Correction

IMMUNOLOGY AND INFLAMMATION

Correction for “IgH isotype-specific B cell receptor expression influences B cell fate,” by Pei Tong, Alessandra Granato, Teng Zuo, Neha Chaudhary, Adam Zuiani, Seung Seok Han, Rakesh Donthula, Akritee Shrestha, Debattama Sen, Jennifer M. Magee, Michael P. Gallagher, Cees E. van der Poel, Michael C. Carroll, and Duane R. Wesemann, which was first published September 18, 2017; 10.1073/pnas.1704962114 (Proc Natl Acad Sci USA 114:E8411–E8420).

The authors note that Fig. 2 appeared incorrectly. The corrected figure and its legend appear below.
Fig. 2. Development and characteristics of IgE+ and IgG1+ mature B cells with the introduction of a prerearranged Igκ(VJκ5). (A) FACS plots show splenic lymphocytes of the indicated mice. Numbers in the plots indicate percentage of gated live CD19+ BCR+ cells (n = 6). (B and C) Dot graphs showing percentage (B) and absolute number (C) of splenic B cells of indicated mice. (D) FACS plots of live CD19+ B220+ CD93− gated lymphocytes from spleens of the indicated mice to identify splenic marginal zone (CD21hi CD23lo) and follicular (CD21int CD23hi) B cells (n = 6). Because Igκe/VJκ5 and Igγ1/γ1VJκ5 mice appear to express higher levels of CD23, the gating is relative within each mouse to identify CD23hi CD21int follicular B cells. Numbers in the plots indicate percentages. (E and F) Total serum IgE (E) and IgG1 (F) concentration measured by ELISA from the indicated mice. Each dot represents individual mice. (G and H) Naive splenic IgE+ and IgG1+ B cells show similar gene expression pattern to WT naive IgM B cells. Microarray analysis of sorted B220+ CD93− CD23hi CD21int (follicular) splenic B cells from IgκWTVJκ5 (IgM) versus Igγ1/γ1VJκ5 (IgG1) mice (G), and IgκWTVJκ5 (IgM) versus Igκe/VJκ5 (IgE) mice (H). Selected chemokine receptor genes (black), splicing factors (green), as well as positive (red) and negative (blue) regulators of plasma cell differentiation are shown. Lines represent cutoffs for genes up- or down-regulated by a fold-change of at least 0.67 (log2). The Pearson correlation coefficient (r) between gene expression levels is given for respective plots (n = 3). (I and J) Flow cytometric histogram plots (I) and summary bar graphs (J) of live BCR+ follicular B cells from the indicated mice analyzed for surface Igκ expression (Left of I and J) and CD79B expression (Right of I and J). Fold median fluorescence intensity (MFI) was calculated by dividing MFI values by the average MFI from IgκM+ from IgκWTVJκ5 mice for each given subset (n = 4–5). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; one-way ANOVA followed by Tukey’s post hoc test. Data are mean values ± SEM. (K) Treemaps showing VH gene segment frequencies in pro- and follicular (Fo) B cells from IgκWTVJκ5, Igκe/VJκ5, and Igγ1/γ1VJκ5 mice. Each block represents combined data from all biologic repeats (n = 5–6). Within a block, each colored box represents one VH segment. The size of the box is directly proportional to the percentage of sequences, which belongs to the VH segment. The same VH segment has the same color in all of the treemaps and the largest boxes contain the VH name. PCR repeats were removed via unique molecular indexing. See also SI Appendix, Figs. S3–S6.
Ig heavy chain (Igh) isotypes (e.g., IgM, IgG, and IgE) are generated as secreted/soluble antibodies (sIg) or as membrane-bound (mIg) B cell receptors (BCRs) through alternative RNA splicing. IgH isotype dictates soluble antibody function, but how mlg isotype influences B cell behavior is not well defined. We examined Igh isotype-specific BCR function by analyzing naturally switched B cells from wild-type mice, as well as by engineering polyclonal Igh1/1γ1 and Igh3/5 mice, which initially produce IgG1 or IgE from their respective native genomic configurations. We found that B cells from wild-type mice, as well as Igh3/5 and Igh3/5 mice, produce transcripts that generate IgM, IgG1, and IgE in an alternative splice form bias hierarchy, regardless of cell stage. In this regard, we found that mlg > mlgγ > mlgγ1, and that these BCR expression differences influence respective developmental fitness. Restrained B cell development from Igh3/5 and Igh3/5 mice was proportional to sig/lg/m ratio and was rescued by enforced expression of the respective mlgns. In addition, artificially enhancing BCR signal strength permitted IgE+ memory B cells—which essentially do not exist under normal conditions—to provide long-lived memory function, suggesting that quantitative BCR signal weakness contributes to restraint of IgE B cell responses. Our results indicate that IgH isotype-specific mlg/BCR dosage may play a larger role in B cell fate than previously anticipated.

Ig heavy chain (Igh) constant region (C\text{H}) isotypes enable coupling of antigen-binding Ig variable regions (\text{V}) to diverse functional contexts. The C\text{H}I exons are arranged in tandem, with C\text{H}1 (encoding the IgM constant region) initially located most proximal to the Ig V\text{H}1 exon, followed by a number of alternative C\text{H}I isotypes (e.g., C\text{H}2, C\text{H}3, and C\text{H}4). Each C\text{H}I is supplied with terminal exon(s) encoding transmembrane and cytoplasmic tail moieties enabling expression of membrane Ig (mIg), which, together with the CD79A and CD79B signaling accessory proteins, form the antigen-binding part of the B cell receptor (BCR) (1). Mutually exclusive alternative splicing can exclude membrane exons to produce secreted Ig (sIg) (2).

Ig V exons of IgH and Ig light (IgL) chains are assembled in bone marrow (BM) progenitor (pro) and precursor (pre) B cells, respectively (3). Productive V\text{H}I and V\text{L} assembly results in IgM expression on the surface of immature B cells, which further develop to mature naïve IgM+ IgG* B cells upon emigration from the BM to the periphery, where they can participate in immune responses. Activated B cells can undergo IgH class switch recombination (CSR), mediated by activation-induced cytidine deaminase (AID). CSR replaces initially expressed IgM with IgG, IgE, or IgA by targeted repositioning of the alternative IgH locus C\text{J}, resulting in permanent deletion of intervening C\text{H}I, C\text{H}2, and C\text{H}3 (4). Following activation, B cells can maintain a general B cell transcriptional program to support the production of long-lived memory B cells, which continue to be dependent upon BCR signals. An alternate fate results from a large shift in the general B cell transcriptional program toward specialization as antibody secreting cells (ASCs) (5).

IgH CSR is associated with different IgH isotype-specific B cell fates following activation (6–12). Investigations into mechanisms underlying how IgH isotype influences BCR/mIg function to date have largely relied upon overexpression and transgenic experiments of monoclonal Ig to identify how differences in protein sequence between IgH isotypes influence BCR signaling (13–18). However, whether differences of endogenous BCR expression from the IgH locus occur between isotypes is not fully defined. To address this, we generated preswitched Igh\text{hdc} and Igh\text{hyc}1/1γ1 mice engineered to produce polyclonal IgE and IgG1 B cells, respectively, to explore the role of IgH isotype on BCR function from native genomic contexts. We identified an isotype-specific BCR expression hierarchy in naïve IgG1 and IgE B cells from preswitched mice that is preserved in B cells after regular activation-induced CSR. We report that IgH isotype-specific BCR expression is an underlying feature that contributes to isotype-specific B cell behaviors.

Results

Generation of Igh\text{hdc} and Igh\text{hyc}1/1γ1 Mice. To explore the degree to which IgH isotype regulates BCR function, we generated Igh\text{hdc} and Igh\text{hyc}1/1γ1 mice engineered to produce polyclonal IgE and IgG1 B cells, respectively. These BCR expression differences contribute to restraint of IgE B cell responses. Our results indicate that IgH isotype-specific mlg/BCR dosage may play a larger role in B cell fate than previously anticipated.

Significance

B cells produce antibodies in the context of immunoglobulin heavy chain (Igh) isotypes (e.g., IgM, IgG, and IgE). Each of these is generated either as secreted proteins or as membrane-bound B cell antigen receptors (BCRs). While much is known about how IgH isotype dictates effector function of soluble antibodies, the role of antibody isotype in the context of BCRs is not well defined. Here we demonstrate that the membrane-bound versions (mlg) of IgM, IgG1, and IgE are produced from their natural genomic loci in a hierarchical fashion, where mRNA transcripts for mlg are always more dominant than mlgG1, which are always more dominant than mlgE, regardless of cell stage. These isotype-specific expression differences contribute to B cell regulation.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The raw sequence data for this study are accessible at the NCBI Sequence Read Archive (SRA) under BioProject accession no. PRJNA394007 with BioSample accession nos. SAMN07347175–SAMN07347206.

P.T. and A.G. contributed equally to this work.

To whom correspondence should be addressed. Email: dwseman@bwh.harvard.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1704962114/-/DCSupplemental.

Published online September 18, 2017 | EB411–EB420
and Ighγ1/y1 mice in an effort to produce native polyclonal IgE+ and IgG1+ B cells, respectively, from natural genomic contexts. The Ighγ1/y1 mice were derived from induced pluripotent stem cells (iPSCs) generated from B-lineage cells that had previously undergone Igh CSR to IgE on an allele that had rearranged a Dγ1 to Jγ1, but that had not yet undergone Vγ to DγJγ assembly (Fig. 1A and SI Appendix, Fig. S1A). The Ighγ1/y1 mice were generated by deletion of a portion of the Cγ1 locus such that the resulting arrangement would be identical to a natural CSR event to Cγ1L. This was achieved by CRISPR-mediated embryonic stem cell (ESC) targeting of the same DNA regions targeted for DNA cleavage events during CSR from Cμ to Cγ1L (Fig. 1A and SI Appendix, Fig. S1B).

**Differential B-Lineage Developmental Competence in Ighγ1/e and Ighγ1/y1 Mice.** Early B cell maturation requires BCR signals to license maturation through developmental stages. Productive assembly of Igκ in the pro-B cell stage leads to surface assembly of the pre-BCR, containing mlgκ, a surrogate light chain complex (SLC), and the CD79A/B proteins. Pre-BCR signaling stimulates Igκ (Igκ or Igλ) V exon assembly in pre-B cells. Productively assembled Igκ produces Igκ or Igκ, which complexes with mlgκ to form IgM, which together with CD79A/B, form the BCR on the surface of immature B cells that provides signals for continued B cell development (19).

We examined the competence of IgE and IgG1 as BCRs to support BCR-dependent developmental steps during early B-lineage cell maturation. We found that Ighγ1/e mouse BM contains abundant B220+CD43+ pre-B cells; however, CD43+ B220+ pre-B, BCR+ immature, and B220+B cell recirculating B cells, were severely reduced in amount (Fig. 1B). In addition, IgE+ B cells in the spleen were nearly undetectable (Fig. 1C and D). Developmental blockade in Ighγ1/y1 mice was not as severe, with detectable BM immature and recirculating B cells (Fig. 1B) and a clear splenic B cell population numbering over 10-fold less compared with wild-type and heterozygous mice (Fig. 1C–E). BM pro-B cells from each mouse expressed levels of IL-7 receptor similar.

---

**Fig. 1.** B cell development in Ighγ1/e and Ighγ1/y1 mice is impaired. (A) Schematic representations of the Ighγ1/e (Top) and Ighγ1/y1 (Bottom) alleles. (B) FACS plots of live CD19+ and B220+ bone marrow cells (Top plots) as well as live B220+CD19+ and BCR− (Bottom plots) mature and recirculating B cells (B220+B−BCR−). (C) FACS plots of splenic lymphocytes showing CD19 expression versus IgM, IgE, or IgG1 from the indicated mice. (D) and (E) Dot graph showing summary statistics of percentages (D), and total number (E), of splenic B cells from the indicated mice. Each dot represents one mouse (n = 4–9). (F) Semiquantitative PCR analyses of Jμ-proximal Vγ7183 and Jμ-distal VγJ558 family rearrangements from sorted bone marrow pro-B cells from indicated mice. Dig5 was amplified as a loading control. Threefold serial dilutions are shown. Results are typical of three experiments. Bands corresponding to rearrangements to various Jμ segments are indicated. (G) FACS plots showing intracellular Igκ, Igκ, and Igγ1 heavy chain in pro-B cells (CD19+ B220+ Igκ− CD43+) from bone marrows of the indicated mice. Results are typical of at least four experiments. (H) Percentage of intracellular Igκ, Igκ, and Igγ1 heavy chain in BM pro-B cells. Each dot represents one mouse (n = 5). (I) Semiquantitative PCR analyses of Vκ Iγ1 rearrangements in magnetically separated bone marrow B220+ cells from indicated mice. Intronic Igκ was amplified as a loading control. Threefold serial dilutions are shown. Bands corresponding to rearrangements to various Jκ segments are indicated on the Left. Results are typical of four experiments. (J) Quantitative PCR analyses of Vκ to Jκ Iγ1 rearrangement relative to β-actin DNA in purified B220+ BM cells from the indicated mice. Expression is shown as fold change relative to wild-type levels. **P < 0.01, ****P < 0.0001; one-way ANOVA followed by Tukey’s post hoc test. Summary data are mean values ± SEM. See also SI Appendix, Figs. S1 and S2.
to wild type (SI Appendix, Fig. S2A) and both had a similar mild reduction of proliferation capacities when stimulated ex vivo with low doses of IL-7, although this did not reach statistical significance (SI Appendix, Fig. S2B). These data suggest that endogenously produced, polyclonal IgE and IgG1 have different levels of BCR fitness in a model of early B cell development, with IgE being the most severely restricted.

IgY1 and IgG: Proteins Are Produced in Pro-B Cells, but Provide Insufficient Stimuli to Induce IgG Assembly. To determine to which degree blockade of B cell development is due to inhibition of VDJr recombination, we assessed the level of IgH VDJr recombination of the two main YH families (proximally positioned 7183, and distally positioned J558 families) on sorted BM B cell progenitors by semiquantitative PCR. IgH/e and IgY1/y1 mice showed similar levels of assembled VDJr compared with wild-type progenitor B cells (Fig. 1F). Despite the preassembled Dij to Jy1 assembly in IgH/e mice, all IgJy were used (Fig. 1F). In addition, flow cytometric analysis of intracytoplasmic IgH expression in pro-B cells demonstrated similar levels of intracellular IgG and IgY1 compared with wild-type pro-B cells expressing IgY (Fig. 1G and H).

In addition, heterozygous IgHr/WT, as well as IgY1/y1 WT heterozygote B cell progenitors show Ig-y1/Igh1 and IgY1/y1 ratios of 1:1 for each (Fig. 1G and H and SI Appendix, Fig. S2 C and D). These data demonstrate both IgM and IgG1 heavy chains are expressed intracytoplasmically at levels very comparable to IgY. Despite this, mature B cells from both IgHr/WT and IgY1/y1 WT mice are essentially all IgM+, suggesting a strong competitive advantage for IgY over IgY1 or IgG in later stages of development.

To determine the degree to which allelic exclusion is affected, we performed quantitative analysis of cells expressing both IgH alleles in IgHr/WT and IgY1/y1 heterozygous mice from developing BM and splenic B cells. While intact allelic exclusion makes IgH production from both alleles scarce (less than 1%), a full break in allelic exclusion would theoretically be indicated by 12.2% of double producers (20), although in practice this may be less due to the ability of IgH mRNA from productively assembled IgH loci to mediate allelic exclusion of homologous loci in the absence of IgH protein (21). Within the pool of IgH-expressing B220+CD3+ BM B-lineage cells, we found 5–8% double IgH1 producers in both IgHr/WT and IgY1/y1 WT heterozygous mice (SI Appendix, Fig. S2 C and D). For splenic B cells, 2–3% express both IgM and IgG1 in IgHr/WT mice, whereas 5–7% are positive for both IgM and IgG in IgHr/WT mice (SI Appendix, Fig. S2 E and F). Of note, cells heterozygous for an allele that can only produce the secreted version of IgM (μMAllele) (22), also contain ~6% of double producers in the spleen (20).

This analysis suggests that IgG1, and to a greater extent, IgE, are unable to mediate normal allelic exclusion.

To determine the degree of IgJ rearrangement in IgHr/WT and IgY1/y1 WT mice, we assessed IgK V-J rearrangement by semi-quantitative PCR as well as the level of rearrangement to Jc1 by qPCR. We found that IgK/e BM B cell IgK rearrangement is very near the Rag2+/− background control with IgY1/y1 Igk rearrangement slightly higher (Fig. 1I and J). Together, these results indicate that, while IgH intracytoplasmic expression reaches wild-type Igk levels, BCR signaling in the context of endogenously produced IgY1 and IgG provides insufficient signaling to stimulate entry into subsequent BCR-dependent cell stages.

IgY1/y1 and IgHr/e: B Cell Development Is Partially Rescued by a Pre-Assembled Igk (VK5). To determine the extent to which introduction of a preassembled Igk can rescue development in IgY1/y1 and IgHr/e B cells, we crossed both IgY1/y1 and IgHr/e mice to the Vκκ5 allele, a natural productive Igk assembly, also produced via B cell to iPSC reprogramming (SI Appendix, Fig. S1C). The presence of the preassembled Vκκ5 resulted in a partial rescue of the developmental blockade observed in IgHr/e mice. IgE+ B cells in IgHr/e/Vκκ5 mice increased in percentage (Fig. 2 A and B), with an ~10-fold increase in splenic B cell number (compare Fig. 1E to Fig. 2C). IgG1+ cells increased ~5-fold in IgHr/e/Vκκ5 mice (compare Fig. 1E to Fig. 2C). We found that mature IgE+ B cells from IgHr/e/Vκκ5 are nearly all of the follicular B cell phenotype (CD19+ B20+ CD95+ CD23+ CD21+), whereas splenic IgG1+ cells from IgY1/y1 and IgY1/y1/Vκκ5 have both follicular and marginal zone (CD19+ B20+ CD95– CD23+ CD21+) populations in the splenic B cell compartment, with an increased percentage of marginal zone B cells compared with wild-type mice (Fig. 2D). The CD19+ IgG1– cells observed in IgY1/y1 spleens (Fig. 1C) expressed CD43 and CD93, consistent with pro-B cells (SI Appendix, Fig. S3A). In addition, despite significant B lymphopenia, IgHr/e mice and IgHr/e/Vκκ5 mice have 100- to 1,000-fold higher serum IgE levels (Fig. 2E) compared with wild-type controls. IgG1 levels in both IgY1/y1 and IgY1/y1/Vκκ5 mice are ~10-fold higher than wild type (Fig. 2F).

Analysis of BM and transitional cell populations demonstrated that the Vκκ5 allele had a large effect increasing the BM populations for IgY1/y1/Vκκ5 mice, particularly immature B cells (SI Appendix, Fig. S3 B–F), whereas in IgHr/e/Vκκ5 mice, the largest effect appeared to be an increase in CD93+ transitional B cells (SI Appendix, Fig. S3 G–I). A 26-d pulse of BrdU in drinking water followed by over a month of normal water (chase) showed that the Vκκ5 rescue had no significant effect on the maintenance of the circulating B cell pool from IgHr/WT, IgHr/e, and IgY1/y1 mice, but did show that both IgHr/e and IgY1/y1/Vκκ5 B cells had shorter half lives than the others by about a week (SI Appendix, Fig. S4A).

Together, these data indicate that the developmental arrest in IgY1/y1 and IgHr/e mice can be partially rescued by a preassembled Igk, but that the amount of IgG1+ and IgE+ B cell levels continue to be moderately and severely restricted, respectively, in the periphery, despite both having productive IgH and IgL expression. Enhanced peripheral B cell numbers with preassembled Igk also suggests that, in general, BCR signal weakness, rather than too much signal strength, contributes to the developmental blockade in IgY1/y1 and IgHr/e mice.

**Mature Naive IgE+ and IgG1- B Cells from IgHr/e/VK5 and IgY1/y1/VK5 Mice, Respectively, Are Transcriptionally Similar to Mature Naive IgM- B Cells.** We considered the possibility that IgG1 and IgE may direct a plasma cell fate by autonomous signaling (9, 11, 12, 15, 16). To address this, we sorted IgM+ follicular B cells from wild-type mice and IgE+ and IgG1+ cells with the same surface phenotype from IgHr/e/VK5 and IgY1/y1/VK5 mice, respectively (SI Appendix, Fig. S4B), to measure gene expression profiles. We found that both IgG1 and IgE B cells align closely to IgM-expressing follicular B cells, with r values of 0.975 and 0.946, respectively (Fig. 2 G and H). We find no differences in genes important for plasma cell differentiation (23) or in core B cell regulatory genes and splicing regulators between IgM, IgE, and IgG1 cells (Fig. 2 G and H). These data indicate that expression of polyclonal IgG1 and IgE from native loci is not sufficient to directly instruct B cells to become plasma cells when expressed from endogenous genomic context.

IgE and IgG1 B Cells Express Low Cell Surface BCR Density. Previous reports have indicated that BCR density influences B cell fate (8, 24–27). In addition, limited BCR density has been proposed to underlie restriction of IgE B cell numbers (8, 27). To explore the mechanism for the differential insufficiencies of endogenously produced, polyclonal IgG1 and IgE in supporting early B cell development and peripheral B cell numbers, we hypothesized that different BCR dosages may play a role in differential IgG1 and IgE B cell behaviors.

To test this, we measured BCR density by cytometrically assessing Igk expression on the cell surface of IgM-expressing B cells from wild-type mice and compared them to IgG1+ and IgE+ B cells from IgHr/e/VK5 and IgHr/e/vK5 mice, respectively.
Because the Ighc/eVJK5 mice only make B cells with the follicular phenotype, we compared the IgE+ follicular B cells to IgG1+ and IgM+ follicular B cells from the other mice. We also assessed BCR density by staining cells for CD79B (also known as Igh), as this forms a part of the BCR for all IgH isotypes (28). We found that both Igk as well as CD79B median fluorescence intensity (MFI) was highest for IgM-expressing naive follicular B cells, and lowest in IgE+ cells, with IgG1-expressing B cells falling in between the two (Fig. 2 I and J). These data suggest that BCR dosage may influence B cell numbers by regulating integrated BCR signaling strength.

BCR-Related Phosphoprotein Analysis in IgM, IgG1, and IgE Cells. We also examined baseline phosphorylation levels of Erk, Syk, and Akt, which are related to BCR signaling (29), by flow cytometry. We found that basal phosphorylation levels of Akt were similar, whereas phospho (p)-Erk, and p-Syk were modestly higher in splenic cells from Ighγ1γ1VJK5 and Ighc/eVJK5 mice compared with IghWTVJK5 mice (SI Appendix, Fig. S4C). These results are consistent with the concept that intrinsic BCR signaling differences exist between IgH isotypes and may be playing a role in the phenotypic differences. However, the degree to which the phenotypic differences are directly related to the BCR is not clear. Also unclear
is whether or not these differences are a cause or an effect of the developmental blockade.

Given the BCR density differences between IgM, IgG1, and IgE, we assessed levels of BCR-related phosphoproteins in a system where expression levels of BCR isotypes from endogenous loci are similar to each other. For this we generated CH12 cell lines expressing IgM, IgG1, or IgE from endogenous loci (SI Appendix, Fig. S4D). We find similar levels of baseline p-Syk, p-Erk, and p-Akt in all three in CH12-IgM and CH12-IgE cells, with higher levels of each in CH12-IgG1 cells (SI Appendix, Fig. S4E). These results suggest that intrinsic differences in isotype-specific BCR signaling likely contribute to B cell behaviors.

**Weaker Developmental Ig Repertoire Selection in IgG and IgE B Cells.**

To explore the degree to which IgG1 and IgE may influence selection of preimmune Ig repertoires, we sorted pro-B and follicular B cells from IghWT/VJ5, Ighγ1/γ1VJ5, and Ighε3/ε3κ5 mice for Ig repertoire sequencing (SI Appendix, Fig. S5A and B). Analysis showed grossly similar Vμ segment use patterns in pro-B cells from each of the genotypes (Fig. 2K and SI Appendix, Fig. S5C). However, the selection patterns between pro-B and follicular B cells varied. In this regard, the Vμ segment Vμ5–2 (also known as 8 μs) is known to be highly used in pro-B cell VDJμ assemblies, but is strongly selected against during B cell development (30). This results in more rare Vμ5–2 use in mature B cells, presumably due to its autoactivity (31, 32). We see Vμ5–2 highly represented in pro-B cells from all genotypes (Fig. 2K and SI Appendix, Figs. S5C and S6 A–C, brown box in Upper Left corner of the treemap plots), and it is selected against in follicular B cells from IghWT/VJ5 and Ighγ1/γ1VJ5 mice, indicating that this aspect of selection is grossly intact in Ighγ1/γ1VJ5 mice. However, the Vμ5–2 gene segment is still the highest used in Ighε3/ε3κ5 follicular B cells (Fig. 2K and SI Appendix, SSC and S6 A–C). This may indicate selection of autoreactive cells capable of overcoming developmental blockade due to otherwise weak signaling. An alternative possibility is that BCR expression may be insufficient to arbitrate BCR-mediated and/or ligand-mediated developmental selection. The fact that overall Vμ use patterns between pro-B and follicular B cells in Ighε3/ε3κ5 is significantly more highly correlated than what is seen in the others suggests the latter is more likely (SI Appendix, Fig. S6 D and E).

Analysis of BM pro-B and splenic follicular B cell Vμ gene segment use in Ighγ1/γ1VJ5 mice also showed a nonsignificant trend toward preservation of the pro-B cell repertoire profile compared to IghWT/VJ5 mice. Postsort cell analysis indicates the preservation of pro-B cell repertoires is not due to early B-lineage cell contamination (SI Appendix, Fig. S5A).

**IgG1 and IgE B Cells Are Moderately and Severely Biased, Respectively, Against the mlg Alternatively Spliced mRNA Variant.** We asked whether differences in alternatively spliced mRNA might contribute to the different BCR density rankings observed in IgG1, IgE, and IgM B cells. Because alternative promoter use can influence alternative splicing (33), as well as mRNA stability (34), we developed an absolute qPCR assay to measure absolute amounts of productive slg and mlg mRNA splice variants by comparing to a known amount of a standard. Productive mRNA transcripts encoding the VDJμ exon together with the Cμ exon are generated from Vμ promoters 5’ to VDJμ exon. Germline (GL) Cμ transcripts are initiated downstream of the VDJμ exon from a noncoding exon 5’ to each Cμ region (called the Iε exon) present at each Igh isotype. B cells constitutively produce transcripts from both Vμ and Iμ promoters, while the other Iμ region promoters (e.g., Iγ1, Iε) are induced upon activation. GL transcripts from Iμ promoters are capped, polyadenylated, and spliced. Previous measurements of slg and mlg splice variants using relative qPCR assessments of 3’ ends of slg and mlg mRNA have not accounted for possible differences of these different promoters on splicing bias (8, 27).

We amplified either Vγ4 or Iγ1, promoter-driven transcripts from wild-type B cells activated for IgG1 and IgE CSR in vitro before using absolute qPCR to measure slg and mlg variants from each pool against their respective standards (SI Appendix, Fig. S7). RNA transcripts produced from Iμ and Iγ1 promoters were relatively more biased toward mlg compared with their Vμ promoter counterparts (SI Appendix, Fig. S8 B–D). In contrast, the slg/mlg ratio was similar between Iε and its Vμ promoter counterpart (SI Appendix, Fig. S8 C). CSR to IgE and IgG1 results in the juxtaposition of Iμ and its Vμ promoter counterpart in the context of Iγ1-Cμ transcripts (SI Appendix, Fig. S8 B–D). These results suggest that promoter use influences alternative splicing biases in the Igh locus, thus supporting a need to isolate and assess transcripts from Vμ promoters to quantify mRNA variants relevant for protein production.

We used Vμ promoter-driven transcript amplification and absolute qPCR assay to assess slg/mlg mRNA ratios in B cell subsets from Ighε3/ε3κ5 and Ighγ1/γ1VJ5 mice. We found that pro-B cells from Ighγ1/γ1VJ5 mice make several hundred to higher slg/mlg than mlg transcript (Fig. 3A and SI Appendix, Fig. S9A). Pro-B cells from Ighγ1/γ1VJ5 mice are also biased to the slg1 mRNA variant at a level of ∼40-fold over mlg1 mRNA (Fig. 3A and SI Appendix, Fig. S9A). The slg/mlg ratios from Ighγ1/γ1VJ5 and Ighε3/ε3κ5 splenic follicular IgG1 and IgE B cells, respectively, showed similar levels to those found in pro-B cells from the respective mice (Fig. 3A). Iγ1 mRNA in wild-type pro-B cells is biased toward the mlg1 splice variant, with an slg/mlg of ∼0.4, while the slg/mlg increased to an ∼1:1 ratio at the mature naïve B cell stage (Fig. 3B).

To evaluate IgH isotype mRNA splicing bias in wild-type cells, we stimulated wild-type B cells to undergo CSR to IgE and IgG1 in vitro (SI Appendix, Fig. S9B). We found that activated, switched B cells are heavily biased toward the slg1 and slg5 splice variants, with IgM mRNA being the most biased toward slg and IgE being the least (Fig. 3B). To determine if similar splicing bias can be detected in memory B cells, we immunized the AID-cre-ERT2 Rosa26-loxP-EYFP pre-B cell reporter mice (6) with sheep red blood cells and sorted IgM+ and IgG1+ memory B cells (Fig. 3A and SI Appendix, Fig. S9A). In memory B cells from Ighγ1/γ1VJ5 mice are also biased to the slg1 mRNA variant at a level of ∼1:1 ratio to the mature naïve B cell stage (Fig. 3B).

To evaluate IgH isotype mRNA splicing bias in wild-type cells, we stimulated wild-type B cells to undergo CSR to IgE and IgG1 in vitro (SI Appendix, Fig. S9B). We found that activated, switched B cells are heavily biased toward the slg1 and slg5 splice variants, with IgM mRNA being the most biased toward slg and IgE being the least (Fig. 3B). To determine if similar splicing bias can be detected in memory B cells, we immunized the AID-cre-ERT2 Rosa26-loxP-EYFP pre-B cell reporter mice (6) with sheep red blood cells and sorted IgM+ and IgG1+ memory B cells (Fig. 3A and SI Appendix, Fig. S9A). In memory B cells from Ighγ1/γ1VJ5 mice are also biased to the slg1 mRNA variant at a level of ∼1:1 ratio to the mature naïve B cell stage (Fig. 3B).

**Ectopic mlg1 or mlg: Rescues B Cell Development in Ighε3/ε3κ5 and mMT Pro-B Cells.** To determine the degree to which developmental blockade at pro-B cell stage is a result of low density of mlg1 and mlg1 in Ighε3/ε3κ5 and Ighγ1/γ1 mice, we retrovirally transduced the membrane form of IgE and IgG1, or empty vector, in developing B-lineage cells ex vivo from Ighε3/ε3κ5 and Ighγ1/γ1 mice, and measured Igκ rearrangement. We included developing B-lineage cells from μMT mice, which have a similar B cell developmental blockade due to inability to produce mlgs (22). Pro-B cells were transduced with vectors expressing GFP alone, or with mlG, mlG1, or mIGE followed by measurement of Igκ assembly and Igκ protein expression. Ighε3/ε3κ5, Ighγ1/γ1, and μMT pro-B cells transduced with GFP alone had minimal Igκ assembly and protein expression; however, enforced expression of mlG and mlG1 was able to induce Igκ gene assembly (Fig. 4 A–C). This resulted in surface Igκ expression in pro-B cells from all three genotypes,
Strengthened PI3K Signaling Can Generate a Memory Response Mediated by IgE+ B Cells. Immunologic IgE memory responses are unlikely to be housed in IgE B cell populations, whereas IgG1 B cells can function in both IgG1 and IgE memory (8, 12). To determine the degree to which IgG1 and IgE B cells in Igfry1/y1 and Igfry1/y1/Cd19+/− mice can participate in a conventional immune response to produce antigen-specific antibodies to immunization, we immunized them with ovalbumin (ova) and assessed IgH isotype-specific serum anti-ova antibodies. We challenged the mice as well to test whether a functional memory response could be detected. For Igfry1/y1 mice, we detected IgG1 and IgE anti-ova responses similar to Igfry1/wt heterozygotes with immunization, with levels increasing in magnitude after rechallenge for IgE responses (Fig. 4 F and G), indicating that the IgG1 cells are competent for germinal center entry and selection as well as CSR to IgE upon activation as expected. In contrast, immunization of Igfry1/y1 mice did not induce any detectable anti-ova IgE response (Fig. 4H), consistent with previous reports of rapid death of IgE+ cells upon activation (8, 9).

We crossed Igfry1/y1/Cd19−/− mice to Cd19cre+ Ptenfl/fl mice for conditional PTEN deletion in B cells with the goal to examine the degree to which strengthened BCR signaling could rescue a functional memory response for IgE+ B cells. PTEN is a negative regulator of PI3K signaling, which is downstream of BCR. When Pten mice are conditionedly deleted in B cells, BCR signaling is bypassed and B cells act as though they are receiving stronger BCR signals (37). Cd19 has also been shown to interact with IgE and negatively regulate IgE responses (16). Igfry1/y1/Cd19cre+ Ptenfl/fl mice have over fivefold more splenic B cells compared with controls at baseline (Fig. 4 I–K), consistent with previous reports of a role of B cell Pten deletion rescuing B cell numbers in the setting of insufficient BCR expression (37). After immunization, clear anti-ova IgE responses were observed in several Igfry1/y1/Cd19cre+ Ptenfl/fl mice with B cell Pten deletion that increases in magnitude upon rechallenge, while five out of six Igfry1/y1/Cd19−/− cre-negative control mice showed no sustained response (Fig. 4L). IgE responses have recently been shown to be increased in the setting of Cd19 haploinsufficiency via an unclear mechanism (16). Since Cd19cre mice are also haploinsufficient for Cd19, this may also contribute to the increased IgE responses in Igfry1/y1/Cd19cre+ Ptenfl/fl mice. Because the magnitude of the Cd19 haploinsufficiency is mild (16), lower Cd19 expression alone in Cd19cre mice is unlikely to explain the large effects observed (Fig. 4L). These results suggest that the lack of functional memory cell capabilities within IgE+ cells may be restored by B cell Pten deletion, implying that weak BCR signaling by IgE, potentially provided by minimal BCR density, plays a key role in limiting IgE+ activation and memory cell functional capacity.

Discussion

IgL isotype plays a major role in defining function of secreted antibodies and can influence BCR function to regulate B cell fate when expressed as miLG. Our data are consistent with a concept that individual IgH isotypes are linked to functionally required for BCR expression (38–40) and was later shown to be a widespread mechanism of gene regulation (41). Alternative splicing for IgH constructs contained a GFP reporter controlled by an internal ribosomal entry site (IRES), GFP expression can provide an indicator of amount of miLG RNA. Cells gated based on low to high expression of GFP showed an expression-dependent increase of the percentage of cells expressing Igx. (Fig. 4 D and E). These results indicate that both IgE and IgG1 can compensate for IgM in delivering a BCR signal sufficient to induce B-lineage maturation in a density-dependent fashion. Because SLC interaction with IgH is known to be functionally required for Igx assembly (36), these results also suggest that IgG1 and IgE can functionally interact with SLC. These findings suggest that insufficient BCR density is likely a contributing factor in the BM blockade seen in Igfry1/+/− and Igfry1/y1 mice.

While miLG and miLG1 could both rescue Ig production, miLM appeared to have an advantage in producing a higher percentage of IgG+ cells, which was more apparent when tested in the context of Igfl1/+/− and µMT pro-B cells (Fig. 4E). We found that this miLM advantage may be related to the ability of miLM to induce proliferation to a greater extent than miLG1 or miLE in the context of pro-B cells from Igfry1/y1 or Igfry1/+/− retrovirally transduced with miLG (SI Appendix, Fig. S10 B and C). The advantage seen by miLM in inducing more proliferation suggests that isotype-intrinsic BCR signaling may contribute as well to the BM blockade seen in Igfry1/− and Igfry1/y1 mice.

Fig. 3. Moderate and severe bias to slg in IgG1 and IgE cells, respectively. (A) Dot graph showing the ratio of slg/mig mRNA expression for Igx (red) and Igx (blue) from B220− BCR− bone marrow (pro/pre) and mature follicular (B220− CD38− B220− CD21+ CD23−) B cells from Igfry1/− and Igfry1/y1 (blue) mouse spleens (n = 4–5). The slg and mig levels, as well as total Ig mRNA levels were determined by absolute qPCR using known levels of standards. (B) Dot graph showing the ratio of slg/mig for Igx (black), Igx (red), and Igx (blue) from the indicated B cells isolated from wild-type (WT) mice. Pro/pre B cells are from B220− BCR− bone marrow. Mature B cells are from magnetically purified B220− splenic cells. Activated B cells were derived by stimulation of magnetically purified B220− splenic cells with anti-CD40 antibody plus IL-4 for 4 d (n = 4–9). (C) Schematic outline (above) of AID-cre-ERT2 Rosa26-loxP-EYFP mice immunized with sheep red blood cells (SRBCs) and induced with tamoxifen as outlined. FACS plots (below) showing gating strategy for flow cytometric sorting of IgM+ and IgG1+ memory B cells shown in D. (D) Dot graph showing slg/mig expression for IgG1+ and IgE+ mice shown in C by the absolute qPCR method described in A. Dots represent individual mice (n = 6). The mig mRNA level was below detection in three IgG1+ cell samples. ***P < 0.0001, two-tailed t-test. Summary data are means ± SEM. See also SI Appendix, Figs. S7–S9.

As the miLG constructs contained a GFP reporter controlled by an internal ribosomal entry site (IRES), GFP expression can provide an indicator of amount of miLG RNA. Cells gated based on low to high expression of GFP showed an expression-dependent increase of the percentage of cells expressing Igx. (Fig. 4 D and E). These results indicate that both IgE and IgG1 can compensate for IgM in delivering a BCR signal sufficient to induce B-lineage maturation in a density-dependent fashion. Because SLC interaction with IgH is known to be functionally required for Igx assembly (36), these results also suggest that IgG1 and IgE can functionally interact with SLC. These findings suggest that insufficient BCR density is likely a contributing factor in the BM blockade seen in Igfry1/− and Igfry1/y1 mice.
bias against mlg splice forms due to higher efficiency polyadenylation at a consensus site upstream of the membrane exons (27). The Cγ1 locus has consensus polyadenylation signals both upstream and downstream of its membrane exons, implicating other regulatory mechanisms involved in producing sIg bias observed in IgG1 B cells. While splicing plays a role in IgH gene expression, other features may contribute to IgG1 isotype-specific BCR density regulation, such as mRNA turn over, translation, surface protein stability, intrinsic signaling differences, as well as endocytosis and BCR recycling. In addition, while IgM appears to be produced...
with relatively higher mlg/slg compared with other IgH isotypes, BCR density has the potential to be even greater when IgD is expressed in addition to IgM, as occurs in mature naïve B cells and in some IgM* memory B cells.

Our finding that retrovirally transduced mlgE and mlgG1 can rescue B cell developmental progression in Ighc/–, Ighγ1/γ1, as well as µMT mice in an expression density-dependent fashion suggests that there may be more functional overlap of autonomous signaling between IgH isotypes than previously anticipated. However, our data are not inconsistent with the concept that differences in autonomous signaling between IgH isotypes contribute to isotype-specific differences in BCR function. BCR dosage levels and autonomous signaling differences likely act together to influence BCR function. Isotype-specific BCR dosage can impact B cell fate by defining signaling needs required to reach functional thresholds through ligand engagement (43). For example, B cells with more dilute BCR isotypes may require higher affinity and/or more cognate antigen availability to reach an integrated BCR signal strength that is similar to cells endowed with higher BCR density (24). The observation that autoreactive Vϕ5–2 was found to be dominant in Ighc/–Vκx5 follicular B cells is consistent with this concept, in that rare IgE cells may have been allowed to develop due to continuous recognition of a putative self-antigen. However, because Vϕ use frequencies between Ighc/–Vκx5 pro-B cells and follicular IgE B cells are so highly correlated (SI Appendix, Fig. S6E), with Vϕ5–2 favored in both, an alternative explanation is that dilute IgE (and perhaps to a lesser extent, IgG1) BCR may render cells relatively deaf to the normal ligand-mediated signals that usually accompany early B cell maturation, resulting in preservation of the pro-B cell Vϕ use pattern (SI Appendix, Fig. S6).

While we know of no other reports of IgE preswitched models, other work has generated preswitched models for IgG (44–47), most with monoclonal IgG specificities. Since Ig specificity alone can substantially impact B cell development (48, 49), it is not clear what aspects of development are due to IgH isotype versus Ig specificity in monoclonal models (44–46, 50). A polyclonal IgG1 preswitched model, called IgHγ1µ1 mice, was produced wherein the intronic polyA site (that regulates splicing to the secreted IgG variant) was deleted to enhance mlgG1 over sIgG1 production (47). In this setting, the IgHγ1µ1 mice have normal peripheral B cell numbers compared with wild-type controls (47), whereas our Ighγ1/γ1 mice are B cell lymphopenic, harboring 10-fold less peripheral B cells than wild-type controls (Fig. 1E). This comparison is consistent with the concept that BCR expression, at least at the mRNA level, plays a role in influencing B cell numbers. The observation of an early BM developing B-lineage cell blockade in IgHγ1µ1 mice suggests that aspects of IgG1 intrinsic signaling contribute to similar blockade in Ighγ1/γ1 mice, consistent with our finding that mlgM provides a pro-B cell proliferative advantage (SI Appendix, Fig. S10 B and C). The fact that the blockade in IgHγ1µ1 mice is much less severe (47) suggests that BCR expression plays a role as well at this stage of development. Isotopy-specific BCR density limits may contribute to functional differences observed in IgG1 and IgE B cells under normal settings, such as limited entry into the memory B cell compartment. The moderate and severe limitations of mlg production for IgG1 and IgE, respectively, are in line with findings showing that IgM-expressing B cells appear to enter the memory compartment at a higher level compared with IgG1-expressing B cells (6, 7, 10), and that IgE-expressing memory B cells are essentially nonexistent (8, 9, 12). In addition, our finding that functional antigen-specific IgE memory carried out by IgE-expressing B cells in the setting of Cd49 hemizygosity and Pten deficiency, is consistent with the concept that weak signaling from IgE BCRs limits IgE memory B cell formation. We conclude that IgH isotype-specific BCR dosage control is a regulatory mechanism in the B cell system.

**Materials and Methods**

**Mice.** The Children's Hospital Boston Animal Care and Use Committee (IACUC) and the Warren Alpert Building, Boston, IACUC approved all experiments. Doxycycline-inducible programmable mice used in these experiments have been described previously (46, 51). Splice B cell lines from transduced mice were cultured in the presence of IL-7 (20 ng/mL). After 2–3 d, cultured BM cells were infected with GFP or mlgH-encoding retroviruses. After two additional days, culture medium was removed and cells were cultured in the presence of IL-7 (20 ng/mL).

**Cell Isolation and Flow Cytometry.** BM cells were flushed from femurs and tibias with ice-cooled staining buffer (PBS supplemented with 2% FBS). Spleen cell suspensions were obtained by gently teasing spleens onto a 70-µm cell strainer. Erythrocytes were depleted using red blood cell lysis buffer (Sigma). Cells were counted using a hemocytometer with exclusion of dead cells with Trypan blue dye. Cells were stained with fluorophore or biotin-conjugated antibodies as described (54, 55) where indicated. The cell sorting was performed on a FACSARia II flow cytometer (BD Biosciences). For the in vivo experiments a FACSAria III cell sorter was used. The IgM, IgG1, and IgE for intracytoplasmic IgH expression was done by using trypsinization followed by fixation/permeabilization as described (54, 55). Data analysis was performed with FlowJo software (v9.9.4). See SI Appendix for further details.

**Cell Culture and CSR Assay.** Splenic and BM cells were isolated by B220 positive selection via magnetic columns (Miltenyi Biotech) according to manufacture instructions. The CH12 B cell line was previously described (56) and provided by Frederick Alt, Boston Children's Hospital, Boston. AID-cre transgenic mouse IgG1 (TOSG1C6, Biolegend). Phosphatase substrate tablets (Sigma) were used. In both assays, standard curves were generated with serial two-fold dilutions of OVA-specific mouse IgE (Cayman Chemical) or OVA-specific mouse IgG1 (SIGMA). Antibodies were used at 2 µg/mL of OVA (Sigma-Aldrich), followed by blocking and incubation with mouse serum dilutions. The same detection antibodies described earlier were used. In both assays, standard curves were generated with serial two- or fourfold dilutions of OVA-specific mouse IgE (Cayman Chemical) or OVA-specific mouse IgG1 (TOSG1C6, Biolegend). Phosphatase substrate tablets (Sigma) were used according to manufacture instructions.

**Immunization.** Male and female Ighγ1/γ1, Ighγ1/WT, Ighγ1/Vκs5, Ighγ1/Vκs5 Pten+/-Cd19cre, and Ighγ1/Vκs5 Pten+/-Cd19cre mice at 6–8 wk were immunized with 50 µg per mouse chicken OVA (Sigma-Aldrich) at days 0, 21, 47, and 73. AID-cre-ERT2 Rosa26-loxp-EYFP mice were immunized with 2 × 106 sheep red blood cells (Colorado Serum Company) at days 0 and 21. AID expression is induced by oral administration with 15 mg tamoxifen per mouse at time point at days 7, 9, 11, and 22.

**ELISA.** Total serum IgE and IgG1 were quantified by sandwich ELISA with the following antibodies: purified anti-mouse IgE (R35-72, BD Biosciences) and alkaline phosphatase conjugated anti-mouse IgE (23G3, Southern Biotech); purified anti-mouse IgG1 (SB77e, Southern Biotech), and alkaline phosphatase conjugated anti-mouse IgG1 (X56, BD Biosciences). To measure serum OVA-specific IgE and IgG1 by ELISA, plates were coated with 20 µg/mL of chicken OVA (Sigma-Aldrich), followed by blocking and incubation with mouse serum dilutions. The same detection antibodies described earlier were used. In both assays, standard curves were generated with serial two- or fourfold dilutions of OVA-specific mouse IgE (Cayman Chemical) or OVA-specific mouse IgG1 (TOSG1C6, Biolegend). Phosphatase substrate tablets (Sigma) were used according to manufacture instructions.

**Overexpression of mlg in Pro-B Cells.** The cDNAs for mlgM, mlgG1, and mlgE were prepared from the CH12 B cell line (mlgM) or CH12-derived B cell lines induced to undergo IgH CSR to IgG1 and IgE and cloned into the pMIG vector (Addgene). BM cells from Ighγ1/γ1/γ1, and µMT mice were cultured in the presence of IL-7 (20 ng/mL). After 2–3 d, cultured BM cells were infected with GFP or mlgH-encoding retroviruses. After two additional days, culture medium was removed and cells were cultured in the presence of BAFF and...
IL-4 for 3 d. Kappa chain rearrangement was analyzed by flow cytometry. See SI Appendix for further details.

Ig \( \mu \) V\( \mu \) Assembly Analysis. Threefold serial dilutions of genomic DNA (100 ng, 30 ng, and 10 ng) were used to perform PCR to analyze Ig heavy chain V\( \mu \)J\( \mu \) and Ig light chain V\( \kappa \)J\( \kappa \) rearrangements. Two main V\( \mu \) families were analyzed (7183 and JSSB) using primers described previously (57). Primers flanking exon 6 of the DilgS5 gene were used as a loading control (SI Appendix, Table S1). V\( \mu \)-J\( \mu \) rearrangement products were PCR amplified using a degenerate V\( \mu \) and the Mar25 primers described previously (58). Primers in the Igk intron were used as a loading control (SI Appendix, Table S1). V\( \kappa \)-J\( \kappa \) rearrangement was also determined by quantitative PCR assay using the degenerate V\( \kappa \) forward primer and a reverse primer complementary to sequences downstream of J\( \kappa \) (J\( \kappa \)-1–2R) as described previously (59, 60). Rearrangement levels measured by qPCR were normalized to the levels of \( \beta \)-actin DNA.

Total RNA Isolation and Gene Expression Analysis. Total RNA was extracted using the TRIzol method (Invitrogen), followed by treatment with RNase-free DNase (Qiagen) and RNasea columns cleanup (Qiagen). Affymetrix Mouse 2.0 ST GeneChips microarray gene expression data were done with the Bioconductor package (R version 3.3.1, Bioconductor version 3.4). The raw data from the .CEL files were normalized and expression matrix was log transformed. Pearson’s correlation coefficient (\( r \)) and fold change in expression level of various genes under different conditions were calculated based on the bivestage average of three biological replicates.

Isolation and Measurement of Membrane Secretory Igh \( \gamma \) mRNA. Cell mRNA was isolated using Dynabeads mRNA DIRECT Micro Purification Kit (ambion/Life Technologies), followed by DNA elimination with gDNA wipeout (Qiagen). Isolated mRNA was reverse transcribed using anchored oligo dT coupled to an universal sequence (E8419). For \( \gamma \)V and Sg\( \gamma \) transcripts were analyzed, the RT step was performed with anchored oligo dT coupled to an universal sequence (SI Appendix, Table S1), followed by a PCR step to amplify either V\( \gamma \) or Sg\( \gamma \) promoter-driven transcripts. For V\( \gamma \) promoters, the V\( \gamma \) segment leader sequence (V\( \gamma \) leader Fw) was used as a forward primer. A mixture of forward primers against all four \( \gamma \) regions and expected sizes confirmed. The final pools were sequenced from both ends of the molecules to a total read length of 250 nt from each end.

Sequencing Data Analysis. The sequences obtained from Illumina MiSeq deep sequencing (nano-run) were run through the standalone Igblast software (version 1.4.0) to identify the V\( \gamma \) segment using reference sequences from IMGT. PCR repeats were filtered using unique molecular identifiers (UMIs) applied during cDNA synthesis. Sequences with the same V\( \gamma \) and the same UMI were considered a single PCR repeat of the same mRNA. Only forward reads (R1) are used as the quality of R2 sequences was poor leading to low merge efficiency. Also, the CD3 regions predicted were not reliable due to low quality intermittent nucleotides, which in turn was required to rule out PCR repeats using the UMIs. By using only the UMIs and the V\( \gamma \)s, we risk losing some unique sequences attached to the same UMI at the cost of excluding all PCR repeats. The frequency of use of each V\( \gamma \) was calculated and compared for IghWTVJ\( \kappa \), Igh\( \kappa \)hV\( \kappa \), and Igh\( \kappa \)h1V\( \kappa \)5 mice. All preprocessing and analysis was done using Bioconductor package v 3.4 (R version 3.3.1).

Data Availability. The raw sequence data for this study are accessible at the NCBI Sequence Read Archive (SRA) under BioProject accession numbers PRJNA394007 with BioSample accession numbers SANN07347175–SAMN07347206.

Statistical Analysis. The \( n \) values in figures and figure legends indicate the number of individual mice, representing biologic replicates. Statistical analysis is described in the figure legends. Studies were not conducted blinded.

ACKNOWLEDGMENTS. We thank Christine Milcarek and Hans Oettgen for critical review of the manuscript and Soren Degn, Elisabeth Carroll, Rupa Kumari, Yuyezhou Chen, Jianxin Li, Colby Devereaux, John Manis, and Thomas Kepler for technical help and advice. This work is supported by the National Institutes of Health Grants AI212394 and AI1113217 to (D.W.R. and N.D.); the National Council for Scientific and Technological Development (CNPq)/Science Without Borders Program, Brazil (to A.G.). D.R.W. holds a Career Award for Medical Scientists from the Burroughs Wellcome Fund and is supported by a New Investigator Award from Food Allergy Research & Education (FARE). This work was also supported by an anonymous gift.

ovalbumin-specific B-cell transnuclear mice show class
ends.

Cell
J Exp Med
Nature
Proc Natl Acad Sci USA
20:293
477:424
Tong et al.
Immunology
7:765
356:154
350:
118:429
J Exp Med
20:313
112:
J Exp Med
recombination.
E8420
domain of human membrane IgE controls apoptotic signaling of the B cell receptor
599–613.
21. Lutz J, et al. (2011) Pro-B cells sense productive immunoglobulin heavy chain re-
arrangement irrespective of polypeptide production. Proc Natl Acad Sci USA 108:
10644–10649.
disruption of the membrane exon of the immunoglobulin mu chain gene. Nature 350:
423–426.
23. Tellier J, et al. (2016) Blimp-1 controls plasma cell function through the regulation of
level determines the fate of developing B lymphocytes: Receptor editing versus se-
25. Wang LD, et al. (2004) Selection of B lymphocytes in the periphery is determined by the
Positive selection of B cells expressing low densities of self-reactive BCRs
causes loss of heavy-chain allelic exclusion.

E457.

29. Rickert RC (2013) New insights into pre-BCR and BCR signalling with relevance to
31. Bellon B, et al. (1987) High frequency of autoantibodies bearing cross-reactive idi-
otypes among hybridomas using VH7183 genes prepared from normal and autoimmune
32. Chen X, Kearney JF (1996) Generation and function of natural self-reactive B lymph-
the genomic level. PloS One 3:e2377.
34. Ayoubi TA, Van De Ven WJ (1996) Regulation of gene expression by alternative
35. Zhang T, et al. (2010) Downstream class switching leads to IgE antibody production by
for rearrangement of the Ig kappa light chain gene in pro-B cell lines. Int Immunol 11:
1195–1202.
the synthesis of membrane and secreted immunoglobulin alpha, gamma and mu
39. Early P, et al. (1980) Two mRNAs can be produced from a single immunoglobulin mu
heavy chains is directed by mRNAs that differ at their 3′ ends. Cell 20:293–301.
42. Park KS, et al. (2014) Transcription elongation factor ELL2 drives Ig secretory-specific
IgD. Immunology 118:429–437.
44. Dougan SK, et al. (2012) IgG1+ ovalbumin-specific B cell transnuclear mice show class
switch recombination in rare allelically included B cells. Proc Natl Acad Sci USA 109:
13739–13744.
45. Dougan SK, et al. (2013) Antigen-specific B cell receptor sensitizes B cells to infection
46. Wesemann DR, et al. (2012) Reprogramming IgH isotype-switched B cells to functional-
47. Waisman A, et al. (2007) IgG1 B cell receptor signaling is inhibited by CD22 and
promotes the development of B cells whose survival is less dependent on Ig alpha/
and role in B cell tolerance and antibody diversification. Immunity 7:765–775.
49. Lam KP, Rajewsky K (1999) B cell antigen receptor specificity and surface density
50. Kumar R, et al. (2015) Antibody repertoire diversification through VH gene re-
placement in mice cloned from an IgA plasma cell. Proc Natl Acad Sci USA 112:
E456–E457.
52. Shinkai Y, et al. (1993) RAG-2-deficient mice lack mature lymphocytes owing to in-
53. Muramatsu M, et al. (2000) Class switch recombination and hypermutation require activa-
tion-induced cytokine deaminase (AID), a potential RNA editing enzyme. Cell
102:553–563.
54. Gallagher MP, Shrestha A, Magee JH, Wesemann DR (2014) Detection of true IgE-
55. Wesemann DR, et al. (2011) Immature B cells preferentially switch to IgE with in-
the synthesis of membrane and secreted immunoglobulin alpha, gamma and mu