are found in bone marrow chimeras in the range of 0.01–1% of T_{reg} cell TCR-transgenic precursors, it seems more likely that TCR competition is the factor that limits T_{reg} cell development in this system. If T_{reg} cell development were to require access to rare complexes of self peptide and major histocompatibility complex (MHC), then decreasing the frequency of progenitor cells with that TCR specificity could radically enhance the probability of encounter with rare self peptide–MHC complexes. Further experiments will be needed to sort this out.

Many indirect results suggest that the T_{reg} cell repertoire consists of self-reactive TCRs. Given that conclusion, it might be predicted that mice transgenic for T_{reg} cell–derived TCRs would show clonal deletion in the thymus. Unexpectedly, Bautista *et al.* detect no evidence of clonal deletion in these mice[1]. On a RAG-deficient background, this TCR transgene gives rise to large populations of $CD4^+$ T cells in the thymus and the periphery. In contrast, another group has reported efficient clonal deletion of $CD4^+$ T cells and almost no detectable $Foxp3^+$ T_{reg} cells in a distinct line of mice transgenic for T_{reg} cell–derived TCRs on a RAG-deficient background, which indicates that T_{reg} cell–derived TCRs are self-reactive. It is possible to imagine a model that can accommodate both results, in which both subtly and overtly self-reactive T cells can give rise to T_{reg} cells (**Fig. 1**). In this model, a certain low threshold of TCR affinity for self peptide–MHC is required for positive selection. With increasing affinity, the propensity to give rise to T_{reg} cells increases but never reaches 100%. At some point, clonal deletion begins to cull self-reactive TCRs from the repertoire. Between the T_{reg} cell–propensity threshold and the clonal-deletion threshold, it is possible for TCRs to appear in both T_{reg} cell and naive T cell repertoires, as is observed in actual experiments. In addition, with TCRs of higher affinity, IL-2 signaling could raise the effective threshold for clonal deletion, creating a window in which a subset of TCRs is found exclusively in the T_{reg} cell repertoire. TCRs in the low to middle regions of the affinity spectrum (such as the receptors cloned by Bautista *et al.*) would give rise to T_{reg} cells with varying frequency. In contrast, TCRs in the high end of the affinity spectrum (such as the receptor cloned before[5]) would be overtly self reactive and thus would be deleted. It will be useful to determine if such high-affinity receptors give rise to T_{reg} cells when present at low frequencies in mixed–bone marrow chimeras. The fact that TCRs from different 'affinity windows' have been cloned in different studies might have to do with how the T_{reg} cells were isolated. In the earlier study, the researchers cloned their T_{reg} cell–derived TCR from an $\alpha\beta$TCR-transgenic strain in which the appearance of $Foxp3^+$ T_{reg} cells depends on endogenous TCRα-chain rearrangement[5]. Thus, the T_{reg} cell receptor is a 'second receptor' on these cells. In this situation, less surface expression of the TCR could have facilitated the 'rescue' of these cells from deletion.

The results of Bautista *et al.* highlight a chief limitation of the use of monoclonal TCR–transgenic mice. The higher frequency of precursor cells expressing a particular TCR clone in these mice masks normal T cell activity during development. Another limitation of TCR-transgenic models is that they tend to allow earlier and stronger TCR expression. If T_{reg} cell development is dependent on the detection of subtle shifts in TCR affinity and/or interaction with unique antigen-presenting cells in a particular environment, TCR-transgenic mice (whether conventional or retrogenic) may not model development appropriately. However, the need to study the activity of cells with fixed TCR specificity remains as crucial as ever. Thus, the field continues to be in need of more-refined TCR-transgenic models.

Overall, this new research highlights the 'instructive' nature of the TCR in T_{reg} cell development but introduces a notable probabilistic element. These seemingly 'fuzzy' rules result in a T_{reg} cell repertoire that, like the repertoire of naive T cells, is diverse and highly adaptable and continually responds to self and environmental cues about where and how to function. These cues are at least as complicated and fascinating as the T_{reg} cell development process itself.

1. Bautista, J. *et al. Nat. Immunol.* **10**, 610–617 (2009).
2. Hsieh, C.S., Zheng, Y., Liang, Y., Fontenot, J.D. & Rudensky, A.Y. *Nat. Immunol.* **7**, 401–410 (2006).
3. Pacholczyk, R. *et al. Immunity* **27**, 493–504 (2007).
4. Burchill, M.A. *et al. Immunity* **28**, 112–121 (2008).
5. DiPaolo, R.J. & Shevach, E.M. *Eur. J. Immunol.* **39**, 234–240 (2009).

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Antigen-processing and presentation pathways select antigenic HIV peptides in the fight against viral evolution

David H Margulies

The evolution of immunodominant epitopes in HIV-1 Gag proteins correlates with quantitative measures of several antigen processing events. Thus, peptides recognized by $CD8^+$ cytolytic T cells are selected by their ability to pass through the antigen processing pathway, as well as by their binding to HLA molecules.

The vertebrate immune system uses different 'arms' that act together to combat viral infection. The cells and receptors of innate and adaptive immunity, including natural killer cells, B lymphocytes (and their protein derivatives, antibodies), T cells and specialized antigen-presenting cells work together to identify viruses before the viruses can even adsorb to cells, to mark infected cells for destruction and/or to limit reactivation of persistently or latently infected cells. Each of these activities waxes and wanes throughout the course of viral exposure and infection. Adaptive cells of the immune system like T cells and B cells repeatedly identify specific antigens; these antigens, called 'immunodominant antigens', have become one focus of efforts to develop better vaccination strategies[1]. In this issue of *Nature Immunology*, Tenzer *et al.*[2] identify parameters that influence the generation and presentation of immunodominant human immunodeficiency virus type 1 (HIV-1) peptides to cytolytic $CD8^+$ T lymphocytes.

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Resistance to chronic viral infections such as HIV can be correlated to some degree with ongoing activity of cytolytic CD8$^+$ T lymphocytes that express αβ T cell antigen receptors that recognize complexes of peptide and major histocompatibility complex (MHC) class I in which the peptides are derived from viral proteins. A major challenge in understanding the variety of immune responses to viral infection has been to identify the parameters, both molecular and cellular, that determine which peptides from which viral proteins are most commonly selected for presentation at the cell surface and to understand how those peptides are generated and loaded onto MHC class I molecules. However, the scientific challenge is not only to understand the rules that govern the generation of immunodominant peptides but also to elucidate the options available to a rapidly evolving virus able to modify the protein from which such peptides derive. Tenzer *et al.* build on previous work showing that wild-type sequences encoding an HIV-1 group-associated antigen (Gag) T cell epitope undergo natural escape mutations that then can ultimately revert back to the wild-type sequence[3]. Here, the authors study the wild-type sequence of the Gag protein p17 as well as seven common natural variants of this sequence to evaluate the influence of several crucial parameters of the MHC class I antigen-processing and antigen-presentation pathway on the detection of each variant peptide by CD8$^+$ T cells. For the sake of completeness, they also analyze epitopes from the p24 Gag protein, but the discussion here is limited to the results obtained with p17.

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Underlying their approach is the view that the final product of this complex antigen-processing and antigen-presentation pathway—that is, the cell surface peptide–MHC class I complex that is subsequently recognized by CD8$^+$ T cells—is generated as a result of the quantitative efficiency of each step of the pathway and that these efficiencies can be examined by reconstitution of the relevant steps *in vitro*. The main steps of the pathway include cytosolic production of peptide precursors, transport of intermediate peptides from the cytosol to the peptide-loading complex of the endoplasmic reticulum by the transporter associated with antigen processing (TAP), and maturation and folding of peptide–MHC class I complexes expressed on the cell surface (**Fig. 1**). Studying all steps of the pathway completely for any given virus (or protein, for that matter) would be a next-to-impossible task, so the authors confine themselves to the merely overwhelming job of studying several steps of the pathway.

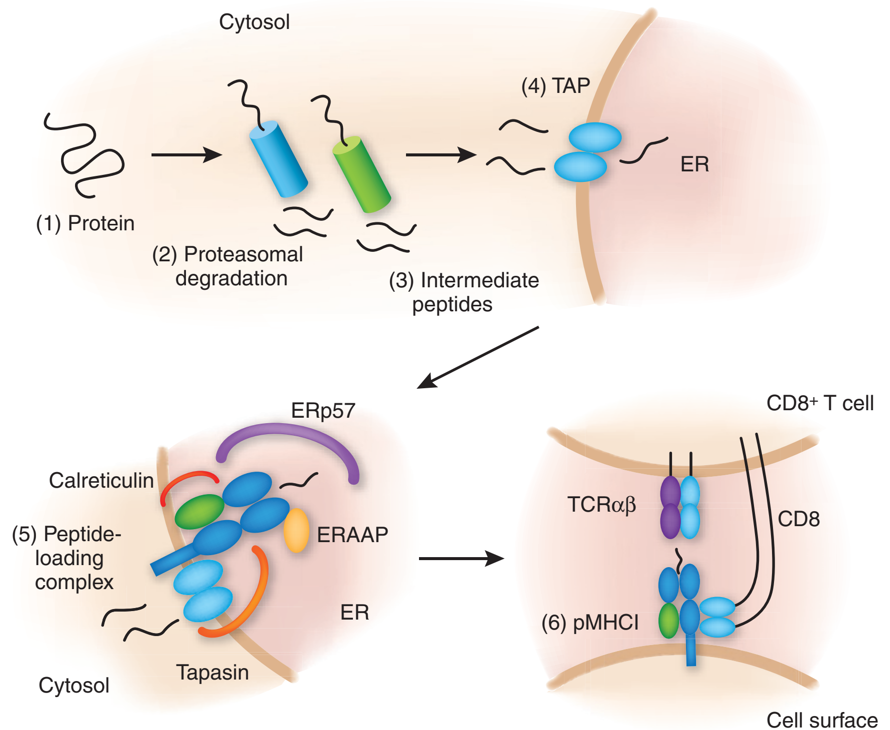

Figure 1 Steps in antigen processing and presentation in the MHC class I pathway. Native, partially unfolded, or denatured protein in the cytosol (1) is further denatured concomitant with proteolytic processing by constitutive or immunoproteasomes (2). In experiments reported in this issue[2], 25–amino acid peptides are used as substrates for isolated proteasomes. Intermediate peptides that result from proteasome degradation (3) then bind TAP (4) and are transported to the peptide-loading complex (5), where the sequential and concerted effects of tapasin, ERp57, calreticulin, MHC class I heavy chain, β_2-microglobulin and ERAAP1,2 lead to the formation of a stable MHC class I–β_2-microglobulin–peptide complex (pMHCI; 6). This complex is transported via the Golgi to the cell surface, where it is available for binding to αβ T cell antigen receptor (αβTCR) and CD8αβ molecules on the surface of CD8$^+$ T cells. ER, endoplasmic reticulum.

Using synthetic 25–amino acid peptides with substitutions representing the sequences commonly found in escape variants, the authors first evaluate the relative abundance of smaller, or intermediate, peptides generated by the proteolytic activity of purified proteasome preparations[4]. These peptides of intermediate length are potentially able to be transported by TAP into the endoplasmic reticulum lumen and to bind by their carboxyl termini to endoplasmic reticulum–resident MHC class I molecules. Using fragment intensity identified by mass spectrometry as an indicator of the abundance of the intermediate peptides produced by proteasomal digestion, the authors find that the wild-type 25–amino acid peptide generates the precursor for the immunodominant peptide presented by HLA-A2 (SLYNTVATL) rather poorly. In addition, they note that different variant 25–amino acid peptide precursors give rise to distinct peptides of intermediate length. Next they measure the affinity of synthetic peptides that represent each of the intermediate peptides for TAP. As TAP-mediated transport is dependent on TAP affinity, peptides with a higher affinity for TAP are expected to be proportionately better represented in the pool of intermediates that reach the peptide-loading complex in the endoplasmic reticulum[5]. After that, they measure the relative efficiency with which ERAAP1,2, an endoplasmic reticulum–lumenal enzyme, trims each intermediate peptide. Finally, they determine the affinity of the presenting HLA molecule for each of the trimmed peptides. They find that cleavage 'preference', TAP binding, ERAAP trimming and HLA affinity all collectively contribute to the relative hierarchy of T cell recognition of the resulting peptide antigen.

In general, the overall hierarchy of T cell recognition of p17 and p24 epitopes correlates with a calculated function of epitope 'abundance' rather than with peptide affinity for HLA molecules. In addition, the detection of differences in the responses of HIV patients to peptide variants with a carbox-

yl-terminal extension of the core epitope prompted structural analyses of this elongated peptide bound to HLA-A2. The crystal structure shows that this elongated p17 core peptide may bind to HLA-A2 in a way that results in the adoption of an unusual 'flipped' orientation by its carboxy-terminal tyrosine residue. Thus, although HLA affinity certainly contributes to the hierarchy of $CD8^+$ T cell recognition, it is not the sole predictor of the cytotoxic T lymphocyte response. Peptide abundance and structure when bound to HLA molecules probably also influence cytotoxic T lymphocyte recognition.

Does the enormous amount of data provided by the analysis by Tenzer *et al.*[2] provide a better sense of the parameters that might control immunodominance? If so, can a sensible theoretical course that might guide the empirical approach to vaccine development be outlined? In terms of the first question, one general take-home message of this study is not completely unexpected: the spectrum of peptides generated from a rather short 25–amino acid peptide precursor after proteasomal processing is very large, and this spectrum can be widely influenced by one to four substitutions in the sequence of the peptide precursor. The finding that different peptide intermediates are transported with different efficiency is also predictable, and the finding that different sequences are trimmed differently by ERAAP1,2 is expected[6]. However, for the second question, the overall analysis emphasizes the naiveté of believing that researchers could look at the sequence of a single wild-type protein, predict—using bioinformatics—its capacity to bind one or several common HLA molecules, and know with assurance exactly which protein sequence to incorporate into a favorite immunogenic vector.

Of course, these findings do not provide a simple guideline for choosing the 'right' peptide every time; HIV-1 is a rapidly mutating virus that can tolerate sequence variation in an essential protein for many generations sufficient to facilitate evasion of an immune response and then 'bounce back' by reverting to the more 'fit' wild-type sequence. Thus, not unlike the solutions to global warming or dependence on foreign oil, the proper immunization for rapidly evolving viruses cannot be a single T cell vaccine but instead must combine many different modalities that, like the 'cooperative arms' of the immune system, work in a complementary way to counter several steps of a pathogen's life cycle.

1. Yewdell, J.W. & Bennink, J.R. *Annu. Rev. Immunol.* **17**, 51–88 (1999).
2. Tenzer, S. *et al. Nat. Immunol.* **10**, 636–646 (2009).
3. Iversen, A.K. *et al. Nat. Immunol.* **7**, 179–189 (2006).
4. Kloetzel, P.M. *Nat. Rev. Mol. Cell Biol.* **2**, 179–187 (2001).
5. Abele, R. & Tampe, R. *Physiology (Bethesda)* **19**, 216–224 (2004).
6. Kanaseki, T., Blanchard, N., Hammer, G.E., Gonzalez, F. & Shastri, N. *Immunity* **25**, 795–806 (2006).

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IL-17A directly inhibits T_H1 cells and thereby suppresses development of intestinal inflammation

Amit Awasthi & Vijay K Kuchroo

T helper type 1 cells (T_H1 cells) serve a dominant function in T cell–mediated colitis. New work reports that interleukin 17A, an effector cytokine required for the development of autoimmune tissue inflammation, directly inhibits T_H1 development by suppressing the expression of key T_H1-associated genes and therefore regulates T_H1 cell–mediated colitis.

Regulation of intestinal immunity is critical for maintaining gut immune homeostasis, as the gut encounters and is exposed to a plethora of environmental and food antigens that can lead to severe immune reactions. Several regulatory mechanisms have evolved to prevent these inflammatory reactions; both $Foxp3^+$ regulatory T cells and type 1 regulatory T cells, which produce IL-10, serve a dominant function in maintaining effector T cell responses[1]. In addition, T helper type 3 cells (T_H3 cells), which produce mainly transforming growth factor-β, mediate tolerance to orally administered antigens[2]. In this issue of *Nature Immunology*, O'Connor *et al.* show that in addition to IL-10 and transforming growth factor-β, IL-17A is crucial in directly inhibiting the T_H1 subset and suppressing gut inflammation[3].

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IL-10 is essential for the immunosuppressive effects of $Foxp3^+$ regulatory T cells and type 1 regulatory T cells in colitis. IL-10-deficient mice develop spontaneous colitis with more T_H1 cells in the inflamed gut[4]. Moreover, IL-10-deficient $Foxp3^+$ regulatory T cells do not protect against colitis triggered by gut flora[5]. T_H1 cells that produce interferon-γ (IFN-γ) are important effector cells in inducing colitis and wasting disease, as blocking IFN-γ at the time of T cell transfer prevents development of colitis. Consistent with the idea that IFN-γ and T_H1 cells are the essential cytokine and effector cells for the induction of colitis in immunodeficient animals, adoptive transfer of T cells from IFN-γ-deficient mice fails to induce disease[6]. In contrast, in other autoimmune disease models such as those that mimic multiple sclerosis (for example, experimental autoimmune encephalomyelitis in mice) and rheumatoid arthritis, IFN-γ is dispensable for the development of autoimmune disease and tissue inflammation. In these models, IFN-γ-deficient and IFN-γ receptor–deficient mice are not resistant to and in fact are more susceptible to autoimmunity and tissue inflammation[7]. Such findings have raised the possibility that another subset of T cells and other cytokines, distinct from T_H1 cells and IFN-γ, are responsible for inducing autoimmune tissue inflammation in these disease models.

Another subset of helper T cells, called 'T_H-17' cells, that produce a panel of cytokines (IL-17A, IL-17F, IL-21 and IL-22) and are distinct from T_H1 or T_H2 cells, have been linked to the induction of autoimmune diseases in mice and humans[7]. In support of the idea of involvement of IL-17 and T_H-17