Viral Protein Fragmentation May Broaden T-Cell Responses to HIV Vaccines

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Classical vaccination approaches that rely on killed, attenuated or subunit preparations have failed in the case of HIV-AIDS. For numerous vaccines that have proven effective against other viral diseases, the primary correlate of protection is the generation of broadly neutralizing antibodies (BnAbs). Many researchers believe that if a highly effective HIV vaccine is ever achieved, it too will be one that generates long lasting humoral immunity in the form of BnAbs (Makedonas and Betts, 2011). Unfortunately, HIV poses many difficulties for this goal, including the masking of HIV envelope protein epitopes by glycans, the conformational flexibility of the HIV envelope, the inaccessibility of many conserved epitopes, the diversion of B-cell responses by non-neutralizing epitopes, and in some cases, the mimicry of host antigens by the HIV epitopes that happen to be both conserved and accessible (Haynes *et al.*, 2012).

In addition to humoral immunity, however, it is widely recognized that a cellular immune response involving cytotoxic $CD8^+$ T-cells (CTL; see panel a of the accompanying figure) also plays a critical role in controlling HIV replication and disease progression (Makedonas and Betts, 2011). For instance, strong, polyfunctional CTL responses (*i.e.*, those involving robust $CD8^+$T-cells that produce more than one cytokine) characterize the so-called 'elite controllers', the tiny fraction of HIV-infected individuals who maintain undetectable viral loads indefinitely and remain disease-free without antiretroviral therapy (Ferrando-Martínez *et al.*, 2012). Consequently, T-cell-based vaccine strategies, as well as combination strategies designed to induce both humoral and T-cell responses, are receiving serious consideration in HIV vaccine research. In a recent study led by Drs. Adel Benlahrech and Steven Patterson of the Imperial College School of Medicine in London, researchers employed a model vaccine based on structural (Gag) proteins of the simian immunodeficiency virus (SIV) to investigate two approaches for potentially broadening the CTL response to vaccination. Among the co-authors of this study were Drs. Steven Self and Fuscheng Li, representing the Vaccine and Infectious Disease Division and the Public Health Sciences Division of the Fred Hutchinson Cancer Research Center.

CTL target and destroy virally-infected cells by recognizing virus-encoded peptides bound to major histocompatibility complex (MHC) class I molecules on the surface of nucleated cells. The peptides

that are presented in this way are primarily generated by proteasomal degradation of proteins expressed by the target cell. However, the CTL that come to dominate any immune response to viral infection recognize a very small proportion of all peptides encoded in a viral genome. This immunodominance by certain CTL is strongly influenced by (*i*) the outcome of proteolytic processing in antigen presenting cells (APC) such as dendritic cells (DC), (*ii*) variation among peptides in affinity for MHC class I molecules, (*iii*) competition in the endoplasmic reticulum among different peptides for binding to MHC class I molecules and (*iv*) additional competition among different CTL for access to cognate epitopes on the surface of DC and other APC. These factors are, therefore, key to the development of T-cell-based HIV vaccines, because a broad CTL response is needed to reduce the likelihood that HIV can escape cellular immunity via mutation.

To broaden the CTL response, Benlahrech *et al*. took two approaches. In the first, they fused the entire Gag protein to ubiquitin, a small regulatory protein which tags other proteins for proteasomal degradation. In their second approach, which focused on reducing both antigenic competition for binding to MHC class I molecules and antigenic competition on the surface of DC, the authors split the Gag protein into seven, 69-92 amino acid fragments and fused each one to ubiquitin. For reference, the authors also investigated full-length unmodified SIV-Gag. In all three cases, the corresponding genes (or mini-gene fragments) were cloned into a replication-defective adenovirus type 5 (Ad5) vaccine vector. The constructs were then tested using an *in vitro*human priming system (described in panel b of the figure). During the experiment, the researchers measured the breadth of the CTL response using the enzyme-linked immunosorbent spot (ELISPOT) assay, and they evaluated cytokine production and the memory phenotype of T-cells by intracellular cytokine staining and flow cytometry.

Benlahrech *et al*. found that, overall, most of the CTL responses were directed against the Ad5 vector. The authors confirmed that fusion of ubiquitin to full-length Gag achieved the desired proteasomal targeting, yet this construct resulted in a reduction in the number of epitopes recognized by CTL compared to full-length, unmodified Gag. In contrast, the authors found that two to six times as many epitopes were recognized in the ubiquitin-fused Gag fragments compared to ubiquitin-fused full-length Gag. Fragmentation and ubiquitination had no effect on T-cell memory differentiation and polyfunctionality. In this novel investigation of combined ubiquitination and protein fragmentation using a human experimental system rather than a mouse model, Benlahrech *et al.* provide encouraging evidence that protein fragmentation may be a worthwhile strategy for improving the breadth of T-cell-based HIV vaccines. However, because linking ubiquitin to full-length

Gag substantially reduced the breadth of the CTL response, follow-up work is now being conducted by the authors to test T-cell responses to Gag protein fragments that are not fused to ubiquitin.

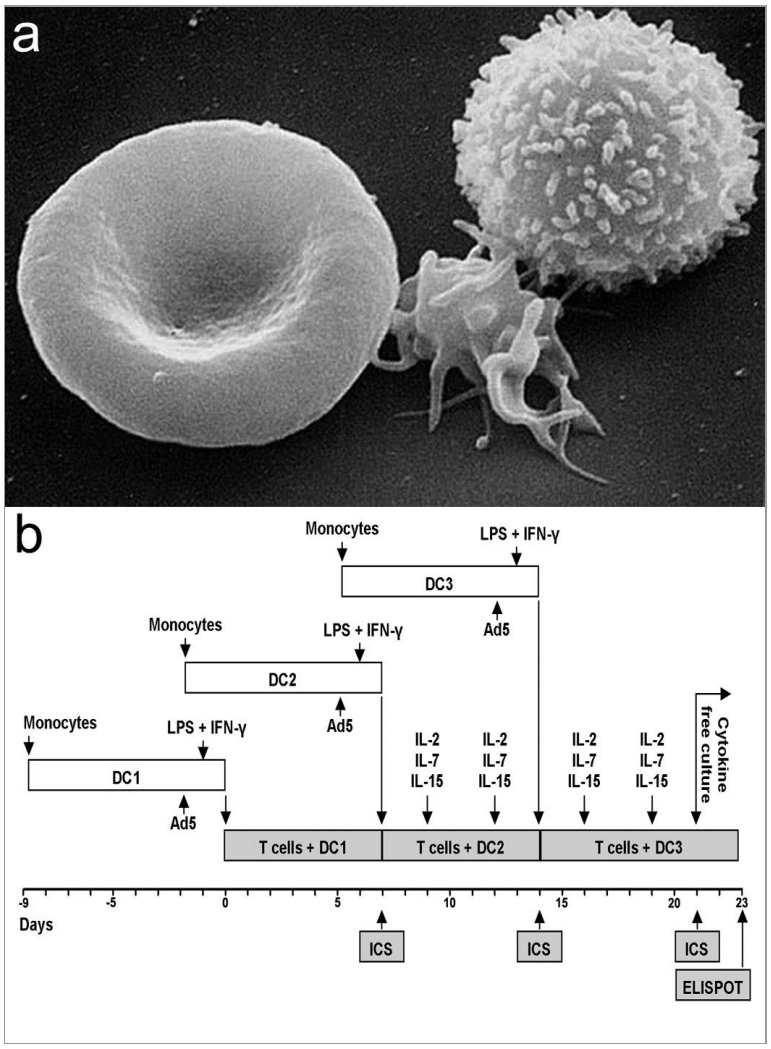
Based on the known effects of different MHC class I alleles on the immunodominance hierarchy with respect to HIV-1, Drs. Li and Self have designed an ingenious strategy in which all unfused Gag segments contain the same number of potential epitopes and are, therefore, allowed to vary in length. According to Dr. Patterson, preliminary results of this follow-up experiment look promising and may provide powerful confirmation that gene fragmentation improves the breadth of T-cell responses.

Benlahrech A, Meiser A, Herath S, Papagatsias T, Athanasopoulos T, Li F, Self S, Bachy V, Hervouet C, Logan K, Klavinskis L, Dickson G, Patterson S. 2012. Fragmentation of SIV-gag vaccine induces broader T cell responses. *PLoS ONE* 7:e48038.

Also see: Ferrando-Martínez S, Casazza JP, Leal M, Machmach K, Muñoz-Fernández MÁ, Viciana P, Koup RA, Ruiz-Mateos E. 2012.Differential gag-specific polyfunctional T cell maturation patterns in HIV-1 elite controllers. *Journal of Virology* 86:3667-3674.

Haynes BF, Kelsoe G, Harrison SC, Kepler TB. 2012. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nature Biotechnology* 5:423-433.

Makedonas G, Betts MR. 2011. Living in a house of cards: re-evaluating $CD8^+$ T-cell immune correlates against HIV.*Immunological Reviews* 239:109-124.



Panel a from the Electron Microscopy Facility at The National Cancer Institute (image downloaded from Wikimedia Commons); panel b modified from Figure S1 in the open-access publication of Benlahrech et al. (2012).

(a) Scanning electron micrograph of a T-cell (right), next to a platelet (center) and red blood cell (left). (b) In vitro protocol for the generation of SIV-specific memory T-cells. Dendritic cells (DC) were generated by culturing human monocytes with two cytokines, GM-CSF and IL-4, for 7 days. DC were then transduced with Ad5 vectors expressing SIV-Gag genes for 24 hours. Next, the DC were matured with bacterial lipopolysaccharide (LPS) and interferon-gamma (IFN-gamma) for an additional 24 hours. DC were subsequently co-cultured with autologous naive T-cells for 7 days, and they were boosted on a weekly basis with Ad5-transduced mature DC. Intracellular cytokine staining (ICS) was performed one day after each boost. ELISPOT assays were performed 23 days after initial T-cell priming, following a resting period of at least 48 hours in cytokine-free medium.