Increasing evidence of the role that CD8+ T cells have in clearing viruses has lead to mounting interest in designing preventive and therapeutic vaccines capable of eliciting or enhancing virus-specific CD8+ T-cell responses. It is hoped that such vaccines will prove useful for both persistent and acute virus infections that are poorly controlled by standard vaccine approaches.

Antiviral CD8+ T cells recognize virus-encoded peptides that bind to major histocompatibility complex (MCH) class I molecules. These viral peptides, generally 8 to 10 amino-acids in length, are predominantly generated from viral gene products through the initial action of proteasomes followed by trimming via aminopeptidases. Proteasomes act through 2 mechanisms: either on antigens synthesized by the antigen presenting cell (APC) through direct presentation, or on antigens acquired by the APC through cross presentation.

Viruses encode between a few thousands and tens of thousands of amino acids and equivalent numbers of potentially immunogenic peptides. Although hundreds of peptides have appropriate sequence to bind MCH1, the bulk of responding CD8+ T cells recognize a tiny fraction of potential epitopes. This phenomenon is termed immunodominance. Figure 1 depicts the processes and filters that contribute to immunodominant responses to vaccinia virus in mice.

Some viral epitopes are dominant, stimulating high frequency T-cell responses, while others are subdominant, stimulating weaker or barely detectable T-cell responses. Antiviral responses to immunodominant and subdominant epitopes form a distinct hierarchy of epitope-specific responses in a naïve host. This hierarchy is remarkably reproducible between individuals.

This immunodominance hierarchy is regulated by various parameters, including the efficiency of antigen (peptide) processing and presentation, the affinity between peptide and the MCH class I, the availability of T cells that recognize the peptide MCH complex, and the competition between T cells on the antigen-presenting cell.

The antigen memory T-cell populations that are important in protection against subsequent infection (and which can be primed by vaccination) have diverse T-cell receptors (TCR) repertoires, but are also intrinsically cross-reactive. The TCR of a CD8+ T cell will discriminate those peptides of 8 to 10 amino acids that are embedded in MCH class I molecules. Crystal structural
studies also documented that only a few TCR contact residues on the peptide presented by MCH molecules are required for activation.

Cross-reactivity of those repertoires may be valuable to the host, considering the large number of potential pathogenic antigens to which one is exposed over a lifetime. Cross-reactivity can compensate for situations with limited TCR repertoires and still allow a normal immune response. Reports of pathogen-specific memory CD8+ T cells recognizing cross-reactive epitopes on different proteins of the same pathogen or proteins from closely related or totally unrelated pathogens have been established.

Immunodominance has been mostly observed in antiviral and antibacterial immune responses, both in mouse models and human diseases. Chen and McCluskey developed a schema illustrating possible mechanisms involved in immunodominance (Figure 2).

**Role of CD8+ T cells during primary HIV infection**

The key role that HIV-1-specific CD8+ T cells have during acute HIV infection has been highlighted by the temporal association between the first emergence of CD8+ cells, the decline of HIV viremia from high levels to the viral set point, the resolution of clinical symptoms of the acute retroviral syndrome and the rapid selection of viral CTL escape variants. In most cases these CD8+ T cells are narrowly directed against a small number of antigenic regions (epitopes) which are typically structured in a clear hierarchical order. Depending on their relative contribution to the overall magnitude of HIV-1-specific CD8+ T-cell responses in a given individual, these HIV-1-specific CD8+ T-cell responses can be classified as dominant, co-dominant or subdominant. This hierarchy of HIV-1-specific CD8+ T cells in acute infection appears to be crucial for the effectiveness of the immune response because specific immunodominant CD8+ T-cell responses have been identified during acute infection and these have been linked to a low ensuing set point viremia. Moreover, rising HIV-1 replication in chronic progressive infection is usually associated with the disintegration of the original pattern of CD8+ T-cell immunodominance. To design vaccines that elicit effective HIV-1-specific CD8+ T-cell responses, a thorough understanding of the factors that determine immunodominance patterns in acute infection therefore appears important.

Of the different components that can impact or alter the selection of immunodominant epitopes in viral infection, 3 major factors have been analyzed most intensely over recent years: the kinetics of viral protein expression, the genetic HLA class I background and the autologous sequence of the inoculated HIV strain.

**Kinetics of viral protein expression**

The kinetics by which viral proteins are expressed, processed and presented by HLA alleles on an infected
cell have recently been identified as a crucial factor in the evolution of immunodominant immune responses in animal models (lymphocytic choriomeningitis virus (LCMV)-infected mice). This may also be important for understanding the hierarchy by which HIV-1-specific T-cell responses evolve during acute HIV-1 infection. Several studies have shown that the expression of viral gene products during the viral life cycle following primary HIV-1 infection occurs with different kinetics and in a distinct chronological order. The early expressed HIV-1 gene products include the regulatory proteins Rev and Tat as well as the accessory protein Nef that can be readily transported from the nucleus to the cytoplasm. By contrast, the structural HIV-1 proteins Pol, Env and Gag, as well as the accessory HIV-1 proteins Vpr, Vpu and Vif, depend on a Rev-mediated nucleocytoplasmatic transport for protein translation into the cytoplasm to occur. These differential patterns of HIV-1 gene expression result in different kinetics of HIV-1 protein biosynthesis and might have important consequences for the temporal evolution of HIV-1-specific T-cell responses in primary HIV-1 infection.

Based on these observations, it has been hypothesized that the early expression of Nef, Rev and Tat during the viral replication cycle results in an accelerated presentation of viral epitopes within these proteins to the immune system and thus might lead to a preferential targeting of these proteins by CD8+ T cells during acute HIV-1 infection. A prominent role for the early expressed proteins Tat and Nef as targets for CD8+ T cells in acute HIV infection in vivo was initially indicated during primary Simian Immunodeficiency Virus (SIV) infection in rhesus macaques. Interestingly, two studies (Allen et al., and O’Connor et al.) demonstrated that Nef and Tat not only elicit a significant proportion of the total magnitude of SIV-specific CD8+ T cells during primary infection but that T-cell epitopes within these early expressed SIV proteins are also the first ones to exhibit CD8+ T cell-induced sequence variations. By contrast, Gag escape mutations are only observed during chronic SIV infection.

An immunodominant role for the early expressed HIV-1 proteins was also described in other recent studies, indicating that HIV-1 Nef elicits the vast majority of CD8+ T-cell responses during primary HIV-1 infection in humans. In contrast to Nef, the overall contribution of the early-expressed HIV-1 gene products Rev and Tat to the total HIV-1-specific CD8+ T-cell response during acute HIV-1 infection was relatively small in studies screening for CD8+ T-cell responses with overlapping peptides derived from HIV-1 clade-specific consensus sequences or laboratory strains of HIV-1.

In summary, the kinetics of viral epitope expression represent only one of the factors determining the selection of immunodominant CD8+ T-cell epitopes in acute HIV-1 infection, and several additional steps during subsequent antigen processing and presentation are likely to also impact immunodominance patterns, although their role in acute HIV-1 infection has not yet been established.

Impact of the genetics: host HLA class I background and selection of immunodominant CD8+ T-cell epitopes

The binding affinity of antigenic peptides to HLA class I molecules is a principal determinant of CD8+ T-cell epitope immunogenicity. HLA class I molecules therefore have a crucial role in selecting the epitopes that can become a dominant target for CD8+ T cells. A direct connection between the genetic HLA class I background and the hierarchy of HIV-1-specific CD8+ T-cell responses in acute HIV-1 infection has been established for a variety of HLA class I alleles (Table 1). Several studies have shown a consistent pattern of immunodominant HIV-1-specific CD8+ T-cell responses in individuals expressing certain HLA class I alleles.

Table 1. Immunodominance patterns in acute infection using optimal CD8+ T-cell epitopes identified during chronic infection. (Lichterfeld et al., 2005)

<table>
<thead>
<tr>
<th>HLA class I type</th>
<th>Epitope</th>
<th>Protein</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>RK9</td>
<td>P24</td>
<td>RLRPGKKK</td>
</tr>
<tr>
<td></td>
<td>KK9</td>
<td>P24</td>
<td>KIRLRPGGK</td>
</tr>
<tr>
<td>A11</td>
<td>QK10</td>
<td>Nef</td>
<td>QVPLRPMTYK</td>
</tr>
<tr>
<td>B7</td>
<td>IL9</td>
<td>gp140</td>
<td>IPRRIRQGL</td>
</tr>
<tr>
<td>B8</td>
<td>FL8</td>
<td>Nef</td>
<td>FLKEKGGGL</td>
</tr>
<tr>
<td>B14</td>
<td>EL9</td>
<td>gp41</td>
<td>ERYLKDQQL</td>
</tr>
<tr>
<td></td>
<td>DA9</td>
<td>P24</td>
<td>DRFYKTLRA</td>
</tr>
<tr>
<td>B27</td>
<td>KK10</td>
<td>p24</td>
<td>KRWILGLNK</td>
</tr>
<tr>
<td>B4001</td>
<td>KL9</td>
<td>Nef</td>
<td>KEKGGLEG</td>
</tr>
<tr>
<td>B51</td>
<td>EL9</td>
<td>Vpr</td>
<td>EAVRHFFPI</td>
</tr>
<tr>
<td>B57/B5801</td>
<td>TW10</td>
<td>P24</td>
<td>TSTLQEIQGW</td>
</tr>
<tr>
<td>Cw12</td>
<td>CC8</td>
<td>Tat</td>
<td>CCFHCQVC</td>
</tr>
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</table>

For example, the 2 HLA-restricted gag epitopes (RK9 and KK9) represent the immunodominant (60% of cases) HLA restricted epitopes during acute HIV infection in individuals expressing HLA-A3. This indicates that in the presence of a given HLA class I allele, single epitopes can be identified that frequently represent the
immunodominant target for HIV-1-specific T cells during acute infection. In general, persons with a given HLA type react to HIV epitopes in a predictable way. However, the factors that enable CD8+ T-cell responses directed against these epitopes to dominate over other epitope-specific responses restricted by the same allele are unclear. Conversely, not all individuals expressing a certain allele target the immunodominant epitope restricted by the respective allele and this has been documented in studies of HIV infection among HLA-identical siblings/twins.

Support for qualitative differences in CTL responses derives from studies of HLA associations with rate of progression in HIV infection. HLA class I molecules such as HLA-B27, HLA-B57 and HLA-B51 have been linked with slow progression, while HLA B8 and HLA B35 have been associated with rapid progression of HIV disease. The different ability of individual HLA alleles to restrict CD8+ T-cell epitopes in acute infection might represent one of the mechanisms accounting for the different speed of disease progression associated with distinct HLA type I classes.

Particular MHC molecules may be associated with slow progression, because, by chance the immunodominant epitopes are relatively invariant. As a result, switching the immunodominance response away from a variable region of the virus towards a highly conserved region has been the focus for vaccine development.

Are immunodominant patterns affected by viral sequence variations in HIV-1?

It is well known that HIV-1 is characterized by a tremendous genetic heterogeneity of circulating strains. Recent data indicate that this is mainly driven by the adaptive immune response, in particular HIV-1-specific CD8+ T cells. An important question is, can viral escape mutants resulting from cell-mediated pressure in an infected individual be transmitted to another host and thus alter the priming of immunodominant CD8+ T-cell responses during acute infection in the newly infected individual? Studies of vertical transmission from HLA-B27 mothers showed that T-cell escape mutation in the immunodominant epitopes of the mothers’ virus (B27-KK10) was stably transmitted into the children. The children were not able to develop immunodominant responses to the same epitopes and thus failed to reach the same level of viral control that is typically observed in carriers of HLA-B27 that target this epitope. Instead, the children generated alternative B27-restricted CD8+ T-cell responses, which remained subdominant. Similar observations were made during primary infection in HLA-A3 subjects who can develop an immunodominant response against two HLA-A3 restricted p17 epitopes (RK9 and KK9). When HLA-A3 subjects were infected with viral strains containing a single amino-acid mutation located within those epitopes, none of these mutant epitopes is targeted by CD8+ cells during primary infection.

In summary, these studies demonstrate that CTL escape mutations selected in one individual can be transmitted and significantly alter the immunodominance of HIV-1-specific CD8+ T-cell responses during acute infection in the new host.

Conclusion

Experimental progress made over recent years has enabled the identification of immunodominant CD8+ T-cell epitopes targeted during acute HIV-1 infection in the context of various HLA haplotypes. In addition, exceptions from these patterns of immunodominance are in many cases linked to the presence of viral sequence variations within otherwise immunodominant epitopes in the infecting virus, thus emphasizing the interplay between the sequence of the autologous virus and the genetic background of the host in determining immunodominance.

A better understanding of this interplay between virus and host genetics will be crucial if rationally designed HIV-1 vaccines and immunotherapeutic interventions are sought. The analysis of the viral resistance pattern developing under sub-optimal antiretroviral therapy has led to the introduction of more rational and efficient combination-therapies that are now highly successful in suppressing viral replication. A more detailed understanding of the viral and host factors that determine the initial immune responses against HIV-1 in a newly infected host might enable one to predict the fine-specificity of the HIV-1-specific CD8+ T-cell response in a given individual, based on the knowledge of the genetic characteristics of both the virus and the host. This will facilitate efforts to use targeted immunotherapy to manipulate the virus-specific T-cell response with the aim of enhancing the ability of the immune system to control viral replication.

To reach this aim, many important questions will have to be answered. Why do certain individuals fail to mount an immune response against otherwise immunodominant epitopes, despite the presence of this epitope in the
autologous viral sequence? Why are certain HLA alleles associated with dominant immune responses during acute infection, whereas others, such as the frequently expressed HLA-A2 allele, are not? What precisely accounts for the apparent discrepancies between different CD8+ T-cell responses in containing viral replication during acute infection? Are some immunodominant responses responsible for the initial control of viral replication, whereas others actively prevent the evolution of broadly directed, more efficient immune responses? Should HIV-1-specific vaccines be designed to only elicit immunodominant immune responses or is a specific pattern of immunodominant and subdominant responses necessary to successfully contain viral replication, as suggested in some studies? Finally, recent data suggest that current immunodominance patterns of HIV-1-specific T-cell responses might be a result of viral evolution, in which preferential selection of epitopes inducing dominant but functionally inefficient CD8+ T-cell responses were made. Therefore, it currently appears that HIV-1 vaccines should not simply aim at recapitulating the immunodominance pattern observed in natural infection, which apparently fails to prevent disease progression in more than 97% of infected individuals. Instead, future vaccine strategies should try to induce alternative clusters of immunodominant and subdominant immune responses that might be more successful in containing viral replication.

—by Gaston Djomand

References Cited


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Network Evaluation

The HIV Vaccine Trials Network (HVTN) Network Evaluation Committee (NEC) annually evaluates the HVTN components of HVTN Core, the Statistical Center for HIV/AIDS Research and Prevention (SCHARP), and the Laboratory Program to assess program effectiveness, help improve program performance, and inform decision making.

The evaluation is designed to help the Network understand how effectively it is performing the tasks and activities along the path to finding an HIV vaccine. The intention is to provide the HVTN with feedback to identify strengths as well as areas for improvement that will help inform programmatic decisions. This article includes data from trials managed by the HVTN and SCHARP and does not include data from trials managed by Merck.

2007 evaluation outcomes

- During 2007, the NEC conducted a comprehensive evaluation of HVTN Core, SCHARP, and the Laboratory Program. The NEC framed the evaluation in terms of the life of a protocol from its initial concept to publication of final results. The NEC evaluated protocol development, protocol implementation, enrollment and demographics, protocol conduct, data and laboratory analysis and publication. Table 1 shows the results of this evaluation. Most of the findings indicate excellent Network performance. Of concern is the amount of enrollment time spent on planned holds (44%), which prompted the NEC to recommend a review of the planned hold schemes included in HVTN protocols. Also, although the discontinuation of vaccination rate is 8% (Table 1), 64% of protocols had discontinuation rates >5% and the NEC recommended that the Network consider the implications of this loss of data to HVTN trials.

- The NEC also evaluated the performance of the 28 Clinical Research Sites (CRSs) that conducted trials between January 1, 2007, and December 31, 2007. The areas evaluated for each site were enrollment, enrollment within a defined period, demographics, visit completion, data management quality, specimen quality, protocol adherence, and protocol implementation (Figures 1-3, pages 7 & 8). Figure 1 shows the trends of enrollment in HIV vaccine trials from 2001 to 2007. Figure 1 indicates that there was a boost in enrollment in 2006 due to the STEP trial (2247 enrolled) followed by a decrease in 2007 (1216 enrolled).

<table>
<thead>
<tr>
<th>Table 1. Summary of 2007 Network performance</th>
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<tr>
<td>Indicator</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Total participants enrolled (2007)</td>
</tr>
<tr>
<td>Percent of women enrolled in HVTN studies</td>
</tr>
<tr>
<td>Percent of non-White participants enrolled at all HVTN sites</td>
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<tr>
<td>Median protocol development time</td>
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<tr>
<td>Percent of total enrollment time spent on planned holds</td>
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<tr>
<td>Percent of sites with all protocols activated with target (standards: 90 days for existing investigational new drug [IND] studies; 120 days for new IND studies) — US sites only</td>
</tr>
<tr>
<td>Number of protocol manuscripts and/or abstracts published</td>
</tr>
<tr>
<td>Percent of total enrolled who did not complete vaccination series</td>
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<tr>
<td>Site development time</td>
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<tr>
<td>Total visits (excluding HVTN 050 and 502)</td>
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<tr>
<td>Mean percent of visits completed across sites</td>
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<tr>
<td>Mean percent of specimens with ≥1m/mL (cell yield)</td>
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<tr>
<td>Mean percent of specimens frozen within eight hours or less</td>
</tr>
<tr>
<td>Mean quality control (QC) rate per 100 pages of CRFs (Standard is ≤10)</td>
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<tr>
<td>Mean percent CRFs faxed to SCHARP within four days</td>
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<tr>
<td>Mean percent QCs resolved within 21 days</td>
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continued on page 7…
Figures 2 and 3 indicate that the number of sites meeting NEC data quality standards, such as quality control queries (QCs) per 100 pages of case report forms (CRFs) and visit completion (standard is ≥ 90%), has dropped since 2006 and will be watched closely. Overall, however, the Network has improved over time in many key areas.

![Figure 1. Number of participants enrolled in HVTN vaccine trials, 2001–2007.](image1)

![Figure 2. Percent of sites meeting data quality performance standards, 2001–2007.](image2)
Due to the success of applying “lean thinking” Rapid Process Improvement (RPI) principles to protocol development and protocol implementation at HVTN Core, and to CRF development at SCHARP, the Evaluation Unit applied the same principles to improve specimen processing and dataset development timelines within the HVTN Laboratory Program. In September 2007, employees from the Laboratory Program, SCHARP, and HVTN Core participated in a weeklong RPI workshop to address these issues. The outcomes were as follows:

- Streamlined the process from data request to data distribution (see Figure 4, page 9)
- Reduced the number of steps in the process by 52%, reduced the number of inspections and reviews by 53%, reduced the number of handoffs by 56%, and reduced the number of queues by 43%
- Developed a specimen processing scheduling system
- Developed a more comprehensive data package
- Created timeline targets for completing specimen processing and data analysis once a set is completed: 12-16 weeks

Figure 3. Percent of sites meeting visit completion performance standards, 2001–2007.
Future directions

The NEC and the HVTN evaluation system, under the leadership of Lindsey Baden, Katy Turner and others, has been one of the great successes of the HVTN. Ellen MacLachlan, who had been working as a Clinical Trials Manager in the Scientific Operations Unit and who earned her Ph.D. degree in Public Health in 2007, assumed the role of Associate Director for Evaluation at HVTN in early January after Katy Turner announced her plans to step down from that position. Ellen has continued the evaluation work started by Katy, Lindsey, and the NEC and has also expanded evaluation into new areas. Ellen emphasizes building new relationships between the NEC, HVTN Core, and the sites in order to successfully undertake and complete collaborative evaluation projects.

To reach this goal, the Evaluation Unit will embark on the following initiatives during 2008:

- The Evaluation Unit is considering working toward per protocol evaluation, where each study developed by HVTN has an evaluation plan that includes NEC-derived standards for performance and where more frequent performance reporting to protocol teams and others is provided.
- The Evaluation Unit also plans to work with the NEC, the Community Education Unit and possibly other DAIDS-funded Networks to implement an evaluation of community education activities. One possible mechanism may be a web-based data collection tool.
- There will be an expansion of the Laboratory Program evaluation to include data from the Laboratory Information Management System (LIMS) and the site-affiliated labs. An example of information that will be collected is international lab data and specimen viability data.
- The Unit wants to work to document best practices at the HVTN sites in various activities, such as best practices in screening and enrollment, clinic management, community education, counseling, media relations and protocol conduct. These will be published on a quarterly basis to highlight innovative work going on in the Network and to increase communication and sharing between HVTN sites.

—by Ellen MacLachlan and Gaston Djomand

Figure 4. Lab specimen processing, analysis, and data distribution.
Upcoming Events

XVIIth International AIDS Conference
Mexico City, Mexico
August 3–8

AIDS Vaccine 2008
Cape Town, South Africa
October 13–16

ICASA
Dakar, Senegal
December 3–7

HVTN Conference
Seattle, WA
November 18–20

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Photos from May 2008 HVTN Conference
Washington, DC

Clockwise from upper left: 1. An array of HVTN Conference-goers show their work on the dance floor. Those caught in the front row are Gabriela Calazans (CER Sao Paulo), Carrie Schonwald (HVTN Community Education Projects Manager), and Jonathan Fuchs (San Francisco Co-PI). 2. The DJ better have the grooves, because Larry Corey’s got the moves. (Note HVTN Event Planner Jill Culver on the right, and don’t forget to thank her for her efforts at the next Conference!). 3. When Yeycy Donastorg (Santo Domingo PI) steps onto the floor, the party gets under way! 4. Ana Olazabal (HVTN Travel Coordinator) shows her grace even at the end of a long day of work. 5. Even an HVTN party isn’t complete without the Macarena...
Send suggestions, questions, and article submissions to:

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