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HIV Immunogens: Affinity Is Key

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Generation of broadly neutralizing antibodies is a key aim of HIV vaccine design, but the precursor B cells are rare. Abbott et al. (2018) report that high affinity and avidity immunogens are required to promote maturation of low frequency B cells in germinal centers.

HIV infection is notorious for the host's poor ability to generate specific neutralizing antibodies against the rapidly mutating virus epitopes. However, some HIV-infected individuals do generate broadly neutralizing antibodies (bNAbs) capable of neutralizing broad strains of HIV virus as a result of a time-consuming process of multiple rounds of somatic hypermutation (SHM) of B cells in germinal centers (Scheid et al., 2011). Significant efforts in the search for effective vaccines are focusing on using immunogens specifically designed to target the precursor unmutated B cells (also known as ''germline-targeting'') so that upon SHM they can ultimately develop into bNAb producers. A notable attempt at this comes from the recent finding that immunogens such as engineered outer domain germline-targeting version 8 (eOD-GT8) are capable of expanding endogenous precursor naive B cells that can generate VRC01 bNAbs through the process of SHM (Briney et al., 2016; Jardine et al., 2016a); these VRC01 bNAbs are capable of binding crucial epitopes of the HIV envelope. However, such precursor VRC01-B cells have been found to be rare in healthy individuals, usually at a frequency of 1 in 400,000 (Jardine et al., 2016b). This raises the question of whether rare VRC01 B cells are able to out-compete other non-VRC01 B cell clones and generate good germinal center responses. Moreover, it would be desirable to know what is the effect of immunogen epitope affinity and avidity when responding B cells are present in low numbers. Abbott et al. now show that only immunogens above a certain affinity and in multimeric form are capable of inducing VRC01 B cell-dominated germinal centers from a starting low VRC01 B cell precursor frequency. Furthermore, these B cells achieve extensive somatic hypermutation after a single immunization.

Abbott et al. (2018) developed a controlled adoptive cell transfer model, in which different numbers of B cells from germline VRC01 heavy and light chain B cell receptor knockin mice ($VRC01^{gHL}$) are transferred into congenic hosts that are subsequently vaccinated with monomeric or multimeric immunogens of defined affinities for $VRC01^{gHL}$ B cells (0.5 μM, 14 μM, and 40 μM) (Jardine et al., 2013), representative of the range of BCR affinities of naïve VRC01-class precursor B cells in humans that ranges from ~0.1 μM to > 100 μM KD (Jardine et al., 2016b). The starting transferred $VRC01^{gHL}$ B cell precursor frequency of 1 in a million was just below the physiological frequency reported in humans (Jardine et al., 2016b). This transfer model allows the rare B cell populations to be tracked so their participation in germinal center and memory responses can be quantified. When germinal center responses were evaluated against a range of immunogen affinities and precursor frequencies, physiological frequencies of precursor B cells generated a poor response, unless the immunogen was of high affinity. Avidity mattered as well, since immunogen monomers elicited several hundred-fold lower responses in comparison to multimers. When physiologically low frequencies of B cells were

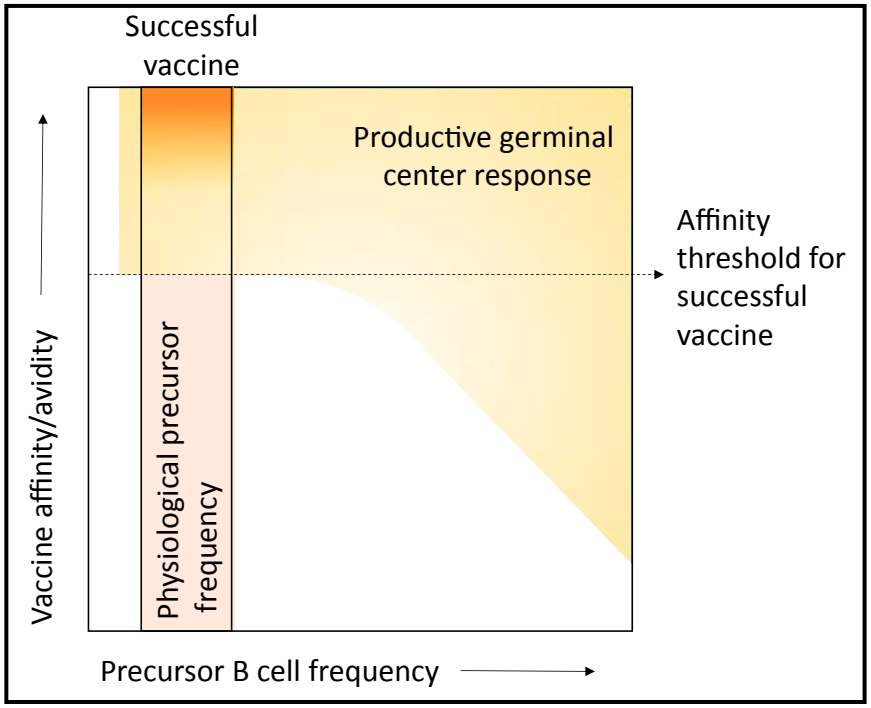

Figure 1. Diagram Depicting the Response of Varying Numbers of Precursor B Cells Capable of Producing bNAbs to HIV Constructs that Stimulate the B Cells with Varying Affinities
Formation of germinal centers in which the precursor B cells can outcompete other endogenous B cells allow acquisition of the desired somatic mutations over time. At the physiologically low precursor frequencies found in humans, an affinity threshold appears essential for a successful immunogen.

transferred and immunized with high-affinity immunogen, a quarter of germinal centers showed immunogen-specific responses. Within these germinal centers, immunogen-specific B cell clones were able to outcompete other clones significantly, implying that once seeded, B cell clones that respond to high affinity immunogens can successfully dominate responses within individual germinal centers, undergo extensive SHM, and form memory cells (Abbott et al., 2018). Needless to say, these are important findings that boost our hope that an HIV vaccine might not be as elusive as until recently thought.

It would be interesting to refine the contribution of immunogen affinities to the recruitment as opposed to clonal expansion within germinal centers. In the current experiments, germinal centers were typically analyzed once fully established at day 8. Looking at earlier time points would allow more accurate quantification of B cells seeding germinal centers. It is possible that high affinity B cells are actually not preferentially recruited to germinal centers but immunogen affinity provides the necessary advantage for clonal competition within germinal centers. In addition, the extrafollicular component of the immune response was not fully analyzed. In experiments with the model antigen hen egg lysozyme, high-affinity naive B cells were driven in large measure toward a large extrafollicular response (Phan et al., 2006). Multimeric antigens also might drive large extrafollicular plasmablast responses, often in a T-independent manner. For example, it was recently shown that the repeat of the *Plasmodium* circumsporozoite protein can drive a short-lived T-independent response (Fisher et al., 2017). The actual affinity of the B cells for the monomeric antigen was found to be quite low, but the repetitive antigen was able to bind multiple antibodies (and perhaps therefore BCRs on the B cell surface) with high avidity. It would be interesting to know whether the multimeric eOD-GT8 antigen likewise stimulates a fulminant extrafollicular response. The circulating antibody produced by such an early response could be beneficial in stimulating completion within the germinal center reaction and might aid the formation of high affinity bNAbs (Zhang et al., 2013).

Interestingly the eOD-GT5 (intermediate affinity) and eOD-GT2 (low affinity) constructs used to elicit the VRC01 bNAbs were also capable of eliciting a significant endogenous mouse B cell response. For the low affinity immunogen, the VRC01^{gHL} cells were often outcompeted by this endogenous response (Abbott et al., 2018). This would not be a desirable outcome for vaccination as it is unlikely that many of these endogenous cells would be able to move through "mutational space" to ultimately become bNAbs, given the very stringent requirements for bNAb activity. On the other hand, when the intermediate affinity construct was used, the VRC01^{gHL} cells then almost always outcompeted the endogenous cells. In coming to dominate the germinal center reaction, these VRC01^{gHL} cells acquired mutations that made them more bNAb-like and further increased their affinity, suggesting that affinity, not stochasticity, drives the maturation of the germinal center reaction (Abbott et al., 2018). These data suggest that an affinity threshold of ~1 μM would be essential for a successful immunogen (Figure 1). This information is valuable as there is little to be done about the frequency of endogenous cells in a vaccinated individual, but we can control the affinity and structure of the immunogen.

What are the implications of this study for HIV vaccine design and success? The results shown here further support that germline-targeting vaccination approaches aimed to elicit bNAbs by targeting rare naive VRC01-B cells present in healthy individuals might be successful: Low starting numbers of precursor B cells will respond, get recruited to germinal centers and outcompete other clones so long as immunogen affinities and avidities are high. At low physiological precursor frequencies of around 1 in a million, the current model predicts low germinal center recruitment rates, especially when immunogen affinities are low. It remains to be tested whether it is the frequency of precursor B cells that matters or the overall number of B cells present in the whole organism. 1 in a million B cells will turn out to be a far smaller absolute number in mice compared to humans, who have higher numbers of total B lymphocytes. Definitive answers in humans will

come from a vaccine trials with eOD-GT8 multimer expected to start in 2018; the first germline-targeting vaccine to be tested.

This study raises further questions that are important for our understanding of germinal center biology and protective HIV antibody responses. It is known that HIV responding B cells are partially self-reactive, binding to lipids such as cardiolipin (Haynes et al., 2005). Does this polyreactivity promote selection of high-affinity clones into germinal center reactions, that would otherwise be destined to become extrafollicular? In the setting of a complex pathogen including an intact HIV virus with multiple low and high affinity epitopes arrayed in highly repetitive patterns, does the multimeric conformation largely override differences in affinity? What is the ceiling to multimer size and epitope distance behond which no further advantage in BCR crosslinking occurs? Furthermore, do larger multimers deviate away from a germinal center response, inducing potent T-independent extrafollicular responses that are less capable of producing long-lived memory responses?

In summary, this study has demonstrated that both precursor frequency and affinity are limiting for optimal competition of VRC01-class B cells in germinal centers. At the low precursor frequency found in humans, a multivalent germline-targeting immunogen with an affinity threshold < 1 μM is expected to successfully provide the advantage VRC01-class B cells need to compete efficiently within GCs, undergo extensive affinity maturation, and become memory B cells. This information informs the nature of a successful HIV vaccine and supports the notion that germline-targeting immunogens might indeed constitute the basis of a long-awaited vaccine.

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A Long-Distance Relay-tionship between Tumor and Bone

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Myeloid cells, including neutrophils, are important regulators of tumor growth and metastasis. In *Science*, Engblom et al. (2017) reveal how lung tumors remotely engage bone-resident cells through a relay mechanism that achieves a sustained supply of tumor-promoting neutrophils.

Aberrant myelopoiesis is a phenomenon shared across many tumor types. In cancer patients, elevated numbers of myeloid cells—both in the circulation and within tumors—frequently correlate with a worse prognosis. These cells not only differ in abundance when compared to healthy individuals, but also display a distorted composition and phenotype (Quail and Joyce, 2013). Moreover, increased mobilization of myeloid progenitors and a skewed differentiation profile toward immature cells are common features of cancer-associated myeloid deviation (Quail and Joyce, 2013). This altered cell pool possesses multiple tumor-promoting properties, including the active suppression of tumor-reactive immune cells and the stimulation of tumor cell proliferation, as well as angiogenic and extracellular matrix remodeling processes (Quail and Joyce, 2013). Neutrophils have emerged as dynamic myeloid cells susceptible to education by tumor cells, which can then regulate cancer progression (Coffelt et al., 2016). In a recent issue of *Science*, Engblom et al. (2017) uncover