present study, IL-4 derived from basophil progenitors in Lin$^-$SHIP-deficient bone marrow cells were reported to mediate IL-3-induced M2 macrophage differentiation in mice (Kuroda et al., 2009). Basophils also produce thymic stromal lymphopoietin (TSLP), which amplifies M2 polarization in allergic airway inflammation (Han et al., 2013). Activated basophils release histamine, which may induce macrophages to express the M2-related chemokine CCL22. Moreover, M2 macrophages can release CCL24, which is a basophil chemoattractant, whereas activated basophils produce CCL3, CCL4, and CCL5, which can attract monocyte-macrophages, favoring their dialog (Figure 1; Biswas and Mantovani, 2010). Importantly, basophils are a main source for early IL-4 production in allergy and a possible initiator of Th2 cell-mediated responses (Sokol and Medzhitov, 2010). This raises the question as to whether basophils display distinct temporal roles in Th2 cell-mediated inflammation, i.e., initiating Th2 cell response in the early stage and attenuating it through M2 macrophages in the later stage. Further work will clarify this and whether the basophil-macrophage interactions described here is a general paradigm or specific to particular Th2 cell-related situations.

How M2 macrophages dampen IgE-CAI was not clear from the present study. Although the contribution of arginase-1, PD-L2, or immunosuppressive cytokines was not favored, efficient antigen uptake and clearance by M2 macrophages making antigen unavailable for basophil activation was suggested. Another possibility arising from the observation that basophils and neutrophils show enhanced recruitment in *Ccr2*$^{-/-}$ skin lesions is whether M2 macrophages themselves may block the recruitment of these cells, thereby attenuating Th2 cell-mediated inflammation. Increased expression of chemokines like CCL24 or CXCL8 by M2 macrophages (or basophils) may downregulate and desensitize their cognate receptors on target cells, blocking their recruitment.

In summary, the present study provides an insight into monocyte-macrophage differentiation during allergic skin inflammation demonstrating inflammatory monocytes to differentiate into M2 macrophages, which then dampen IgE-CAI. Importantly, the M2 skewing of macrophages was mediated by basophils, emphasizing that basophil-macrophage crosstalk presents a unique paradigm in macrophage polarization, which merits further investigation. Finally, it is tempting to speculate whether modulating basophil activation may be a potential strategy to "reprogram" aberrant macrophages in disease settings, including cancer, where polarized macrophages have an ambivalent role.

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# HIV-1 Vaccines: Let's Get *Physical*

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**Ferguson et al. (2013) use applied physics to quantitate the fitness of HIV-1 Gag based on sequence variability across the protein. This enables a new approach to vaccine design that focuses CD8$^+$ T cell responses on fitness-constrained parts of Gag.**

Considerable effort has been put into defining the immune responses induced by HIV-1 infection that are associated with greatest viral control, with the aim of reproducing the most effective responses by prophylactic vaccination. Human leukocyte antigen (HLA) Class I proteins determine the specificity of CD8$^+$ T cell responses and, given that certain HLA alleles are enriched in individuals who exhibit marked HIV-1 control (elite controllers), the specificity of these T cell responses must be important for virus control. HIV-1 CD8$^+$ T cell responses are first observed in acute

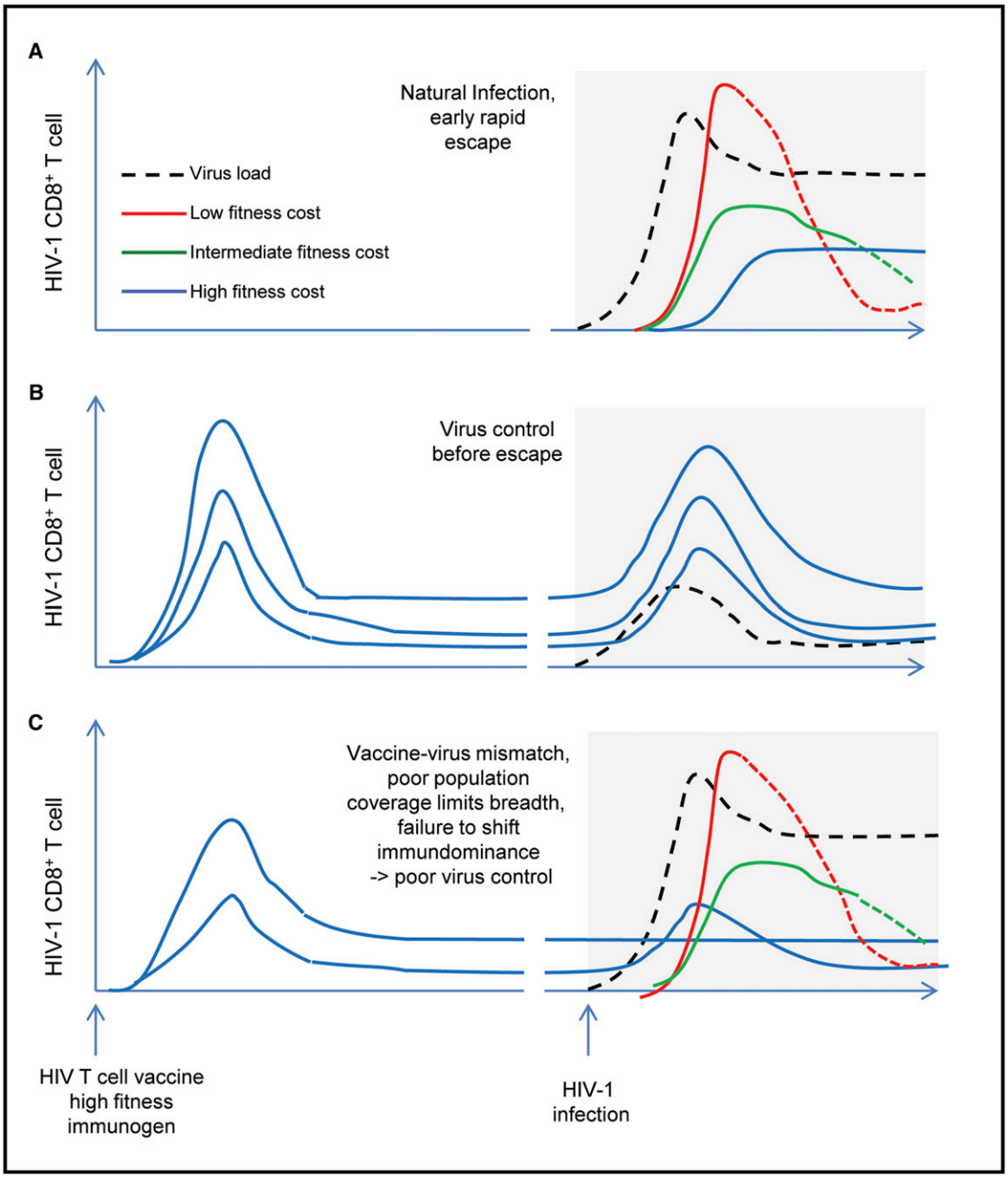


**Figure 1. Shifting HIV-1-Specific CD8 T Cell Immundominance through Vaccination**

(A) HIV $CD8^+$ T cell responses (solid colored lines) induced following HIV-1 infection expand unevenly. The immunodominant $CD8^+$ T cell response often targets highly variable regions of the HIV-1 proteome resulting in rapid escape (dotted) and limited fitness cost to the virus. T cells targeting more fitness constrained HIV-1 regions from which escape is slow are also targeted but are often subdominant in acute infection. Some early virus control is observed in most subjects but elite control (<50 cp/ml) is observed in <1% of individuals.

(B) One approach to HIV-1 vaccination is to redirect $CD8^+$ T cell immundominance to regions of HIV-1 where escape imparts a significant fitness cost. The aim is that following HIV-1 infection, preexisting vaccine-induced T cell responses would dominate over those induced by infection and $CD8^+$ T cell selection pressure maximized because virus escape would be constrained, resulting in greater virus control.

(C) Vaccination is unable to shift immunodominance in acute infection because of limited recognition of the infecting virus, poor population coverage because epitopes numbers are limited in the immunogen, lower T cell frequencies, and/or escape, so virus control will not be effective.

HIV-1 infection, expanding strongly as initial virus load (VL) declines (Figure 1, top). The level of virus load set point is established in the first 3–6 months of infection. This implies that the first HIV-1 $CD8^+$ T cell responses in elite controllers must contribute strongly to the lower VLs ultimately observed. The converse is that in the majority of HIV-1 infected individuals who have higher VLs, the first $CD8^+$ T cell responses induced after infection fail to exert sufficient VL control.

Vaccines may be able to correct the latter.

Why are some HIV-1-specific T cell responses more effective than others? $CD8^+$ T cells select escape mutants during acute and chronic HIV infection. These may be selected very rapidly or more slowly. The strongest antiviral pressure, resulting in rapid virus escape, is exerted by the T cells that are immunodominant, in the classical sense that they are the highest magnitude responses at that stage of infection (Liu et al., 2013). $CD8^+$ T cell immunodominance hierarchies shift as HIV-1 infection progresses largely because of escape mutations. Changing immunodominance patterns are unique in every individual, but people who share HLA alleles often target the same immunoprevalent epitopes though these may not be immunodominant. Another key factor in defining HIV-1 virus levels and the rate of virus escape is the replicative fitness of HIV-1 itself. Some T cell epitopes lie in regions of the HIV-1 proteome that are so functionally constrained, they endure $CD8^+$ T cell pressure for years without escape. Not surprisingly, these epitopes are conserved in sequence at the population level and are often immunoprevalent amongst individuals expressing protective HLA alleles (Liu et al., 2013). If such conserved epitopes could be identified in people who do not have protective HLA types, they could be targeted by vaccines.

So far, quantitative measures of HIV-1 fitness have only been possible in vitro and have focused on specific epitopes and individual amino acids. In this issue of *Immunity*, Ferguson et al. (2013) bring applied physics to the study of HIV-1 to quantitate the fitness of HIV-1 in silico. Their aim was to define the fitness through examining whole HIV-1 proteins, not just reactive epitopes, which is important because mutations outside reactive T cell epitopes also impact virus fitness and the ability of T cells to select escape mutations (Crawford et al., 2007). Their approach enables them to consider the effect of multiple, ongoing mutations across a protein with the view to more accurately quantifying the "fitness landscape" of the protein. Ferguson et al. (2013) developed an algorithm by using amassed sequence data for HIV-1, largely from the chronic stage of infection. They focused on Gag proteins because of the large numbers of sequences available and evidence that Gag is the target of the most protective T cell responses. They simultaneously examined the frequency of having the most common amino acid at each position of the protein versus any other residue. Fitness predictions were made from the relative frequency of these combinations in the sequence database across the proteome. Thus the most common sequence patterns with the lowest "energy" are the most fit. Despite the assumptions made, that mutation at any position in and around a T cell epitope has equal impact on T cell recognition and that a fitness cost is associated with all amino acids that are not consensus, they were able to validate their model by showing strong correlations between fitness landscape predictions and existing in vitro fitness data looking at the impact of specific escapes on virus replication in vitro. These findings may have an interesting application in studies of primary HIV-1 infection. No study has yet fully explained the correlation between VL decline in acute infection and the specificity, selective pressure, or functional properties of the $CD8^+$ T cells. The "quantitative landscape" approach should enable the intrinsic fitness of the founder virus, certainly a major contributor to virus load (Goepfert et al., 2008; Mostowy et al., 2012), to be factored in. Broader application for this approach may be to identify novel sites of fitness cost in other HIV-1 proteins and in variable viruses such as HCV where less in vitro fitness data are available.

The authors employed their method to design novel $CD8^+$ T cell immunogens for vaccination. Their rationale, as also proposed by others, is to induce immunodominant $CD8^+$ T cell responses to epitopes that are immunoprevalent and are slow to escape because of the fitness cost associated with mutation. Following HIV-1 infection, early T cell immunodominance would be shifted to these vaccine-induced T cell responses, which would then control VL prior to virus escape (Figure 1, middle). The authors examined many iterations of vaccine immunogens proposing a 113aa immunogen containing 12 T cell epitopes that best accommodated their three criteria, fitness cost on escape, maximum coverage of their target population set at 45% of Europeans with the most common HLA types, and minimization of the number of epitopes.

How does this epitope-based approach differ from, and possibly add to, other $CD8^+$ T cell immunogen designs? It is strongly distinctive from whole-protein immunogens. These approaches use whole virus proteins to maximize population level coverage and T cell breadth. The whole protein antigen is likely to be processed in the same manner as the natural HIV antigen in infected cells. They do not contain artificial linker sequences or junctional sequences that may produce non-HIV-1 epitopes and possibly distort immunodominance following vaccination. However, compared with more focused vaccine designs, any single whole protein immunogens is a far from perfect match for infecting virus and, as in natural infection, is unlikely to induce early immundominant T cell responses against fitness constrained epitopes. However, the whole-protein approach has been improved by the design of mosaic immunogens, which include two or more alternative sequences at variable sites. These have been shown in macaques to maximize the breadth (number epitopes targeted) and depth (cross reactivity to epitope variants) of the T cell responses induced by vaccination to afford better coverage of highly diverse incoming viruses (Barouch et al., 2010). Although increased breadth of T cell response does not guarantee that the immunodominant T cell responses see the most fitness constrained epitopes, if mosaic vaccines could induce T cell responses that eradicated infected cells very early following HIV-1 infection, as has been described in 50% of vaccinated monkeys by Hansen et al. (2011), this might not matter.

The approach of Ferguson et al. also differs from other immunogen designs that consist of conserved regions of HIV-1 proteome rather than specific T cell epitopes (Létourneau et al., 2007; Rolland et al., 2007). Those approaches aim to limit vaccine induced T cell responses to sites of HIV-1 that are most sequence constrained but do not limit coverage to specific HLA haplotypes. Like Ferguson et al., they aim to ensure that early immunodominant $CD8^+$ T cell responses are made to conserved epitopes (Figure 1, middle), in contrast to natural infection where these are usually

delayed and subdominant (Figure 1, top). A related immunogen design considers immunoprevalent epitopes associated with lower VL setpoints (Mothe et al., 2011). Here, VL setpoint could be considered the cumulative output of $CD8^+$ T cell mediated effects on virus fitness. These epitopes often occur in the more conserved regions of HIV-1, such as Gag proteins so that virus escape from these immunogens would also be associated with fitness costs. Given the common focus of these approaches on conserved regions of HIV-1, it is not surprising that several epitopes identified in the Ferguson et al., epitope string have also been identified in these other immunogen designs. However, some new, including more variable, epitopes were defined. In vitro testing of the fitness impact of mutations in these sites would be further validation of the Ferguson et al. approach and would also inform whether these epitopes should be included in other designs. Overall, the strength of conserved immunogen designs would be to shift immunodominace to key epitopes that prevent escape, arguably allowing a wider time window following natural infection for vaccination induced T cells to control VL. The weakness is that T cell breadth may be limited or skewed to non-HIV-1 epitopes and therefore result in poor population-level immunogenicity.

Ultimately, all HIV-1 vaccine designs will work best when the founder virus is very close to the consensus sequence, but we know that does not always happen. Indeed, even conserved epitopes in founder viruses often contain both escape and compensating mutations, generated in the patient's sexual partner (who has a different HLA type) (Goepfert et al., 2008), which may take months or years to revert. Moreover, it is not at all clear whether shifting T cell immunodominance though vaccination can be successfully achieved by a nonpersisting vaccine that sets up T cell memory several months to years previously. In the worst-case scenario, the immunogenic stimulus of an incoming infection may override these responses with little beneficial effect (Figure 1, bottom). The best case would be that the most favorable T cell responses become the first T cell responses after infection (Figure 1, middle), which occurs only rarely following natural infection (Figure 1, top) even in those with protective HLA types. Success of T cell vaccines against HIV-1 therefore depends not only on immunogen design but also on whether the vaccine vectors employed can induce and sustain sufficient frequencies and qualities of $CD8^+$ T cells to enable early virus control. The advent of applied physics to help address these difficult challenges is welcome.

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