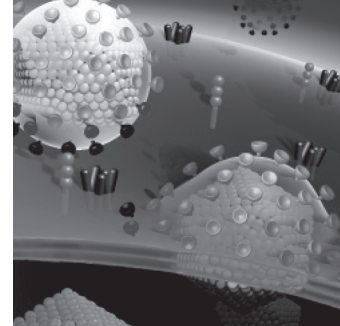


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Are affinity-enhanced T cells the future of HIV therapy?

“... one can imagine that the final decision on which affinity-enhanced TCR to advance through the clinic to be based on a balance between anti-HIV-1 activity, survival of transduced T-cell population after infusion and safety.”

An effective HIV-1 vaccine has eluded the research community for more than 25 years. Neutralizing antibodies, the key effector in most, if not all, current vaccines [1], have proved difficult to generate against HIV-1 [2]. Therefore, much effort has been devoted to developing vaccines that induce effective T-cell responses. However, with the failure of the STEP and Phambili trials (HVTN 502 and 503) in which vaccine-induced T-cell responses were unable to reduce infection rates [2], faith in the ability of T cells to protect and/or control HIV-1 replication long term has been shaken. In this editorial we will examine the merits of generating an HIV-1-specific T-cell response by introducing enhanced HIV-1-specific T-cell receptors (TCRs) into a large population of autologous CD8⁺ T cells and re-infusing these TCR-transduced CD8⁺ T cells to create what can be termed an instant vaccine. Our preliminary data suggests that this is a promising approach as an HIV-1 therapy [3] and may also provide crucial information to guide the development of effective traditional vaccines.

HIV-1 infection induces a potent T-cell response that is responsible for an initial drop in viral load [4,5]. In greater than 99% of all cases the immune response fails to control HIV-1 replication and a chronic infection that leads to immune dysfunction ensues. Three key concepts influence our understanding of how a chronic immune response differs from an acute/effective immune response: escape, exhaustion and polyfunctionality. HIV-1 has shown a tremendous ability to mutate and escape from immune responses. Cytotoxic T lymphocyte (CTL) escape has one of two consequences: if the virus incurs no fitness cost by CTL escape, then progression to AIDS occurs more rapidly [6–8]; however, if CTLs force HIV-1 to retain a mutation that results in a significant loss of viral fitness, long-term control of HIV-1 replication

can occur [9–11]. Ideally, adoptively transferred T cells should select for immune escape variants with reduced viral fitness. Unfortunately, initial attempts to adoptively transfer antigen-specific T cells for treatment of HIV-1 disease preceded our current understanding of viral escape [10] and how to best expand T cells for adoptive T-cell therapy [12,13], and CTL escape and rapid progression was observed [8]. Moreover, the degree by which human leukocyte antigen (HLA) influences HIV-1 immune escape and survival is now fully coming to light [14]. When simian immunodeficiency virus first transitioned to humans, it faced enormous selection pressure as it encountered the human immune system. Given that HLA-A2 is the most prevalent HLA allele worldwide [15], it is thought that HIV-1 rapidly evolved so that HLA-A2-restricted T-cell responses would be ineffective [16], similar to what is observed in other lentiviral infections [17–19]. Thus, HLA-A2-expressing individuals are likely at a disadvantage to benefit from traditional vaccines because HIV-1 has evolved to avoid or escape from effective HLA-A2-restricted responses, and thus the vast majority of HLA-A2-restricted responses are not protective. By contrast, HLA-B27- and HLA-B57-restricted responses, especially those targeting HIV-1_{GAG}, do correlate with reduced viral load and thus HLA-B27 and HLA-B57 are called protective alleles [20,21]. Importantly, individuals harboring these alleles are highly over-represented in elite control cohorts [22], suggesting that under rare circumstances HIV-1 infection can be effectively controlled by a protective T-cell response.

T-cell exhaustion has been most thoroughly studied using the lymphocytic choriomeningitis virus (LCMV) model. Mice clear wild-type LCMV infection rapidly and protective memory develops. By contrast, a two amino-acid substitution mutant of LCMV (Clone 13) is not cleared and a chronic, persistent infection ensues [23].

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In the face of chronic antigen exposure, T cells undergo a distinct differentiation program and progressively lose their effector functions [24]. High levels of programmed death (PD)-1 expression mark these exhausted T cells, and blockade of PD-1/PD-L1 interactions restores function to exhausted T cells [25]. HIV-1-specific T cells undergo a similar differentiation program [26–28] and IFN- γ is one of the last effector functions lost. This discovery is unfortunate because most vaccine-induced T-cell responses have been measured by IFN- γ enzyme-linked immunosorbent spot assays. As a result, investigators now use multiparameter flow cytometry techniques to measure multiple T-cell effector functions (IL-2 production, TNF- α production and CD107 α mobilization, among others). In fact, there are strong correlations between the number of polyfunctional HIV-1-specific T cells, control of viral load and disease progression [29,30], suggesting that it would be highly desirable to induce a polyfunctional T-cell response in patients. Unfortunately, means to induce polyfunctional responses in the CD4 T-cell help-depleted environment of HIV infection have remained elusive.

Our recent data suggests that engineered TCRs might provide a means of generating HIV-1-specific polyfunctional T-cell responses [3]. T cells recognize antigen by means of an interaction dominated by the specific binding of the α/β TCR to peptide fragments presented on the cell surface by MHC, also known as HLA in humans [31]. MHC class I molecules present short peptides, typically 8–12 amino-acid residues in length, bound in a groove formed by the $\alpha 1$ and $\alpha 2$ domains of the membrane-anchored HLA heavy chain, which is also associated with $\beta 2$ -microglobulin [32]. Through somatic gene rearrangement, the immune system produces a vast variety of random TCR specificities, including self-antigen-reactive and nonreactive receptors. Thymic selection narrows the peripheral TCR repertoire, eliminating cells with inept or autoreactive TCRs. Thus, peripheral T-cell populations are enriched for TCRs with the potential to recognize foreign peptides presented by self HLAs. Accumulated evidence derived from studies with recombinant TCRs indicate that TCR/peptide MHC of antigen-responsive T cells fall within a narrow range, approximately K_D 0.1–250 μ M [3,33]. This affinity range is thought to represent a compromise, allowing T cells to respond to antigenic peptides while remaining tolerant to self-antigens. The critical parameter determining the T-cell activation threshold is likely to be the off-rate, for instance,

the duration of TCR/peptide MHC engagement. This is exemplified by the demonstration that, of two ligands with similar K_D , only the one with the slower off-rate acts as an agonist [34]. The TCR binds MHC peptide antigen through three loop regions on each of the α and β chains, the complementarity determining regions (CDRs). CDR3s are mainly involved in interactions with the peptide antigen, CDR2s with the MHC heavy chain. In most TCRs, CDR1s have limited interaction with the peptide and/or HLA heavy chain [35]. In recent years, molecular evolution methodology has been developed for TCRs, enabling the selection of mutated receptors with increased affinities for antigen [36,37]. TCRs with slower off-rates of binding have been demonstrated to enhance the ability of T cells to recognize cells with low levels of antigen presentation and to improve their cytotoxic capacity [38]. However, for each antigen and TCR involved, there appears to be an optimal off-rate of binding, beyond which further improvements in the off-rate do not improve T-cell functionality.

A key question in the field is whether T cells function because of, or in spite of, their low affinity for antigen. B cells offer an interesting comparison. Most B cells start off with a relatively low affinity for their cognate antigen. However, as the immune response progresses, B cells mutate their B-cell receptor via somatic hypermutation and clones that have the highest affinity for antigen survive, a process called affinity maturation. This process can augment antibody affinity for antigen by several orders of magnitude and it is clearly a component of why antibodies are exquisitely specific and effective mediators of the immune response [39]. T cells, by contrast, did not develop such an effective affinity maturation process. However, with the aid of molecular evolution methodology we can now ask how T cells behave with affinities for antigen that rival B-cell affinity for antigen. Our studies show that T cells expressing a high affinity TCR are more effective than T cells expressing a wild-type affinity TCR. In particular, these T cells generate a much more polyfunctional response to antigen that was highlighted by high levels of IL-2 production [3]. Thus, these T cells have the potential to act as ‘lone rangers’ in that they can proliferate and survive in the absence of CD4 T-cell help. Additionally, T-cells transduced with high affinity TCRs were able to control HIV-1 infection at much lower effector to target ratios, supporting their use in adoptive T-cell therapy. Most importantly, these high affinity TCRs were able to control the replication of viruses that had

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escaped the natural HLA-A2-response [3]. Thus, it appears by augmenting the affinity HLA-A2-restricted TCRs we were able to convert a nonprotective response to a protective response.

Thus, adoptively transferred T cells engineered to express a high affinity HIV-1 TCR are poised to circumvent many of the limitations associated with therapeutic HIV-1-specific vaccines and natural HIV-1-specific T-cell responses. TCR-transduced CD8⁺ T-cell populations are likely to include cells that have not started down the pathway to exhaustion. It is currently not known whether T cells expressing a high affinity HIV-1-specific TCR are more or less susceptible to exhaustion or retain their polyfunctional nature *in vivo* but in any case multiple infusions of TCR-transduced T cells can be performed to keep fresh soldiers on the front lines of HIV-1 defense. Moreover, as noted earlier, high affinity TCRs can still see disguised HIV-1 that have escaped from the natural HLA-A2-restricted response. Given the history of HIV-1, it is foolhardy to predict that HIV-1 will not eventually escape this added pressure but it is reasonable to hope that these additional disguises HIV-1 must add to avoid the high affinity HLA-A2-restricted response will exact an overall fitness cost and result in less HIV-1 replication. Moreover, additional T cells transduced with other HLA-A2-restricted TCRs could be introduced creating a combination T-cell therapy cocktail that, in theory, could exert enough immune pressure to control HIV-1. Last, if TCR-transduced T cells are able to allow the immune system to regain control, the study of these cells will provide great insight into how to design the next series of HIV-1 vaccines.

Fortunately, many of the hurdles of using lentivirally transduced T cells for clinical use have been overcome [40]. But use of high affinity

TCRs to redirect T cells to HIV-1 does pose some additional questions and concerns: for one, does enhanced affinity lead to loss of specificity? Some affinity-enhanced tumor-specific TCRs recognize HLA-A2 in the absence of cognate antigen [41], suggesting that these TCRs are recognizing other peptides. Using similar models to one that detected cross-reactivity in the tumor-specific TCRs, we were unable to detect loss of antigen specificity using the HIV-1-specific TCRs, but it is difficult, if not impossible, to evaluate every single HLA-A2-restricted antigen present in a human being. Whether this finding represents a difference between viral and tumor-specific TCRs remains to be seen. Additionally, augmented TCR affinity may affect T-cell survival *in vivo*. Thus, one can imagine that the final decision on which affinity-enhanced TCR to advance through the clinic to be based on a balance between anti-HIV-1 activity, survival of transduced T-cell population after infusion and safety.

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