


COMMENTARY

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## B-cell restriction – an alternative piece to the puzzle

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### ABSTRACT

Effective vaccination is based on three critical aspects of the B-cell response towards infectious agents: (i) that B-cells can generate specific antibodies towards a vast molecular diversity of antigens; proteins, sugars, DNA and lipids. There seems to be no limit to the ability to raise antibodies to everything. (ii) once stimulated, B-cells can perfect their antibodies through affinity maturation to complement every nook and cranny of the epitope and (iii) that the pathogen remains genetically stable and does not change to any great extent. Thus, antibodies produced against the vaccine and subsequent boosts recognize the viral virulent field isolates in future encounters and effectively knock them out. However, some vaccine targets, such as flu virus and HIV, are extremely genetically dynamic. The rapid genetic drift of these viruses renders them moving targets which assist in their ability to evade immune surveillance. Here we postulate that in the case of hyper-variable pathogens the B-cell response actually might be “too good”. We propose that restricting B-cell activities may prove effective in counteracting the genetic diversity of variant viruses such as flu and HIV. We suggest two levels of “B-cell restriction”: (i) to focus the B-cell response exclusively towards neutralizing epitopes by creating epitope-based immunogens; (ii) to restrict affinity maturation of B-cells to prevent the production of overly optimized exquisitely specific antibodies. Together, these “B-cell restrictions” provide a new modality for vaccine design.

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Training the immune system with vaccines has proven as an enormously effective means to prevent disease and save lives.<sup>1</sup> Since Jenner, millions and millions of children worldwide are protected yearly against diseases that in many countries and societies are now almost forgotten threats of the past. The general principle of vaccination is rather simple and straightforward; train our immune systems with dummy pathogens to gain experience and protection in the event that we encounter virulent forms in the future. Yet, while some vaccines work, others do not.

### Vaccines that work

So long as pathogens remain genetically stable, vaccines prove amazingly effective. The measles, mumps and rubella vaccines afford lifelong protection and have changed little since they were first licensed.<sup>2</sup> Smallpox has been vaccine-eradicated<sup>3-5</sup> and polio is just about there.<sup>3</sup> Much of the success of these vaccines lies in their ability to deposit and store reservoirs of trained B-cells able to produce fine-tuned, highly efficient neutralizing antibodies.<sup>1</sup> Consequently, future encounters with virulent field virus-isolates are met with an immediate cross-reactive secondary response perfected and ready to knock-out the intrusion.

Three properties of the B-cell response contribute much towards successful prophylaxis.

### *The ability to produce highly specific and discriminating lead antibodies ... to everything*

Tonegawa discovered the ability of chromosomes to rearrange and produce VJ/VDJ junctions.<sup>6</sup> This breakthrough

cracked the long-lasting perplexing conundrum of how, with so few open reading frames in our genome, we are able to produce millions and millions of unique antibodies, each the product of a distinct B-cell clone. The wealth of antibodies is further increased by the introduction of P and N nucleotides at the combinatorial junctions. Thus, there seems to be no limit to the diversity of antibodies we are able to produce. Antigens can be proteins, nucleic acids,<sup>7,8</sup> sugars<sup>9,10</sup> as well as lipids.<sup>11-13</sup> So long as there is even marginal affinity of the B-cell receptor (BCR) for an epitope of the immunogen, clonal expansion is launched and ever-improved antibodies are generated.

- **The ability to perfect exquisitely specific antibodies.** Somatic hyper-mutation accompanied with multiple rounds of immunogen/B-cell encounters are the steps that lead to affinity maturation.<sup>14</sup> An initial binding event of a pathogen by a B-cell launches clonal expansion and the production of lead IgM antibodies. Initially, the efficacy of these relatively broad-spectrum weak binders to counteract invading pathogens lies in the avidity gained by the deca-valency of IgM.<sup>15</sup> However, as AID-mediated mutagenesis of the variable domains kicks in, the fit of the CDR loops to the idiosyncrasies of the epitope being bound gradually improves. Mutation, followed by the selection of those B-cell clones that gain ever-increasing affinity, drive the antibody to perfection and the ability to continually clear the pathogen as its concentration ever decreases. Ultimately, an optimized

antibody is produced whose paratope neatly complements the nooks and crannies of the epitope with precision, exhibiting binding affinities of  $KD < 10^{-9}$ – $10^{-10}$  M.<sup>14,16–18</sup>

- **The deposit and recall of mature memory cells.** This process of developing perfected B-cell responses takes time. However, when naturally encountering a virulent pathogen, this time can be critical, giving the pathogen an opportunity to replicate and establish a life-threatening infection. Vaccination, on the other hand, affords our immune system the chance to study harmless versions of the pathogen, elicit clonal expansion of select B-cells and the ability to go through repeated rounds of somatic hypermutation to perfect affinity matured antibodies.<sup>1</sup> The success of vaccination lies in the option to train effective B-cells and store mature memory cells in the absence of disease and to recall them upon demand. The archive of perfected memory B-cell clones ensures that future encounters with virulent field isolates of the pathogen are met with an immediate B-cell frenzy secreting perfectly matched antibodies that intercept the pathogens and knock them out before infection sets in and disease ensues.

## Evasion

Despite an impressive collection of pathogens for which vaccination has proven remarkably effective, some viruses simply do not comply.<sup>19–22</sup> All attempts to produce protective vaccines against HIV have failed and influenza continues to be a very challenging adversary.<sup>19–22</sup> There are numerous different strategies employed by viruses to evade immunity. The following examples are some tactics viruses use to interfere with the ability of B-cells to mount potent long-lasting cross-reactive neutralizing antibodies.

- **Concealing neutralizing epitopes.** Antibody-mediated neutralization is based on the B-cell targeting of critical neutralizing epitopes of the virus. Viruses, for example, gain entry to their target host-cells by exploiting cell surface-proteins that serve as “receptors”. Obviously, the “receptor binding site” (RBS) of the viral spike protein thus, presents a good neutralizing epitope.<sup>23–26</sup> Antibodies that bind and thus occlude the RBS prevent the virus from being able to associate with the target cell surface. Hence, keeping the RBS concealed reduces its immunogenicity. The CD4 binding site of HIV-1 gp120 tends to be buried in a crypt and is certainly much less accessible than the surrounding surfaces of the spike protein.<sup>27</sup> Therefore, generally prime neutralizing epitopes are less available to B-cell scrutiny.
- **Shadowing neutralizing surfaces.** Glycan-shields are another strategy employed by some viruses.<sup>28–30</sup> Viral spike proteins are typically highly glycosylated. The glyco-moieties loom over the surface of the virus and shadow the access to the underlying spike amino-acid surfaces. Thus, access to the more immunogenic aspects of the spike is limited by steric hindrance imposed by the

multiple glycan branches stemming from strategically positioned N or O glycosylation sites of the spike.<sup>28,30,31</sup>

- **Display of multiple non-neutralizing epitopes.** The viral surface contains only a select, very few, truly critical neutralizing epitopes which may be hidden or shadowed. That being said, there is an extensive accessible surface of the spike that can and does interact with B-cells. The enormous diversity of B-cells that can complement literally any and every antigenic surface of the virus thus leads to a plethora of generally useless antibodies. Indeed, HIV and influenza stimulate a robust serological response upon infection; however, the vast majority of antibodies produced do little to counteract the invasion.<sup>26,32</sup> Flooding the system with impotent antibodies can reduce viral load to some degree but does not really perturb the infectious process. A special case of distracting targets is realized by shedding “chaff” soluble spike components.
- **Dispensing chaff and false targets.** Viral spikes often contain multiple subunits. In HIV-1, for example, gp160 is cleaved to produce membrane-anchored gp41 which is non-covalently associated with soluble gp120. The mature spike is then assembled to form a trimer consisting of three gp41/gp120 monomers. The buried inner-surfaces where the gp41/gp120 monomers meet to produce trimers do not contain neutralizing epitopes, as these surfaces are never exposed in the native functional spike.<sup>33–35</sup> However, shedding of monomeric gp120s into the serum reveals the previously buried surfaces of the spike.<sup>36</sup> B-cells, that target these “decoy” false targets of the virus, are dissuaded and immunity is evaded by the chaff-like shed gp120.<sup>33</sup>
- **Dynamic genetic variations.** The success of polio and smallpox vaccines lies in that these viruses tend to be genetically stable. However, HIV and influenza exhibit extensive genetic instability. These viruses have evolved unique mechanisms to increase genetic variation. The RNA-DNA-RNA cycling of the HIV genome via reverse transcriptase is highly error-prone.<sup>37</sup> Influenza benefits from both genetic drift and shift.<sup>20,38</sup> These dynamic instabilities produce vast amounts of variant viruses, many of which may be non-productive progeny. However, with each replicative cycle of the virus some amino acid compositional variations – mutations, are produced that do not compromise viral infection. These mutant virus variants may not be recognized by the antibodies previously raised against the original viral infectious isolate and thus, offer an escape route for the virus and the ability to evade immunity.

## Cases where the B-cell response might be too good

The dynamic dialogue between pathogens and our immune defenses leads to serial rounds of co-evolution. Our B-cells encounter vast repertoires of epitopes presented by a virus. The virus evolves to best conceal its most vulnerable targets while in turn presents multiple distracting non-neutralizing epitopes; banking on the fact that B-cells indiscriminately bind

and respond to everything.<sup>39</sup> Multiple antibodies are produced, most of which, do not hit critical neutralizing epitopes. Those that do, however, initiate rounds of somatic hypermutations and go on to optimize their affinity to precisely target and complement the founder infectious strain of virus.<sup>14,40–42</sup> As affinity-maturation proceeds, the antibodies focus and gain perfection for that specific strain initially encountered, all the while losing ability to bind divergent viral genetic variants yet to emerge.<sup>14</sup> Perfection of highly target-specific neutralizing affinity-matured antibodies goes hand in hand with loss of broad cross-reactivity that allows recognition of ever variant members of the evolving viral swarm. Perfection of potent exquisitely specific neutralizing antibodies opens the back door for viral mutations to escape scrutiny and go on to spread infection and havoc.<sup>43</sup>

Curiously, two strong points of B-cells physiology, the ability to (i) bind and react to everything and (ii) affinity-perfect their antibodies, provide some advantage for the most challenging virus targets, those that are hypervariable, constantly changing while luring B-cells in false directions. Paradoxically, what might prove to be the best strategy for production of vaccines towards hyper-variant viruses like HIV and influenza is to *restrict* the B-cell response!

### B-cell restriction #1

In view of the seemingly endless capacity of B-cells to generate antibodies towards every nuance of an infectious virus, it would make sense to restrict and focus the immune response exclusively towards neutralizing epitopes. It certainly makes sense to invest all B-cell energy in producing exceptionally effective antibodies, antibodies that ensure that each binding event cripples the virus and directly interferes with infectivity. So, the first restriction of the B-cell response towards viruses should prevent the production of antibodies to non-neutralizing surfaces of the virus. We should try to focus on the immune response such that every antibody counts.

There are a number of approaches that can be used to restrict and focus the B-cell response.

#### Shielding irrelevant surfaces

Assuming that one has mapped the preferred neutralizing surfaces of a given virus, it might be possible to accentuate these targets by occluding and shielding otherwise distracting surfaces. Strategically placing glyco-moiety to shield surfaces of the spike protein has been proposed so to leave only the neutralizing surfaces exposed and subject to B-cell scrutiny.<sup>44,45</sup> In essence, the use of trimeric spike proteins, as in the case of SOSIP trimers for HIV-1, ensures that the inner surfaces of gp120 “chaff decoys” are avoided.<sup>46–48</sup>

#### Subunit vaccines

One means to better focus the response towards select neutralizing epitopes would be to remove all otherwise distracting antigens. Subunit vaccines are the first step towards this goal. Instead of vaccinating with intact Pertussis bacterium (some 2000 proteins), a-cellular vaccines present to our immune system a handful of only the most relevant Pertussis antigens.<sup>49,50</sup>

Similarly, Hepatitis B surface antigen is a good example of a successful subunit vaccine.<sup>51–53</sup> Recently, the Human Papilloma Virus subunit vaccine illustrates how the use of recombinant L1 capsid protein can reduce the occurrence of cervical cancer.<sup>54–57</sup> If one is able to dissect out the neutralizing epitope exclusively, this could lead to the ultimate form of targeted vaccination, i.e., epitope-based vaccines.<sup>58</sup>

### Epitope-based vaccines

For this, the nature of the neutralizing epitope first needs to be mapped with precision. Then systematic removal of all other viral surfaces should leave only the desired epitope as an immunogen. Obviously, this is not a simple task, as most epitopes are discontinuous and highly conformational.<sup>59</sup> It should not be expected that by simply expressing the correct peptide sequences corresponding to the epitope, the native conformation of a functional epitope surface would be achieved. We have addressed this task of “epitope reconstitution” by implementing a novel combinatorial approach.<sup>60</sup> The RBS of SARS Coronavirus (SARS CoV) is contained in a sub-domain of the viral spike of about 200aa. The actual surface that contacts the viral receptor is less than 40 amino acid residues situated on two discontinuous antiparallel beta strands.<sup>60,61</sup> Using combinatorial linkers to bridge the two strands of the bona fide RBS has enabled the affinity selection of functionally reconstituted neutralizing epitopes of the virus.<sup>60</sup> This same approach has since been found effective for the reconstitution of neutralizing epitopes of MERS Coronavirus and Dengue virus (not published). Focusing the immune response on exclusively neutralizing epitopes may be particularly important in Dengue prevention as it may obviate the development of antibody-dependent enhancement (ADE).<sup>62,63</sup>

Hence, the first level of B-cell restriction that should improve vaccines is to discard all irrelevant and distracting pathogen antigens and focus the B-cell response exclusively where it counts, towards neutralizing epitopes.

### B-cell restriction #2

Curiously, the perfection of antibodies via affinity maturation might be over-doing it in some cases. The evolution of the humoral immune system may not have been able to predict hypervariable pathogens such as HIV-1 as well as shifting and drifting viruses like flu.<sup>64,65</sup> Thus driving antibodies to perfection by gaining extreme specificity and high affinity ensures the ability to clear pathogens as their concentrations dwindle below the initial KD of the first generation of antibodies. Paradoxically, this is precisely a boon for dynamically changing virus opponents. The natural first generation response is a bit fuzzy in its recognition and more tolerant of epitope variation. There is a need to recognize and handle any virus that may infect us. Therefore, germline responses tend to be more cross-reactive, loose-fitting paratopes that bind enough to launch clonal expansion. The multiple rounds of perfection that follow produce the ultimate-fit antibody targeting the initial infectious virus. Gain of specificity and affinity, however, is at the expense of loss of flexibility, tolerance and cross-

reactivity. Thus, as the virus accumulates mutations and genetic variants, it gradually morphs to slowly escape the range of recognition of the perfected and high-affinity antibody that matured.<sup>66–68</sup>

If only one could restrict the B-cell response it might prove to be the answer to dynamic genetic drift!

If one could stimulate early B-cell lineages yet prevent their further affinity maturation, “fuzzy vaccines” would be possible. Fuzzy-Vaccines would be those that do *not* train our system to forge ahead and develop antibodies with single variant specificity. Rather, the preferred germline B-cells would be activated but not allowed to affinity mature beyond a certain point, thus gaining the ability to cope not only with the infectious isolate but also with emerging variants yet to appear.

Hence, the second mode of B-cell restriction should teach our system to be happy with cross-reactive antibodies, mediocre to some degree, but precisely what is needed to counteract genetic drift and emerging virus variation.

How to produce such “fuzzy vaccines” is a challenge worth meeting.

## Final remarks

The immune system has evolved to effectively counteract and eliminate infectious viruses. Vaccination has been the most effective medical intervention since immunity was first discovered. However, for some viruses, the paradigm of training our immune system with a discrete immunogen faithfully representing the native pathogen in its entirety simply does not work. The evolution of “virus fast moving-targets”, hypervariable species that evade the canonical “expected” B-cell response, presents a challenge to the vaccine industry. Here is where next-generation biotechnological ingenuity must step in. Our understanding of the natural B-cell response is extensive. One means to outwit HIV-1 and counter influenza is to play outside the box where virus evolution cannot anticipate. Paradoxically, one way of achieving this goal is by B-cell restriction.

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