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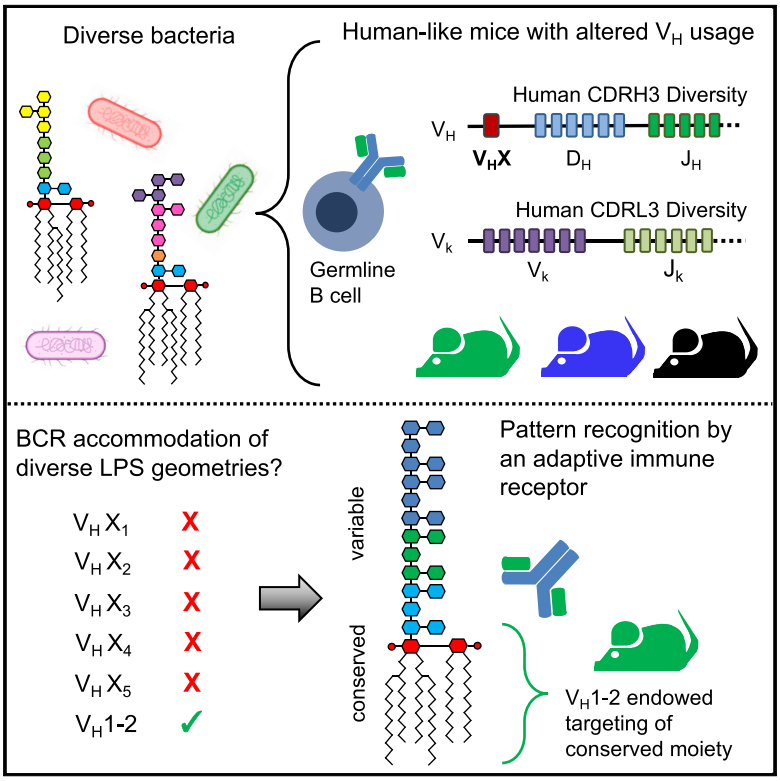
Cell Reports

A Single Human V_H-gene Allows for a Broad-Spectrum Antibody Response Targeting Bacterial Lipopolysaccharides in the Blood

Graphical Abstract



Highlights

- Transgenic mice alter V_H-gene contribution to human-like BCR repertoires
- Specific human V_H-genes are needed to engage bloodborne bacterial LPS *in vivo*
- Germline-endowed antigen recognition targets a universally conserved LPS moiety

Authors

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In Brief

The ligand binding surface of antibodies is generated by diverse gene-encoded and hypervariable loops. Sangesland et al. demonstrate that certain gene-encoded loops are needed for human antibodies to accommodate the shapes of some bacterial antigens and naturally tune for recognition of conserved microbial features therein.

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Report

A Single Human V_H-gene Allows for a Broad-Spectrum Antibody Response Targeting Bacterial Lipopolysaccharides in the Blood

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SUMMARY

B cell receptors (BCRs) display a combination of variable (V)-gene-encoded complementarity determining regions (CDRs) and adaptive/hypervariable CDR3 loops to engage antigens. It has long been proposed that the former tune for recognition of pathogens or groups of pathogens. To experimentally evaluate this within the human antibody repertoire, we perform immune challenges in transgenic mice that bear diverse human CDR3 and light chains but are constrained to different human V_H-genes. We find that, of six commonly deployed V_H sequences, only those CDRs encoded by IGHV1-2*02 enable polyclonal antibody responses against bacterial lipopolysaccharide (LPS) when introduced to the bloodstream. The LPS is from diverse strains of gram-negative bacteria, and the V_H-gene-dependent responses are directed against the non-variable and universal saccrolipid substructure of this antigen. This reveals a broad-spectrum anti-LPS response in which germline-encoded CDRs naturally hardwire the human antibody repertoire for recognition of a conserved microbial target.

INTRODUCTION

The adaptive immune system generates diverse sets of antibodies through the recombination of B cell receptor (BCR) gene segments. For each germline BCR, affinity to incoming antigen is provided by complementarity determining regions (CDRs) that are either directly encoded by antibody variable (V) genes (CDR1 and CDR2) or assembled *de novo* through stochastic N-junctional diversification of antibody D and J segments (CDR3). Most BCR diversity is concentrated within the antibody heavy-chain CDR3 (CDRH3), which occupies a central position within the germline antigen binding site (Glanville et al., 2009; Schroeder and Cavacini, 2010). CDRH3 loops vary highly in length, sequence, and shape, and because of this diversity, they are the principal determinants of antigen specificity (Kuroda et al., 2008; Morea et al., 1998; North et al., 2011; Oliva et al., 1998; Saada et al., 2007; Schroeder and Cavacini, 2010; Shirai et al., 1999; Wedemayer et al., 1997; Xu and Davis, 2000). The remaining CDRs are genetically encoded and structurally restricted to a few canonical loop conformations that have generally been considered functionally redundant (Al-Lazikani et al., 1997; Martin and Thornton, 1996; North et al., 2011), although some bacterial and viral proteins bear natural affinities for V-gene-specific features outside the antigen binding surface, triggering non-productive/superantigen B cell responses (Silverman and Goodyear, 2006; Villar et al., 2016). In the current study, we harnessed mouse genetics to mechanistically interrogate natural antigen specificities endowed by human antibody genes *in vivo*. Our results show that despite their limited diversity, human heavy chain variable (V_H) genes are not functionally redundant and that specific germline-encoded input is both needed and tuned to accommodate conserved bacterial structures when present in the bloodstream.

It has long been hypothesized that the encoded CDR repertoire was shaped to endow antigen-specific recognition of conserved microbial features (Chothia and Lesk, 1987; Chothia

et al., 1989; Cohn and Langman, 1990). Although this hypothesis has been refined and applied to account for human antibody specificities (Henry Dunand and Wilson, 2015; Lerner, 2011; Lonberg, 2005), experimental evaluation of germline-encoded recognition of recurrent microbial patterns has largely been explored within mice in which genetic manipulation has enabled mechanistic studies *in vivo* (Chen et al., 2009; Mi et al., 2000). Early reports noting V_H-gene bias in hybridomas reactive to phosphorylcholine (PC), a lipid head group displayed by bacterial and eukaryotic cell membranes (Crews et al., 1981; Gearhart et al., 1981), revealed a germline-encoded target specificity that is now recognized as a core feature of natural antibodies: circulating polyreactive immunoglobulins that are produced constitutively by murine B1 cells and provide important bacterial defense activities (Chen et al., 2009; Grönwall et al., 2012; Holodick et al., 2017; Mi et al., 2000; Savage and Baumgarth, 2015). Natural antibodies with V_H-gene-biased polyreactive substrate specificities have also been identified in humans that, like their murine counterparts, are produced from specialized B cell subsets (Grönwall et al., 2012; Nguyen and Baumgarth, 2016). However, in these cases, the microbial targets do not serve as the natural B cell priming agents, because natural antibodies are produced constitutively, possibly through BCR ligation by polyreactive self-antigens (Grönwall et al., 2012; Holodick et al., 2017; Savage and Baumgarth, 2015).

In this study, we sought to mechanistically evaluate whether conventional B cell responses from the human antibody repertoire (triggered by BCR ligation with exogenous antigen) bear natural specificities for conserved microbial substrate. Individual germline antibody CDRs have already shown clear evidence of differing utilities, because CDR sequences are differentially deployed following immune exposure to certain antigens (Brorson et al., 1999; Hardy et al., 1989; Henry Dunand and Wilson, 2015; Hwang et al., 2014; Kirik et al., 2017; Lerner, 2011; Lonberg, 2005; Pape et al., 2018; Schickel et al., 2017; Wang et al., 2014; Willis et al., 2013), most recently observed for antibody responses to SARS-CoV-2 within convalescent individuals (Cao et al., 2020; Robbiani et al., 2020; Wec et al., 2020b). In humans, such germline-endowed affinity for antigen can also seed genetically reproducible or public broadly neutralizing antibody (bnAb) responses against numerous pathogens, including influenza virus, HIV, hepatitis C virus, hepatitis B virus, and yellow fever virus (Avnir et al., 2014; Flyak et al., 2018; Golsaz-Shirazi et al., 2015; Hehle et al., 2020; Joyce et al., 2016; Lerner, 2011; Lingwood et al., 2012; Lucas et al., 1991; Pappas et al., 2014; Scheid et al., 2011; Tzarum et al., 2019; Wec et al., 2020a; Wheatley et al., 2015; Wu et al., 2011; Zhou et al., 2013, 2015). We have experimentally demonstrated that such public epitope targeting by human BCRs enables vaccine amplification of pathway-reproducible antibody responses that broadly neutralize influenza virus (Sangesland et al., 2019). However, much of the work defining natural antigen substrate for human antibodies has relied on retrospective analyses of immune responses, in which patterns in antibody gene usage are first identified and then subsequently defined through biochemical activities *in vitro*.

To mechanistically evaluate human antibody V_H-gene contribution to the polyclonal response within the living organism, we engineered transgenic animals that harbored human CDRH3 and light-chain (LC) diversities but were simultaneously constrained to different user-defined V_H-genes (encoding CDRH1 + CDRH2). Using this approach, we defined the *in vivo* V_H-gene contribution to extrafollicular responses against bacterial antigens in the blood, simple antiseptic B cell reactions that enable rapid humoral targeting of bacterial lipids and carbohydrates and in which response titers reflect antigen recognition by germline BCRs (Cerutti et al., 2013; Garraud et al., 2012; Limet et al., 1987, 1989; MacLennan et al., 2003; Shishido et al., 2012). We observed that specific V_H-gene input was needed to engage and respond to lipopolysaccharide (LPS), a defining antigen of gram-negative bacteria. Notably, we found that the human V_H-gene IGHV1-2*02, known for genetically endowing public bnAb responses against HIV (Gristick et al., 2016; Scheid et al., 2011; Wu et al., 2011; Zhou et al., 2013, 2015), was required to accommodate this key glycolipid antigen when prepared from diverse bacterial sources. We then mapped this V_H-endowed polyclonal target specificity to the conserved substructure of LPS. Animals using non-IGHV1-2 V_H-genes failed to accommodate LPS, despite bearing similar CDRH3 and LC diversities, B cell frequencies, and capacity to respond to other thymus-independent (TI) and thymus-dependent (TD) antigen geometries when present in the blood. Hence, we conclude that despite their limited diversity, individual human antibody V_H sequences are not functionally redundant and that specific germline-encoded CDRs naturally endow the human antibody repertoire with a broad-spectrum response targeting a conserved bacterial feature.

RESULTS

A System to Mechanistically Evaluate Human V_H-gene Contribution to BCR Antigen Recognition *In Vivo*

We have previously shown that mice homozygous for the HC2 locus are fully constrained to user-defined usage of single human V_H-genes while containing a humanized CDRH3 length distribution and amino acid composition that is similar to that of the human repertoire (Sangesland et al., 2019). By systematically altering the V_H sequence, this system provides an opportunity to mechanistically evaluate human V_H-gene contribution to the polyclonal antibody response within the living organism. The number and frequency of B and T lymphocytes, antibody response titers, and somatic hypermutation within these mice are comparable to wild-type (WT) C57BL/6 (Sangesland et al., 2019). We previously used the HC2 locus to fully constrain the antibody response to single human V_H-genes: IGHV1-2*02 or IGHV1-69*01, in which antibody LC diversity was provided by the murine repertoire (Sangesland et al., 2019).

To more closely recapitulate the human repertoire in the present study, we replaced the murine LC repertoire with KCo5, an engineered human kappa chain locus, as previously established for the HC2 platform (Fishwild et al., 1996; Lonberg, 2005; Xu and Davis, 2000) (Figure 1A). As expected, deep sequencing of the immunoglobulin M (IgM) BCR repertoires of these transgenic mice, termed IGHV1-2 + hIgK and IGHV1-69 + hIgK, revealed CDRH3 length and amino acid distributions that were similar to each other (>13.0 × 10^6 aligned BCR reads per genotype) and to the human CDRH3 profile, as mined from a recent human IgM repertoire analysis (DeWitt et al., 2016) (Figures 1B and 1C).

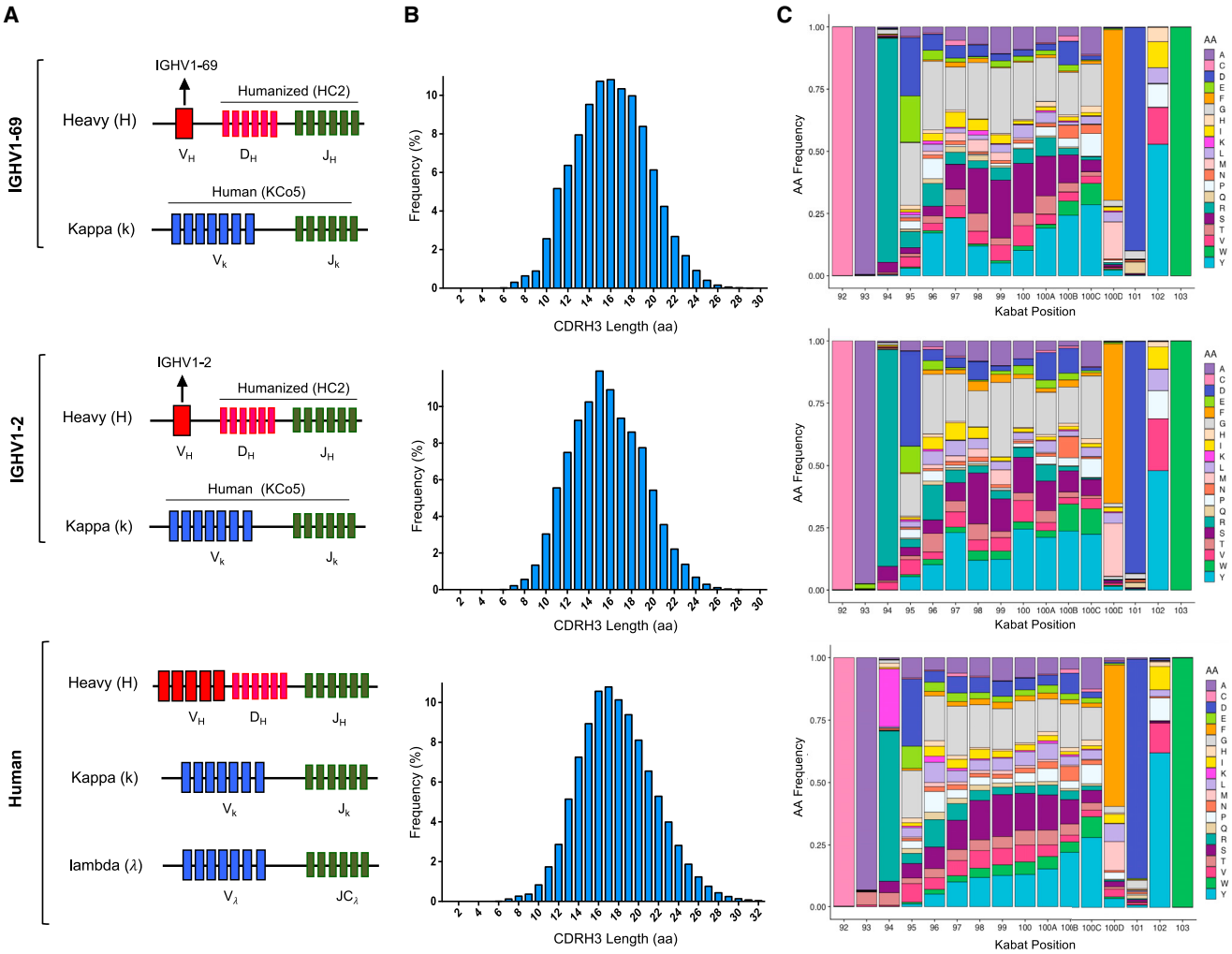

Figure 1. Transgenic Mice Are Constrained to User-Defined Human V_H-genes and Unconstrained Humanized CDRH3 Diversity

(A) Illustration of the antibody heavy-chain and LC loci the IGHV1-69 + hIgK mice and IGHV1-2 + hIgK mice, as well as in humans.

(B) CDRH3 length distribution in a IGHV1-2 and IGHV1-69 restricted mouse compared with humans. BCR sequences obtained from naive IgM B cells from spleen (>13 million BCR reads for one animal from each genotype). The human repertoire from one individual was mined from publicly available data (DeWitt et al., 2016).

(C) CDRH3 amino acid composition from the deep sequencing in (B).

Hence, these animals provided a system to experimentally vary human antibody V_H-gene usage to mechanistically evaluate V_H contribution to antigen engagement in the context of an otherwise unconstrained polyclonal human-like BCR repertoire. We then applied this approach to evaluate B cell antigen recognition within the rapid blood-based extrafollicular antibody responses, normally triggered by bacterial lipids and carbohydrates (Cerutti et al., 2013; Garraud et al., 2012; Limet et al., 1987, 1989; MacLennan et al., 2003; Shishido et al., 2012).

Transgenic Animals Have Similar Frequencies of B Lymphocytes

Antiseptic responses to bloodborne bacterial lipids and carbohydrates are primarily TI IgM arising from B cells residing in the marginal zone (MZ) of the spleen (Cerutti et al., 2013; Garraud et al., 2012; MacLennan et al., 2003; Mond et al., 1995; Vos et al., 2000).

We measured the frequencies and total numbers of B cells ($CD3^-/CD19^+$), T cells ($CD3^+/CD19^-$), and MZ B cells ($CD3^-/CD19^+/B220^+/MHCII^+/CD23^{high}/CD23^{low}$) (Garraud et al., 2012; Kanayama et al., 2005) within our transgenic animals (Figures 2A and 2B). We found that the animal genotypes contained similar numbers and frequencies of these immune cell subsets. Furthermore, to confirm that the intravenously (i.v.) introduced bacterial antigens trafficked to and perfused the extrafollicular space, we i.v. injected fluorescent bacterial LPS and then imaged the CD169 macrophage surface boundary layer encapsulating the spleen follicles. We found that the fluorescent antigen perfused the MZ and was captured within this space in both transgenic mouse genotypes (Figure 2C). Hence, B cells from our mice bearing human antibody repertoires with different V_H-genes appeared similarly poised to initiate humoral responses against extrafollicular antigenic stimuli, particularly bacterial LPS.

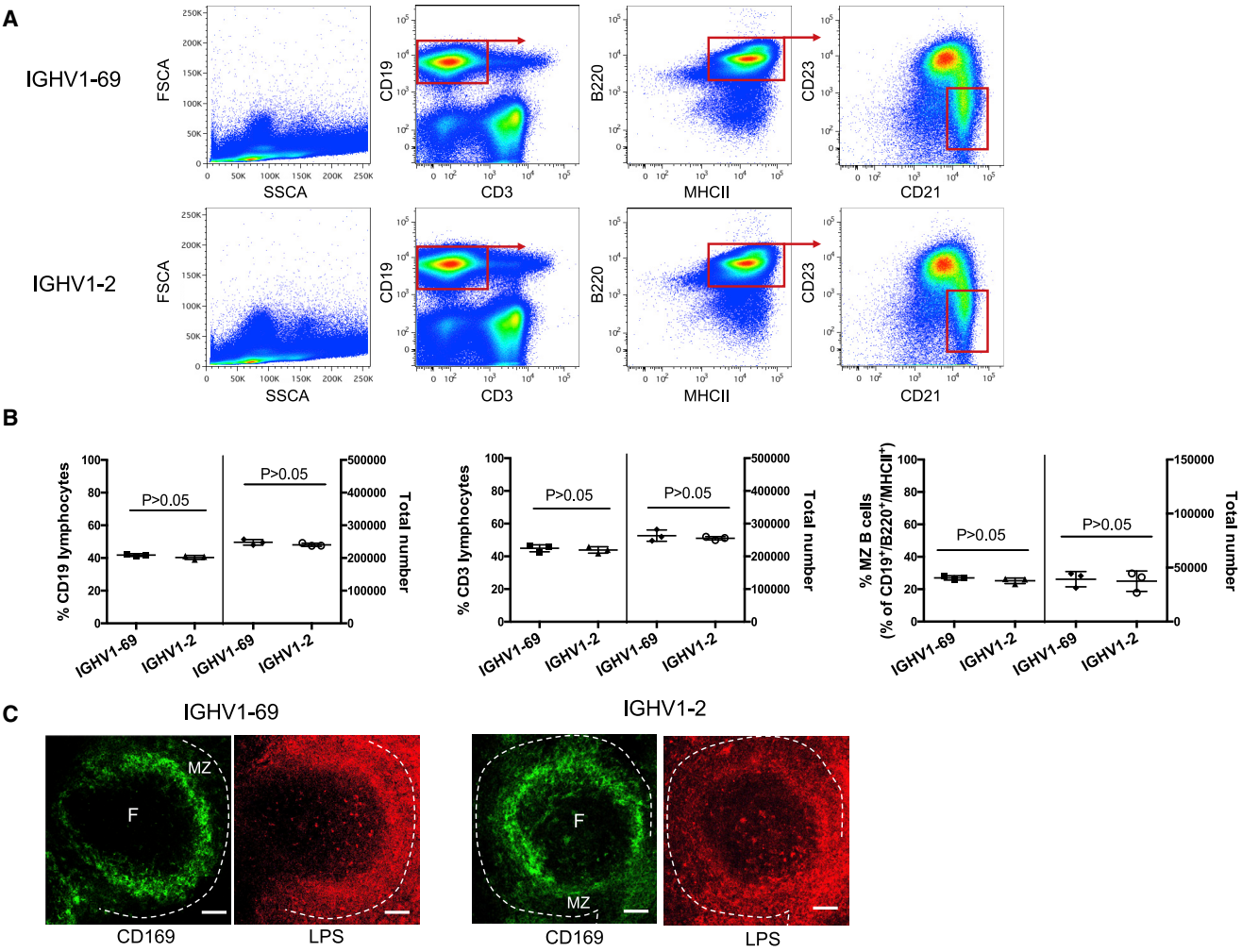

Figure 2. Varying the V_H Sequence Does Not Alter B Cells Numbers or Antigen Perfusion of the Extrafollicular Space

(A) Representative gating strategy on lymphocytes harvested from the spleens of IGHV1-69 and IGHV1-2 mouse genotypes. CD3- and CD19-positive cells were gated negatively against a blue live/dead marker. Singlets were defined by forward scatter height (FSCH) versus forward scatter area (FSCA). MZ B cells were defined as $CD3^-/CD19^+/B220^+/MHCII^+/CD23^{low}/CD21^+$, as shown by the gating scheme in red.

(B) Frequency and number of CD3 T cells, CD19 B cells, and $CD3^-/CD19^+/B220^+/MHCII^+/CD23^{low}/CD21^+$ (MZ) B cells were quantified in IGHV1-69 versus IGHV1-2 mice ($p > 0.05$, two-sided Student's t test, n = 3 mice per genotype).

(C) To ensure that LPS perfused the MZ in both animal genotypes, fluorescent LPS (red) was injected into the bloodstream, and its collection in the spleen MZ was monitored 1 h later by immunofluorescence. Green indicates the CD169 metallophilic macrophage layer demarking the separation of spleen follicles (F) and extrafollicular MZ (marked by the dotted line). Scale bar, 50 μm.

A Specific Human V_H Sequence Is Needed to Accommodate Bacterial LPS *In Vivo*

Extrafollicular IgM responses emanating from spleen MZ B cells reflect antigen accommodation by germline BCRs (Cerutti et al., 2013; Garraud et al., 2012; MacLennan et al., 2003; Mond et al., 1995; Shishido et al., 2012; Vos et al., 2000) and can be can be tracked 3, 7, and 14 days after i.v. immune challenge (Cerutti et al., 2013; Chappell et al., 2012; MacLennan et al., 2003; Rubtsov et al., 2005). Moreover, sequential immunization with TI antigen will not boost antibody response titers, because there is no T cell help to coordinate B cell germinal center formation and development of B cell memory (Mond et al., 1995; Tittle and Rittenberg, 1980; Vos et al., 2000).

We found that both IGHV1-2 and IGHV1-69 mice produced robust extrafollicular IgM responses against TNP-ficoll, a standard TI-2 antigen that elicits antibodies through BCR crosslinking only, in the absence of innate immune receptor stimulation (Mond et al., 1995; Öner et al., 2016; Tittle and Rittenberg, 1980; Vos et al., 2000) (Figure 3A). We observed the same result following immunization of HCo12 mice, which use the same human CDRH3 and LC diversity but deploy five common human V_H-genes (IGHV5-51 + IGHV3-23 + IGHV3-30 + IGHV1-69 +IGHV4-34) (Figures 3A and 3B). All mouse genotypes also produced robust IgM responses against ovalbumin when this control TD antigen was administered i.v. (Figure 3A). Surprisingly, however, only the IGHV1-2 mice generated bloodborne

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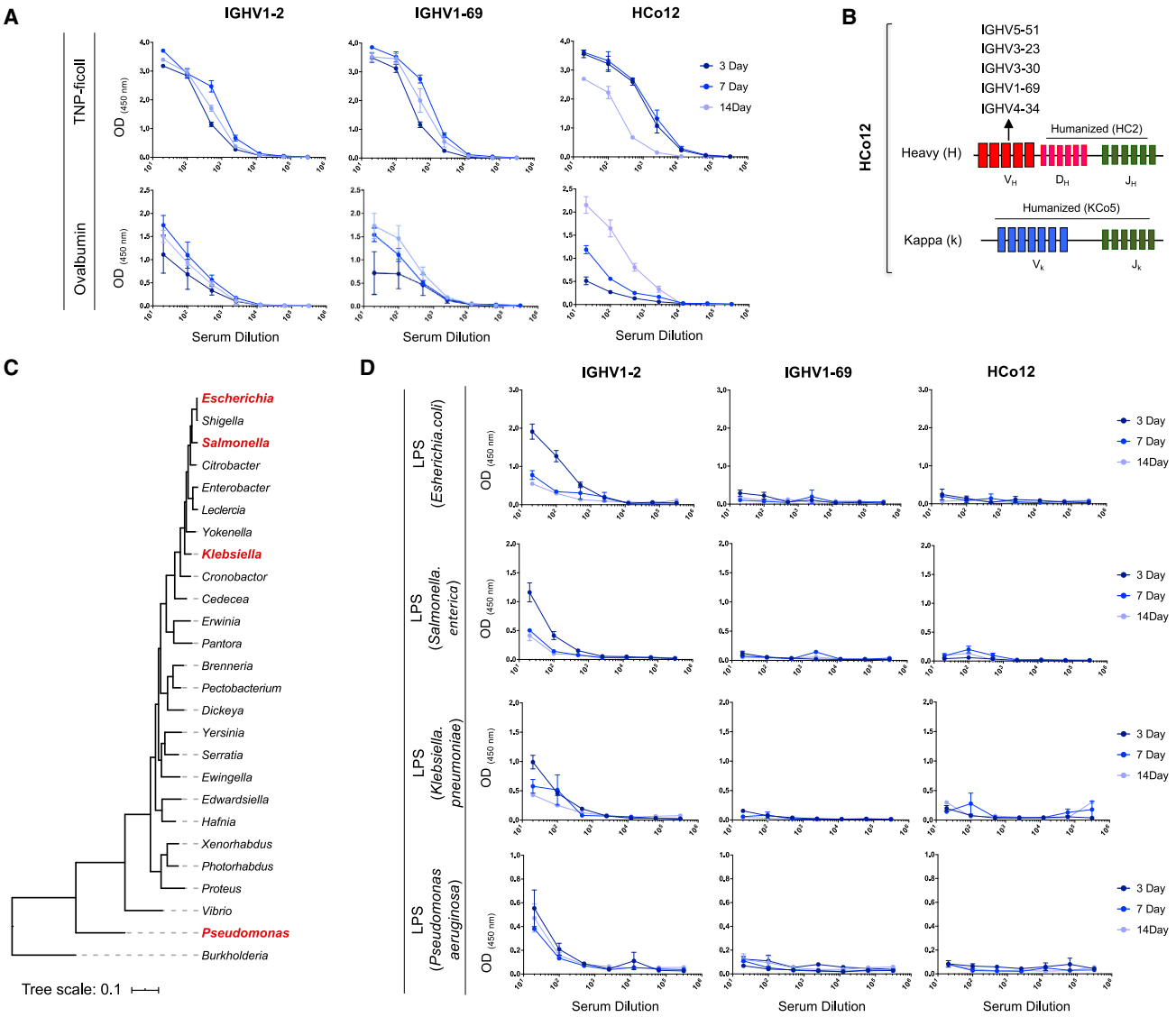

Figure 3. Of Six Commonly Used Human V_H Sequences, Only IGHV1-2 Endows the Capacity to Accommodate Divergent LPS Structures when Present in the Bloodstream

(A) Extrafollicular IgM responses to bloodborne antigens measured 3, 7, and 14 days post-immune challenge (mean ± SD, n = 3 per genotype). This includes responses to TNP-ficoll, a conventional TI antigen, and ovalbumin, a common (TD) antigen. Pre-immune readings were subtracted from these curves.

(B) Illustration of the antibody heavy-chain and LC loci of HCo12 mice, which bear the same CDR3 and LC diversity except that they deploy five common human V_H sequences: IGHV5-51, IGHV3-23, IGHV3-30, IGHV1-69, and IGHV4-34.

(C) Phylogenetic reconstruction of the gram-negative bacteria. This tree spans two bacterial orders (Pseudomonadales and Enterobacterales) and was rooted in *Burkholderia cepacia* of the order Burkholderiales. The scale bar refers to the average number of mutations per position.

(D) IgM responses against LPS, the principle TI antigen from gram-negative bacteria. LPS was prepared from bacteria spanning the orders Pseudomonadales and Enterobacterales (mean ± SD, n = 3 per genotype). Pre-immune readings were subtracted from these curves.

IgM responses against LPS, which were prepared from diverse bacterial strains spanning two phylogenic orders: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella enterica* (Figures 3B and 3C). This demonstrated that despite housing comparable levels of CDRH3 and LC diversities and B cells receptive to other TI and TD antigen geometries, polyclonal accommodation of diverse LPS structures was functionally reliant on CDRs encoded by IGHV1-2.

V_H-gene Hardwired Antibody Specificity for LPS Maps to the Conserved Saccrolipid Core of This Antigen

Given that V_H-gene-dependent immunoreceptor activity was observed for diverse sources of LPS, we hypothesized that IGHV1-2-encoded CDRs may endow target solutions that recognize conserved features of this antigen, perhaps the conserved saccrolipid substructure of bacterial LPS (Figure 4A). To test this, we immunized our animals with a uniform Kdo2-lipid

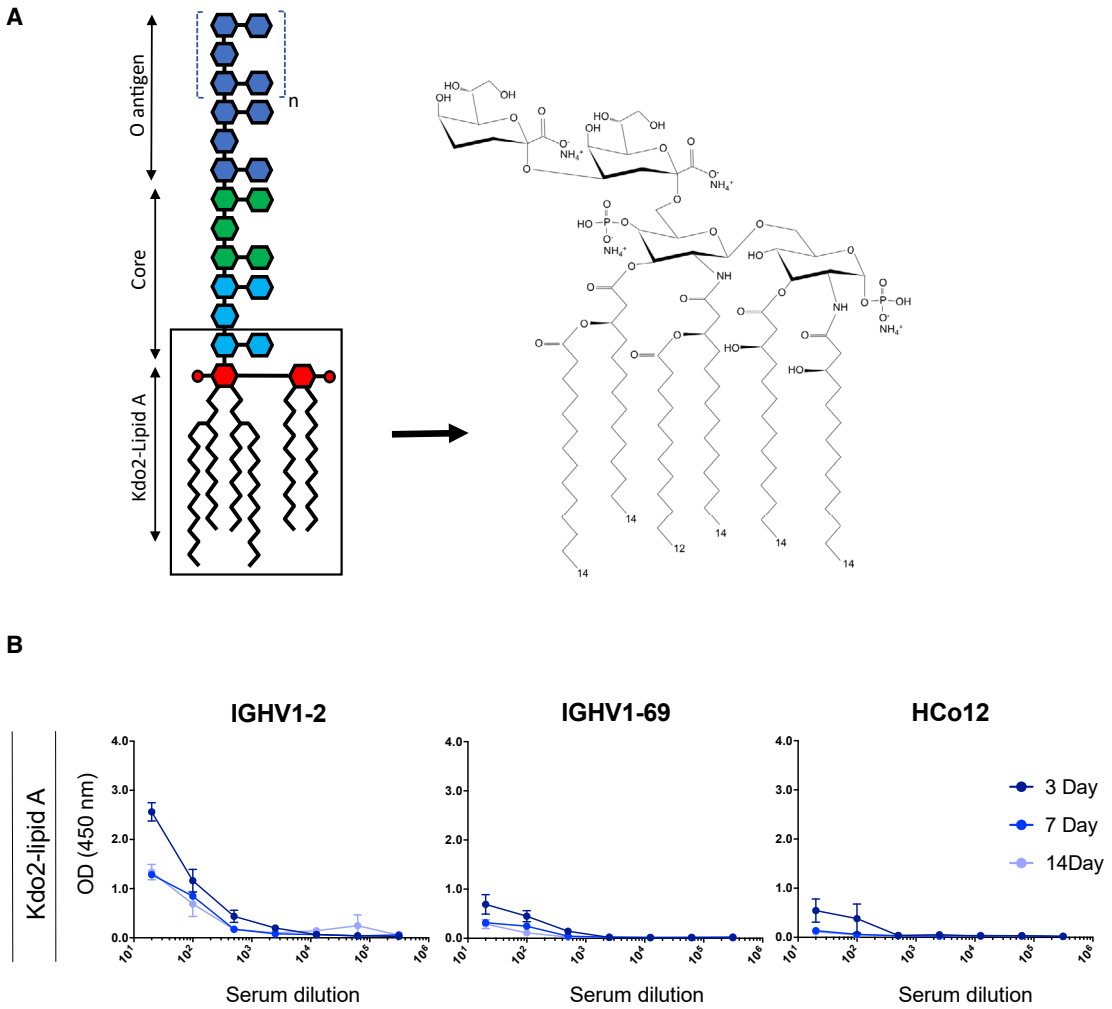

Figure 4. IGHV1-2 Endows Innate-like BCR Specificity for the Conserved Saccrolipid Core of Bacterial LPS

(A) Kdo2-lipid A (Di[3-deoxy-D-manno-octulosonyl]-lipid A) from is a conserved LPS substructure and is the most homogeneous and potent LPS preparation available (Saito et al., 2010) (the defined hydrocarbon chain lengths are marked).

(B) Extrafollicular IgM responses against Kdo2-lipid A when introduced into the bloodstream of IGHV1-2, IGHV1-69, and HCo12 animals. IgM responses were monitored 3, 7, and 14 days post-immune challenge (mean ± SD, n = 3 per genotype). Pre-immune readings were subtracted from these curves.

A preparation with chemically homogeneous acyl chain structures, and consequently the most potent LPS-based TLR4 agonist yet described, reported as 10-fold higher than conventional LPS preparations (Saito et al., 2010). As with the heterogeneous LPS inocula, we observed V_H-gene-dependent accommodation of Kdo2-lipid A *in vivo*, as evidenced by robust IgM responses in IGHV1-2 animals and dampened immunogenicity in the IGHV1-69 and HCo12 genotypes (Figure 4B). We therefore surmised that the natural polyclonal B cell target specificity endowed by the IGHV1-2 sequence mapped to the conserved, universal building block of bacterial LPS.

DISCUSSION

Genetically hardwired specificities for conserved microbial patterns are hallmarks of the innate immune system and have long been proposed for B cell antigen receptors (Chothia and Lesk, 1987; Chothia et al., 1989; Cohn and Langman, 1990; Henry Dunand and Wilson, 2015; Hwang et al., 2014; Lerner, 2011; Lonberg, 2005). However, such germline-endowed affinities for specific features common to pathogens or groups of pathogens have been difficult to mechanistically prove *in vivo*. This is because the ligand binding surface of the BCR is composed of a chimeric display of encoded and non-encoded CDRs unique to each lymphocyte clone (Glanville et al., 2009; Kuroda et al., 2008; Morea et al., 1998; Oliva et al., 1998; Saada et al., 2007; Schroeder and Cavacini, 2010; Shirai et al., 1999; Wedemayer et al., 1997; Xu and Davis, 2000). How then can mechanistic causality be assigned to any one component *in vivo*? Much of the work defining natural human antibody substrate has relied on retrospective analyses of human immune responses in which patterns in antibody gene usage are first identified and then biochemically evaluated *in vitro*. In this study, we harnessed mouse genetics to mechanistically interrogate BCR antigen specificities

endowed by individual human antibody genes. We engineered a system in which all antibody CDRs were humanized, allowing unconstrained diversity with the exception of those encoded by the V_H sequence, which were 100% restricted and systematically varied. Through this approach, we could experimentally define human V_H-gene contribution to the polyclonal antigen response as a whole and as a function of immune challenge within the living organism.

We evaluated this parameter within extrafollicular B cell responses to bloodborne LPS, simple and rapid antiseptic reactions in which antibody response titers reflect germline BCR engagement capacity (Cerutti et al., 2013; Garraud et al., 2012; MacLennan et al., 2003; Shishido et al., 2012). Our results indicated that of six common human V_H sequences, only IGHV1-2 could support robust polyclonal accommodation of LPS, which was prepared from diverse sources spanning two phylogenic orders of gram-negative bacteria. Importantly, this was achieved by genetically endowing for recognition of the non-variable saccrolipid core of this antigen, revealing a broad-spectrum antiseptic response against a conserved microbial feature, akin to the pattern recognition receptor activities of the innate immune system.

In a pioneering study, Xu and Davis (2000) reported that a single V_H-gene mouse generated antibodies against any protein antigen through CDRH3 diversity alone, functionally establishing this motif as the principle determinant of B cell antigen recognition *in vivo*. The centrally positioned CDRH3 supplies the human BCR repertoire with $>10^6$ times the diversity of antibody V-gene-encoded CDRs and is thought to enable target solutions against any antigenic surface (Glanville et al., 2009; Kuroda et al., 2008; Morea et al., 1998; Oliva et al., 1998; Saada et al., 2007; Schroeder and Cavacini, 2010; Shirai et al., 1999; Wedemayer et al., 1997; Xu and Davis, 2000). Diverse CDRH3- and LC-associated targeting solutions have been observed for antibody responses against LPS and other bacterial antigens (Haji-Ghassemi et al., 2015; Nguyen et al., 2003) and would also be expected for the polyclonal anti-LPS responses we observe. However, we demonstrate a deterministic feature that functionally gates the polyclonal response as a whole, namely, that specific human V_H-gene input is obligate for engaging bloodborne LPS, despite the presence of equivalent CDRH3 and LC diversities across our transgenic mouse genotypes. The LPS response deficits occurred in animals that were otherwise able to respond to control TI and TD antigens and were not explained by the capacity to perfuse the spleen MZ with LPS or the number of responding B cells in residence. LPS is also a potent agonist for Toll-like receptor 4 (TLR4) and a powerful B cell adjuvant (Pone et al., 2012; Schweighoffer et al., 2017), and it is particularly potent in the Kdo2-lipid A format (Saito et al., 2010). The failure to accommodate these antigen geometries, even in the presence of such TLR stimulation, underscores our conclusions that despite their limited diversity, human V_H-genes are not functionally redundant and that some human antiseptic responses are germline encoded.

The amino acid sequence XXGG(S/T/G) within the CDRH2 loops of mouse immunoglobulins has previously been reported as a lipid A binding motif (Haji-Ghassemi et al., 2016). This motif is present in the CDRH2 from IGHV1-2*02; however, it is also present within IGHV3-23*01, contained within HCo12 mice, which did not respond to bloodborne LPS. Consequently, LPS binding through this motif may not be broadly transplantable to the human V_H-genes of our study, although differential expression of V_H sequences within the HCo12 mouse also cannot be ruled out.

In conclusion, we demonstrate that conventional B cell responses can be tuned by human-gene-endowed affinities for a conserved microbial pattern, akin to the receptors of the innate immune system. This discovery was enabled by manipulating mouse genetics to experimentally define V_H-gene contribution to B cell antigen recognition within an otherwise unrestricted human-like antibody repertoire. Beyond its potential clinical importance in combating sepsis, a germline-encoded anti-LPS response may reflect a primordial/evolutionarily retained capacity that, in the case of IGHV1-2*02, could have provided a foundation for later unanticipated germline utilities, such as the non-random use of this V_H sequence in human bnAbs against HIV (Gristick et al., 2016; Scheid et al., 2011; Wu et al., 2011; Zhou et al., 2013, 2015).

STAR★METHODS

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AUTHOR CONTRIBUTIONS

M.S., A.S.Y., and D.L. designed the research studies; M.S., A.S.Y., L.R., S.W.K., A.L.Z., G.J.G., M.R.H., R.M.B., M.Q.-C., D.R., N.L., D.K., A.K.S.,

and D.L. performed the research; M.S., A.S.Y., D.K., A.K.S., and D.L. analyzed the data; and M.S. and D.L. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Al-Lazikani, B., Lesk, A.M., and Chothia, C. (1997). Standard conformations for the canonical structures of immunoglobulins. J. Mol. Biol. *273*, 927–948.

Avnir, Y., Tallarico, A.S., Zhu, Q., Bennett, A.S., Connelly, G., Sheehan, J., Sui, J., Fahmy, A., Huang, C.Y., Cadwell, G., et al. (2014). Molecular signatures of hemagglutinin stem-directed heterosubtypic human neutralizing antibodies against influenza A viruses. PLoS Pathog. *10*, e1004103.

Bolotin, D.A., Poslavsky, S., Mitrophanov, I., Shugay, M., Mamedov, I.Z., Putintseva, E.V., and Chudakov, D.M. (2015). MiXCR: software for comprehensive adaptive immunity profiling. Nat. Methods *12*, 380–381.

Brorson, K., Thompson, C., Wei, G., Krasnokutsky, M., and Stein, K.E. (1999). Mutational analysis of avidity and fine specificity of anti-levan antibodies. J. Immunol. *163*, 6694–6701.

Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C., et al. (2020). Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. Cell *182*, 73–84.e16.

Cerutti, A., Cols, M., and Puga, I. (2013). Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat. Rev. Immunol. *13*, 118–132.

Chappell, C.P., Draves, K.E., Giltiay, N.V., and Clark, E.A. (2012). Extrafollicular B cell activation by marginal zone dendritic cells drives T cell-dependent antibody responses. J. Exp. Med. *209*, 1825–1840.

Chen, Y., Khanna, S., Goodyear, C.S., Park, Y.B., Raz, E., Thiel, S., Grönwall, C., Vas, J., Boyle, D.L., Corr, M., et al. (2009). Regulation of dendritic cells and macrophages by an anti-apoptotic cell natural antibody that suppresses TLR responses and inhibits inflammatory arthritis. J. Immunol. *183*, 1346–1359.

Chothia, C., and Lesk, A.M. (1987). Canonical structures for the hypervariable regions of immunoglobulins. J. Mol. Biol. *196*, 901–917.

Chothia, C., Lesk, A.M., Tramontano, A., Levitt, M., Smith-Gill, S.J., Air, G., Sheriff, S., Padlan, E.A., Davies, D., Tulip, W.R., et al. (1989). Conformations of immunoglobulin hypervariable regions. Nature *342*, 877–883.

Cohn, M., and Langman, R.E. (1990). The protecton: the unit of humoral immunity selected by evolution. Immunol. Rev. *115*, 11–147.

Crews, S., Griffin, J., Huang, H., Calame, K., and Hood, L. (1981). A single VH gene segment encodes the immune response to phosphorylcholine: somatic mutation is correlated with the class of the antibody. Cell *25*, 59–66.

DeWitt, W.S., Lindau, P., Snyder, T.M., Sherwood, A.M., Vignali, M., Carlson, C.S., Greenberg, P.D., Duerkopp, N., Emerson, R.O., and Robins, H.S. (2016). A Public Database of Memory and Naive B-Cell Receptor Sequences. PLoS ONE *11*, e0160853.

Fishwild, D.M., O'Donnell, S.L., Bengoechea, T., Hudson, D.V., Harding, F., Bernhard, S.L., Jones, D., Kay, R.M., Higgins, K.M., Schramm, S.R., and Lonberg, N. (1996). High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice. Nat. Biotechnol. *14*, 845–851.

Flyak, A.I., Ruiz, S., Colbert, M.D., Luong, T., Crowe, J.E., Jr., Bailey, J.R., and Bjorkman, P.J. (2018). HCV Broadly Neutralizing Antibodies Use a CDRH3 Disulfide Motif to Recognize an E2 Glycoprotein Site that Can Be Targeted for Vaccine Design. Cell Host Microbe *24*, 703–716.

Garraud, O., Borhis, G., Badr, G., Degrelle, S., Pozzetto, B., Cognasse, F., and Richard, Y. (2012). Revisiting the B-cell compartment in mouse and humans: more than one B-cell subset exists in the marginal zone and beyond. BMC Immunol. *13*, 63.

Gearhart, P.J., Johnson, N.D., Douglas, R., and Hood, L. (1981). IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. Nature *291*, 29–34.

Glanville, J., Zhai, W., Berka, J., Telman, D., Huerta, G., Mehta, G.R., Ni, I., Mei, L., Sundar, P.D., Day, G.M., et al. (2009). Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. Proc. Natl. Acad. Sci. USA *106*, 20216–20221.

Golsaz-Shirazi, F., Amiri, M.M., Bahadori, M., Bayat, A.A., Mohammadi, H., Farid, S., Maddah, M., Khoshnoodi, J., Zarnani, A.H., Jeddi-Tehrani, M., and Shokri, F. (2015). Molecular Characterization of Murine Monoclonal Antibody Variable Regions Specific for Hepatitis B Surface Antigen. Viral Immunol. *28*, 425–433.

Gristick, H.B., von Boehmer, L., West, A.P., Jr., Schamber, M., Gazumyan, A., Golijanin, J., Seaman, M.S., Fätkenheuer, G., Klein, F., Nussenzweig, M.C., and Bjorkman, P.J. (2016). Natively glycosylated HIV-1 Env structure reveals new mode for antibody recognition of the CD4-binding site. Nat. Struct. Mol. Biol. *23*, 906–915.

Grönwall, C., Vas, J., and Silverman, G.J. (2012). Protective Roles of Natural IgM Antibodies. Front. Immunol. *3*, 66.

Haji-Ghassemi, O., Blackler, R.J., Martin Young, N., and Evans, S.V. (2015). Antibody recognition of carbohydrate epitopes. Glycobiology *25*, 920–952.

Haji-Ghassemi, O., Müller-Loennies, S., Rodriguez, T., Brade, L., Grimmecke, H.D., Brade, H., and Evans, S.V. (2016). The Combining Sites of Anti-lipid A Antibodies Reveal a Widely Utilized Motif Specific for Negatively Charged Groups. J. Biol. Chem. *291*, 10104–10118.

Hardy, R.R., Carmack, C.E., Shinton, S.A., Riblet, R.J., and Hayakawa, K. (1989). A single VH gene is utilized predominantly in anti-BrMRBC hybridomas derived from purified Ly-1 B cells. Definition of the VH11 family. J. Immunol. *142*, 3643–3651.

Hehle, V., Beretta, M., Bourgine, M., Ait-Goughoulte, M., Planchais, C., Morisse, S., Vesin, B., Lorin, V., Hieu, T., Stauffer, A., et al. (2020). Potent human broadly neutralizing antibodies to hepatitis B virus from natural controllers. J. Exp. Med. *217*, e20200840.

Henry Dunand, C.J., and Wilson, P.C. (2015). Restricted, canonical, stereotyped and convergent immunoglobulin responses. Philos. Trans. R. Soc. Lond. B Biol. Sci. *370*, 20140238.

Hogan, B., Beddington, R., Costantini, F., and Lacy, E. (1994). Manipulating the Mouse Embryo, A Laboratory Manual, 2nd edition (Cold Spring Harbor Laboratory Press).

Holodick, N.E., Rodríguez-Zhurbenko, N., and Hernández, A.M. (2017). Defining Natural Antibodies. Front. Immunol. *8*, 872.

Huerta-Cepas, J., Serra, F., and Bork, P. (2016). ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. Mol. Biol. Evol. *33*, 1635–1638.

Hwang, K.K., Trama, A.M., Kozink, D.M., Chen, X., Wiehe, K., Cooper, A.J., Xia, S.M., Wang, M., Marshall, D.J., Whitesides, J., et al. (2014). IGHV1-69 B cell chronic lymphocytic leukemia antibodies cross-react with HIV-1 and hepatitis C virus antigens as well as intestinal commensal bacteria. PLoS ONE *9*, e90725.

Hyatt, D., Chen, G.L., Locascio, P.F., Land, M.L., Larimer, F.W., and Hauser, L.J. (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics *11*, 119.

Joyce, M.G., Wheatley, A.K., Thomas, P.V., Chuang, G.Y., Soto, C., Bailer, R.T., Druz, A., Georgiev, I.S., Gillespie, R.A., Kanekiyo, M., et al.; NISC Comparative Sequencing Program (2016). Vaccine-Induced Antibodies that Neutralize Group 1 and Group 2 Influenza A Viruses. Cell *166*, 609–623.

Kanayama, N., Cascalho, M., and Ohmori, H. (2005). Analysis of marginal zone B cell development in the mouse with limited B cell diversity: role of the antigen receptor signals in the recruitment of B cells to the marginal zone. J. Immunol. *174*, 1438–1445.

Kirik, U., Persson, H., Levander, F., Greiff, L., and Ohlin, M. (2017). Antibody Heavy Chain Variable Domains of Different Germline Gene Origins Diversify through Different Paths. Front. Immunol. *8*, 1433.

Kuroda, D., Shirai, H., Kobori, M., and Nakamura, H. (2008). Structural classification of CDR-H3 revisited: a lesson in antibody modeling. Proteins *73*, 608–620.

Lerner, R.A. (2011). Rare antibodies from combinatorial libraries suggests an S.O.S. component of the human immunological repertoire. Mol. Biosyst. *7*, 1004–1012.

Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. *47* (*W1*), W256–W259.

Limet, J., Plommet, A.M., Dubray, G., and Plommet, M. (1987). Immunity conferred upon mice by anti-LPS monoclonal antibodies in murine brucellosis. Ann. Inst. Pasteur Immunol. *138*, 417–424.

Limet, J.N., Bosseray, N., Garin-Bastuji, B., Dubray, G., and Plommet, M. (1989). Humoral immunity in mice mediated by monoclonal antibodies against the A and M antigens of Brucella. J. Med. Microbiol. *30*, 37–43.

Lingwood, D., McTamney, P.M., Yassine, H.M., Whittle, J.R., Guo, X., Boyington, J.C., Wei, C.J., and Nabel, G.J. (2012). Structural and genetic basis for development of broadly neutralizing influenza antibodies. Nature *489*, 566–570.

Lonberg, N. (2005). Human antibodies from transgenic animals. Nat. Biotechnol. *23*, 1117–1125.

Lonberg, N., Taylor, L.D., Harding, F.A., Trounstine, M., Higgins, K.M., Schramm, S.R., Kuo, C.C., Mashayekh, R., Wymore, K., McCabe, J.G., et al. (1994). Antigen-specific human antibodies from mice comprising four distinct genetic modifications. Nature *368*, 856–859.

Louie, D.A.P., and Liao, S. (2019). Lymph Node Subcapsular Sinus Macrophages as the Frontline of Lymphatic Immune Defense. Front. Immunol. *10*, 347.

Lucas, A.H., Langley, R.J., Granoff, D.M., Nahm, M.H., Kitamura, M.Y., and Scott, M.G. (1991). An idiotypic marker associated with a germ-line encoded kappa light chain variable region that predominates the vaccine-induced human antibody response to the *Haemophilus influenzae* b polysaccharide. J. Clin. Invest. *88*, 1811–1818.

MacLennan, I.C., Toellner, K.M., Cunningham, A.F., Serre, K., Sze, D.M., Zúñiga, E., Cook, M.C., and Vinuesa, C.G. (2003). Extrafollicular antibody responses. Immunol. Rev. *194*, 8–18.

Martin, A.C., and Thornton, J.M. (1996). Structural families in loops of homologous proteins: automatic classification, modelling and application to antibodies. J. Mol. Biol. *263*, 800–815.

Mi, Q.S., Zhou, L., Schulze, D.H., Fischer, R.T., Lustig, A., Rezanka, L.J., Donovan, D.M., Longo, D.L., and Kenny, J.J. (2000). Highly reduced protection against *Streptococcus pneumoniae* after deletion of a single heavy chain gene in mouse. Proc. Natl. Acad. Sci. USA *97*, 6031–6036.

Mond, J.J., Lees, A., and Snapper, C.M. (1995). T cell-independent antigens type 2. Annu. Rev. Immunol. *13*, 655–692.

Moran, I., Grootveld, A.K., Nguyen, A., and Phan, T.G. (2019). Subcapsular Sinus Macrophages: The Seat of Innate and Adaptive Memory in Murine Lymph Nodes. Trends Immunol. *40*, 35–48.

Morea, V., Tramontano, A., Rustici, M., Chothia, C., and Lesk, A.M. (1998). Conformations of the third hypervariable region in the VH domain of immunoglobulins. J. Mol. Biol. *275*, 269–294.

Nazarov, V.I., Pogorelyy, M.V., Komech, E.A., Zvyagin, I.V., Bolotin, D.A., Shugay, M., Chudakov, D.M., Lebedev, Y.B., and Mamedov, I.Z. (2015). tcR: an R package for T cell receptor repertoire advanced data analysis. BMC Bioinformatics *16*, 175.

Nguyen, T.T., and Baumgarth, N. (2016). Natural IgM and the Development of B Cell-Mediated Autoimmune Diseases. Crit. Rev. Immunol. *36*, 163–177.

Nguyen, H.P., Seto, N.O., MacKenzie, C.R., Brade, L., Kosma, P., Brade, H., and Evans, S.V. (2003). Germline antibody recognition of distinct carbohydrate epitopes. Nat. Struct. Biol. *10*, 1019–1025.

North, B., Lehmann, A., and Dunbrack, R.L., Jr. (2011). A new clustering of antibody CDR loop conformations. J. Mol. Biol. *406*, 228–256.

O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., et al. (2016). Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. *44* (*D1*), D733–D745.

Oliva, B., Bates, P.A., Querol, E., Avilés, F.X., and Sternberg, M.J. (1998). Automated classification of antibody complementarity determining region 3 of the heavy chain (H3) loops into canonical forms and its application to protein structure prediction. J. Mol. Biol. *279*, 1193–1210.

Öner, E.T., Hernández, L., and Combie, J. (2016). Review of Levan polysaccharide: From a century of past experiences to future prospects. Biotechnol. Adv. *34*, 827–844.

Pape, K.A., Maul, R.W., Dileepan, T., Paustian, A.S., Gearhart, P.J., and Jenkins, M.K. (2018). Naive B Cells with High-Avidity Germline-Encoded Antigen Receptors Produce Persistent IgM^+ and Transient IgG^+ Memory B Cells. Immunity *48*, 1135–1143.e4.

Pappas, L., Foglierini, M., Piccoli, L., Kallewaard, N.L., Turrini, F., Silacci, C., Fernandez-Rodriguez, B., Agatic, G., Giacchetto-Sasselli, I., Pellicciotta, G., et al. (2014). Rapid development of broadly influenza neutralizing antibodies through redundant mutations. Nature *516*, 418–422.

Pone, E.J., Zhang, J., Mai, T., White, C.A., Li, G., Sakakura, J.K., Patel, P.J., Al-Qahtani, A., Zan, H., Xu, Z., and Casali, P. (2012). BCR-signalling synergizes with TLR-signalling for induction of AID and immunoglobulin class-switching through the non-canonical NF-κB pathway. Nat. Commun. *3*, 767.

Robbiani, D.F., Gaebler, C., Muecksch, F., Lorenzi, J.C.C., Wang, Z., Cho, A., Agudelo, M., Barnes, C.O., Gazumyan, A., Finkin, S., et al. (2020). Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature, Published online June 18, 2020. https://doi.org/10.1038/s41586-020-2456-9.

Rubtsov, A., Strauch, P., Digiacomo, A., Hu, J., Pelanda, R., and Torres, R.M. (2005). Lsc regulates marginal-zone B cell migration and adhesion and is required for the IgM T-dependent antibody response. Immunity *23*, 527–538.

Saada, R., Weinberger, M., Shahaf, G., and Mehr, R. (2007). Models for antigen receptor gene rearrangement: CDR3 length. Immunol. Cell Biol. *85*, 323–332.

Saito, O., Svensson, C.I., Buczynski, M.W., Wegner, K., Hua, X.Y., Codeluppi, S., Schaloske, R.H., Deems, R.A., Dennis, E.A., and Yaksh, T.L. (2010). Spinal glial TLR4-mediated nociception and production of prostaglandin E(2) and TNF. Br. J. Pharmacol. *160*, 1754–1764.

Sangesland, M., Ronsard, L., Kazer, S.W., Bals, J., Boyoglu-Barnum, S., Yousif, A.S., Barnes, R., Feldman, J., Quirindongo-Crespo, M., McTamney, P.M., et al. (2019). Germline-Encoded Affinity for Cognate Antigen Enables Vaccine Amplification of a Human Broadly Neutralizing Response against Influenza Virus. Immunity *51*, 735–749.

Savage, H.P., and Baumgarth, N. (2015). Characteristics of natural antibody-secreting cells. Ann. N Y Acad. Sci. *1362*, 132–142.

Scheid, J.F., Mouquet, H., Ueberheide, B., Diskin, R., Klein, F., Oliveira, T.Y., Pietzsch, J., Fenyo, D., Abadir, A., Velinzon, K., et al. (2011). Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science *333*, 1633–1637.

Schickel, J.N., Glauzy, S., Ng, Y.S., Chamberlain, N., Massad, C., Isnardi, I., Katz, N., Uzel, G., Holland, S.M., Picard, C., et al. (2017). Self-reactive VH4-34-expressing IgG B cells recognize commensal bacteria. J. Exp. Med. *214*, 1991–2003.

Schroeder, H.W., Jr., and Cavacini, L. (2010). Structure and function of immunoglobulins. J. Allergy Clin. Immunol. *125* (*Suppl 2*), S41–S52.

Schweighoffer, E., Nys, J., Vanes, L., Smithers, N., and Tybulewicz, V.L.J. (2017). TLR4 signals in B lymphocytes are transduced via the B cell antigen receptor and SYK. J. Exp. Med. *214*, 1269–1280.

Shirai, H., Kidera, A., and Nakamura, H. (1999). H3-rules: identification of CDR-H3 structures in antibodies. FEBS Lett. *455*, 188–197.

Shishido, S.N., Varahan, S., Yuan, K., Li, X., and Fleming, S.D. (2012). Humoral innate immune response and disease. Clin. Immunol. *144*, 142–158.

Silverman, G.J., and Goodyear, C.S. (2006). Confounding B-cell defences: lessons from a staphylococcal superantigen. Nat. Rev. Immunol. *6*, 465–475.

Sunagawa, S., Mende, D.R., Zeller, G., Izquierdo-Carrasco, F., Berger, S.A., Kultima, J.R., Coelho, L.P., Arumugam, M., Tap, J., Nielsen, H.B., et al. (2013). Metagenomic species profiling using universal phylogenetic marker genes. Nat. Methods *10*, 1196–1199.

Tittle, T.V., and Rittenberg, M.B. (1980). IgG B memory cell subpopulations: differences in susceptibility to stimulation by TI-1 and TI-2 antigens. J. Immunol. *124*, 202–206.

Tobias, P.S., Soldau, K., and Ulevitch, R.J. (1989). Identification of a lipid A binding site in the acute phase reactant lipopolysaccharide binding protein. J. Biol. Chem. *264*, 10867–10871.

Trombetta, J.J., Gennert, D., Lu, D., Satija, R., Shalek, A.K., and Regev, A. (2014). Preparation of Single-Cell RNA-Seq Libraries for Next Generation Sequencing. Curr. Protoc. Mol. Biol. *107*, 4.22.1–4.22.17.

Tzarum, N., Giang, E., Kong, L., He, L., Prentoe, J., Augestad, E., Hua, Y., Castillo, S., Lauer, G.M., Bukh, J., et al. (2019). Genetic and structural insights into broad neutralization of hepatitis C virus by human VH1-69 antibodies. Sci. Adv. *5*, eaav1882.

Villar, R.F., Patel, J., Weaver, G.C., Kanekiyo, M., Wheatley, A.K., Yassine, H.M., Costello, C.E., Chandler, K.B., McTamney, P.M., Nabel, G.J., et al. (2016). Reconstituted B cell receptor signaling reveals carbohydrate-dependent mode of activation. Sci. Rep. *6*, 36298.

Vos, Q., Lees, A., Wu, Z.Q., Snapper, C.M., and Mond, J.J. (2000). B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. Immunol. Rev. *176*, 154–170.

Wang, C., Liu, Y., Xu, L.T., Jackson, K.J., Roskin, K.M., Pham, T.D., Laserson, J., Marshall, E.L., Seo, K., Lee, J.Y., et al. (2014). Effects of aging, cytomegalovirus infection, and EBV infection on human B cell repertoires. J. Immunol. *192*, 603–611.

Wec, A.Z., Haslwanter, D., Abdiche, Y.N., Shehata, L., Pedreño-Lopez, N., Moyer, C.L., Bornholdt, Z.A., Lilov, A., Nett, J.H., Jangra, R.K., et al. (2020a). Longitudinal dynamics of the human B cell response to the yellow fever 17D vaccine. Proc. Natl. Acad. Sci. USA *117*, 6675–6685.

Wec, A.Z., Wrapp, D., Herbert, A.S., Maurer, D.P., Haslwanter, D., Sakharkar, M., Jangra, R.K., Dieterle, M.E., Lilov, A., Huang, D., et al. (2020b). Broad neutralization of SARS-related viruses by human monoclonal antibodies. Science, Published online June 15, 2020. https://doi.org/10.1126/science.abc7424.

Wedemayer, G.J., Patten, P.A., Wang, L.H., Schultz, P.G., and Stevens, R.C. (1997). Structural insights into the evolution of an antibody combining site. Science *276*, 1665–1669.

Wheatley, A.K., Whittle, J.R., Lingwood, D., Kanekiyo, M., Yassine, H.M., Ma, S.S., Narpala, S.R., Prabhakaran, M.S., Matus-Nicodemos, R.A., Bailer, R.T., et al. (2015). H5N1 Vaccine-Elicited Memory B Cells Are Genetically Constrained by the IGHV Locus in the Recognition of a Neutralizing Epitope in the Hemagglutinin Stem. J. Immunol. *195*, 602–610.

Willis, J.R., Briney, B.S., DeLuca, S.L., Crowe, J.E., Jr., and Meiler, J. (2013). Human germline antibody gene segments encode polyspecific antibodies. PLoS Comput. Biol. *9*, e1003045.

Wu, X., Zhou, T., Zhu, J., Zhang, B., Georgiev, I., Wang, C., Chen, X., Longo, N.S., Louder, M., McKee, K., et al.; NISC Comparative Sequencing Program (2011). Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science *333*, 1593–1602.

Xu, J.L., and Davis, M.M. (2000). Diversity in the CDR3 region of V(H) is sufficient for most antibody specificities. Immunity *13*, 37–45.

Yassine, H.M., Boyington, J.C., McTamney, P.M., Wei, C.J., Kanekiyo, M., Kong, W.P., Gallagher, J.R., Wang, L., Zhang, Y., Joyce, M.G., et al. (2015). Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. Nat. Med. *21*, 1065–1070.

Zhou, T., Zhu, J., Wu, X., Moquin, S., Zhang, B., Acharya, P., Georgiev, I.S., Altae-Tran, H.R., Chuang, G.Y., Joyce, M.G., et al.; NISC Comparative Sequencing Program (2013). Multidonor analysis reveals structural elements, genetic determinants, and maturation pathway for HIV-1 neutralization by VRC01-class antibodies. Immunity *39*, 245–258.

Zhou, T., Lynch, R.M., Chen, L., Acharya, P., Wu, X., Doria-Rose, N.A., Joyce, M.G., Lingwood, D., Soto, C., Bailer, R.T., et al.; NISC Comparative Sequencing Program (2015). Structural Repertoire of HIV-1-Neutralizing Antibodies Targeting the CD4 Supersite in 14 Donors. Cell *161*, 1280–1292.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rat Anti-CD19-PerCP-Cy5.5	BioLegend	Cat#115533; RRID: AB_2259869
Rat Anti-B220-FITC	BD Biosciences	Cat#553087; RRID: AB_394617
Rat Anti-MHCII-BV510	BioLegend	Cat#107636; RRID: AB_2734168
Rat Anti-CD3-BV785	BioLegend	Cat#100232; RRID: AB_2562554
Rat-Anti-CD23-PE/Cy7	BioLegend	Cat#101614; RRID: AB_2103036
Rat-Anti-CD169-FITC	BioRad	Cat#MCA884FT; RRID: AB_1100895
Rat Anti-CD21/CD35-BV421	BD Biosciences	Cat#562756; RRID: AB_2737772
Goat Anti-IgM-HRP	Southern Biotech	Cat#1021-05; RRID: AB_2794240
Chemicals, Peptides, and Recombinant Proteins		
LIVE/DEAD Fixable Blue Dead Cell Stain	Thermo Fisher	Cat#L34961
Sigma Adjuvant System	Sigma-Aldrich	Cat#S6322
KAPA HiFi HotStart ReadyMix	KAPA Biosystems	Cat#KK2602
Maxima H Minus Reverse Transcriptase	Thermo Fisher	Cat#EP0751
HotStarTaq Plus DNA Polymerase	QIAGEN	Cat#203603
RLT Buffer	QIAGEN	Cat#79216
Collagenase D (Roche collagenase D)	Roche	Cat#11088858001
ACK lysing buffer	Lonza	Cat#10-548E
Tissue-Tek O.C.T Compound	Sakura	Cat#4583
Background Sniper Blocking Reagent	Biocare Medical	Cat#902-966-082317
LPS (*E.coli* Serotype 055:B5, Fluor568 conjugate)	Thermofisher	Cat#L23352
LPS (*Escherichia coli* O55:B5)	Sigma	Cat#L2880
LPS (*Klebsiella pneumoniae*)	Sigma	Cat#L4268
LPS (*Pseudomonas aeruginosa* 10)	Sigma	Cat#L9143
LPS (*Salmonella enterica* serotype typhimurium)	Sigma	Cat#L6511
kdo2-Lipid A	Avanti Polar Lipids	Cat#699500P
TNP-ficoll	Biosearch Technologies	Cat#F-1300-10
Ovalbumin	Biosearch Technologies	Cat#O-1000-100
Tetramethylbenzidine (TMB)	Sigma	Cat#T0440
Critical Commercial Assays		
MiSeq Reagent Kit, V2 500 cycles	Ilumina	Cat#MS-102-2003
Mouse B Cell Isolation Kit II	Miltenyi	Cat#130-104-443
Deposited Data		
IgM HC sequences from IGHV1-69+hIgK and IGHV1-2+hIgK mice	This Paper	GEO: GSE154285 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154285
Experimental Models: Organisms/Strains		
Mouse: IGHV1-69*01+hIgK	This Paper	N/A
Mouse: IGHV1-2*02+hIgK	This Paper	N/A
Mouse: HCo12	This Paper	N/A
Oligonucleotides		
Primers for Bulk HC Amplification	Sangesland et al., 2019	N/A
Software and Algorithms		
FlowJo v.9.3.2	TreeStar	https://www.flowjo.com; RRID: SCR_008520
Prism 7	GraphPad	https://www.graphpad.com; RRID: SCR_002798

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
MiXCR	MI Laboratory	https://milaboratory.com/software/mixcr/; RRID:SCR_018725
Immunarch (v0.5.5)	Nazarov et al., 2015	https://zenodo.org/record/3613560#.XwpXkS3Myoh
Prodigal	Oak Ridge National Laboratory	http://www.ornl.gov; RRID: SCR_011936
fetchMG (v1)	Sunagawa et al., 2013	https://motu-tool.org/fetchMG.html
ETE3 (v3.1.1)	Huerta-Cepas et al., 2016	http://etetoolkit.org
iTOL (v5)	Letunic and Bork, 2019	https://itol.embl.de; RRID:SCR_018174
Other		
96 well microtiter plates	Fisher Scientific	Cat#439454

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for reagents should be directed to and will be fulfilled by Daniel Lingwood (dlingwood@mgh.harvard.edu).

Materials Availability

There are restrictions to the availability of the IGHV1-69, IGHV1-2 and HCo12 mice due to a MTA with Bristol-Myers Squibb.

Data and Code Availability

Antibody sequences from this study were deposited in GEO (GSE154285).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Transgenic Mice

All transgenic mice were obtained as a gift to D.L. from Bristol-Myers Squibb (Redwood City, CA). The animals were generated using a pre-established strategy that fully constrains to user-defined human V_H segments, while allowing for normal and random recombination with diverse human D_H and J_H segments, as encoded by the HC2 locus (Fishwild et al., 1996; Lonberg, 2005; Lonberg et al., 1994; Sangesland et al., 2019) (Figure 1). We have previously demonstrated that murine N-junctional diversification on the HC2-encoded D_H and J_H genes produces a germline CDRH3 repertoire in antigen naive IgM B cells that is similar to humans (Fishwild et al., 1996; Lonberg, 2005; Lonberg et al., 1994; Sangesland et al., 2019). Full IgH restriction to IGHV-69*01 or IGHV1-2*02 was also previously demonstrated using this approach (Sangesland et al., 2019), however, the light chain repertoire in those animals was provided by the murine kappa and lambda genes (Sangesland et al., 2019). The animals in the present study were further humanized by replacing the murine LC repertoire with the human kappa chain-encoded KCo5 cassette (= IGHV1-2 + hIgK and IGHV1-69 + hIgK), which provides for near full diversity in the human kappa locus (Fishwild et al., 1996; Lonberg, 2005; Xu and Davis, 2000). HCo12 animals were also deployed where the mice bear the same LC repertoire and humanized diversity in CDRH3, but are modified to include five common human V_H-gene segments (IGHV5-51, IGHV3-23, IGHV3-30, IGHV1-69, and IGHV4-34) and have previously been applied in antibody drug discovery, where they have generated high-affinity therapeutic antibodies (US Patent 7247301). All transgenic animals were generated per standard techniques as described by Hogan et al. (1994). The animals were maintained within Ragon Institute's HPPF barrier facility and all experiments were conducted with institutional IACUC approval (MGH protocol 2014N000252). In this study, both male and female animals, aged 6-10 weeks, were used.

METHOD DETAILS

BCR sequencing

CDRH3 diversity within the transgenic mice was measured by deep sequencing the BCRs from antigen naive IgM B cells in bulk according to our previously deployed method (Sangesland et al., 2019; Trombetta et al., 2014). Briefly, we enriched BCR libraries from B cell whole transcriptome amplification (WTA) products using the Smart-Seq2 protocol, as previously described (Sangesland et al., 2019; Trombetta et al., 2014). Following WTA, two 0.8x (v/v) SPRI bead-based cleanups and cDNA quantitation/normalization, we PCR amplified the BCR heavy chains (FR3 to CDR3) [HotStarTaq Plus; QIAGEN], using with a forward primer specific for the FR3 region within the V_H-gene of interest (final concentration: 0.5 μM) and reverse primers against the heavy constant regions (final concentration: 1μM) (Sangesland et al., 2019). These primers were attached to the Illumina P7 (FR3 region) and P5 (constant region) as

described previously (Sangesland et al., 2019). Following BCR amplification, SPRI cleanup was performed, and we quantified and normalized the amplicons to 0.2–0.5 ng/μL. We then amplified the heavy chain sequences further using step-out PCR with the Illumina sequencing adapters [Kapa HiFi HotStart ReadyMix; Kapa Biosystems]. We sequenced these PCR products (FR3 through CDRH3) and aligned the resulting paired end FASTQs using MiXCR (Bolotin et al., 2015). Within this alignment step, we applied the following parameter: OvParameters.geneFeatureToAlign = {FR3Begin:Vend}, and constrained alignment to heavy chain matches only. We trimmed low sequence quality reads using the default settings within MiXCR. To measure the CDRH3 diversity in the human repertoire, we mined data from Donor 1A within a recent study of naive IgM BCR sequencing (DeWitt et al., 2016). Using custom scripts within immunarch (v0.5.5; https://zenodo.org/record/3613560#.XnzYjdNKiJQ) tcR (Nazarov et al., 2015), we reconfigured the sequenced naive IgM BCRs and the mined human data to generate frequency plots of CDRH3 length and assigned Kabat numbering for amino acid composition of 16aa length CDRH3s.

Flow cytometry

Mouse spleens were gently disrupted in R10 (RPMI with 10% FBS) in the presence of

1mg/ml collagenase D (Roche collagenase D) and then filtered through a 70μm cell strainer. The splenocytes were resuspended within ACK lysis buffer to remove red blood cells (Lonza) and then washed with PBS. The cells were first stained with Blue viability dye (0.025 mg/ml; Thermofisher) and then master mixes containing the following fluorescent antibodies: CD19 PerCP-Cy5.5; B220-FITC; MHCII-BV510; CD23-PECy7; CD21/CD35-BV421; CD3-BV785. Each antibody was used at a final dilution of 1:100 within the master mix. The samples were then run on a 5 laser LSR Fortessa (BD Biosciences) and data were analyzed using FlowJo software version 9.3.2 (TreeStar).

Imaging antigen capture in the spleen

To confirm that our transgenic mice could capture bacterial LPS within the spleen marginal zone, we intravenously injected 15μg fluorescent LPS (*E.coli* Serotype 055:B5, Fluor568 conjugate, Thermofisher) and then removed the spleens at 1 after the injection. We prepared the spleens for immunostaining by submerging ~5mm thick pieces within O.C.T Compound (Tissue-Tek; Sakura) and then freezing within a metal container that contained isobutene but was surrounded by liquid nitrogen. We then made tissue sections from the frozen block (40 μm) using a cryostat. These samples were air-dried overnight and then fixed in ice-cold acetone for 10 min. Following rehydration in PBS, we blocked the sections using "Background sniper" (Biocare) within a within a dark humid chamber and then incubated the chamber with anti-CD169-FITC diluted 1:500 in PBS containing 0.1% Tween20). This stained metallophilic macrophages to delineate the follicular versus extrafollicular space (Louie and Liao, 2019; Moran et al., 2019). The sections were then washed with PBS in a coplin jar (3x, five min each) and then slide-mounted using ProLong Gold Antifade (Invitrogen). Images of the fluorescent LPS and FITC were then acquired using a Zeiss Elyra PS.1 confocal/super resolution microscope.

Immune challenge with bloodborne antigens

Antigens were injected intravenously (IV) in 100μl inoculums containing: 15μg LPS (preparations from *Escherichia. coli, Klebsiella pneumoniae*, Pseudomonas *aeruginosa, or Salmonella enterica;* Sigma); 30μg TNP-ficoll (Biosearch Technologies); 25μg ovalbumin (Biosearch Technologies); or 15μg of kdo2-Lipid A (Di[3-deoxy-D-manno-octulosonyl]-lipid A, Avanti Polar Lipids). Ovalbumin was adjuvanted with 50% w/v Sigma adjuvant, as described previously (Sangesland et al., 2019; Yassine et al., 2015). Blood was sampled pre-immunization and 3, 7 and 14 days post immunization.

ELISA

Serum antibody responses to LPS were measured according to a previously described method (Tobias et al., 1989). Briefly, 96 well microtiter plates were first coated with 30 μg/ml LPS in carbonate buffer (100mM Na_2CO_3, 20mM EDTA, pH 9.6) at 100μl/well for 3 h at 37°C. The plates were then washed (3x) with water and then dried overnight in a desiccator. Excess binding sites were then blocked by addition of 10mg/ml BSA in HS buffer (50mM HEPES, 0.15mM NaCl, pH 7.4) at 200μl/well. After blocking, the sera (pre-bleed and immune sera) were added through serial dilution in HS buffer containing 1mg/ml BSA, starting at 1:20 (v/v). This was allowed to bind for 3 h at 37°C. The plate was then washed (3x) with HS buffer containing 1mg/ml BSA and then incubated with anti-IgM-HRP at 1:5000 dilution in HS buffer + 1mg/ml BSA. The wells were then washed (3x) as above and developed by addition of TMB substrate (Sigma) followed by quenching with 1N sulphuric acid. The plates were then read at 450nm using the Teacan Infinite m1000 Pro microplate absorbance reader (Männedorf, Switzerland).

For the other antigens, 200ng/well of each antigen was coated to the microtiter plates by overnight incubation at 4°C. The plates were blocked with 2% BSA in PBS for 1 h and then washed (3x) with PBS. After blocking, the pre and post immune sera was serially diluted into PBS [starting at 1:20 (v/v)] and then added to the wells and incubated for 1 h. The plates were washed (3x) in PBS containing 0.5% Tween 20 (PBST) and incubated with the anti-IgM-HRP diluted at 1:5000. The wells were then washed in PBST and developed using TMB substrate followed by quenching by sulphuric acid, as detailed above.

Phylogenetic reconstruction

Genomes for type strains were downloaded from NCBI refSeq (O'Leary et al., 2016). Genes were called using Prodigal (Hyatt et al., 2010). Forty universal single copy marker genes were identified using fetchMG v1.0 (Sunagawa et al., 2013). ETE3 v3.1.1

(Huerta-Cepas et al., 2016) was used to produce a phylogenetic reconstruction. The tree was rooted to *Burkholderia cepacia* ATCC 25416 and visualized using iTOL v5 (Letunic and Bork, 2019).

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analysis was performed using Prism Graphpad software. Sample sizes of animals and specific tests to determine statistical significance used are indicated in the methods and corresponding figure legends. Data were considered statistically significant at $p < 0.05$.