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Current Opinion in Virology

Strategies to guide the antibody affinity maturation process

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Antibodies with protective activity are critical for vaccine efficacy. Affinity maturation increases antibody activity through multiple rounds of somatic hypermutation and selection in the germinal center. Identification of HIV-1 specific and influenza-specific antibody developmental pathways, as well as characterization of B cell and virus co-evolution in patients, has informed our understanding of antibody development. In order to counteract HIV-1 and influenza viral diversity, broadly neutralizing antibodies precisely target specific sites of vulnerability and require high levels of affinity maturation. We present immunization strategies that attempt to recapitulate these natural processes and guide the affinity maturation process.

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Current Opinion in Virology 2015, **11**:137–147

This review comes from a themed issue on **Preventive and therapeutic vaccines**

Edited by **Mansun Law**

For a complete overview see the Issue and the Editorial

Available online 24th April 2015

http://dx.doi.org/10.1016/j.coviro.2015.04.002

1879-6257/Published by Elsevier B.V.

Affinity maturation is the process by which antibodies gain increased affinity, avidity, and anti-pathogen activity and is the result of somatic hypermutation (SHM) of immunoglobulin genes in B cells, coupled to selection for antigen binding (Figure 1). This iterative process occurs in germinal centers (GCs), structures within secondary lymphoid tissues [1]. The resulting antibodies can be highly mutated from their germline-encoded counterparts, with increases of several orders of magnitude in affinity for antigen compared to the corresponding naïve B cell receptors (BCRs) [2].

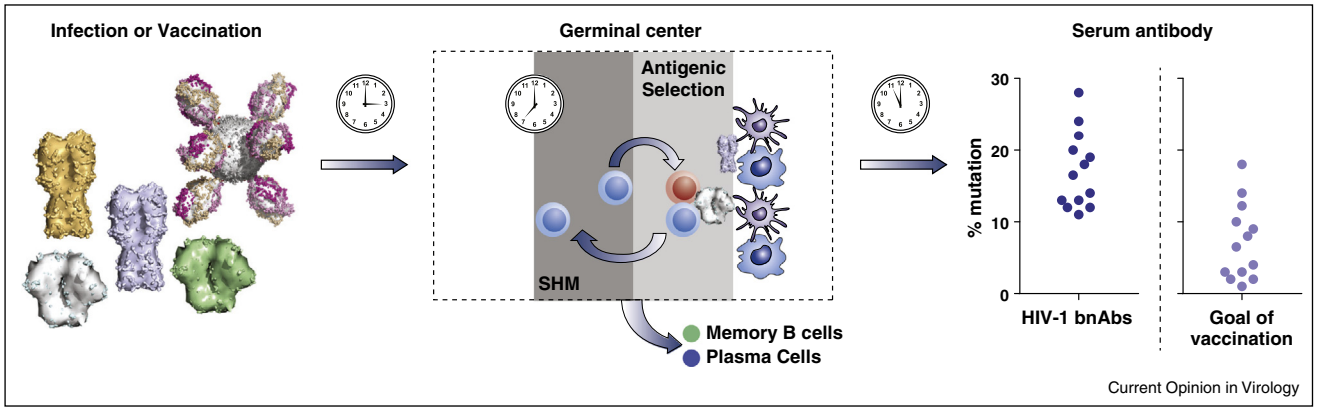
Why would affinity maturation need to be guided? In many cases, particularly for highly variable pathogens such as influenza and HIV-1, the antibodies typically elicited by vaccination or infection are poorly functional or insufficiently cross-reactive against multiple viral variants. Only a subset of antibodies that bind viral proteins can neutralize the virus, and an even smaller fraction is broadly neutralizing (cross-reactive). B cell selection is driven by affinity to the antigen that is presented in the germinal center, not by functionality that may be desirable in a vaccine context or measured *in vitro*, such as neutralization of heterologous viral strains [3,4]. In many studies of HIV antibodies in which multiple variants of a neutralizing antibody lineage were identified [5,6••,7••,8–11], each lineage had members with broad cross-reactivity and others with poor activity, despite the antibodies containing similar levels of SHM. Thus, increasing SHM generated increasing functionality for some sub-lineages, but went 'off track' for others (Penny Moore, personal communication and [11]) while the combined effects of broadly and poorly neutralizing antibodies are only recently being appreciated [12]. Therefore, there is currently much discussion in the literature about how to guide affinity maturation.

On a guided journey, it is important to know where we want to go, start out headed in the right direction, not get lost along the way, and know when we have arrived at the desired destination. In this article, we will discuss recent findings regarding HIV-1 and influenza antibodies, new concepts for appropriate immunogen design and presentation, and strategies for priming and guiding the immune system along the maturation pathway.

Where do we want to go?

Antibodies can perform numerous antiviral functions, including neutralization of free virus, as well as Fc-requiring functions such as antibody-dependent cell-mediated cytotoxicity (ADCC). There are natural examples of differing ways to achieve potency and cross-reactivity: via a single antibody lineage that accounts for nearly all of the serum breadth and potency [9,13], or by a collection of antibodies that collectively provide the observed breadth [14–16]. The required levels of SHM and affinity maturation may vary from target to target — for example, influenza neutralizing antibodies average 5–10% mutation from their germline genes [17,18•], while some classes of HIV-1 broadly neutralizing antibodies show mutation levels of 15–20% [6••] and others show upwards of 30% mutation [13]. Even among the most highly mutated antibodies, not all of the mutations are required for full activity [18•,19,20], and levels over 20% may be difficult to achieve by vaccination; therefore we suggest a goal of mutation levels closer to 5–20% for antibodies that target specific and multiple sites of vulnerability (Figure 1).

Figure 1



Overview of affinity maturation. Left, Naïve or memory B cells are activated by exposure to viral antigens by infection or vaccination. Center, Activated naïve or memory B cells migrate to germinal centers within secondary lymphoid tissues such as lymph nodes [111,112]. There, B cells cycle between a dark zone, where they undergo mutation and proliferate, and a light zone, where they undergo selection [1]. In the light zone, B cells compete for antigen on follicular dendritic cells, internalize the antigen, and present it to T follicular helper cells. The B cells with highest affinity internalize the most antigen, conferring an advantage in obtaining T cell help which in turn regulates survival, dwell time, and number of cycles of selection [105,106]. Approximately 90% of selected cells return to the dark zone and repeat the cycle, while the remaining 10% exit to serve as memory cells or plasma cells [113]. Right, After sufficient time passes for multiple rounds of germinal center selection, the resulting antibodies may be highly mutated from their naïve precursors. While chronic infection may result in mutation levels upwards of 30% as seen in HIV-1 broadly neutralizing antibodies (bNAbs) [22], mutations of 5–20% may provide sufficient maturation to be effective [17,18•], and is more readily achieved by vaccination.

Starting in the right direction

The initial immune response is likely to be crucial in starting antibody lineages along the path to highly functional mature antibodies. The initial naïve B cell repertoire is large and highly diverse following VDJ recombination and selection against self-reactivity [2]. Naïve BCRs that target specific sites, or have certain characteristics such as utilizing a specific VH gene or displaying a long CDR H3, may be better suited than others to mature into highly functional antibodies [6••,21].

While most antibodies concentrate antigen-contacting amino acids in the CDR H3 (encoded by the VDJ junction), two groups of highly cross-reactive antibodies against influenza and HIV-1 bind primarily using the CDR H2, which is entirely encoded by the VH gene. Broadly neutralizing antibodies targeting the CD4-binding site (CD4bs) on the HIV-1 Envelope glycoprotein (Env) preferentially utilize the VH1-2*02 gene [8,21,22] or the VH1-46 gene [8] while those targeting the conserved influenza HA stem region utilize certain alleles of the VH1-69 gene [18•,23]. These genes contain critical binding motifs [18•,21,23,24] but also undergo SHM leading to increased affinity and neutralization breadth [18•,19,21].

In addition, broadly cross-reactive antibodies that utilize the more typical CDR H3 recognition mode have been noted. In the case of HIV-1, many such antibodies display a characteristic elongated CDR H3 that is used to penetrate the extensive glycan shield found on the HIV-1 Env molecule [11,25,26•,27]. For influenza HA, the head region encodes significant diversity, yet a number of cross-reactive antibodies have been identified that target the receptor binding domain (RBD) within the head domain by using an aromatic residue located on the CDR H3 to mimic the HA-receptor interactions [28–31]. Importantly, antibodies with these modes of recognition have been isolated from multiple donors, indicating a common solution leading to broad neutralization.

This leads us to the hypothesis that immunogens that can bind to naïve BCRs with favorable genetic properties can trigger the initial development of broadly neutralizing antibodies. In addition, there are other sites targeted by broadly neutralizing antibodies in multiple donors, such as the glycan-V3 or the membrane proximal external region of HIV-Env [9,13,32–36], that do not share genetic characteristics. Immunogens that bind to naïve BCRs of multiple unrelated lineages may be more difficult to engineer, but would also have great potential as vaccines.

Guiding maturation

Following initial activation of B cells with preferred properties (such as BCR precursors of neutralizing antibodies or those that target known sites of vulnerability), further immunization will be required to drive the maturation process toward a refined set of broadly neutralizing antibodies. There have been a significant number of studies [18•,22,37,38] that have mapped the evolution of broadly neutralizing antibodies in both HIV-1 and influenza. More recent studies have mapped the co-evolution of