The 2019 Optimal HIV CTL epitopes update: Growing diversity in epitope length and HLA restriction

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Over the 20 years since it's first edition, the Los Alamos National Laboratory HIV Immunology database's "Optimal CTL epitope list" has grown from a mere 60 epitopes in 1995 to a collection of close to 350 epitopes for which specific HLA class I restriction and optimal peptide length have been experimentally defined. The collective information, gathered from many laboratories worldwide and oftentimes with generous contributions of unpublished data, has allowed the field to better characterize the T-cell response to HIV infection, to gain important insights into mechanisms of viral evolution and viral adaptation to the host immune surveillance and to critically support different HIV vaccine designs (1-4). It has also helped to refine HLA binding-motifs and facilitated structural and molecular analyses of T cell receptors involved in pathogen-specific immune responses. Over the years, we have tried to adjust inclusion criteria for this listing to the standards and technical advances made in the field, in order to maximize coverage of described epitopes while not negatively impacting the accuracy of information on their HLA restriction and definition of optimal epitope length. However, the extensive degree of HLA binding promiscuity, the potential role of T cells with non-classical HLA restriction and data emerging from epitope elution analyses continuously challenge our guidelines for inclusion. Thus, every update of the "Epitope A-list" has seen more or less extensive modifications, based on newly adopted criteria, new data published, and removal of previously included epitope sequences that were shown to be inaccurate.

The 2019 update adheres to the inclusion criteria which require i) experimental demonstration of HLA class I restriction (through specific presentation on antigen presenting cells expressing the defined HLA allele(s) or by stable tetramer formation and staining in flow cytometry assays) and ii) the definition of the shortest, most potent epitope sequence, ideally defined by using end-point dilutions of serial single-amino acid truncations. Aside from adding almost 40 new epitopes in this update, we have also reviewed the HLA information for epitopes that only had 2-digit HLA class I allele information. In several cases, we have thus been able to identify the actual presenting 4-digit class I allele(s) from the published literature, leading to the inclusion of some epitopes with assigned restrictions to different subtypes of the same 2-digit allele. This has been the case in the past, for alleles such as HLA-A*02, -A*26, -B*27 and –B*44, where different subtypes present the same epitope, and has been extended now to alleles such as HLA-B*14 and others, where detailed studies, additional HLA typing or re-analyses of existing data have shown presentation by one or more of the different 4-digit subtypes (5,6). The distinction of restricting HLA subtypes is especially important for those alleles where the binding preferences vary between subtypes (most prominently the HLA-B*15 and A68 loci) and/or have been linked to distinctively different HIV disease outcomes (such as HLA-B*35 and B*58). A few, mainly older epitopes, for which allele subtype information was not available remain listed with only a 2-digit HLA restriction, which does not imply that they are presented across different subtypes of the given HLA allele.

Also of relevance for an accurate reflection of targeted T-cell epitopes in HIV and for the definition of optimal epitopes are the emerging data on epitope length variants. While many elution studies have assessed peptides from regular antigen presenting cells expressing all three classical HLA-A, -B and –C loci, some laboratories have developed systems where only a single allele is expressed and the peptide-loaded class I molecules are being secreted in the culture supernatant (7). This ensures that a) only epitopes from a single allele are being studied and b) epitope processing intermediates, which are generally captured in whole-cell approaches, are unlikely to confound the analyses (8,9). In one of these studies, epitopes from HLA- A*1101-secreting, HIV infected and uninfected cells

were eluted and analyzed (9). While the majority of epitopes eluted were in the expected range of known HLA-A*11 restricted epitopes (9-11mers), there was a substantial number of ligands that were between 12 and 15 amino acids in length, with some eluted sequences being even longer. Importantly, this was the case for both, host protein ligands and HIV-derived peptides, suggesting that HIV infection did not grossly change the processing machinery in regards to the length of peptides produced. In addition, about 90% of the eluted peptides had a C-terminal anchor consisting of Lysine or Arginine, again in line with published motif data for HLA-A*1101 and not significantly different between ligands originating from self or from viral proteins. There was also remarkable consistency in the anchor position at the B-pocket, suggesting that some of the longer epitopes may be presented as bulged ligands (10). Regardless of the final structural characteristics of the HLA-peptide complex though, it is quite likely that T-cell receptors (TCR) would be available that can recognize structures with very long peptides. Indeed, further immunogenicity analyses in the HLA-A*1101 study above indicated broad in-vitro reactivity of these longer peptides (9). Still, it remains largely unclear whether HIV infection induced distinct T-cell populations with separate specificity for each different epitope length variant or whether the observed responses are due to cross-reactive T-cells recognizing different length variants or embedded epitopes common to the reactive peptides. In the absence of detailed TCR or cross-reactivity analyses, the experimental definition of the “optimal” HLA-A*1101 epitope(s) may be considerably complicated by potential heterogeneous T cells population each recognizing a different epitope length-variant. The use of T cell clones could overcome this issue, but it may prove very labor intensive to isolate and expand clones to different length variants. In addition, the definition of “optimal” epitope length may need to be seen in the context of how responses to the different length variants contribute to virus control in vivo. Assessing antigen processing efficacies and binding affinities to the presenting HLA molecules and understanding viral evolution pathways in response to immune pressure exerted on these different length variants may provide a more holistic picture of what the “optimal” length of the presented epitope may be.

For the present listing of epitopes, we have attempted to analyze available data in cases where we identified conflicting reports on epitope length and have used access to overlapping peptide screens and HLA footprint data to best support the final inclusion of a specific epitope. In some cases, sufficient evidence was available to unequivocally call two, partly overlapping or embedded epitopes as distinct entities, each inducing in natural infection its own specific T-cell clonotypes. However, there are generally insufficient data available to discern cross-reactivity from truly separate responses towards partly overlapping or embedded epitopes. Yet, it may be interesting and necessary to identify and clarify these situations since a precise epitope landscape is essential for proper definition of HLA motifs and correct interpretation of HIV CTL escape data. The latter has been subject of a publication (11), that has reported multipronged targeting of the same epitope region in the context of one single HLA class I allele. Without detailed knowledge of the precise epitope boundaries, the interpretation of subsequent CTL escape and HLA footprint analyses will be flawed. But the issue goes evidently well beyond just describing epitope sequences for a listing as the present one since the targeting of the same region of the viral protein(s) with partly overlapping CTL specificities may complicate viral immune evasion considerably and mediate superior viral control in vivo. Just how extensive this issue is, is highlighted by our own analyses of potential "shifted" epitopes around defined optimal epitopes: by screening just 5 amino acids up- and downstream of each described optimal epitope for suitable anchor positions satisfying the allele-specific binding motif and allowing for an epitope length of 9 to 12 amino acids, the number of potential independent epitopes that could bind the given HLA class I molecule and induce T cell responses to the same region increased by more than 120 % (Silva-Arrieta et al, unpublished). Of course, responses to such shifted epitopes will be measured when using longer test peptides; but further, detailed epitope mapping would be required to discriminate responses to the different epitope length variants overlapping the known optimal epitope.

The presence of shifted epitopes also has potential implications on HIV vaccine design. If it turns out that targeting the same region of the viral proteome by T cells with specificities for partly overlapping or embedded epitopes is critical to avoid viral immune escape, then it

would likely be advantageous to induce such responses by using longer epitope sequences rather than just sequences truncated down to the most potent, shortest epitopes, i.e. optimal epitope. Hence, vaccine-immunogen sequences based on optimal epitope strings may not perform as well as desired since the virus could more readily evade this single T cell specificity. The notion that overlapping epitope responses may drive viral evolution in vivo is also supported by the identification of HLA footprints in flanking sequences of optimally defined epitopes. These have only occasionally been attributed to the occurrence of effective antigen processing escape mutations (12,13) and a renewed look at defined HLA footprints close to described optimal epitopes may help to elucidate the impact of multiple T cell specificities on the same epitope region.

In light of recent reports that suggest a potential role for virus-specific T cells with non-classical HLA restriction, we have also considered the inclusion of T cell epitopes presented by HLA-E alleles and other non-classical class I molecules as well as epitopes presented by HLA class II molecules but targeted by CD8+ T cells (14-16). We feel that the experimental data on these responses are too preliminary at this time and the relevance to HIV infection too uncertain to warrant wide inclusion in the A-list. We will however keep on monitoring the literature and include epitopes with non-classical restriction as they become fully defined. Another source of "non-classical epitopes" may be those encoded in alternative and antisense reading frames of HIV. The existence of immunogenic epitopes in both settings has repetitively been demonstrated but the epitopes have rarely been defined to the optimal epitope level (17-21). Although their antiviral effects need to be still determined, frameshifted epitopes may still be expressed, processed and presented in cells that do not contain replication competent virus (as are many other "conventional" epitopes) and contributing to the shaping of the virus-specific T cell response and, in turn, the proviral landscape (22). Finally, other non-classical epitopes may include those that have been described as HLA-class I binders important for NK cell interaction but which may not necessarily induce a T cell response (23,24). While they could be critical for NK activity and NK-mediated elimination of infected cells, they likely escape current screening approaches and one would need to define additional experimental criteria to include them in the A-list.

As always, we are grateful to the numerous laboratories that continue to provide additional information and unpublished data to compile the current update and apologize if we should have overlooked some epitope sequences that merit inclusion. We appreciate any further additional information on new epitopes until the next update, especially also if investigators wish to contribute data on epitope length that may conflict with current entries in this 2019 update.

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