Discovering protective CD8 T cell epitopes—no single immunologic property predicts it!
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Once a burgeoning field of study, over the past decade or so, T cell epitope discovery has lost some luster. The contributory factors per chance are the general notion that any newly discovered epitope will reveal very little about an immune response and that knowledge of epitopes are less critical for vaccine design. Despite these notions, the breadth and depth of T cell epitopes derived from clinically important microbial agents of human diseases largely remain ill defined. We review here a flurry of recent reports that have re-visited the field. These reports reveal that epitope discovery is an essential step toward rational vaccine design and critical for monitoring vaccination efficacy. The new findings also indicate that neither immunogenicity nor immunodominance predict protective immunity. Hence, an immunogenic epitope is but a peptide unless proven protective against disease.

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Introduction: prevention is better than cure
We all agree with the age-old adage ‘prevention is better than cure’. Vaccination has accomplished this for many infectious diseases, thereby significantly reducing morbidity and mortality. Yet several current scourges defy our best efforts at effective vaccine development. The poor success of the much anticipated vaccine trials against human immunodeficiency virus/acquired immunodeficiency disease syndrome (HIV/AIDS), tuberculosis (TB) and malaria causes pause to re-think strategies for knowledge-based vaccine design and vaccination. Even though many agree that an effective vaccine should target both humoral and cellular arms of the adaptive immune system, most effort is invested in vaccine-induced antibody-mediated protective immunity. Recent years have seen increased focus on developing T cell-targeted vaccines. T cell-targeted vaccine development poses two technical challenges: one pertains to difficulties associated with the discovery of critical T cell targets comprised of microbial epitopes that are efficiently and abundantly presented during a natural infection. Another challenge pertains to defining protective epitopes in humans, which in most cases can be learnt only by indirect approaches that include protection studies in surrogate animal models and/or establishing correlates of protection in humans. Knowledge of such protective epitopes will facilitate the design of novel vaccines and the generation of critical reagents to track the host T cell response to vaccines in real time.

Refinement of existing techniques and/or the development of newer methods enhance studies of biologic processes with increased sensitivity, specificity and reproducibility. Since the revelation that MHC restriction entailed intracellular processing of proteins to short peptides and their cell surface presentation by MHC molecules to T cells, numerous approaches have been developed to identify T cell epitopes [1]. Herein we briefly review a few of these approaches, starting with the characterization of naturally processed epitopes (Box 1) to the recent advances in proteogenomics approaches for the discovery of alloreactive and tumor-specific T cell epitopes.

Many ways to discover T cell epitopes
The different approaches to discover T cell epitopes have been reviewed recently [1] and, hence, not all are belabored here. The most popular of these is algorithm-based epitope prediction coupled with biochemical and immunologic validation. From the large collection of all known MHC-restricted peptides and epitopes deposited in the Immune Epitope Data Base (IEDB: [27]) and SYFPEITHI [28], epitope prediction algorithms have been developed. NetMHC-3.0 — an artificial neural network-based prediction algorithm — allows rapid identification of microbial T cell epitopes [29]. Epitope prediction is high-throughput and effective for microbes with small proteomes such as those of viruses the largest of which express ~250–300 open reading frames (ORFs). Experiments
Box 1 A walk down memory lane with Stan Nathenson et al.

The 1980s and 1990s were exciting times for students of antigen processing and presentation and T cell biology. By this time immunologists and geneticists had established that the antigen(s) coded by the Major histocompatibility complex (Mhc) controlled allogeneic skin and tumor graft rejection both in mice and men [27,33]. As well, the 1970s witnessed the first descriptions of MHC restriction [14,34] — a process that controlled host T and B cell responses to proteins, viruses, and bacteria. These two seemingly distinct immunologic recognition processes needed a biochemical definition. By the late 1970s and early 1980s, Nathenson and colleagues had devised ways to cleave MHC class I molecules from cell surfaces and adapted a radiochemical method which, coupled with Edman degradation, unveiled the first primary structure of his favorite MHC molecule — H2-K. Immediately thereafter, primary structures of several other MHC molecules were determined [85,90].

Having unraveled the primary structures of several mouse and human MHC class I and class II molecules, the stage was set to elucidate the biochemical basis of MHC restriction. Prior to this, from the works of Unanue and colleagues, it was known that the activities of T lymphocytes were intimately linked to their interactions with macrophages, whose purpose was to process antigens [9,89,107]. So also, it was known that nucleo-cytoplasmic proteins, notably the SV40 T antigen and influenza A nucleoprotein and derived peptides, or proteins deliberately delivered to the cytosol by fusion of non-replicative influenza A virus or by osmotic shock (e.g., ovalbumin) were targets of class I-restricted CD8 T cells [11,12,13,14,15,16]. The *in vitro* binding studies that followed [16,17] and the solution of the three-dimensional structure of an HLA class I molecule — HLA-A2 [16,17*], revealed that the MHC was a receptor for processed peptides with a single binding site. The question now became, what sorts of peptides do MHC molecules bind and display to T cells *in vivo*? This was a burning question for MHC and T cell enthusiasts in the mid to late 1980s and early 1990s.

The radiochemical approach — invented to determine the amino acid sequences of peptides and proteins that were available in limited quantities [77] — returned yet another time to unveil the biology of MHC molecules. The first three-dimensional structure of a HLA-A2:01 had revealed that the binding site was occupied by a conglomerate of ligands whose identities eluded Bjorkman, Strominger, Wiley and colleagues [19]. The general notion was that not a few or several but numerous peptides were bound in that A2:01 antigen-binding groove indicating that the isolation of associated ligands in sufficient quantities to permit amino acid sequence determination by Edman method would be challenging. Hence, Nathenson and Grada Van Bleek reasoned that if cells infected with a virus that shuts off host protein synthesis (a la vesicular stomatitis virus, VSV) were tagged with radiolabelled amino acids, the tag would get incorporated into newly synthesized viral proteins. The peptides processed from the radiolabelled viral proteins would then be available for binding to MHC class I molecules. Such peptides could then be isolated from the restricting class I molecule and subjected to Edman sequencing. Indeed, the skilled execution of this experiment revealed one of the first naturally processed peptide antigens isolated from an MHC molecule: the VSV N protein-derived RGYVYQGL [20]! Concurrently, Rammensee and colleagues deploying a completely different approach, had extracted specific influenza virus-derived peptides from whole infected cells and determined the identities of the two distinct peptides that were presented by H2-K and H2-D molecules [21,22,23,24]. All of these studies culminated in a molecular definition of MHC restriction.

These initial reports were shortly followed by direct sequencing of individual peptides eluted from MHC with the aid of mass spectrometry [25,26]. Advances in mass spectrometers and proteomics technologies and platforms have since paved the way to directly elucidate the amino acid sequences of antigenic peptides. The nature of naturally processed peptide antigens derived from numerous re-emerging and newly emerging pathogens — for example, Dengue, Marburg, Ebola, *Mycobacterium tuberculosis*, *Plasmodium vivax*, and *P. falciparum* — yet remains. This knowledge is a prerequisite to track protective immunity in experimental models and in vaccine trials.

Whilst algorithms rapidly predict T cell epitopes, it neither predicts whether such peptides are presented during a natural infection nor their immunogenicity unless empirically determined [30–32]. The development of several transgenic (tg) mice expressing major HLA class I alleles [33,34] provides a preclinical, small animal model to validate the immunologic properties of the putative epitopes [30,34]. Comparative analysis showed that there is some but not complete overlap between CD8 T cell epitopes recognized by immune HLA tg mouse and vaccinated volunteers, suggesting that with some limitations, such a model is suitable for studying HLA class I restricted immune epitopes [34,35].

Discovery of T cell epitopes from larger microbes such as *M. tuberculosis* and *Plasmodium* spp. by using prediction algorithms would be challenging because the expressed genome of these microbes can encode ~4000–6000 proteins. Sette and colleagues have found that the smallpox vaccine — that is, vaccinia virus (VACV), which encodes ~250 ORFs — yielded an unwieldy number of putative epitopes that are homologous to variola proteinome and are presented by the six major HLA class I supertypes (see Ref. [36]) using predictive IC50 algorithm. To narrow the focus, a cut-off of the top ten best binding peptides per VACV protein per supertype was set, thereby yielding ~6055 predicted epitopes. Of these, T cell-based validation unveiled 48 CD8 T cell epitopes recognized by VACV-immunized volunteers [37]. Modeling on this approach and scaling-up to account for the larger proteomes of mycobacterium and plasmodium in comparison to VACV, one would expect over one million putative CD8 T cell epitopes. In actuality however, epitopes presented by bacteria-infected or parasite-infected cells would be expected to be narrower as compared to those displayed upon viral infections ([38] and our unpublished observation). This is perhaps because viruses translate their ORFs and some their ARFs (alternate reading frames) on host ribosomes. DRiPs (defective ribosomal products) generated from the translation of ORFs and ARFs are a substantial source of antigenic peptides [39,40]. In contrast, bacteria and parasites translate their genomes on their own ribosomes, wherein DRiPs may be
lost to rapid degradation and, hence, are unavailable for presentation. This may explain why an earlier study reported only three overlapping *M. tuberculosis*-derived naturally processed epitopes [38]. Functional validation of such a large number of predicted epitopes would be challenging, requiring newer approaches that can rapidly and precisely inform immune epitopes/vaccine candidates.

In this regard it is noteworthy that several groups have recently reported a proteogenomic approach that allows T cell epitope discovery from species with large proteomes such as ours and mice. This approach has led to the discovery of several cancer-specific as well as minor histocompatibility alloantigen-derived CD8 T cell epitopes [41**,42**,43**,44**,45**]. Proteogenomic approach entails first defining the tumor transcriptome in relationship to the same individual’s non-cancerous genome or transcriptome in order to identify non-synonymous single nucleotide polymorphisms (nsSNP). The translated mutant proteome is subjected to T cell epitope prediction using NetMHC-3.0. This information then allows the search for variant peptides within the material eluted from a given MHC molecule using the mass spectrometry experiment called multiple reaction monitoring (MRM). From the resulting naturally processed tumor epitopes, immunogenicity was predicted *in silico* with both immunogenicity and protection validated *in vivo* [41**,42**,43**]. Or alternatively, the proteogenomics approach can involve first an in-depth analysis of MHC associated self peptidome or ligandome — the collection of peptides derived from self proteins associated with a test MHC molecule. The potential variation within each peptide that is caused by nsSNP is ascertained from the genomes or transcriptomes of allogeneic or cancer cells and validated in immunologic assays [44**,45**].

An adaptation of this approach would be to determine the *in vivo* microbial transcriptome and/or proteome during a natural infection — for example, the translated proteome of pre-erythrocytic stage plasmodium induced within infected hepatocytes — to focus on in proteins that contain potential T cell epitopes [46]. Such an approach combines the relative ease of transcriptome/proteome determination, the rapidity of epitope prediction, and the ever-increasing sensitivity of mass spectrometers for the discovery of naturally processed T cell epitopes.

**Many determinants are presented yet only a few are recognized**

Several T cell epitopes are known to emerge from a single microbial protein. For example, the simian virus-40 (SV-40) large T antigen contains one H2Kb-restricted and three H2Dd-restricted epitopes [47]. Similarly, multiple T cell epitopes are known to emerge from a single microbe; for example, several H2b-restricted epitopes are presented during a natural influenza A virus (IAV) infection of C57BL/6 mouse [48] and HLA-restricted HIV epitopes (http://www.hiv.lanl.gov). Despite these and numerous other similar studies (e.g. [30]) the breadth and depth of microbial determinants displayed by an MHC class I molecule remain unknown.

A few groups employed a proteomics approach to answer this question: peptides associated with HLA class I molecules expressed by uninfected and VACV-infected cells were eluted and their sequences determined by mass spectrometry [34*,49,50]. The emerging data indicated that numerous VACV-derived peptides/proteins were processed and presented by HLA class I molecules during a natural infection (e.g., Table 1; [34*,49,50]). Despite the presentation of numerous peptide determinants or the existence of numerous predicted epitopes, upon infection immune T cells arise only against a subset of these peptides (Table 1; [34*,37]). Moreover, the identification of naturally processed determinants precisely informed immune epitopes and vaccine candidates because immune T cells recognized a large fraction of stably presented VACV-derived peptides and/or conferred protective immunity upon epitope vaccination [34*]. Nevertheless, there was only partial overlap between immune epitopes identified by the two approaches — algorithm-based prediction versus elution and proteomics — indicating that a combination of both approaches as in the proteogenomic approach could be powerful in the initial identification of potentially protective CD8 T cell epitopes.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Summary of naturally processed CD8 T cell epitopes</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HLA</th>
<th>Total peptide sequences</th>
<th>VACV derived homologous to VARV</th>
<th>Immune epitopes: eluted versus predicted epitopes</th>
<th>CD8 T cell reactive (human/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*02:01</td>
<td>~2500</td>
<td>109</td>
<td>17/25</td>
<td>31/18 (9)**</td>
</tr>
<tr>
<td>B*07:02</td>
<td>~1200</td>
<td>65</td>
<td>2/8</td>
<td>15/10 (7)**</td>
</tr>
</tbody>
</table>

VACV, vaccinia virus; VARV, variola virus — the agent of smallpox.

** See Refs. [30,34*,37,49,51,52].

*** Large majority were host cell-derived self-peptides. Peptides (our unpublished data).

**** Common epitopes recognized by human and mouse CD8 T cells.
One question that emerged from the afore studies is ‘how does the host benefit from presenting so many determinants by a given HLA class I molecule (Table 1; [34,49,50])?’ Presentation of a broad array of VACV determinants might underlie cross-protective immunity against heterologous poxviral infections and might underlie the success of vaccinating against smallpox with cowpox virus or VACV. Yet another answer might lie in human CD8 T cell response to VACV. Several groups have reported that vaccinated volunteers expressing the same HLA class I molecule recognize different subsets of partially overlapping VACV-derived epitopes, suggesting a variegated pattern of recognition (e.g., Table 2; [34,37,51,52]). Therefore, it is possible that the presentation of numerous class I-restricted determinants underlies the recognition of at least one epitope (see Table 2; [34,37,51,52]). A population-wide study is needed to test this hypothesis. As well, such studies could lead to an understanding of population genetics of variegated responses. Together they have the potential to inform vaccine design and vaccination strategies and, hence, are worthy of investment.

**The sensitive yet cross-reactive TCR: an oxymoron?**

The T cell receptor (TCR) is very sensitive: it is capable of recognizing and responding to one-to-ten molecules of an antigen [53,54]. Additionally, it can discriminate between two peptides differing by a methylene group or a methyl and a hydroxyl group in an accessory anchor — for example, H4 minor histocompatibility alloantigens [55,56]. This sensitivity coupled with a rather loose ‘recognition logic’ with which the TCR interfaces its cognate antigen — the p/MHC [57–59] — makes it highly cross reactive.

The estimated frequency of T cell cross reactivity to unrelated peptides is 1/30,000 [60]. In search of the H4 alloantigen using a pep-scan approach, we discovered that an H4\(^\beta\)-reactive CD8 T cell line recognized ~100 different peptides [61] — that is, mimotopes — yet did not yield the primary structure of the actual epitope [55,56]. This was not peculiar to the alloreactive TCR because the SV-40 epitope-4 specific and herpes simplex virus 1 gB-reactive T cell clones showed extensive cross reactivity as well. A common feature between the mimotopes recognized by the three CD8 T cell clones was they contained a TCR-specific recognition motif consisting of one or two conserved putative solvent exposed residues that can be contacted by the receptor. At the other extreme, a single autoimmune TCR has recently been shown to recognize over a million different peptides within a broad cross-reactivity profile [62]. Such cross reactivity is not peculiar to MHC class I-restricted TCRs as several class II-restricted TCRs were shown to cross react in a similar manner (see [63\*,64] and references therein). The cross-reactive feature of the TCR further underscores the critical need for comprehensive immunologic validation of an identified epitope. Furthermore, inclusion of structural features of p/MHC as well as TCR-p/MHC binding and interactions (e.g. [57–59,65]) into newer iterations of algorithms can enhance their predictive power [63\*].

**Immunogenicity and immunodominance: it ain’t what it used to be!**

A large number of T cell epitopes have populated the IEDB and other data bases. Even for pathogens with relatively small genomes such as HIV there are zillions of known immune epitopes that arise from ORFs and even ARFs (http://www.hiv.lanl.gov). Nonetheless, in many cases, which of these epitopes form potent targets for vaccination requires further characterization. The correlates of protective immunity in humans are largely unknown, but the prevailing view is that epitope immunogenicity and immunodominance might be the best predictors of protective T cell responses. Immunogenicity is assessed as the ability to recruit the naive precursors into the immune response upon epitope immunization. Immunodominance is a property of the adaptive immune response to complex antigens wherein antigen-specific

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**Table 2**

Variegated pattern of naturally processed B*07:02-restricted epitope recognition by smallpox vaccines

<table>
<thead>
<tr>
<th>Amino acid sequence**</th>
<th>ORF</th>
<th>B*07:02-positive volunteers$</th>
<th>Immunoresponse</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPYEGKRF</td>
<td>E9L</td>
<td>456</td>
<td></td>
</tr>
<tr>
<td>FPRSLMIF</td>
<td>L4R</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>SPNSHFHLL</td>
<td>A3L</td>
<td>291; 823</td>
<td></td>
</tr>
<tr>
<td>FPRMDVFSF</td>
<td>B8R</td>
<td>534; 539; 673</td>
<td></td>
</tr>
<tr>
<td>RPRAIRFL</td>
<td>E2L</td>
<td>367</td>
<td></td>
</tr>
<tr>
<td>RPNHHTIDR</td>
<td>N2L</td>
<td>576</td>
<td></td>
</tr>
<tr>
<td>APASSLLPAL</td>
<td>A4L</td>
<td>392</td>
<td></td>
</tr>
<tr>
<td>FPSTVIFNDI</td>
<td>E9L</td>
<td>332; 689</td>
<td></td>
</tr>
<tr>
<td>VPITGSKLL</td>
<td>G2R</td>
<td>158; 974; 278; 736</td>
<td></td>
</tr>
<tr>
<td>YPSKNEYI</td>
<td>A11R</td>
<td>1317</td>
<td>438</td>
</tr>
<tr>
<td>LPSNVEIKAI</td>
<td>I16L</td>
<td>1317</td>
<td>438</td>
</tr>
<tr>
<td>ILYLEIEI</td>
<td>A20R</td>
<td>722</td>
<td></td>
</tr>
<tr>
<td>NPSTKVYALL</td>
<td>ESR</td>
<td>291; 1725</td>
<td>681</td>
</tr>
<tr>
<td>NPVLKILLL</td>
<td>B25R</td>
<td>824; 2235</td>
<td></td>
</tr>
</tbody>
</table>

* See Ref. [47] for details.
** Anchor residues are in bold.
$ Anchor spot-forming cells over background per million volunteer peripheral blood mononuclear cells. Intensity of red, hierarchy within an individual; green, no response.
lymphocytes respond disproportionately to the different epitopes on the antigen. This feature of the immune response is more accentuated in inbred strains of mice than in outbred populations such as ours. Hence, immunodominance has been extensively studied in mice—for example, CD8 responses to SV-40 T antigen, IAV, lymphocytic choriomeningitis virus and VACV (e.g. Refs. [47,48,66–68]). Multiple host factors—including the kinetics and dynamics of epitope generation and presentation, p/MHC stability, a diverse and functional T cell repertoire, precursor frequency, TCR avidity/dwell-time for cognate p/MHC and T cell competition for epitopes—control immunodominance [69–71].

A recent advance, which involves p/MHC tetramer-based enrichment allows enumeration of the naïve CD8 T cell precursors bearing antigen-specific TCR [69]. Many studies have shown that the magnitude of immune T cell response is roughly proportional to the naïve precursor frequency. Some immune epitopes violate this rule, however. Thus, despite relatively high naïve precursor frequency and high immunogenicity—as assessed by peptide immunization—several epitopes yielded subdominant CD8 T cell responses to VACV infection in mice, and vice versa (see Table 3). In the most striking case, a highly immunogenic B*07:02-restricted epitope in mice that has a very high naïve precursor frequency elicited a poor CD8 T cell response of low magnitude during viral infection (Table 3). This was due to poor and late epitope presentation [34*]. Furthermore, challenge studies in mice with VACV and the mousepox agent ectromelia virus revealed that subdominant epitopes can also elicit protective immunity as do immunodominant epitopes (Table 3; [31*,34*]). Hence, neither immunodominance nor immunogenicity predicted the most protective epitopes when the entire panel was assessed (Table 3). In mice, therefore, the protective capability of individual T cell response upon epitope immunization might be a complex interplay between efficient processing of epitopes from cognate antigen, the presence of naïve precursors, as well as temporality and duration of epitope presentation by microbe-infected cells—none of which can be predicted by currently available algorithms.

Immunodominant T cell responses are observed in humans as well, wherein a preferential recognition of a particular epitope by a majority of the subjects within the cohort tested, even if the magnitude of the response is low, is considered immunodominance. Strikingly, however, several groups have reported that vaccinated volunteers expressing the same HLA class I molecule recognize different subsets of partially overlapping VACV-derived epitopes, suggesting a variegated pattern of recognition (e.g., Table 2). Hence, a clearly defined immunodominant VACV epitope(s) was not seen within the three study populations. That notwithstanding, a clear hierarchic response to the different epitopes was observed within each individual tested (e.g., Table 2; [34*,37,51]). This finding suggests that the combination of HLA class I molecules (HLA haplotypes) can control CD8 T cell response. Indeed, immunodominance hierarchy was altered depending

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**Table 3**

<table>
<thead>
<tr>
<th>Amino acid sequence**</th>
<th>ORF</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (hour)</th>
<th>Precursor frequency (#/mouse)</th>
<th>Magnitude of response to VACV % (mean)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Magnitude of response to peptide % (mean)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Protective epitope&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPRPDTVRHL</td>
<td>A34R40-90</td>
<td>1.52</td>
<td>2465</td>
<td>4.0–10 (6.81)</td>
<td>11.6–22.6 (15.0)</td>
<td>++</td>
</tr>
<tr>
<td>RPSTRNFFEL</td>
<td>D1R609-817</td>
<td>2.93</td>
<td>1892</td>
<td>4.0–8.7 (6.28)</td>
<td>1.2–27.4 (13.9)</td>
<td>+</td>
</tr>
<tr>
<td>FPPKNDTVSF</td>
<td>B8F30-79</td>
<td>5.2</td>
<td>1472</td>
<td>1.0–4.9 (2.38)</td>
<td>16.6–51.5 (35.0)</td>
<td>+++</td>
</tr>
<tr>
<td>MPAYIRNTL</td>
<td>J6R303-311</td>
<td>5.44</td>
<td>521</td>
<td>0.3–1.8 (0.86)</td>
<td>11.9–51.4 (32.6)</td>
<td>+++</td>
</tr>
<tr>
<td>HPRHYATVM</td>
<td>D1R666-694</td>
<td>6.47</td>
<td>308</td>
<td>0.2–1.6 (0.7)</td>
<td>4.1–14.5 (10.1)</td>
<td>+</td>
</tr>
<tr>
<td>SPSSNHILL</td>
<td>A3L192-200</td>
<td>5.46</td>
<td>134</td>
<td>0.3–1.3 (0.67)</td>
<td>14.6–34.5 (21.6)</td>
<td>+++</td>
</tr>
<tr>
<td>FPTWLTISI</td>
<td>ISL237-245</td>
<td>0.54</td>
<td>nd</td>
<td>0.01–0.8 (0.35)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>FPPKMSLSIF</td>
<td>LAR37-45</td>
<td>6.08</td>
<td>6104</td>
<td>0.05–0.3 (0.17)</td>
<td>84.1–92 (86.5)</td>
<td>–</td>
</tr>
<tr>
<td>LPKEYSSEL</td>
<td>DSP375-383</td>
<td>7.44</td>
<td>1240</td>
<td>0.07–0.3 (0.16)</td>
<td>8.3–22.6 (16.1)</td>
<td>++</td>
</tr>
<tr>
<td>ANPNSRFV1</td>
<td>F4L14-14</td>
<td>8.81</td>
<td>668</td>
<td>0.03–0.5 (0.15)</td>
<td>17.7–75.2 (53.6)</td>
<td>+++</td>
</tr>
<tr>
<td>RPMSSLSTTI</td>
<td>O1L335-344</td>
<td>5.31</td>
<td>nd</td>
<td>0.0–0.4 (0.14)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>RPRDAIRFL</td>
<td>E2L216-224</td>
<td>4.81</td>
<td>126</td>
<td>0.03–0.2 (0.11)</td>
<td>22.4–44.3 (24.5)</td>
<td>+</td>
</tr>
<tr>
<td>RPQKHTIDIL</td>
<td>N2L104-113</td>
<td>4.73</td>
<td>nd</td>
<td>0.02–0.3 (0.1)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>FPYKGDGRVF</td>
<td>E9L526-534</td>
<td>6.17</td>
<td>nd</td>
<td>0.01–0.1 (0.05)</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

**See Ref. [34*] for details.

**Listed in the order of hierarchy, see last column.

<sup>a</sup> Half-life of p/MHC stability.

<sup>b</sup> Range and mean of splenic responder CD8 T cells elicited by VACV infection.

<sup>c</sup> Range and mean of splenic responder CD8 T cells elicited by peptide immunization; nd, not determined.

<sup>d</sup> Protection from lethal intranasal VACV challenge of mice prime boosted with the test peptide: –, non-protective; + weakly protective — low VACV burden yet sustained weight loss as compared to mock control; ++, highly protective — low VACV burden and weight loss when compared to mock control.
on the mouse MHC haplotype and genetic background. This outcome was explained by alterations in naïve precursor frequency of CD8 T cell responders against VACV epitopes within the inbred versus F1 mice tested [66]. A recent paradigm-shifting study by Picker and colleagues uncovered a role for immunoregulation in shaping antiviral response that suggested a new avenue for epitope discovery: immunization with genetically engineered cyto-megalovirus vectors induced a broad, protective SIV-specific CD8 T cell response that targeted unconventional (MHC class II restricted) and promiscuous (presented by multiple MHC alleles) epitopes [72**]. If violation of rules of MHC restriction is more common than previously thought, then efforts so far will have underestimated the breadth of immune epitopes and missed protective antigens. The interplay between antigen presentation, measurable T cell response parameters, and microbial pathogenesis will be a matter for continued investigation to enable protective epitope discovery. The poor success of the recent T cell targeted vaccine trials against HIV/AIDS, malaria, and TB [73–76] clearly signal the need for re-evaluating current strategies for protective T cell epitope discovery.

**Note added in proof**

In their report, which appeared after the submission of this review, Jenkins and colleagues (RW Nelson et al. Immunity 42: 95–107; 2015) addressed why TCRs are cross reactive and what the consequences might be. TCR cross reactivity to multiple different peptides was determined by sharing five residues within nonameric peptides (see also Refs. [61,63**]). Such cross reactivity deleted a substantial pool of self reactive T cells and, thereby, reduced the size of the peripheral T cell repertoire reactive toward an antigen. As well, the maintenance of a cross reactive peripheral T cell repertoire pre-disposed the host to autoimmunity while responding to microbial infections.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- **of outstanding interest**


An up-to-date review of the various methods used to identify and validate MHC-restricted T cell epitopes. Pros and cons of each method are discussed. Several methods — overlapping peptide scan, T-CAD (T cell antigen discovery), ATLAS (AnTigen Lead AcquisitionSystem), among others — and references to them, not discussed herein, can be found in this single source.


An easy-to-read narrative of the history and science behind the discovery and biology of the Mhc — from ‘groping the elephant’ to molecular science of MHC-restriction and allograft rejection. It is as much of the science as of the people that helped write this history.


- Refs. [3**.*
- [4].


See annotation to Ref. [3**].


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31. Remakus S, Rubio D, Ma X, Sette A, Sigal LJ: Memory CD8+ T cells specific for a single immunodominant or subdominant determinant induced by peptide-dendritic cell immunization protect from an acute lethal viral disease. J Virol 2012, 86: 9748-9759. This work demonstrates three features of memory CD8 T cells that help understand protective immunity to poxviruses in a homotypic model using ECTV — the agent of mousepox — infection of mice; firstly, NK cell-mediated immunity to ECTV was dispensable in the presence of memory CD8 T cells, secondly, memory CD8 T cell response to a single epitope conferred protection in a challenge model, and thirdly, subdominant memory CD8 T cell response to a single epitope could do the same. These findings suggested that the focus on immunodominant antigens for vaccine design may not be a wise strategy.

Haeyfar SM, Williams SJ, Sette A, Benkock JR et al.: Identification of poxvirus CD8+ T cell determinants to enable rational design and characterization of smallpox vaccines. J Exp Med 2005, 201:95-104. This report describes the generation and use of several HLA class I transgenic mice indicated in the title. The transgene construct encodes for the respective HLA α102 domains fused with a mouse α3 domain and covalently linked to human β2-microglobulin. The resulting transgene was introgressed into mouse H2 class I deficient background. The combination of mouse α3 domain and endogenous mouse class I deficiency encourages interactions between the recombinant HLA class I molecule and mouse DB molecules during T cell development and effector differentiation. These transgenic mice make a powerful small animal model for HLA-restricted CD8 T cell epitope discovery and validation as well as for pre-clinical protection studies.

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Erickson JJ, Boyd KL, McAfee KJ et al.: Discovering naturally processed antigenic determinants that confer protective T cell immunity. J Clin Invest 2013, 123:1976-1987. This comprehensive proof-of-principle study identified potent CD8 T cell targets from a complex virome. It reports large-scale discovery and immunologic characterization of naturally processed antigenic determinants of VACV that are presented by five frequent HLA class I alleles that represent four major class I supertypes (see Ref. [36]). Immunologic characterization revealed that, (a) memory CD8 T cell response to a single epitope conferred protection in an intranasal VACV challenge model; (b) immunodominance did not strictly segregate with naïve precursor CD8 T cell frequency; and (c) a sub-dominant memory CD8 T cell response to a single epitope conferred protection to intranasal VACV challenge. Hence, naturally processed HLA class I-restricted epitopes informed targets for CD8 T cell-based protective immunity.

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Pawashe AP, Blanchard T, McMahon D, Sidney J, Sette A et al.: Genomic and bioinformatic profiling of mutational neoepitopes reveals new rules to predict anticancer immunogenicity. J Exp Med 2014, 211:2231-2249. Refs. [41* 42*43*] describe the proteogenomic approach to tumor-specific CD8 T cell epitope discovery. This version of the proteogenomic approach entailed first defining the tumor transcriptome in relationship to the same individual’s non-cancerous genome or transcriptome. Such an analysis led to the identification of nsSNP. The translated mutant
proteome (mutome, in some corners) was subjected to T cell epitope prediction using NetMHC-3.0 algorithm. This information then allowed the search for variant peptides within the material eluted from a given MHC molecule in MRM experiments. From the resulting naturally processed tumor peptides, immunogenicity was predicted in silico with both immunogenicity and presentation validated in vivo. Ref. [40-42] also demonstrated that immunization with novel cancer epitopes coupled with checkpoint blockade treatment can be powerful immunotherapy against cancers.


See annotation to Ref. [41].


See annotation to Ref. [41].


Refs. [44,45] describe the discovery of novel cancer-specific and allogeneic-derived CD8+ T cell epitopes using a modification of the proteogenomics approach described above. In this approach, an in-depth analysis of HLA class I-associated self-peptide/ligandome was performed first. Then the potential variation within each peptide that was caused by snSNPs was ascertained from the genomes or transcriptomes of allogeneic or cancer cells and validated in immunologic assays.


See annotation to Ref. [44].


63. Birnbaum ME, Mendoza JL, Sethi DK, Dong S, Glavirle J, Dobbins J, Ozkan E, Davis MM, Wucherpenf WW, Garcia KC: Deconstructing the peptide-MHC specificity of T cell recognition. Cell 2014, 157:1073-1087. This study revisited a baffling property of the TCR — high cross-reactivity against the backdrop of high sensitivity (Refs. [53,54]). To define the molecular basis of this recognition logic, a novel experimental model consisting of yeast pMHC display libraries was coupled with deep sequencing to identify the ligands for the test TCRs. The emerging data revealed that TCR cross-reactivity was not as extreme but involved hundreds (see also Ref. [56] and not billions as previously thought). The primary structures of the ligands indicated that a good number of peptides had a TCR-specific recognition motif consisting of conserved amino acid residues. From this deconstruction of the TCR-pMHC recognition logic, an algorithm was developed that facilitated the prediction of ligands for autoreactive TCRs. Powerful new iterations of epitope prediction algorithms can emerge by inclusion of these findings with the structural constraints in MHC binding peptides (see Ref. [65]) to the currently used algorithms.


This paradigm-shifting study reports how modified viral vectors can impact the quality of the T cell response to the protein(s) encoded by the recombinant gene. In so doing, novel CD8 T cell epitopes are unveiled and elicit responses that violate the rules of MHC restriction. Whether this is of common occurrence needs to be discovered. Should it be, an in-depth understanding of microbial pathogenesis will become critical more than ever for protective epitope discovery. So also, efforts invested so far on epitope discovery will have underestimated the breadth of immune epitopes and will have missed protective antigens.


