



# HHS Public Access

Author manuscript

*Curr Top Microbiol Immunol.* Author manuscript; available in PMC 2020 September 11.

Published in final edited form as:

*Curr Top Microbiol Immunol.* 2020 ; 428: 165–180. doi:10.1007/82\_2019\_156.

## B Cells Carrying Antigen Receptors Against Microbes as Tools for Vaccine Discovery and Design

Deepika Bhullar, David Nemazee

Department of Immunology and Microbiology, The Scripps Research Institute, 10550 North Torrey Pines Rd, IM29, La Jolla, CA 92037, USA

### Abstract

Can basic science improve the art of vaccinology? Here, we review efforts to understand immune responses with the aim to improve vaccine design and, eventually, to predict the efficacy of human vaccine candidates using the tools of transformed B cells and targeted transgenic mice carrying B cells with antigen receptors specific for microbes of interest.

### 1 Introduction

Vaccinology is, and has always been, a crucial field for public health and for basic research. Early vaccine studies led to the discovery of antibodies, which were identified by their ability to neutralize microbial toxins upon passive transfer to naive animals. While some well-known vaccines are capable of eradicating important human pathogens, we lack adequate vaccines for many, perhaps most, human pathogens. For these challenging pathogens, the classical empirical approach to vaccinology fails, necessitating better knowledge of the microbes and the basic science of successful immune responses.

When a vaccine does not work, it is often difficult to determine the reason. Basic studies in B cell biology can have an impact on finding a way forward. One facet of this approach is in the discovery of good vaccine targets. The isolation of neutralizing antibodies derived from the B cells of infected patients through the use of methodologies such as phage display, hybridoma technology, and single-cell antibody gene cloning has identified crucial epitopes toward which one can focus vaccine responses. As described in the accompanying chapter by Dennis Burton and colleagues, this approach has been fruitful in the identification of neutralizing epitopes to pathogens with high diversity such as HIV, which require broadly neutralizing antibodies (bnAbs) that neutralize many substrains.

### 2 Recurrent Clonotypes

Besides their value in identifying epitopes of vulnerability, bnAb sequences provide information about the reproducibility of desired antibody responses. In many cases where antibody responses to defined epitopes have been studied in detail, certain VH or VL genes and often VH/VL pairs are recurrently selected (Nisonoff and Ju 1976; Crews et al. 1981;

Wysocki et al. 1985; Kaartinen et al. 1983; Gearhart et al. 1981). This is most apparent in inbred animals, where the appearance of recurrent “clonotypes” was apparent many years ago from isoelectric focusing and idiotype studies (Sigal et al. 1977; Sher and Cohn 1972; Lieberman et al. 1974). In early studies in mice, certain responses were useful in mapping VH gene alleles that conferred specificity, for example, to microbial cell wall components such as phosphorylcholine (Lieberman et al. 1974). A particular VL gene was also associated with the anti-influenza A response of mice (Clarke et al. 1990). It was appreciated that recurrent responses were associated with high precursor frequency (Sigal et al. 1977) and sometimes microbial resistance (Mi et al. 2000). In the lightly mutated human antibody responses to bacteria, recurrent responses have been identified for the capsular polysaccharides of *Streptococcus pneumoniae* 23F (Zhou et al. 2002) and *Haemophilus influenzae* (Lucas and Reason 1999). In the case of Hib, lack of a particular VK gene has been linked to disease susceptibility (Feeney et al. 1996; Nadel et al. 1998). Human neutralizing antibodies to the cytomegalovirus AD-2S1 epitope appear to use lightly mutated versions of a single VH/VL pair and make contacts largely with non-mutated residues (Thomson et al. 2008). Remarkably, the recurrent use of particular VH or VL genes also occurs in certain classes of heavily mutated human bnAbs to highly diverse microbes, including the CD4 binding site of HIV (Zhou et al. 2013; Wu et al. 2011; Bonsignori et al. 2012; Zhou et al. 2010) and the stem region of influenza hemagglutinin (Sui et al. 2009; Ekiert et al. 2011; Wrammert et al. 2011; Whittle et al. 2011; Dreyfus et al. 2012; Lingwood et al. 2012; Kashyap et al. 2008; Throsby et al. 2008; Whittle et al. 2014). Identification of V-gene recurrence likely requires analysis of well-defined epitopes. A recent deep sequencing study of dengue-exposed donors failed to find VH or VL dominance in the population as a whole but could identify CDRH3 motifs shared between independent infected donors, suggesting a convergent evolution in this case (Parameswaran et al. 2013).

## 2.1 From BnAb Sequences to “Germline” BnAb B Cells

The identification of reproducible bnAbs from humans also defines the receptor of the B cell making this desirable response. Although the typical bnAb sequence is mutated by activation-induced cytidine deaminase (AID)-catalyzed diversification, it is usually possible to infer the antibody sequence prior to mutation using sequence analysis programs (Gaeta et al. 2007; Alamyar et al. 2012; Ye et al. 2013; Russ et al. 2015). This so-called germline (gl)-bnAb in turn defines the B cell receptor (BCR) carried by the naive B cell giving rise to that bnAb. In a real sense, vaccines must target gl-bnAb BCRs and further promote and select their appropriate mutants, the bnAbs. Although these bnAbs can differ widely, as discussed above, reproducible responses are of particular interest for responses that are difficult to elicit. Accordingly, in vitro models of B cells carrying bnAbs and gl-bnAbs as BCRs have proven to be useful tools in vaccine research (Lingwood et al. 2012; Ota et al. 2012; Jardine et al. 2013, 2015; Hoot et al. 2013; McGuire et al. 2014a, b). Unlike free antibody, BCRs on the B cell surface are topologically constrained by the plasma membrane and associated with other cell surface molecules. An additional constraint in naive B cells is that IgM lacks the hinge region present in IgG antibodies. Activation by antigen of B cells carrying bnAb or gl-bnAb BCRs provides a stringent test for the ability of vaccine candidates or other antigens to stimulate B cells. B cells carrying bnAbs and gl-bnAbs as BCRs thus provide in vitro models to evaluate and design vaccine biologically active immunogens.

One way such models are generated experimentally is by the transfection of B cell tumor lines with the desired antibody genes carrying the membrane form of the H-chain. These cells have many similarities to the B cells found in vivo in their biochemical triggering through the BCR. Owing to polar residues in its transmembrane domain, the membrane form of antibody does not normally come to the cell surface unless associated with the signal transducer complex Ig- $\alpha/\beta$  (CD79a/CD79b) (Venkitaraman et al. 1991). In fact, B cell transfection experiments were critical in the discovery of Ig- $\alpha/\beta$  (Hombach et al. 1990). Ig- $\alpha$  and Ig- $\beta$  are B cell-restricted transmembrane proteins carrying in their cytoplasmic domains the so-called ITAM (immunoreceptor tyrosine activation motif: YxxL/Ix<sub>(6-8)</sub>YxxL/I) common to many activating receptors in leukocyte biology, including the CD3 components of the T cell receptor (Reth 1989). These tyrosines become phosphorylated by src family kinases upon activation, leading to a cascade of events including recruitment of the tyrosine kinase Syk and the activation of additional enzymes and second messengers (Reth 1992). PLC $\gamma$  in particular is responsible for initiating Ca<sup>++</sup> mobilization, which is a convenient early readout. Later steps in activation in primary B cells include the upregulation of surface markers such as CD69 and CD86 (Cambier and Monroe 1984; Hara et al. 1986; Lenschow et al. 1994), which promote T cell interactions and whose upregulation is often also mimicked in transduced B cell lines stimulated by ligands that ligate the BCR.

### 3 B Cell Tumor Models for the Candidate Vaccine Antigen Response

A number of laboratories have used B cell lines transfected with vectors encoding HIV bnAbs BCRs to evaluate candidate vaccine antigens for bioactivity and to assess novel ligands for HIV gl-bnAb BCRs (Ota et al. 2012; Jardine et al. 2013; Jardine et al. 2015; Hoot et al. 2013; McGuire et al. 2014, 2013; Doores et al. 2013). These studies reinforced the finding for soluble IgG bnAbs that reversion of these mutated antibodies to the inferred germline sequence eliminates binding by demonstrating the lack of effective bioactivity. One surprise in these studies was that HIV virions were poorly stimulatory even to B cells carrying bnAb receptors (Ota et al. 2012) a result that was subsequently supported by studies in b12 transgenic mice (Ota et al. 2013). Less surprising is the fact that highly multimeric forms of antigen such as nanoparticles carrying repeating subunits, conjugates on virus-like particles, or liposome mounted antigens were most effective in vitro (Ota et al. 2012; Doores et al. 2013) (Ingale et al. in press). McGuire, Stamatatos, and colleagues have carried out extensive studies using gl-bnAb-expressing B cell lines to investigate the role of carbohydrate associated with HIV Env in limiting the functional access to bnAb and gl-bnAb BCRs on the B cell surface (Hoot et al. 2013; McGuire et al. 2014, 2013). An important conclusion from these studies was that certain wild-type envelopes could be recognized by gl-bnAb BCRs provided that one or more key N-glycosylation sites flanking the site of vulnerability were eliminated. Studies on cells carrying membrane-bound anti-influenza hemagglutinin antibodies have also been carried out (Lingwood et al. 2012; Weaver et al. 2016). Interestingly, these investigators were able to express membrane IgG bnAbs and gl-bnAbs on 293 cells, a non-lymphoid cell line that is easy to transfect and lacks Ig- $\alpha/\beta$ . It is unclear why the BCR is able to come to the plasma membrane in this context, and the cells cannot signal as in a B cell activation assay. Nonetheless, the system has been

useful in assessing some aspects of BCR/antigen interactions (Lingwood et al. 2012; Weaver et al. 2016).

## 4 Immunoglobulin Transgenic and Knock-in Mice for Vaccine Research

Rearranged immunoglobulin genes were among the first genes to be used in the generation and study of transgenic mice (Brinster et al. 1983; Storb et al. 1986; Storb 1987). Technically, this is carried out using microinjection into the male pronucleus of a recently fertilized egg (zygote). These first-generation transgenics inserted the microinjected DNA randomly into the genome, usually in multicopy arrays, which led to varied and often nonphysiological expression patterns. Researchers quickly realized that the technology requires careful transgene design to include appropriate regulatory elements in cis and careful selection of transgenic lines with appropriate expression. The early studies, along with related knockout studies, supported a model of feedback suppression of antibody gene expression: expression in developing B cells of an active, pre-rearranged transgenic immunoglobulin gene would tend to suppress or prevent endogenous antibody gene rearrangement (Ritchie et al. 1984; Weaver et al. 1985; Nussenzweig et al. 1987; Kitamura and Rajewsky 1992; Rusconi and Kohler 1985; Hagman et al. 1989; Betz et al. 1993). Transgenes expressing antibody H/L pairs not only could lead to expression of predefined antibody to an antigen of interest, but also suppressed other specificities by promoting B cell development and blocking endogenous rearrangements (Rusconi and Kohler 1985), leading in some instances to mice with virtually monoclonal B cell populations (Goodnow et al. 1988; Nemazee and Burki 1989; Russell et al. 1991). These “conventional” antibody transgenic mice have proven to be very useful in the study of B cell development and self-tolerance (Goodnow et al. 1988; Nemazee and Burki 1989; Russell et al. 1991; Erikson et al. 1991; Arnold et al. 1994; Borrero and Clarke 2002; Carsetti et al. 1995; Kenny et al. 1991; Brink et al. 1992; Gay et al. 1993; Tiegs et al. 1993; Fulcher and Basten 1994; Hayakawa et al. 1999; Chumley et al. 2000; Hayakawa et al. 2003; Foster et al. 1997; Shlomchik et al. 1993) and in the response to microbial antigens such as LCMV, VSV, and influenza (Seiler et al. 1998; Martin et al. 2001; Carmack et al. 1991, 1990). However, these models had limitations for the analysis of immunity, such as an inability to undergo H-chain class switching (owing to a lack of downstream H-chain genes), and their multicopy nature, which made analysis of aspects such as somatic mutation difficult (Betz et al. 1993; O’Brien et al. 1987). The usefulness of these conventional Ig transgenic models for vaccine design has been mainly in aiding research into the T cell-independent immune response, in facilitating visualization of the responding cells, and in the analysis of bystander activation (Seiler et al. 1998; Senn et al. 2003).

### 4.1 Knock-in Mice

More recently, antibody gene “knock-in” mice was developed, in which the antibody transgenes of interest are targeted to the physiological locus (Chen et al. 1995; Taki et al. 1993; Luning Prak and Weigert 1995; Pelanda et al. 1997; Cascalho et al. 1996; Sonoda et al. 1997; Pewzner-Jung et al. 1998; Litzemberger et al. 2000; Phan et al. 2003; Hangartner et al. 2003; Berland et al. 2006; Hangartner et al. 2006). Targeting to the immunoglobulin locus provides more physiological genetic control, allowing such key features such as robust

somatic mutation and class switching. However, despite this fairly good physiological control, the antibody genes introduced by targeting can be eliminated in developing B cells by physiological receptor editing and in preB cells by VH replacement (Chen et al. 1995; Luning Prak and Weigert 1995; Pelanda et al. 1996; Casellas et al. 2001) or by the nonphysiological use of the targeted VH element as an acceptor of DH invasion (Taki et al. 1993; Cascalho et al. 1996; Golub et al. 2001; Koralov et al. 2006). These latter phenomena involve the recombination by upstream VH or DH elements to a conserved heptamer signal sequence site within the knock-in coding region (TACTGTG), which is present in many germline VH regions of mouse and human. Such rearrangements are typically destructive, leading to expression of the alternate IgH allele (Chen et al. 1995; Luning Prak and Weigert 1995; Casellas et al. 2001; Taki et al. 1995). An upshot of these recombinations is that B cells in knock-in mice are rarely monoclonal, and in some extreme cases the transgene-encoded specificity is barely expressed (Pelanda et al. 1997; Chen et al. 1997). When the B cells are autoreactive, negative selection can occur by several mechanisms, including apoptosis, anergy, or receptor editing in the bone marrow (reviewed in) Nemazee 2006; Cambier et al. 2007; Shlomchik 2008; Goodnow et al. 2005. Receptor editing typically results in ongoing L-chain gene recombination, which can displace a functional L-chain gene or inactivate it, leading to its functional replacement.

Among the strengths of the knock-in technology is that it allows one to identify BCRs that fail to support B cell development. Developmental failure can occur if the BCR is sufficiently autoreactive or if the antibody chain in question has other structural defects that prevent proper folding or association with the partner chain. The effects of autoreactivity on B cell development have been extensively studied in models designed for the purpose (reviewed in) Nemazee 2006; however, increasing evidence suggests that some desirable, even broadly neutralizing, antibody specificities to HIV may be negatively selected (Verkoczy and Diaz 2014; Haynes et al. 2005; Verkoczy et al. 2011; Doyle-Cooper et al. 2013; Chen et al. 2013; Finton et al. 2013; Yang et al. 2013). Given the high safety standards for human vaccines, epitopes that require such negatively selected specificities might be undesirable. In the case of the well-known anti-HIV gp41 broadly neutralizing antibodies 2F5 and 4E10, it has been proposed that specific intracellular self-ligands promote negative selection (Finton et al. 2013; Yang et al. 2013). Definitive testing of this hypothesis should be possible by assessing the phenotype of 2F5 and 4E10 knock-in mice in which the cognate epitopes are eliminated, in which negative selection is predicted to be relieved.

Knock-in antibody mice have been useful in studies of microbial resistance, viral evasion, and vaccinology. Ig H-only or H/L knock-in models have been generated using antibodies specific for *Streptococcus pneumoniae* (Taki et al. 1995; Hu et al. 2002), VSV (Hangartner et al. 2003), LCMV (Hangartner et al. 2003; Hangartner et al. 2006), and HIV (Jardine et al. 2015; Ota et al. 2013; Verkoczy et al. 2011; Doyle-Cooper et al. 2013; Chen et al. 2013; Finton et al. 2013; Verkoczy et al. 2010; Verkoczy et al. 2013; Dosenovic et al. 2015; Zhang et al. 2016). Several of these studies involved the analysis of mice carrying antibody H-chains from neutralizing antibodies (usually mutated) paired with random mouse L-chains (Jardine et al. 2015; Ota et al. 2013; Hangartner et al. 2003; Hangartner et al. 2006; Finton et al. 2013; Hu et al. 2002; Dosenovic et al. 2015). Other studies involved knock-in mice expressing both H- and L-chains derived from neutralizing Abs (Ota et al. 2013; Verkoczy et

al. 2011; Doyle-Cooper et al. 2013; Chen et al. 2013). More recently, mice have been generated to express H or H + L genes encoding the inferred non-mutated precursors of HIV broadly neutralizing antibodies (Jardine et al. 2015; Dosenovic et al. 2015; Zhang et al. 2016). These last allow one to assess the ability of experimental vaccination to mature the response appropriately, starting with a defined gene of known potential. These recent studies have indicated the outlines of a priming and possible booster vaccination pathway to elicit antibodies to the CD4 binding site on HIV Env (Jardine et al. 2015; Dosenovic et al. 2015). What makes these studies unique is that the priming immunogen used was targeted to a specific human gene VH1-2\*02 and a particular length and sequence in the CDRL3 loop of L-chain. Testing such vaccines that are intended for human vaccination in small animals was not possible without a knock-in or comparable approach.

It is important to keep in mind some of the limitations of these knock-in models for vaccine research. A high precursor frequency of B cells expressing the BCR of a neutralizing antibody facilitates many analyses, but also provides B cells at super-physiological copy number. Moreover, in many models, some of the transgenic Ig is spontaneously secreted, which could affect certain analyses, such as by providing preimmune resistance to infection (Hangartner et al. 2006). Fortunately, these deficiencies can be readily overcome by transfer of limiting B cell numbers to adoptive recipients prior to vaccination (Ota et al. 2013; Hangartner et al. 2006).

#### 4.2 Nuclear Transfer Mice

A more recent way to generate mouse models carrying defined receptors is to clone mice from the nuclei of lymphocytes (Hochedlinger and Jaenisch 2002; Kirak et al. 2010). This feat has been achieved many times by a small number of laboratories. When the lymphocytes come from immunized individuals, the approach permits the isolation of antigen-specific cells (Kirak et al. 2010; Dougan et al. 2012). For example, Kirak et al. generated transnuclear mice with T cells specific for *Toxoplasma gondii*. The resulting mice have predefined receptors, though for B lymphocytes these are often “pre-switched” to downstream H-chain isotypes (Dougan et al. 2012; Kumar et al. 2015), and the receptors can still be modified by receptor editing (Gerdes and Wabl 2004) or VH replacement at the proB cell stage (Kumar et al. 2015). Although remarkably useful for a range of basic studies, the nuclear transfer approach is somewhat laborious and does not allow the type of precise pre-engineering of antibody sequence that is feasible in the knock-in approach.

### 5 Hu/SCID Mice in Vaccine Research

A final aspect of contemporary vaccine research concerns the increasing “humanization” of mouse models. One approach is to reconstitute immunodeficient mice with human hematopoietic cells. However, the humoral immune responses of such chimeras are so far suboptimal, which limits the use of these models to study the immune response to vaccination (Villaudy et al. 2014; Karpel et al. 2015). On the other hand, such models have proved to be useful in the analysis of passive antibody immunity and the mutational escape of microbes such as HIV (Karpel et al. 2015; Hur et al. 2012; Klein et al. 2012).

## 5.1 Mice and Rats Carrying the Full Complement of Human Ig Genes

An alternative approach that has proved fruitful in making humanized antibodies is the use of mice with inactivated endogenous antibody genes engineered to carry large transgenes encoding human immunoglobulin loci that are composed of many or all gene segments (reviewed in) Bruggemann et al. 2015. Immune responses in these animals seem to work most efficiently if the human H-chain VDJ elements are placed upstream of constant regions of the host (Pruzina et al. 2011; Osborn et al. 2013; Green 2014; Ma et al. 2013), presumably because the Fc portions of the antibodies interact properly with the mouse FcRs and complement. These models would be ideal for many human vaccine studies in which particular V genes are targeted for priming, as discussed above. As a practical matter, however, the mice in question are not easily accessible through normal scientific exchange, owing to their remarkable commercial value for the generation of monoclonal antibodies. And so these models have sadly had little impact so far in the basic science of vaccinology.

## References

- Alamyar E, Duroux P, Lefranc MP, Giudicelli V (2012) IMGT((R)) tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. *Methods Mol Biol* 882:569–604 [PubMed: 22665256]
- Arnold LW, Pennell CA, McCray SK, Clarke SH (1994) Development of B-1 cells: segregation of phosphatidyl choline-specific B cells to the B-1 population occurs after immunoglobulin gene expression. *J Exp Med* 179:1585–1595 [PubMed: 8163938]
- Berland R, Fernandez L, Kari E, Han JH, Lomakin I, Akira S, Wortis HH, Kearney JF, Ucci AA, Imanishi-Kari T (2006) Toll-like receptor 7-dependent loss of B cell tolerance in pathogenic autoantibody knockin mice. *Immunity* 25:429–440 [PubMed: 16973388]
- Betz AG, Rada C, Pannell R, Milstein C, Neuberger MS (1993) Passenger transgenes reveal intrinsic specificity of the antibody hypermutation mechanism: clustering, polarity, and specific hot spots. *Proc Natl Acad Sci USA* 90:2385–2388 [PubMed: 8460148]
- Bonsignori M, Montefiori DC, Wu X, Chen X, Hwang KK, Tsao CY, Kozink DM, Parks RJ, Tomaras GD, Crump JA, Kapiga SH, Sam NE, Kwong PD, Kepler TB, Liao HX, Mascola JR, Haynes BF (2012) Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: implications for vaccine design. *J Virol* 86:4688–4692 [PubMed: 22301150]
- Borrero M, Clarke SH (2002) Low-affinity anti-Smith antigen B cells are regulated by anergy as opposed to developmental arrest or differentiation to B-1. *J Immunol* 168:13–21 [PubMed: 11751941]
- Brink R, Goodnow CC, Crosbie J, Adams E, Eris J, Mason DY, Hartley SB, Basten A (1992) Immunoglobulin M and D antigen receptors are both capable of mediating B lymphocyte activation, deletion, or anergy after interaction with specific antigen. *J Exp Med* 176:991–1005 [PubMed: 1402669]
- Brinster RL, Ritchie KA, Hammer RE, O'Brien RL, Arp B, Storb U (1983) Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice. *Nature* 306:332–336 [PubMed: 6417544]
- Bruggemann M, Osborn MJ, Ma B, Hayre J, Avis S, Lundstrom B, Buelow R (2015) Human antibody production in transgenic animals. *Arch Immunol Ther Exp (Warsz)* 63:101–108 [PubMed: 25467949]
- Cambier JC, Monroe JG (1984) B cell activation. V. Differentiation signaling of B cell membrane depolarization, increased I-A expression, G0 to G1 transition, and thymidine uptake by anti-IgM and anti-IgD antibodies. *J Immunol* 133:576–581 [PubMed: 6610706]

- Cambier JC, Gauld SB, Merrell KT, Vilen BJ (2007) B-cell anergy: from transgenic models to naturally occurring anergic B cells? *Nat Rev Immunol* 7:633–643 [PubMed: 17641666]
- Carmack CE, Shinton SA, Hayakawa K, Hardy RR (1990) Rearrangement and selection of VH11 in the Ly-1 B cell lineage. *J Exp Med* 172:371–374 [PubMed: 2358782]
- Carmack CE, Camper SA, Mackle JJ, Gerhard WU, Weigert Mg (1991) Influence of a V kappa 8 L chain transgene on endogenous rearrangements and the immune response to the HA(Sb) determinant on influenza virus. *J Immunol* 147, 2024–2033 [PubMed: 1909739]
- Carsetti R, Kohler G, Lamers MC (1995) Transitional B cells are the target of negative selection in the B cell compartment. *J Exp Med* 181:2129–2140 [PubMed: 7760002]
- Cascalho M, Ma A, Lee S, Masat L, Wabl M (1996) A quasi-monoclonal mouse. *Science* 272:1649–1652 [PubMed: 8658139]
- Casellas R, Shih TA, Kleinewietfeld M, Rakonjac J, Nemazee D, Rajewsky K, Nussenzweig MC (2001) Contribution of receptor editing to the antibody repertoire. *Science* 291:1541–1544 [PubMed: 11222858]
- Chen C, Nagy Z, Prak EL, Weigert M (1995) Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing. *Immunity* 3:747–755 [PubMed: 8777720]
- Chen C, Prak EL, Weigert M (1997) Editing disease-associated autoantibodies. *Immunity* 6:97–105 [PubMed: 9052841]
- Chen Y, Zhang J, Hwang KK, Bouton-Verville H, Xia SM, Newman A, Ouyang YB, Haynes BF, Verkoczy L (2013) Common tolerance mechanisms, but distinct cross-reactivities associated with gp41 and lipids, limit production of HIV-1 broad neutralizing antibodies 2F5 and 4E10. *J Immunol*
- Chumley MJ, Dal Porto JM, Kawaguchi S, Cambier JC, Nemazee D, Hardy RR (2000) A VH11 V kappa 9 B cell antigen receptor drives generation of CD5+ B cells both in vivo and in vitro. *J Immunol* 164:4586–4593 [PubMed: 10779761]
- Clarke SH, Staudt LM, Kavaler J, Schwartz D, Gerhard WU, Weigert MG (1990) V region gene usage and somatic mutation in the primary and secondary responses to influenza virus hemagglutinin. *J Immunol* 144:2795–2801 [PubMed: 2108213]
- Crews S, Griffin J, Huang H, Calame K, 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 [PubMed: 6791833]
- Doores KJ, Huber M, Le KM, Wang SK, Doyle-Cooper C, Cooper A, Pantophlet R, Wong CH, Nemazee D, Burton DR (2013) 2G12-expressing B cell lines may aid in HIV carbohydrate vaccine design strategies. *J Virol* 87:2234–2241 [PubMed: 23221565]
- Dosenovic P, von Boehmer L, Escolano A, Jardine J, Freund NT, McGuire AT, Kulp DW, Oliveira T, Scharf L, Pietzsch J, Gray MD, Cupo A, van Gils MJ, Yao KH, Liu C, Gazumyan A, Seaman MS, Bjorkman PJ, Sanders RW, Moore JP, Stamatatos L, Schief WR, Nussenzweig MC (2015) Immunization for HIV-1 broadly neutralizing antibodies in human Ig knockin mice. *Cell* 161:1505–1515 [PubMed: 26091035]
- Dougan SK, Ogata S, Hu CC, Grotenbreg GM, Guillen E, Jaenisch R, Ploegh HL (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 [PubMed: 22869725]
- Doyle-Cooper C, Hudson KE, Cooper AB, Ota T, Skog P, Dawson PE, Zwick MB, Schief WR, Burton DR, Nemazee D (2013) Immune tolerance negatively regulates B cells in knock-in mice expressing broadly neutralizing HIV antibody 4E10. *J Immunol*
- Dreyfus C, Laursen NS, Kwaks T, Zuijdgheest D, Khayat R, Ekiert DC, Lee JH, Metlagel Z, Bujny MV, Jongeneelen M, van der Vlugt R, Lamrani M, Korse HJ, Geelen E, Sahin O, Sieuwerts M, Brakenhoff JP, Vogels R, Li OT, Poon LL, Peiris M, Koudstaal W, Ward AB, Wilson IA, Goudsmit J, Friesen RH (2012) Highly conserved protective epitopes on influenza B viruses. *Science* 337:1343–1348 [PubMed: 22878502]
- Ekiert DC, Friesen RH, Bhabha G, Kwaks T, Jongeneelen M, Yu W, Ophorst C, Cox F, Korse HJ, Brandenburg B, Vogels R, Brakenhoff JP, Kompier R, Koldijk MH, Cornelissen LA, Poon LL, Peiris M, Koudstaal W, Wilson IA, Goudsmit J (2011) A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science* 333:843–850 [PubMed: 21737702]



- Erikson J, Radic MZ, Camper SA, Hardy RR, Carmack C, Weigert M (1991) Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. *Nature* 349:331–334 [PubMed: 1898987]
- Feeney AJ, Atkinson MJ, Cowan MJ, Escuro G, Lugo G (1996) A defective V $\kappa$ A2 allele in Navajos which may play a role in increased susceptibility to haemophilus influenzae type b disease. *J Clin Invest* 97:2277–2282 [PubMed: 8636407]
- Finton KA, Larimore K, Larman HB, Friend D, Correnti C, Rupert PB, Elledge SJ, Greenberg PD, Strong RK (2013) Autoreactivity and exceptional CDR plasticity (but not unusual polyspecificity) hinder elicitation of the anti-HIV antibody 4E10. *PLoS Pathog* 9:e1003639 [PubMed: 24086134]
- Foster MH, Liu Q, Chen H, Nemazee D, Cooperstone BG (1997) Anti-laminin reactivity and glomerular immune deposition by in vitro recombinant antibodies. *Autoimmunity* 26:231–243 [PubMed: 9543184]
- Fulcher DA, Basten A (1994) Reduced life span of anergic self-reactive B cells in a double-transgenic model. *J Exp Med* 179:125–134 [PubMed: 8270860]
- Gaeta BA, Malming HR, Jackson KJ, Bain ME, Wilson P, Collins AM (2007) iHMMune-align: hidden Markov model-based alignment and identification of germline genes in rearranged immunoglobulin gene sequences. *Bioinformatics* 23:1580–1587 [PubMed: 17463026]
- Gay D, Saunders T, Camper S, Weigert M (1993) Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med* 177:999–1008 [PubMed: 8459227]
- Gearhart PJ, Johnson ND, Douglas R, Hood L (1981) IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. *Nature* 291:29–34 [PubMed: 7231520]
- Gerdes T, Wabl M (2004) Autoreactivity and allelic inclusion in a B cell nuclear transfer mouse. *Nat Immunol* 5:1282–1287 [PubMed: 15516926]
- Golub R, Martin D, Bertrand FE, Cascalho M, Wabl M, Wu GE (2001) VH gene replacement in thymocytes. *J Immunol* 166:855–860 [PubMed: 11145660]
- Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, Brink RA, Pritchard-Briscoe H, Wotherspoon JS, Loblay RH, Raphael K et al. (1988) Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* 334, 676–682 [PubMed: 3261841]
- Goodnow CC, Sprent J, Fazekas de St GB, Vinuesa CG (2005) Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* 435:590–597 [PubMed: 15931211]
- Green LL (2014) Transgenic mouse strains as platforms for the successful discovery and development of human therapeutic monoclonal antibodies. *Curr Drug Discov Technol* 11:74–84 [PubMed: 23978036]
- Hagman J, Lo D, Doglio LT, Hackett JJ, Rudin CM, Haasch D, Brinster R, Storb U (1989) Inhibition of immunoglobulin gene rearrangement by the expression of a lambda 2 transgene [published erratum appears in *J Exp Med* 1989 Aug 1;170(2):619]. *J Exp Med* 169, 1911–1929 [PubMed: 2499652]
- Hangartner L, Senn BM, Ledermann B, Kalinke U, Seiler P, Bucher E, Zellweger RM, Fink K, Odermatt B, Burki K, Zinkernagel RM, Hangartner H (2003) Antiviral immune responses in gene-targeted mice expressing the immunoglobulin heavy chain of virus-neutralizing antibodies. *Proc Natl Acad Sci USA* 100:12883–12888 [PubMed: 14569006]
- Hangartner L, Zellweger RM, Giobbi M, Weber J, Eschli B, McCoy KD, Harris N, Recher M, Zinkernagel RM, Hangartner H (2006) Nonneutralizing antibodies binding to the surface glycoprotein of lymphocytic choriomeningitis virus reduce early virus spread. *J Exp Med* 203:2033–2042 [PubMed: 16880253]
- Hara T, Jung LK, Bjorndahl JM, Fu SM (1986) Human T cell activation. III. Rapid induction of a phosphorylated 28 kD/32 kD disulfide-linked early activation antigen (EA 1) by 12-o-tetradecanoyl phorbol-13-acetate, mitogens, and antigens. *J Exp Med* 164:1988–2005 [PubMed: 2946796]
- Hayakawa K, Asano M, Shinton SA, Gui M, Allman D, Stewart CL, Silver J, Hardy RR (1999) Positive selection of natural autoreactive B cells. *Science* 285:113–116 [PubMed: 10390361]
- Hayakawa K, Asano M, Shinton SA, Gui M, Wen LJ, Dashoff J, Hardy RR (2003) Positive selection of anti-thy-1 autoreactive B-1 cells and natural serum autoantibody production independent from bone marrow B cell development. *J Exp Med* 197:87–99 [PubMed: 12515816]

- Haynes BF, Fleming J, St Clair EW, Katinger H, Stiegler G, Kunert R, Robinson J, Scarce RM, Plonk K, Staats HF, Ortel TL, Liao HX, Alam SM (2005) Cardiophilin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. *Science* 308:1906–1908 [PubMed: 15860590]
- Hochedlinger K, Jaenisch R (2002) Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* 415:1035–1038 [PubMed: 11875572]
- Hombach J, Tsubata T, Leclercq L, Stappert H, Reth M (1990) Molecular components of the B-cell antigen receptor complex of the IgM class. *Nature* 343:760–762 [PubMed: 2304550]
- Hoot S, McGuire AT, Cohen KW, Strong RK, Hangartner L, Klein F, Diskin R, Scheid JF, Sather DN, Burton DR, Stamatatos L (2013) Recombinant HIV envelope proteins fail to engage germline versions of anti-CD4bs bNAbs. *PLoS Pathog* 9:e1003106 [PubMed: 23300456]
- Hu L, Rezanka LJ, Mi QS, Lustig A, Taub DD, Longo DL, Kenny JJ (2002) T15-idiotype-negative B cells dominate the phosphocholine binding cells in the preimmune repertoire of T15i knockin mice. *J Immunol* 168:1273–1280 [PubMed: 11801665]
- Hur EM, Patel SN, Shimizu S, Rao DS, Gnanapragasam PN, An DS, Yang L, Baltimore D (2012) Inhibitory effect of HIV-specific neutralizing IgA on mucosal transmission of HIV in humanized mice. *Blood* 120:4571–4582 [PubMed: 23065154]
- Jardine J, Julien JP, Menis S, Ota T, Kalyuzhnyi O, McGuire A, Sok D, Huang PS, Macpherson S, Jones M, Nieuwsma T, Mathison J, Baker D, Ward AB, Burton DR, Stamatatos L, Nemazee D, Wilson IA, Schief WR (2013) Rational HIV immunogen design to target specific germline B cell receptors. *Science* 340:711–716 [PubMed: 23539181]
- Jardine JG, Ota T, Sok D, Pauthner M, Kulp DW, Kalyuzhnyi O, Skog PD, Thinnis TC, Bhullar D, Briney B, Menis S, Jones M, Kubitz M, Spencer S, Adachi Y, Burton DR, Schief WR, Nemazee D (2015) HIV-1 VACCINES. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. *Science* 349:156–161 [PubMed: 26089355]
- Kaartinen M, Griffiths GM, Markham AF, Milstein C (1983) mRNA sequences define an unusually restricted IgG response to 2-phenyloxazolone and its early diversification. *Nature* 304:320–324 [PubMed: 6877353]
- Karpel ME, Boutwell CL, Allen TM (2015) BLT humanized mice as a small animal model of HIV infection. *Curr Opin Virol* 13:75–80 [PubMed: 26083316]
- Kashyap AK, Steel J, Oner AF, Dillon MA, Swale RE, Wall KM, Perry KJ, Faynboym A, Ilhan M, Horowitz M, Horowitz L, Palese P, Bhatt RR, Lerner RA (2008) Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. *Proc Natl Acad Sci USA* 105:5986–5991 [PubMed: 18413603]
- Kenny JJ, Stall AM, Sieckmann DG, Lamers MC, Finkelman FD, Finch L, Longo DL (1991) Receptor-mediated elimination of phosphocholine-specific B cells in x-linked immune-deficient mice. *J Immunol* 146:2568–2577 [PubMed: 1901880]
- Kirak O, Frickel EM, Grotenbreg GM, Suh H, Jaenisch R, Ploegh HL (2010) Transnuclear mice with predefined T cell receptor specificities against *Toxoplasma gondii* obtained via SCNT. *Science* 328:243–248 [PubMed: 20378817]
- Kitamura D, Rajewsky K (1992) Targeted disruption of mu chain membrane exon causes loss of heavy-chain allelic exclusion [see comments]. *Nature* 356:154–156 [PubMed: 1545868]
- Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, Mouquet H, Spatz LA, Diskin R, Abadir A, Zang T, Dorner M, Billerbeck E, Labitt RN, Gaebler C, Marcovecchio PM, Incesu RB, Eisenreich TR, Bieniasz PD, Seaman MS, Bjorkman PJ, Ravetch JV, Ploss A, Nussenzweig MC (2012) HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* 492:118–122 [PubMed: 23103874]
- Koralov SB, Novobrantseva TI, Konigsmann J, Ehlich A, Rajewsky K (2006) Antibody repertoires generated by VH replacement and direct VH to JH joining. *Immunity* 25:43–53 [PubMed: 16860756]
- Kumar R, Bach MP, Mainoldi F, Maruya M, Kishigami S, Jumaa H, Wakayama T, Kanagawa O, Fagarasan S, Casola S (2015) Antibody repertoire diversification through VH gene replacement in mice cloned from an IgA plasma cell. *Proc Natl Acad Sci USA* 112:E450–457 [PubMed: 25609671]

- Lenschow DJ, Sperling AI, Cooke MP, Freeman G, Rhee L, Decker DC, Gray G, Nadler LM, Goodnow CC, Bluestone JA (1994) Differential up-regulation of the B7-1 and B7-2 costimulatory molecules after Ig receptor engagement by antigen. *J Immunol* 153:1990–1997 [PubMed: 7519638]
- Lieberman R, Potter M, Mushinski EB, Humphrey W Jr, Rudikoff S (1974) Genetics of a new IgVH (T15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J Exp Med* 139:983–1001 [PubMed: 4131516]
- Lingwood D, McTamney PM, Yassine HM, Whittle JR, Guo X, Boyington JC, Wei CJ, Nabel GJ (2012) Structural and genetic basis for development of broadly neutralizing influenza antibodies. *Nature*
- Litzenburger T, Bluthmann H, Morales P, Pham-Dinh D, Dautigny A, Wekerle H, Iglesias A (2000) Development of myelin oligodendrocyte glycoprotein autoreactive transgenic B lymphocytes: receptor editing in vivo after encounter of a self-antigen distinct from myelin oligodendrocyte glycoprotein. *J Immunol* 165:5360–5366 [PubMed: 11046072]
- Lucas AH, Reason DC (1999) Polysaccharide vaccines as probes of antibody repertoires in man. *Immunol Rev* 171:89–104 [PubMed: 10582166]
- Luning Prak E, Weigert M (1995) Light chain replacement: a new model for antibody gene rearrangement. *J Exp Med* 182:541–548 [PubMed: 7629511]
- Ma B, Osborn MJ, Avis S, Ouisse LH, Menoret S, Anegon I, Buelow R, Bruggemann M (2013) Human antibody expression in transgenic rats: comparison of chimeric IgH loci with human VH, D and JH but bearing different rat C-gene regions. *J Immunol Methods* 400–401:78–86
- Martin F, Oliver AM, Kearney JF (2001) Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity* 14:617–629 [PubMed: 11371363]
- McGuire AT, Hoot S, Dreyer AM, Lippy A, Stuart A, Cohen KW, Jardine J, Menis S, Scheid JF, West AP, Schief WR, Stamatatos L (2013) Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. *J Exp Med* 210:655–663 [PubMed: 23530120]
- McGuire AT, Dreyer AM, Carbonetti S, Lippy A, Glenn J, Scheid JF, Mouquet H, Stamatatos L (2014a) HIV antibodies. Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies. *Science* 346:1380–1383 [PubMed: 25504724]
- McGuire AT, Glenn JA, Lippy A, Stamatatos L (2014b) Diverse recombinant HIV-1 Envs fail to activate B cells expressing the germline B cell receptors of the broadly neutralizing anti-HIV-1 antibodies PG9 and 447–52D. *J Virol* 88:2645–2657 [PubMed: 24352455]
- Mi QS, Zhou L, Schulze DH, Fischer RT, Lustig A, Rezanka LJ, Donovan DM, Longo DL, Kenny JJ (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 [PubMed: 10811914]
- Nadel B, Tang A, Lugo G, Love V, Escuro G, Feeney AJ (1998) Decreased frequency of rearrangement due to the synergistic effect of nucleotide changes in the heptamer and nonamer of the recombination signal sequence of the V kappa gene A2b, which is associated with increased susceptibility of Navajos to *Haemophilus influenzae* type b disease. *J Immunol* 161:6068–6073 [PubMed: 9834090]
- Nemazee D (2006) Receptor editing in lymphocyte development and central tolerance. *Nat Rev Immunol* 6:728–740 [PubMed: 16998507]
- Nemazee DA, Burki K (1989) Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature* 337:562–566 [PubMed: 2783762]
- Nisonoff A, Ju S (1976) Studies of a cross-reactive idiotype associated with anti-para-azophenylarsenate antibodies of A/J mice. *Ann Immunol (Paris)* 127:347–356 [PubMed: 786141]
- Nussenzweig MC, Shaw AC, Sinn E, Danner DB, Holmes KL, Morse HC, Leder P (1987) Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin mu. *Science* 236:816–819 [PubMed: 3107126]
- O'Brien RL, Brinster RL, Storb U (1987) Somatic hypermutation of an immunoglobulin transgene in kappa transgenic mice. *Nature* 326:405–409 [PubMed: 3104790]

- Osborn MJ, Ma B, Avis S, Binnie A, Dilley J, Yang X, Lindquist K, Menoret S, Iscache AL, Ouisse LH, Rajpal A, Anegon I, Neuberger MS, Buelow R, Bruggemann M (2013) High-affinity IgG antibodies develop naturally in Ig-knockout rats carrying germline human IgH/Igkappa/Iglambda loci bearing the rat CH region. *J Immunol* 190:1481–1490 [PubMed: 23303672]
- Ota T, Doyle-Cooper C, Cooper AB, Huber M, Falkowska E, Doores KJ, Hangartner L, Le K, Sok D, Jardine J, Lifson J, Wu X, Mascola JR, Poignard P, Binley JM, Chakrabarti BK, Schief WR, Wyatt RT, Burton DR, Nemazee D (2012) Anti-HIV B cell lines as candidate vaccine biosensors. *J Immunol*. 189:4816–4824 [PubMed: 23066156]
- Ota T, Doyle-Cooper C, Cooper AB, Doores KJ, Aoki-Ota M, Le K, Schief WR, Wyatt RT, Burton DR, Nemazee D (2013) B cells from knock-in mice expressing broadly neutralizing HIV antibody b12 carry an innocuous B cell receptor responsive to HIV vaccine candidates. *J Immunol*
- Parameswaran P, Liu Y, Roskin KM, Jackson KK, Dixit VP, Lee JY, Artiles KL, Zompi S, Vargas MJ, Simen BB, Hanczaruk B, McGowan KR, Tariq MA, Pourmand N, Koller D, Balmaseda A, Boyd SD, Harris E, Fire AZ (2013) Convergent antibody signatures in human dengue. *Cell Host Microbe* 13:691–700 [PubMed: 23768493]
- Pelanda R, Schaal S, Torres RM, Rajewsky K (1996) A prematurely expressed Ig(kappa) transgene, but not V(kappa)J(kappa) gene segment targeted into the Ig(kappa) locus, can rescue B cell development in lambda5-deficient mice. *Immunity* 5:229–239 [PubMed: 8808678]
- Pelanda R, Schwers S, Sonoda E, Torres RM, Nemazee D, Rajewsky K (1997) Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversification. *Immunity* 7:765–775 [PubMed: 9430222]
- Pewzner-Jung Y, Friedmann D, Sonoda E, Jung S, Rajewsky K, Eilat D (1998) B cell deletion, anergy, and receptor editing in “knock in” mice targeted with a germline-encoded or somatically mutated anti-DNA heavy chain. *J Immunol* 161:4634–4645 [PubMed: 9794392]
- Phan TG, Amesbury M, Gardam S, Crosbie J, Hasbold J, Hodgkin PD, Basten A, Brink R (2003) B cell receptor-independent stimuli trigger immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. *J Exp Med* 197:845–860 [PubMed: 12668643]
- Pruzina S, Williams GT, Kaneva G, Davies SL, Martin-Lopez A, Bruggemann M, Vieira SM, Jeffs SA, Sattentau QJ, Neuberger MS (2011) Human monoclonal antibodies to HIV-1 gp140 from mice bearing YAC-based human immunoglobulin transloci. *Protein Eng Des Sel* 24:791–799 [PubMed: 21810921]
- Reth M (1989) Antigen receptor tail clue. *Nature* 338:383–384 [PubMed: 2927501]
- Reth M (1992) Antigen receptors on B lymphocytes. *Annu Rev Immunol* 10:97–121 [PubMed: 1591006]
- Ritchie KA, Brinster RL, Storb U (1984) Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in kappa transgenic mice. *Nature* 312:517–520 [PubMed: 6438533]
- Rusconi S, Kohler G (1985) Transmission and expression of a specific pair of rearranged immunoglobulin mu and kappa genes in a transgenic mouse line. *Nature* 314:330–334 [PubMed: 3920529]
- Russ DE, Ho KY, Longo NS (2015) HTJoinSolver: human immunoglobulin VDJ partitioning using approximate dynamic programming constrained by conserved motifs. *BMC Bioinformatics* 16:170 [PubMed: 26001675]
- Russell DM, Dembic Z, Morahan G, Miller JF, Burki K, Nemazee D (1991) Peripheral deletion of self-reactive B cells. *Nature* 354:308–311 [PubMed: 1956380]
- Seiler P, Kalinke U, Rulicke T, Bucher EM, Bose C, Zinkernagel RM, Hengartner H (1998) Enhanced virus clearance by early inducible lymphocytic choriomeningitis virus-neutralizing antibodies in immunoglobulin-transgenic mice. *J Virol* 72:2253–2258 [PubMed: 9499083]
- Senn BM, Lopez-Macias C, Kalinke U, Lamarre A, Isibasi A, Zinkernagel RM, Hengartner H (2003) Combinatorial immunoglobulin light chain variability creates sufficient B cell diversity to mount protective antibody responses against pathogen infections. *Eur J Immunol* 33:950–961 [PubMed: 12672061]
- Sher A, Cohn M (1972) Inheritance of an idiomotype associated with the immune response of inbred mice to phosphorylcholine. *Eur J Immunol* 2:319–326 [PubMed: 4117282]

- Shlomchik MJ (2008) Sites and stages of autoreactive B cell activation and regulation. *Immunity* 28:18–28 [PubMed: 18199415]
- Shlomchik MJ, Zharhary D, Saunders T, Camper SA, Weigert Mg (1993) A rheumatoid factor transgenic mouse model of autoantibody regulation. *Int Immunol* 5, 1329–1341 [PubMed: 8268138]
- Sigal NH, Pickard AR, Metcalf ES, Gearhart PJ, Klinman NR (1977) Expression of phosphorylcholine-specific B cells during murine development. *J Exp Med* 146:933–948 [PubMed: 302315]
- Sonoda E, Pewzner-Jung Y, Schwers S, Taki S, Jung S, Eilat D, Rajewsky K (1997) B cell development under the condition of allelic inclusion. *Immunity* 6:225–233 [PubMed: 9075923]
- Storb U (1987) Transgenic mice with immunoglobulin genes. *Annu Rev Immunol* 5:151–174 [PubMed: 3109454]
- Storb U, Pinkert C, Arp B, Engler P, Gollahon K, Manz J, Brady W, Brinster RL (1986) Transgenic mice with mu and kappa genes encoding antiphosphorylcholine antibodies. *J Exp Med* 164:627–641 [PubMed: 2425036]
- Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, Santelli E, Stec B, Cadwell G, Ali M, Wan H, Murakami A, Yammanuru A, Han T, Cox NJ, Bankston LA, Donis RO, Liddington RC, Marasco WA (2009) Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* 16:265–273 [PubMed: 19234466]
- Taki S, Meiering M, Rajewsky K (1993) Targeted insertion of a variable region gene into the immunoglobulin heavy chain locus [see comments]. *Science* 262:1268–1271 [PubMed: 8235657]
- Taki S, Schwenk F, Rajewsky K (1995) Rearrangement of upstream DH and VH genes to a rearranged immunoglobulin variable region gene inserted into the DQ52-JH region of the immunoglobulin heavy chain locus. *Eur J Immunol* 25:1888–1896 [PubMed: 7621865]
- Thomson CA, Bryson S, McLean GR, Creagh AL, Pai EF, Schrader JW (2008) Germline V-genes sculpt the binding site of a family of antibodies neutralizing human cytomegalovirus. *EMBO J* 27:2592–2602 [PubMed: 18772881]
- Throsby M, van den Brink E, Jongeneelen M, Poon LL, Alard P, Cornelissen L, Bakker A, Cox F, van Deventer E, Guan Y, Cinatl J, ter Meulen J, Lasters I, Carsetti R, Peiris M, de Kruijff J, Goudsmit J (2008) Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM + memory B cells. *PLoS ONE* 3:e3942 [PubMed: 19079604]
- Tiegs SL, Russell DM, Nemazee D (1993) Receptor editing in self-reactive bone marrow B cells. *J Exp Med* 177:1009–1020 [PubMed: 8459201]
- Venkitaraman AR, Williams GT, Dariavach P, Neuberger MS (1991) The B-cell antigen receptor of the five immunoglobulin classes. *Nature* 352:777–781 [PubMed: 1881434]
- Verkoczy L, Diaz M (2014) Autoreactivity in HIV-1 broadly neutralizing antibodies: implications for their function and induction by vaccination. *Curr Opin HIV and AIDS* 9:224–234 [PubMed: 24714565]
- Verkoczy L, Diaz M, Holl TM, Ouyang YB, Bouton-Verville H, Alam SM, Liao HX, Kelsoe G, Haynes BF (2010) Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. *Proc Natl Acad Sci USA* 107:181–186 [PubMed: 20018688]
- Verkoczy L, Chen Y, Bouton-Verville H, Zhang J, Diaz M, Hutchinson J, Ouyang YB, Alam SM, Holl TM, Hwang KK, Kelsoe G, Haynes BF (2011) Rescue of HIV-1 broad neutralizing antibody-expressing B cells in 2F5 VH × VL knockin mice reveals multiple tolerance controls. *J Immunol* 187:3785–3797 [PubMed: 21908739]
- Verkoczy L, Chen Y, Zhang J, Bouton-Verville H, Newman A, Lockwood B, Scarce RM, Montefiori DC, Dennison SM, Xia SM, Hwang KK, Liao HX, Alam SM, Haynes BF (2013) Induction of HIV-1 broad neutralizing antibodies in 2F5 knock-in mice: selection against membrane proximal external region-associated autoreactivity limits T-dependent responses. *J Immunol* 191:2538–2550 [PubMed: 23918977]
- Villaudy J, Schotte R, Legrand N, Spits H (2014) Critical assessment of human antibody generation in humanized mouse models. *J Immunol Methods* 410:18–27 [PubMed: 24952244]

- Weaver D, Costantini F, Imanishi-Kari T, Baltimore D (1985) A transgenic immunoglobulin mu gene prevents rearrangement of endogenous genes. *Cell* 42:117–127 [PubMed: 3926323]
- Weaver GC, Villar RF, Kanekiyo M, Nabel GJ, Mascola JR, Lingwood D (2016) In vitro reconstitution of B cell receptor-antigen interactions to evaluate potential vaccine candidates. *Nat Protoc* 11:193–213 [PubMed: 26741406]
- Whittle JR, Zhang R, Khurana S, King LR, Manischewitz J, Golding H, Dormitzer PR, Haynes BF, Walter EB, Moody MA, Kepler TB, Liao HX, Harrison SC (2011) Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin. *Proc Natl Acad Sci USA* 108:14216–14221 [PubMed: 21825125]
- Whittle JR, Wheatley AK, Wu L, Lingwood D, Kanekiyo M, Ma SS, Narpala SR, Yassine HM, Frank GM, Yewdell JW, Ledgerwood JE, Wei CJ, McDermott AB, Graham BS, Koup RA, Nabel GJ (2014) Flow cytometry reveals that H5N1 vaccination elicits cross-reactive stem-directed antibodies from multiple Ig heavy-chain lineages. *J Virol* 88:4047–4057 [PubMed: 24501410]
- Wrarmert J, Koutsouanos D, Li GM, Edupuganti S, Sui J, Morrissey M, McCausland M, Skountzou I, Hornig M, Lipkin WI, Mehta A, Razavi B, Del Rio C, Zheng NY, Lee JH, Huang M, Ali Z, Kaur K, Andrews S, Amara RR, Wang Y, Das SR, O'Donnell CD, Yewdell JW, Subbarao K, Marasco WA, Mulligan MJ, Compans R, Ahmed R, Wilson PC (2011) Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* 208:181–193 [PubMed: 21220454]
- Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, Chen X, Longo NS, Louder M, McKee K, O'Dell S, Peretto S, Schmidt SD, Shi W, Wu L, Yang Y, Yang ZY, Yang Z, Zhang Z, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Simek M, Burton DR, Koff WC, Doria-Rose NA, Connors M, Mullikin JC, Nabel GJ, Roederer M, Shapiro L, Kwong PD, Mascola JR (2011) Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* 333:1593–1602 [PubMed: 21835983]
- Wysocki LJ, Margolies MN, Huang B, Nemazee DA, Wechsler DS, Sato VL, Smith JA, Gefter ML (1985) Combinational diversity within variable regions bearing the predominant anti-p-azophenylarsonate idiotype of strain A mice. *J Immunol* 134:2740–2747 [PubMed: 2982953]
- Yang G, Holl TM, Liu Y, Li Y, Lu X, Nicely NI, Kepler TB, Alam SM, Liao HX, Cain DW, Spicer L, Vandeberg JL, Haynes BF, Kelsoe G (2013) Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. *J Exp, Med*
- Ye J, Ma N, Madden TL, Ostell JM (2013) IgBLAST: an immunoglobulin variable domain sequence analysis tool. *Nucleic Acids Res* 41:W34–40 [PubMed: 23671333]
- Zhang R, Verkoczy L, Wiehe K, Munir Alam S, Nicely NI, Santra S, Bradley T, Pemble CWt., Zhang J, Gao F, Montefiori DC, Bouton-Verville H, Kelsoe G, Larimore K, Greenberg PD, Parks R, Foulger A, Peel JN, Luo K, Lu X, Trama AM, Vandergrift N, Tomaras GD, Kepler TB, Moody MA, Liao HX, Haynes BF (2016) Initiation of immune tolerance-controlled HIV gp41 neutralizing B cell lineages. *Sci Transl Med* 8, 336ra362
- Zhou J, Lottenbach KR, Barenkamp SJ, Lucas AH, Reason DC (2002) Recurrent variable region gene usage and somatic mutation in the human antibody response to the capsular polysaccharide of *Streptococcus pneumoniae* type 23F. *Infect Immun* 70:4083–4091 [PubMed: 12117915]
- Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, Kwon YD, Scheid JF, Shi W, Xu L, Yang Y, Zhu J, Nussenzweig MC, Sodroski J, Shapiro L, Nabel GJ, Mascola JR, Kwong PD (2010) Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science* 329:811–817 [PubMed: 20616231]
- Zhou T, Zhu J, Wu X, Moquin S, Zhang B, Acharya P, Georgiev IS, Altae-Tran HR, Chuang GY, Joyce MG, Do Kwon Y, Longo NS, Louder MK, Luongo T, McKee K, Schramm CA, Skinner J, Yang Y, Yang Z, Zhang Z, Zheng A, Bonsignori M, Haynes BF, Scheid JF, Nussenzweig MC, Simek M, Burton DR, Koff WC, Program NCS, Mullikin JC, Connors M, Shapiro L, Nabel GJ, Mascola JR, Kwong PD (2013) Multidonor analysis reveals structural elements, genetic determinants, and maturation pathway for HIV-1 neutralization by VRC01-class antibodies. *Immunity* 39:245–258 [PubMed: 23911655]