domain. XENP8253 is a chimeric murine surrogate for XmAb7195 containing an engineered human IgG1 Fc domain including S267E and L328F mutations to enhance binding to human Fc γ RIIb (as in Table I). To boost total IgE responses, mice were immunized with 40 μ g of chicken ovalbumin (Sigma-Aldrich, St Louis, Mo) in complete Freund's adjuvant (Chondrex, Redmond, Wash) injected intraperitoneally on day 2. Blood was drawn by retro-orbital sinus puncture on days indicated to assess mouse free and total IgE and total IgG levels by using ELISA.

Quantitation of serum level of, and mouse immune response to, anti-IgE antibodies in human Fc γ RIIb transgenic mice

To quantify the serum concentrations of XENP8252 and XENP8253, plates were coated with goat antihuman IgG Fc-specific antibody (Jackson ImmunoResearch). Antibodies were detected by using europium-labeled antihuman kappa chain-specific antibody (Invitrogen) and signals were read by using the Wallac Envision reader (PerkinElmer). To assess the formation of mouse anti-XENP8252 or anti-XENP8253 antibodies in immunocompetent C57BL/6 mice, dissociation-enhanced lanthanide fluorescent immunoassay-based bridging assays were used. Unlabeled drugs were used for capture, and europium-labeled drugs were used for detection.

Statistical analyses

Statistical significance was determined with Prism software (GraphPad Software, La Jolla, Calif) using 1-way ANOVA with Dunnett posttest for multiple comparisons versus vehicle; the exception to this is the second huSCID study, in which repeated-measures 1-way ANOVA was used. Levels of significance were defined as $P < .05$ (*), $P < .01$ (**), and $P < .001$ (***).

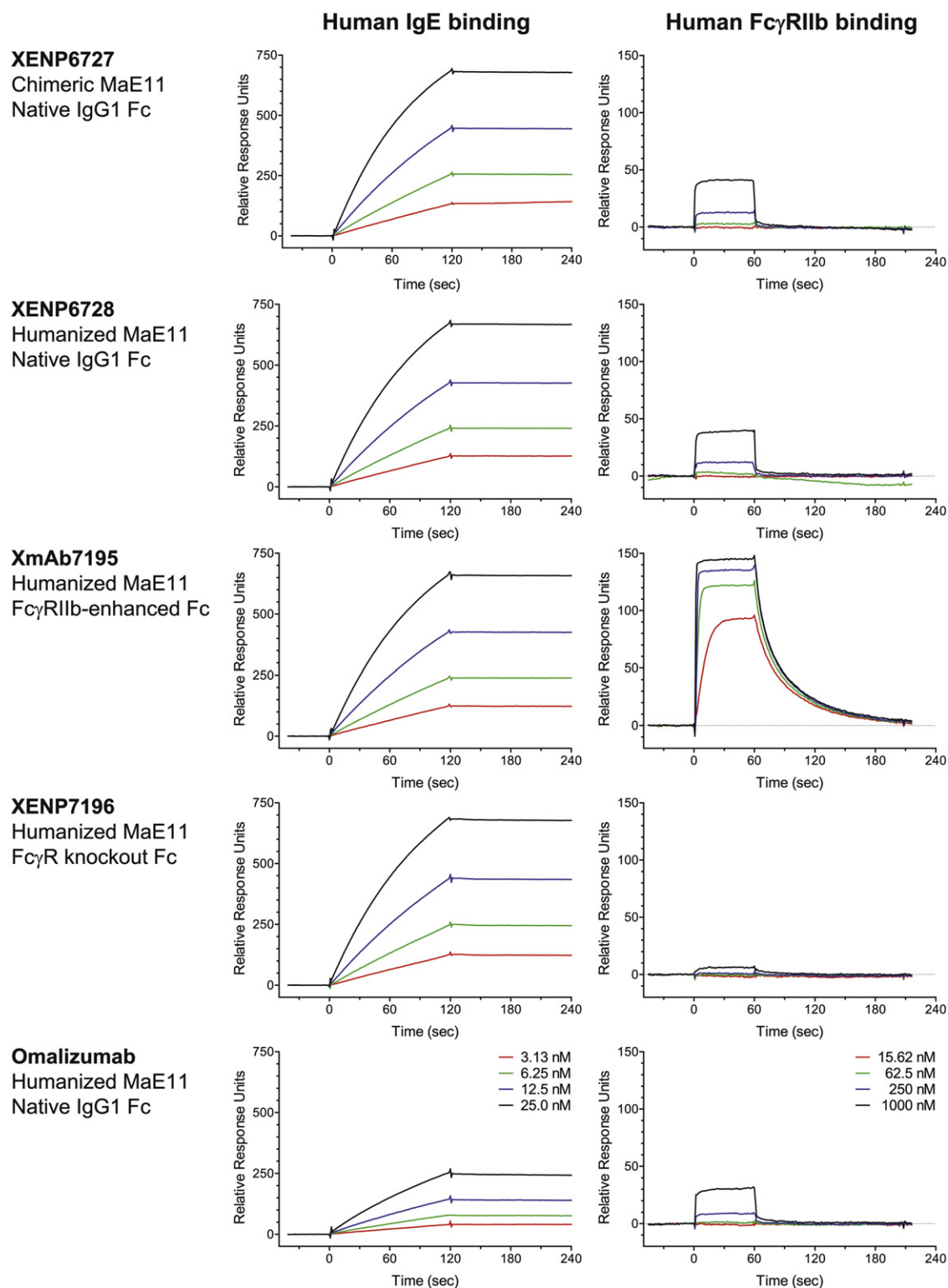


FIG 1. Increased affinity for soluble human IgE and FcγRIIb of XmAb7195 relative to omalizumab. Surface plasmon resonance traces of Fv domain of full-length antibody binding to IgE (*left panel*) and of Fc domain of full-length antibody binding to Fc inhibitory receptor FcγRIIb (*right panel*). See [Table 1](#) for affinity determinations. Binding curves for IgE were performed at 3.13, 6.25, 12.5, and 25.0 nM; binding curves for FcγRIIb were performed at 15.62, 62.5, 250, and 1000 nM.

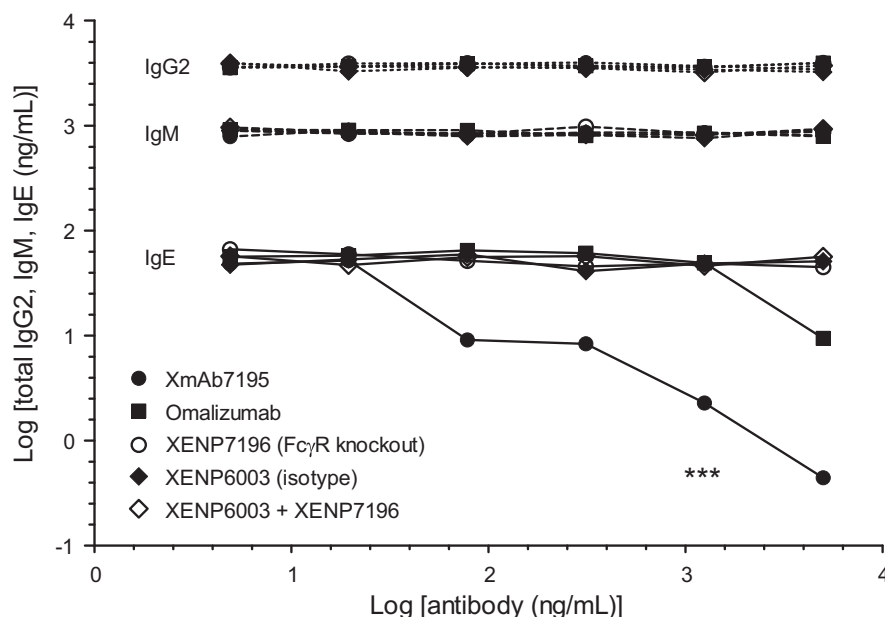


FIG 2. Inhibition of IgE but not IgG2 or IgM production by XmaB7195 in cultured human PBMCs. PBMCs were cultured for 14 days to stimulate the differentiation of B cells into plasma cells and subsequent production of immunoglobulins. Results shown represent 1 study of PBMCs from a single healthy donor and are representative of at least 4 independent experiments using PBMCs from 4 donors. Dose responses were fit by using linear regression of log-transformed data, and slopes and standard errors were calculated for each group. A 1-way ANOVA with Dunnett posttest for multiple comparisons was then used to compare all groups versus isotype control XENP6003. The suppressive effect of XmaB7195 is significant for IgE but not for IgM or IgG2 (*** $P < .001$); if unlabeled, not significant ($P > .05$).

RESULTS

Humanization and Fc engineering generated an antibody with higher affinity for human IgE and FcγRIIb relative to omalizumab

The mouse antihuman IgE antibody MaE11 is the parent of the humanized antibody rhumAb-E25,^{6,7} also known as omalizumab (Xolair). To avoid the stimulation of antibody-induced cross-linking of FcεRI and consequent mast cell degranulation, MaE11 was selected because it does not bind to IgE already bound to FcεRI.^{4,29} MaE11 also binds to IgE BCR as well as to soluble IgE.⁸ Because our strategy to activate FcγRIIb signaling requires antibody binding to IgE BCR but precludes binding to FcεRI-bound IgE, we likewise used MaE11 as our starting point for humanization and Fc engineering. We first fused the Fv domain of MaE11 to human IgG1 Fc to make the chimeric antibody XENP6727 (see Table 1 for a description of all antibodies generated). We next humanized XENP6727 by using the method of string content optimization²¹ to create XENP6728, an antibody with a slightly higher human sequence identity than that of omalizumab (83% for XENP6728 vs 80% for omalizumab). We next incorporated amino acid modifications S267E and L328F in the Fc region of XENP6728 to generate high-affinity binding to FcγRIIb,¹⁸ which resulted in the therapeutic candidate XmaB7195. These mutations reduce affinity to FcγRIIIa, a key activating receptor, to undetectable levels and do not increase affinity for activating receptors FcγRI and FcγRIIa (H131 allele) relative to native IgG1 Fc.^{18,23} Finally, to create a control antibody useful in assessing Fcγ receptor interactions, we mutated XENP6728 to form XENP7196, which contains G236R and L328R mutations in its Fc domain that abolish binding to Fcγ receptors.¹⁹ By using surface plasmon resonance, we assessed the

binding affinity for both human IgE and FcγRIIb of these 4 antibodies relative to omalizumab (Fig 1 and Table I). XENP6728 and XmaB7195, humanized via string optimization, have an IgE binding affinity 5.3-fold tighter than that of omalizumab and 1.5-fold tighter than that of the MaE11 parent. The observed 0.39 nM binding affinity of omalizumab for human IgE is comparable to the reported affinity of 0.17 nM.⁷ Binding affinities of omalizumab, XENP6727, and XENP6728 for human FcγRIIb were weaker than 3 μM, as expected for the interaction of native IgG1 with this low-affinity Fc receptor. In contrast, the observed 7.7 nM K_D affinity of the Fc domain of XmaB7195 for FcγRIIb was at least 400-fold greater than the affinity of the Fc domains of chimeric MaE11 and omalizumab, owing to the introduction of Fc substitutions S267E and L328F.^{18,23}

Coengagement of FcγRIIb with IgE BCR inhibited IgE but not IgG or IgM secretion in human PBMCs

To assess the effects of Fc-engineered anti-IgE antibodies on the activation and differentiation of B cells expressing different immunoglobulin isotypes, we quantified IgE, IgG, and IgM secretion from human PBMC cultures stimulated with IL4 and anti-CD40 antibody (Fig 2).³⁰ IgE levels were suppressed in a dose-dependent manner by XmaB7195 but not by XENP7196, the knockout control lacking binding to Fcγ receptors, or by XENP6003, the anti-respiratory syncytial virus isotype control with the same Fc domain as XmaB7195. The lack of IgE suppression by the combination of isotype and FcγR knockout controls, which together match the high Fc and Fv affinities of XmaB7195 for IgE and FcγRIIb, respectively, demonstrates that selective inhibition requires coengagement of FcγRIIb with IgE BCR by a

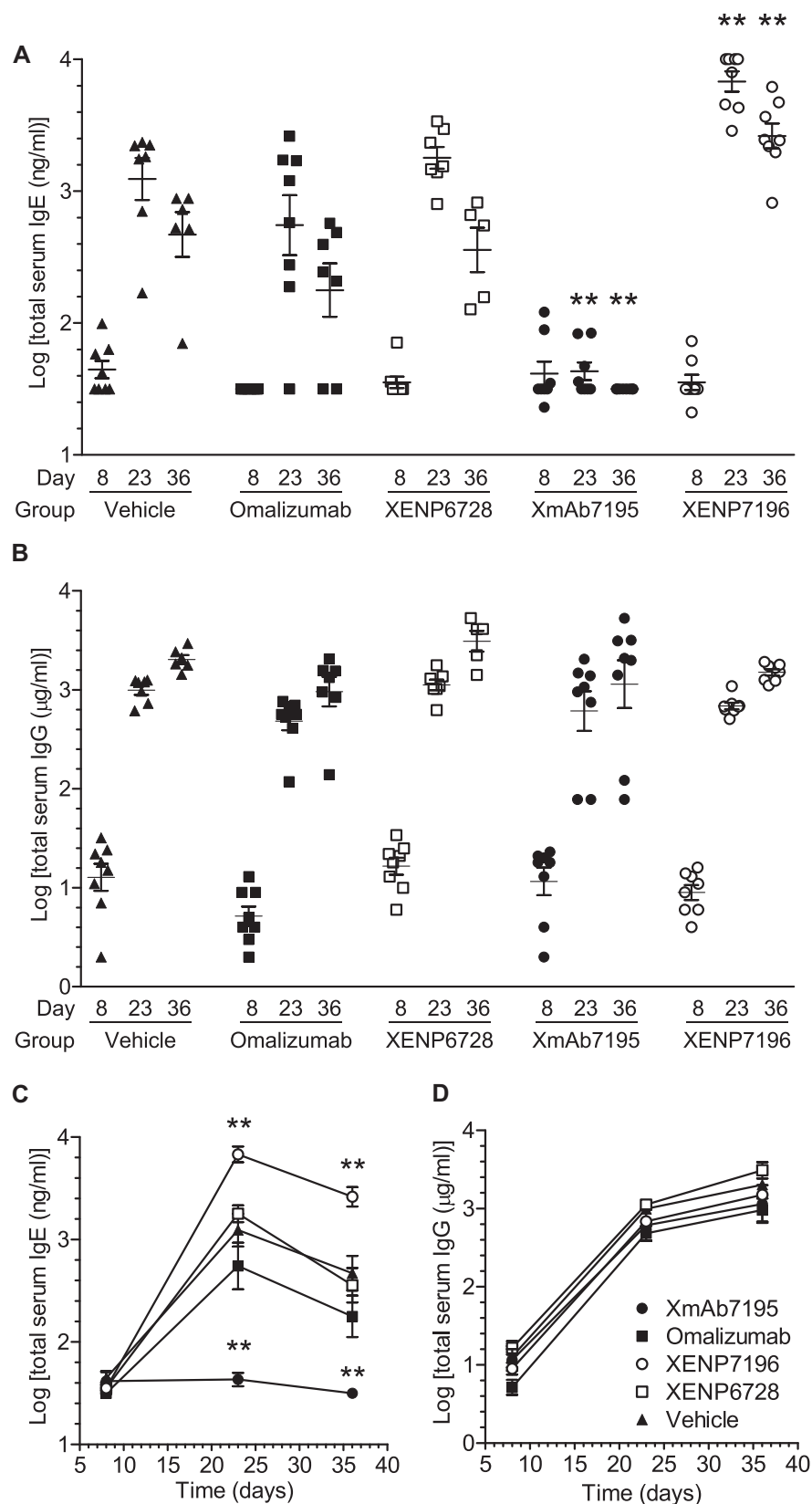


FIG 3. Suppression of human IgE but not IgG production by XmAb7195 in huSCID mice. PBMCs were engrafted on day 0, antibodies (10 mg/kg) were given on days 9 and 24, and total human IgE and IgG levels were measured in mouse serum at days 8, 23, and 36. **A**, Total serum IgE levels. **B**, Total serum IgG levels. **C** and **D**, Data for **A** and **B**, respectively, plotted as the time course of group averages. Results are representative of at least 4 similar experiments. Data are for 8 mice per group, and error bars represent mean $\pm$ SEM

single antibody (as opposed to independent engagement of both receptors by different antibodies). Omalizumab suppressed IgE only at the highest concentration tested, probably because of its weaker binding to FcγRIIb. To address whether inhibition by XmAb7195 was specific to IgE secretion, we also examined IgG2 and IgM production in the same human PBMC cultures. The IgG2 subtype was measured to avoid assay interference from the antibodies tested, which were all of the IgG1 subtype. Notably, no antibodies affected IgG2 or IgM production at any concentration, further demonstrating that high-affinity engagement of FcγRIIb on naive IgM⁺ or class-switched IgG⁺ B cells is not sufficient to suppress their activation.

XmAb7195 selectively suppressed IgE but not IgG or IgM production in huSCID mice

Like omalizumab, XmAb7195 is designed for human use and does not cross-react with mouse Fcγ receptors or IgE, precluding its assessment in common murine models of allergy. The huSCID mouse is well established as a surrogate system to partially recapitulate human immune responses³¹; therefore, we elected to use this model to assess the *in vivo* activity of therapeutic antibodies and controls. The baseline levels of both IgE and IgG at 8 days postengraftment with 3×10^7 human PBMCs (Fig 3) indicated that subsequent higher signals were due to immunoglobulin secretion by newly differentiated human plasma cells. Vehicle-treated mice showed high serum levels of both IgE (Fig 3, A and C) and IgG (Fig 3, B and D) by 23 days postengraftment, reaching near-physiological levels (greater than 6 μg/mL for IgE and greater than 1 mg/mL for IgG), with IgE decreasing and IgG increasing at day 36. Other groups were treated with 10 mg/kg of antibodies 9 days after engraftment. XmAb7195 strongly suppressed IgE production by 29-fold relative to vehicle, while anti-IgE antibodies possessing native IgG1 Fc domains (omalizumab and XENP6728) did not. Notably, XENP7196, the control for XmAb7195 and XENP6728 that lacks Fc binding to FcγRIIb, stimulated IgE production *in vivo* by 6-fold relative to vehicle. This result suggests that the cross-linking of BCR in the absence of FcγRIIb coengagement activates IgE⁺ B cells, increasing plasma cell number and consequently IgE production. Because omalizumab and XENP6728 did not cause this effect, this result also implies that the native IgG1 Fc domains of these antibodies are capable of some regulation of IgE production, presumably through their weak affinity for FcγRIIb. In contrast to the markedly diverse effects on total IgE production caused by antibodies possessing different Fc domains, production of total IgG remained unaffected by any antibody (Fig 3, B and D), further demonstrating that the stimulation of this B-cell inhibitory pathway was absolutely dependent on high-affinity coengagement of FcγRIIb with IgE BCR.

To explore the temporal relationship between total versus free IgE levels, we again tested XmAb7195 and omalizumab in the huSCID model with additional isotype and Fc controls. Mice received a single 10 mg/kg dose of antibody 8 days after engraftment with PBMCs, and we measured changes in free and total human IgE levels over 5 weeks. Fig 4, A shows that both

omalizumab and XmAb7195 rapidly sequestered free IgE to comparable levels, as expected; after 1 hour, free IgE was 24-fold and 28-fold lower, respectively, relative to vehicle. XENP4546 and XENP6003, the anti-respiratory syncytial virus isotype controls for omalizumab and XmAb7195, respectively, were not effective, indicating that antibody activity required binding to free IgE. In contrast, the effects of XmAb7195 and omalizumab on total IgE levels (Fig 4, B) were markedly different. XmAb7195 suppressed total IgE levels by at least 13-fold (to below assay detection limits); in contrast, omalizumab increased total IgE levels by 2-fold. The small peak of total IgE levels detectable for 3 days after XmAb7195 treatment may be due to IgE-secreting plasma cells formed prior to drug treatment that are exhausted by day 16, when no total IgE is detectable in this group. The modest increase in total IgE induced by omalizumab may be due to its stabilization in omalizumab/IgE immune complexes or due to omalizumab-mediated cross-linking of IgE BCR leading to B-cell activation and increased IgE production. A single treatment with XmAb7195 resulted in a substantially prolonged suppression of free IgE levels for at least 20 days; in contrast, IgE levels in omalizumab-treated mice increased markedly by 3 days after treatment (Fig 4, A). The sustained suppression of free IgE levels by XmAb7195 (a 60-fold reduction vs omalizumab at day 22) is likely due to its inhibition of IgE production as seen in Fig 4, B. This long-term inhibition of total IgE levels by XmAb7195 when given several days before the peak of IgE production is in agreement with its putative B cell-suppressing mechanism of action. However, to exclude the possibility that differences in drug exposure might have contributed to the disparate effects of omalizumab and XmAb7195, we also measured serum levels of each antibody at the same time points. Omalizumab and XmAb7195 showed similar peak concentrations and overall exposure (Fig 4, C). Taken together with the lack of activity of 2 isotype control antibodies (Fig 4, A and B), these results suggest that the enhanced suppression of free and total IgE by XmAb7195 relative to omalizumab can be attributed to significantly greater affinity for FcγRIIb, as opposed to greater drug levels.

Mice shown in Figs 3 and 4 were treated with XmAb7195 or omalizumab well before the peak of IgE production in the huSCID model, and thus reflect prophylactic drug dosing in a clinical setting. To better replicate the anticipated treatment of an existing allergic human IgE response, we therefore extended these studies by waiting 21 days after PBMC engraftment for plasma cells to fully differentiate before dosing with therapeutic antibodies. Omalizumab and XmAb7195, dosed twice weekly for 14 days, again efficiently sequestered free IgE, while the isotype control XENP6003 remained ineffective (Fig 5, A). In contrast, XmAb7195 reduced total IgE levels by 36-fold after 1 week of dosing and 79-fold after 2 weeks of dosing, while the effect of omalizumab was not significantly different from that of the vehicle (Fig 5, B). Total human IgG2 levels, in contrast, remained largely unaffected by any antibody (Fig 5, C). This demonstrates that even when human plasma cells are well established *in vivo*, as indicated by human IgE levels of approximately 1 μg/mL in all groups at 3 weeks postengraftment, FcγRIIb coengagement with IgE BCR can effectively suppress IgE but not IgG

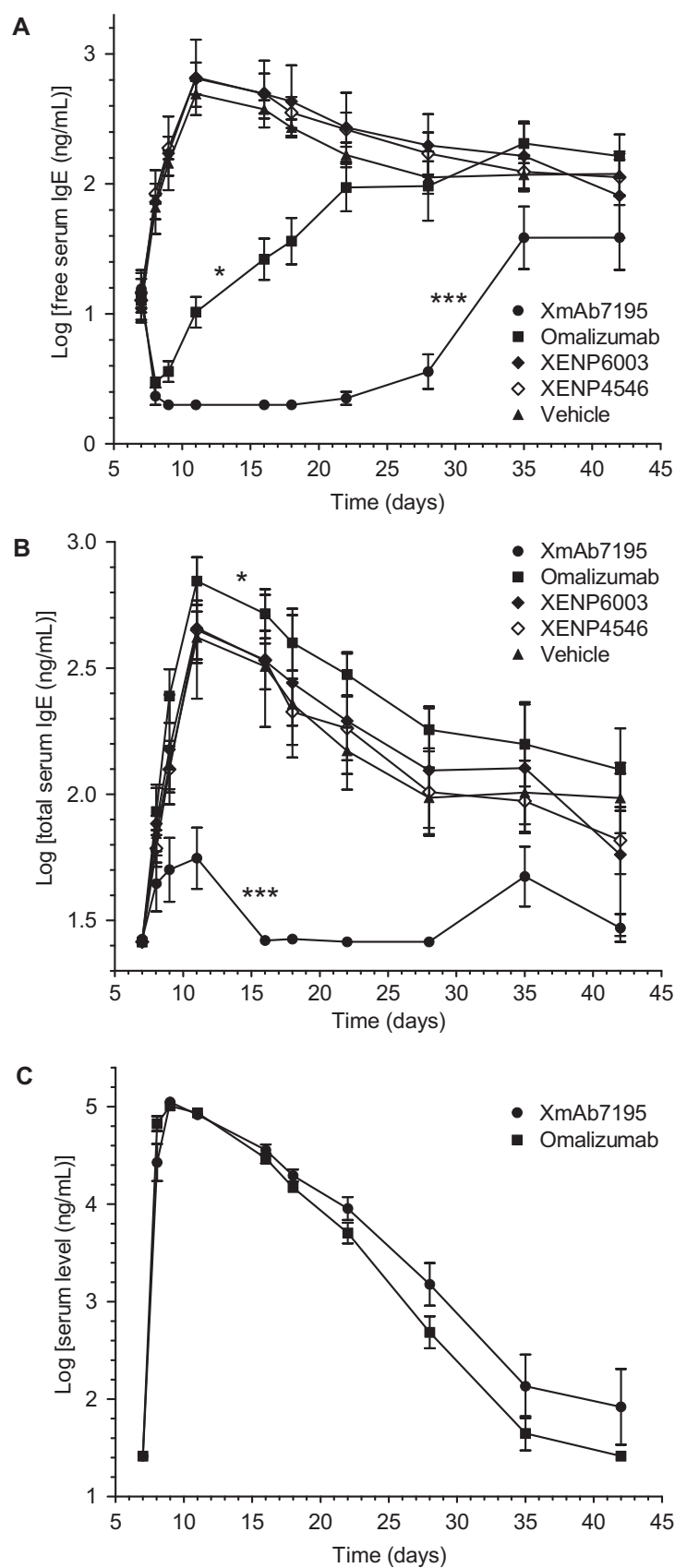


FIG 4. Suppression of free and total human IgE levels by XmAb7195 in huSCID model. PBMCs were engrafted on day 0, blood was drawn on day 7 to determine baselines, antibodies (10 mg/kg) were given once on day 8, and free and total human IgE levels in mouse serum were measured at times shown (day 8)

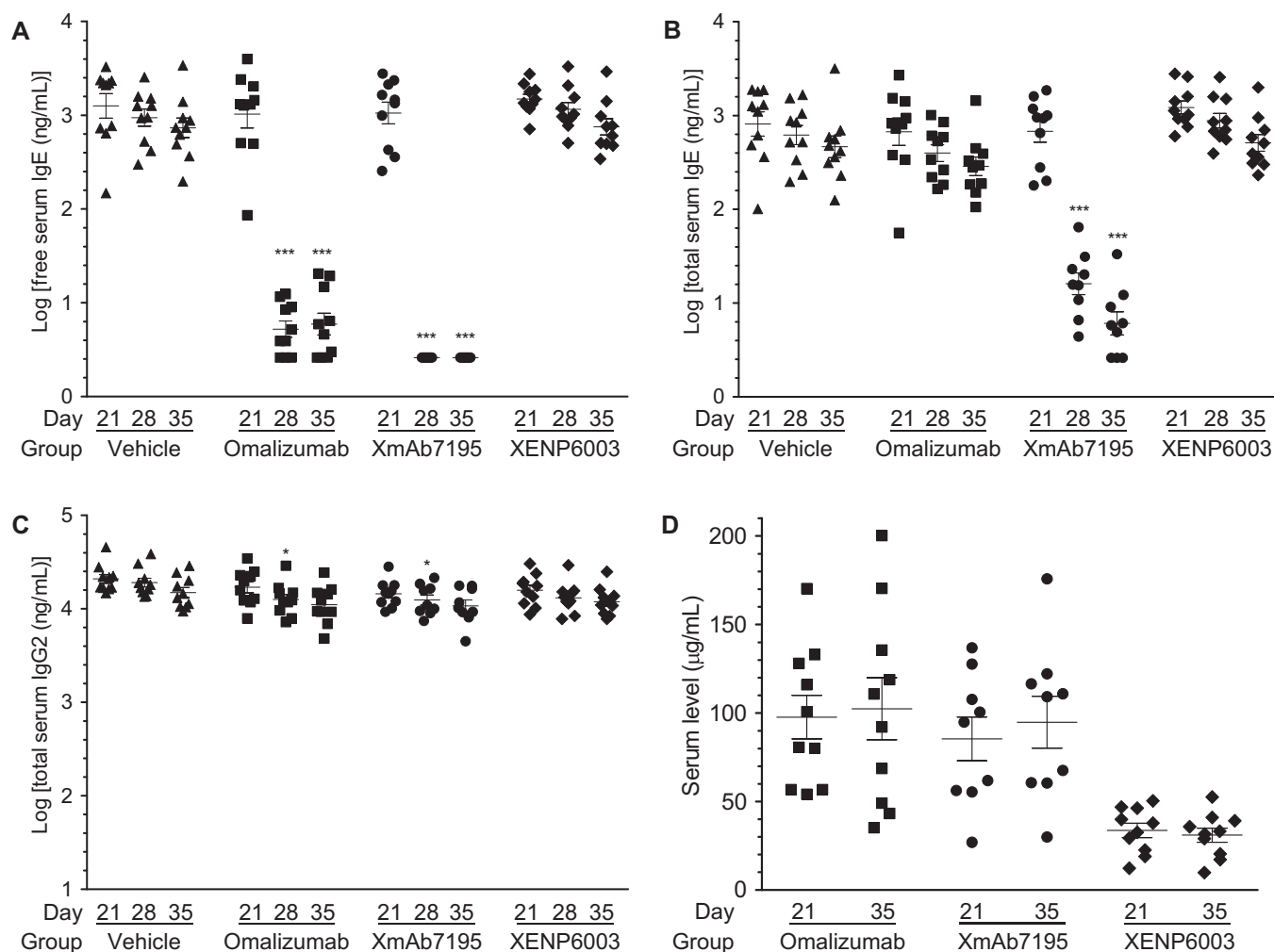


FIG 5. Suppression of IgE in established huSCID model with high baseline IgE levels. Antibodies were dosed twice weekly for 14 days starting immediately after blood was drawn on day 21. **A**, Free human IgE levels. **B**, Total human IgE levels. **C**, Total human IgG2 levels. **D**, Drug levels in day 35 serum. Data are for 10 mice per group, and error bars represent mean $\pm$ SEM of log-transformed values. *P* values in **A**, **B**, and **C** represent 1-way ANOVA with Dunnett posttest for multiple comparisons (vs vehicle at the same time point); **P* < .05; ****P* < .001; if unlabeled, not significant (*P* > .05). Values below assay limit of quantitation in **A** and **B** (2.6 ng/mL) are plotted at this baseline.

production. As in Fig 4, the contrasting effects of omalizumab and XmAb7195 on total IgE levels cannot be attributed to differences in drug half-life or exposure, because serum levels of these antibodies were similar (Fig 5, D).

To further investigate the selectivity of XmAb7195 for IgE⁺ B cells, we quantified soluble IgM, IgG, and IgE levels in another huSCID study. As a nonselective positive control, we included the Fc-engineered antibody XmAb5871.^{18,19} XmAb5871 shares an identical FcγRIIb affinity-enhanced IgG1 Fc domain with XmAb7195, but its Fv domain binds to the BCR coreceptor CD19 that is widely expressed on B cells. XmAb5871 significantly suppressed the production of human IgM, IgG, and IgE in huSCID mice (Fig 6). In contrast, XmAb7195 had no effect

on soluble IgM or IgG production yet suppressed total IgE levels more effectively than did XmAb5871. These results demonstrate that Fc-mediated engagement of FcγRIIb can block the activation of multiple B-cell isotypes if targeted to the appropriate BCR component whereas the anti-IgE variable domain of XmAb7195 confers specificity for those B cells fated to differentiate into IgE-secreting plasma cells.

XmAb7195 surrogate suppressed total IgE levels in C57BL/6 mice transgenic for human FcγRIIb

Given that XmAb7195 and omalizumab do not cross-react with mouse IgE, we next generated murine surrogates for

blood was drawn 1 h after antibody injection). **A**, Free IgE levels. **B**, Total IgE levels. **C**, Drug levels in serum. Data are for 8 mice per group, and error bars represent mean $\pm$ SEM of log-transformed values. *P* values in **A** and **B** represent repeated-measures 1-way ANOVA with Dunnett posttest for multiple comparisons (vs vehicle); ****P* < .001; ***P* < .01; **P* < .05; if unlabeled, not significant (*P* > .05). Values below assay limits of quantitation in **A** and **B** (2 and 26 ng/mL, respectively) are plotted at these baselines.

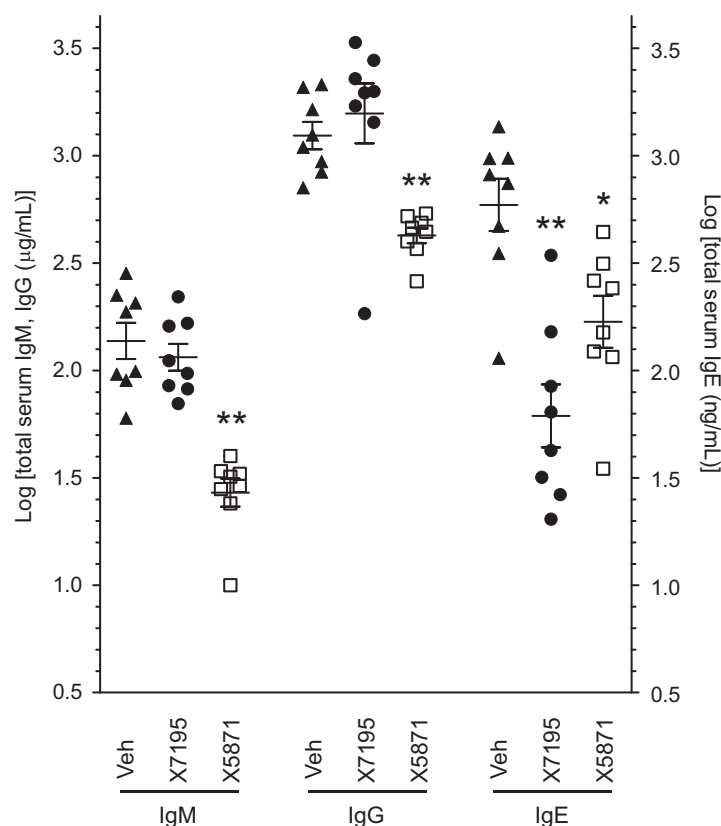
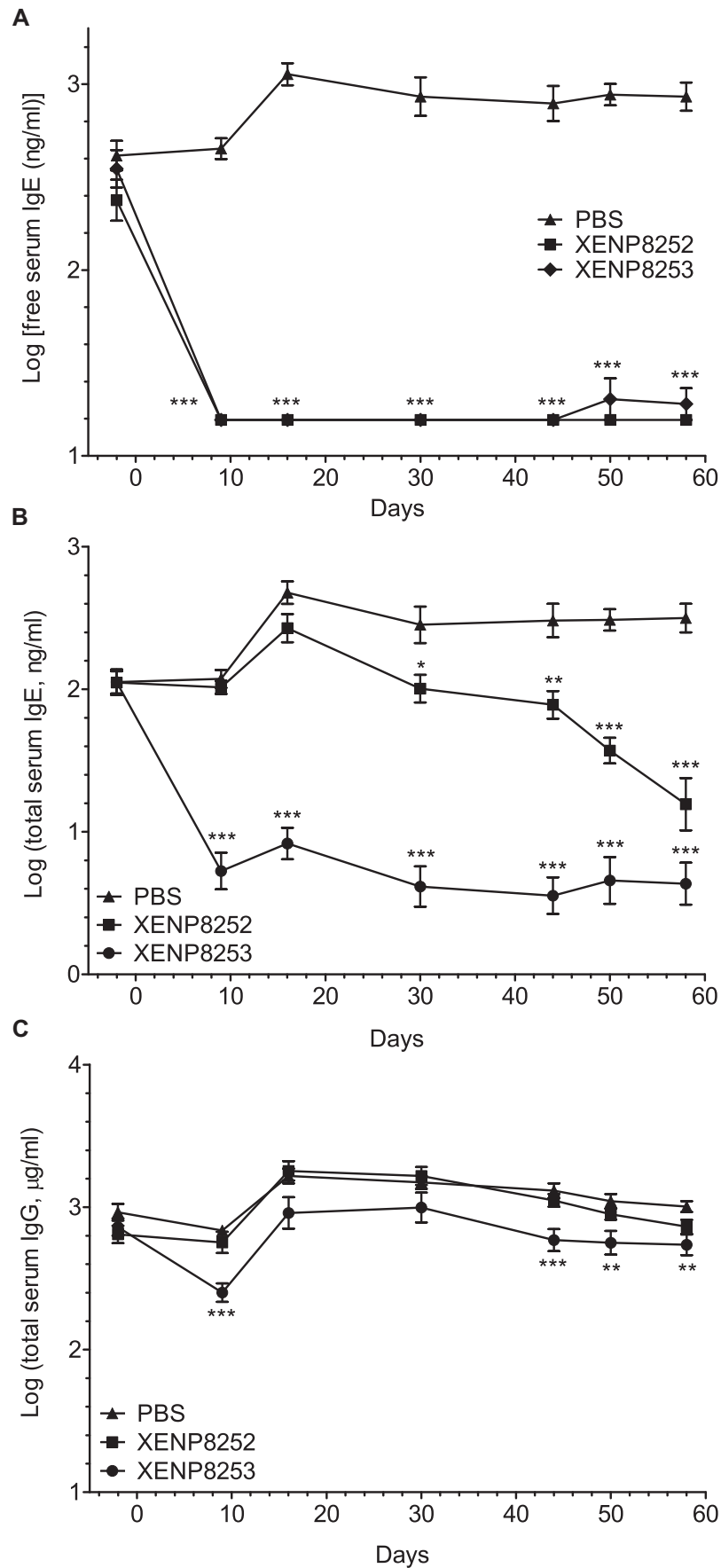


FIG 6. Selective and nonselective reduction of human IgE, IgG, and IgM in huSCID model. Mice received a single 10 mg/kg dose of antibodies 8 days after engraftment with human PBMCs, and serum was collected 14 days later. Data are for 8 mice per group, and error bars represent mean $\pm$ SEM of log-transformed values. *P* values represent 1-way ANOVA with Dunnett posttest for multiple comparisons (vs vehicle for the same isotype); ***P* < .01; **P* < .05; if unlabeled, not significant (*P* > .05). Data for vehicle and XmaB5871 controls have been previously reported.¹⁹

investigation in C57BL/6 mice transgenic for human Fc γ RIIb.^{25,26} XENP8252 is a chimeric surrogate for omalizumab containing its native human IgG1 Fc domain. XENP8253 is a chimeric surrogate for XmaB7195 containing its identical human IgG1 Fc domain modified for high affinity to Fc γ RIIb. Both antibodies contain identical rat antimouse IgE Fv domains derived from R1E4, a commonly used surrogate for omalizumab that sequesters murine IgE.²⁷ In transgenic mice treated twice weekly with surrogate antibodies, free murine IgE levels were rapidly suppressed by the first measured time point (9 days after dosing) and remained suppressed throughout the 58-day study (Fig 7, A). Similar to the results for XmaB7195 in the huSCID model, XENP8253 suppressed total murine IgE levels to below detection limits by day 9 (Fig 7, B), demonstrating that Fc γ RIIb engagement remained effective in immunocompetent mice possessing the full complement of activating Fc γ receptors. Total mouse IgE levels were also reduced at later time points by XENP8252, although the extent of suppression did not approach that of XENP8253 at any time point. The delayed activity of XENP8252 may be mediated by an Fc-dependent cell-mediated cytotoxic effect or by the weak interaction of native IgG1 Fc with human Fc γ RIIb. Production of total murine IgG remained high throughout the study, although levels in XENP8253-treated mice fell by day 10 and then stabilized, remaining consistently lower than in XENP8252-treated mice (Fig 7, C). This effect of the surrogate antibody, which was not observed for human IgG using

XmaB7195 *in vitro* or *in vivo* (Figs 2, 3, 5, 6), raises the possibility of an Fc γ RIIb-dependent effect on IgG⁺ B cells or plasma cells in this model; elucidation of a potential mechanism will require further studies using isotype control antibodies and nontransgenic mice.

The human Fc domains of both XENP8252 and XENP8253 are foreign to C57BL/6 mice and thus have the potential to be immunogenic. This raises the possibility that the resulting mouse antihuman Fc antibodies might cross-link XENP8252 or XENP8253 bound to IgE⁺ B cells, thereby activating IgE BCR and stimulating rather than suppressing IgE production. Several mice in the XENP8252- and XENP8253-treated groups in Fig 7 developed antidrug antibodies. Notably, by day 50, one XENP8253-treated mouse generated antibody levels 45-fold higher than the group average (the second strongest response was only 3.5-fold above the group average), and immunogenicity in this mouse correlated with rapid drug clearance (not shown). Despite such antidrug immune responses, suppression of total serum IgE levels was maintained during 58 days of dosing with XENP8253 (Fig 7, B). Interestingly, the mouse raising the strongest immune response did exhibit a small rebound in free serum IgE level at days 50 and 58 (Fig 7, A); however, no drug-treated mice showed free or total serum IgE levels that reached or exceeded the levels found in vehicle groups at any time point. These results with surrogate antibodies suggest that immune responses to the Fc domains of omalizumab and XmaB7195 have the



potential to partially neutralize antibody activity, but will not paradoxically stimulate activation of IgE⁺ B cells.

XmAb7195 suppressed total IgE levels induced by polyclonal anti-IgE-mediated IgE⁺ B-cell activation

To explore the ability of XmAb7195 to block a robust human IgE⁺ B-cell response, we treated huSCID mice with polyclonal antihuman IgE to cross-link IgE BCR (Fig 8). IgE⁺ B cells were activated in a dose-dependent manner by chicken anti-IgE, which increased levels of total human IgE. Under all conditions, XmAb7195 suppressed this effect (Fig 8, A). Notably, in a second study, XmAb7195 again suppressed this polyclonal IgE response while the FcγR knockout control XENP7196 did not (Fig 8, B). In agreement with our other huSCID studies, these results demonstrate that FcγRIIb coengagement with IgE BCR can suppress IgE⁺ B cells even when they are strongly activated. These results also suggest that a possible immune response against XmAb7195 in clinical use may be suppressed by its intrinsic suppression of B cells with cognate BCR.

DISCUSSION

The clinical efficacy of omalizumab attests to the role of high IgE levels in the pathogenesis of allergy and asthma. However, IgE sequestration by omalizumab does not address the underlying disease etiology: activation and differentiation of IgE⁺ B cells to generate IgE-secreting plasma cells. On the contrary, omalizumab impedes the naturally rapid clearance of IgE and thereby increases total IgE levels for months after cessation of therapy.^{2,10,11} Therefore, alternatives to IgE sequestration, including suppression of IgE production, have been explored in animal models. For example, several groups have developed therapeutic antibodies that, in contrast to omalizumab, bind only to transmembrane IgE to stimulate apoptosis of IgE⁺ B cells.^{8,12} An anti-IgE gene therapy has also been reported to suppress the activation of IgE⁺ B cells in mice.²⁷ If these approaches prove capable of successfully depleting IgE⁺ B cells in humans, thereby blocking replenishment of IgE-secreting plasma cells, then total IgE levels may gradually diminish over time, reducing IgE occupancy on FcεRI and thereby desensitizing mast cells and basophils. However, the failure of such approaches to sequester free IgE is likely to result in a greatly delayed onset of action relative to omalizumab, and they are therefore expected to complement rather than replace the therapeutic use of this antibody.

We reasoned that a combination strategy that retains the sequestration efficiency of omalizumab while also suppressing IgE production may have advantages as a long-term therapy for allergy and asthma. By exploiting the interaction of the antibody Fc domain with FcγRIIb, we have developed a therapeutic antibody that blocks IgE⁺ B-cell differentiation and also sequesters soluble IgE without binding to IgE complexed with FcεRI.

FcγRIIb is the sole inhibitory receptor for the Fc domain of IgG antibodies and, because of its expression on B cells, macrophages, and dendritic cells, plays a key role in modulating immune responses. FcγRIIb has been characterized largely on the basis of its interactions with IgG Fc domains, and limited information exists on the role it plays in the modulation of IgE⁺ B-cell responses; however, it has been reported that the Fc domain of IgE can bind to IgG receptors including FcγRIIb, FcγRIIa, and FcγRIIIa on mast cells, with affinities comparable to those of IgG Fc.^{16,17} A role for FcγRIIb in suppressing IgE responses is also demonstrated by the exacerbation of allergic rhinitis and asthma reported in FcγRIIb knockout mice.^{32,33} By stimulating the coengagement of FcγRIIb and surface IgE, XmAb7195 can target B cells as soon as they class-switch to express IgE BCR. However, because XmAb7195 also exhibits an enhanced ability to sequester soluble IgE relative to omalizumab, its therapeutic activity is not contingent on the gradual elimination of plasma cells, in contrast to approaches that do not target soluble IgE.^{8,12}

FcγRIIb is also expressed on plasma cells, and Xiang et al³⁴ demonstrated that antibody-mediated cross-linking of plasma cell FcγRIIb can induce apoptosis. In addition, our results using the surrogate antibody XENP8253 show that total IgG levels were partially suppressed (Fig 7, C), but then stabilized over 8 weeks. This observation raises the possibility that in immunocompetent (but not huSCID) mice, FcγRIIb engagement in the absence of IgE⁺ BCR binding may target plasma cells or B cells expressing other Ig isotypes. This finding for the surrogate raises the possibility that the Fc domain of XmAb7195 could have direct effects on human plasma cells as well as on B cells. However, the following data argue against this hypothesis: (1) the Fc domain of XmAb7195 binds monovalently and cannot cross-link plasma cell FcγRIIb (see Table I); (2) the avidity effect caused by bivalent binding to surface IgE is diminished on plasma cells, in which BCR is absent or greatly reduced^{35,36}; (3) XmAb7195 suppressed IgE production in huSCID mice when given several days before peak plasma cell output of IgE, suggesting that the primary target is IgE⁺ B cells; (4) XmAb7195 had no effect on serum IgM or IgG levels, indicating that these plasma cell populations are not targeted; and (5) the isotype control antibody XENP6003, which engages FcγRIIb with identical affinity to XmAb7195, was inactive in all *in vitro* and *in vivo* models.

As with any therapeutic antibody, the possibility exists for an immune response to XmAb7195 in clinical use. Notably, our results using the murine surrogate XENP8253 (Fig 7) demonstrate that suppression of both free and total IgE levels persisted throughout 8 weeks of chronic dosing, even in mice that developed antihuman Fc immune responses. These results in immunocompetent mice, combined with results showing that XmAb7195 can suppress human B-cell activation induced by a strong polyclonal anti-IgE stimulus in huSCID mice (Fig 8), suggest that high-affinity coengagement of FcγRIIb with IgE BCR may suppress the potential immunogenicity of XmAb7195 in humans,

FIG 7. Efficacy of mouse anti-IgE surrogates in C57BL/6 mice transgenic for human FcγRIIb. XENP8252 and XENP8253 are surrogates for omalizumab and XmAb7195, respectively. Mice were treated with vehicle or 10 mg/kg antibodies twice weekly starting on day 0. **A**, Free mouse IgE levels. **B**, Total mouse IgE levels. **C**, Total mouse IgG levels. Data are for 10 mice per group, and error bars represent mean ± SEM of log-transformed values. Results are representative of 2 similar experiments. *P* values represent repeated-measures 1-way ANOVA with Dunnett posttest for multiple comparisons (vs vehicle); ****P* < .001; ***P* < .01; **P* < .05; if unlabeled, not significant (*P* > .05). Values below assay limits of quantitation in A and B (15.6 and 2.6 ng/mL, respectively) are plotted at these baselines.

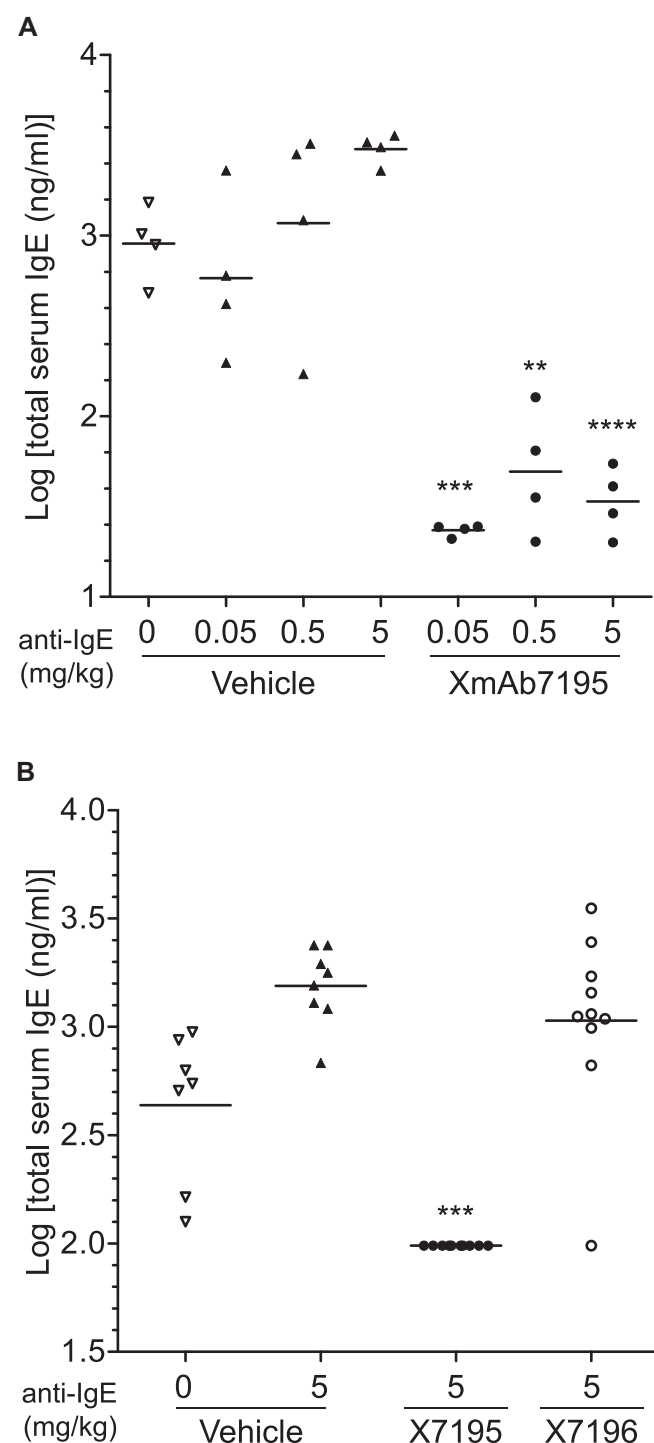


FIG 8. IgE⁺ B-cell activation by polyclonal anti-IgE antibody and its suppression by XmAb7195 in huSCID mice. **A**, Increasing concentrations of polyclonal anti-IgE activate human B cells and stimulate human IgE production (measured 20 days after engraftment and 11 days after anti-IgE treatment). XmAb7195 (10 mg/kg) suppresses IgE production under all anti-IgE treatment conditions. **B**, Increase in total IgE levels induced by polyclonal anti-IgE is suppressed by XmAb7195 but not by FcγR knockout control XENP7196. *P* values in **A** represent Student *t* tests versus vehicle at the same time point and in **B** represent 1-way ANOVA with Dunnett posttest for multiple comparisons versus vehicle; ***P* < .01; ****P* < .001; *****P* < .0001; if unlabeled, not significant (*P* > .05). Values below assay limits of quantitation in **A** and **B** (20 and 98 ng/mL, respectively) are plotted at these baselines.

especially in comparison to therapeutic antibodies with native Fc domains.

Our results using isotype and Fc control antibodies also shed new light on the pharmacology of omalizumab, which contains a native IgG1 Fc domain and therefore interacts with multiple Fcγ receptors including the activating receptors FcγRI, FcγRIIa, and FcγRIIIa as well as FcγRIIb. By comparing the effects of omalizumab to those of XmAb7195, its native IgG1 control, and its control lacking binding to Fcγ receptors, it is evident that antibody cross-linking of IgE BCR in the absence of FcγRIIb interaction increases serum IgE levels. The difference in total IgE levels observed for omalizumab and the FcγR knockout antibody control suggests that omalizumab's clinical effects may consist of a complex mixture of IgE sequestration coupled with the engagement of inhibitory and activating Fcγ receptors on B cells and other leukocytes. Our evidence for a signaling component of omalizumab activity in the huSCID models extends the report from Ota et al indicating that R1E4, an antimouse IgE used as a surrogate for omalizumab, activates calcium mobilization in mouse B cells in addition to sequestering IgE.²⁷

In summary, XmAb7195 is predicted to sequester IgE as well as, or more efficiently than, an existing anti-IgE therapeutic antibody. More importantly, the additional mechanism of action mediated by the engagement of its Fc domain with FcγRIIb may block the differentiation of IgE⁺ B cells, with a consequent sustained reduction in IgE-secreting plasma cells. It is plausible that these dual mechanisms may be synergistic in the clinic, with a reduction in total IgE production facilitating a sustained and more effective sequestration of remaining free IgE during antibody therapy of allergy and allergic asthma.

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Clinical implications: These results suggest that the long-term suppression of IgE production coupled with IgE sequestration is feasible by using a therapeutic antibody and may be an effective treatment for allergic asthma.

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