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

Deepika Bhullar and David Nemazee

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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

D. Bhullar · D. Nemazee (✉)
Department of Immunology and Microbiology, The Scripps Research Institute,
10550 North Torrey Pines Rd, IM29, La Jolla, CA 92037, USA
e-mail: nemazee@scripps.edu

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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-α/β (CD79a/CD79b) (Venkitaraman et al. 1991). In fact, B cell transfection experiments were critical in the discovery of Ig-α/β (Hombach et al. 1990). Ig-α and Ig-β are B cell-restricted transmembrane proteins carrying in their cytoplasmic domains the so-called ITAM (immunoreceptor tyrosine activation motif: YxxL/Ix$_{(6-8)}$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). PLCγ in particular is responsible for initiating Ca^{++} 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-α/β. 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; Litzenburger 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.

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