Antiviral T cell responses: phalanx or multipronged attack?

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Around 700 BCE, a new military formation called the phalanx was established in ancient Greece: a tight column of heavy infantry carrying long spears, or pikes, used in a single prong of attack. Later, in the battle of Marathon described by Herodotus, the Greeks learned the advantages of multipronged attacks, a strategy still used in modern warfare. Is the immune system similar in its approach to combating pathogens or tumors?

Concrete evidence that a diverse, multipronged T cell response is more effective than a single-pronged response in controlling viral infection in vivo in humans is quite limited. Two recent papers in the *JEM* describe the T cell response to human cytomegalovirus (hCMV) and point out that successful outcomes, with control of viremia, are correlated with a more polyclonal and diverse response (1, 2).

$CD8^+$ T cell responses to hCMV

hCMV infects over 50% of the human population. Although hCMV encodes ~200 gene products (3), the cellular immune response is thought to be focused on two proteins, IE-1 and pp65. ~80% of hCMV-specific $CD8^+$ T cells are estimated to target these two proteins (4), but with new epitopes being discovered at an ever-increasing rate, these figures may change. The $CD8^+$ T cell response is critical for maintenance of clinical "latency." Suppression of $CD8^+$ T cell responses leads to viral replication and disease, whereas adoptive transfer of hCMV-specific $CD8^+$ T cells results in reconstitution of effective cellular immunity (5).

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pp65 is an abundant tegument protein produced as an early and late gene product. It is considered the major target of hCMV-specific cytolytic T lymphocytes (CTLs) based on classical cytotoxicity assays. Prior to MHC tetramer technology and cytokine-based assays, CTLs specific for IE-1 were not well appreciated. IE-1 is an immediate early gene product with a key role in transactivation of other viral genes. Several hCMV gene products interfere with MHC-I and MHC-II antigen presentation (6). pp65 itself blocks presentation of IE-1 peptides via the MHC class I pathway and inhibits expression of genes associated with the induction of interferon responses (3). It is therefore possible that IE-1–specific responses require cross-presentation by an uninfected cell to avoid the inhibitory effects of pp65 (7). It has not been clear what the biological role of IE-1– versus pp65-specific responses might be, but the fact that hCMV has evolved a strategy to avoid IE-I–specific T cell responses suggests an important role for these cells in control of viral infection.

This conclusion was recently supported by a paper in the *JEM* by Bunde et al. (1). These investigators examined reactivation of latent viral infection in immune-suppressed patients, which is a major clinical problem in the field of transplantation. In 27 transplant patients on immunosuppressive drugs, they found a correlation between an early $CD8^+$ T cell response to IE-1 and protection against hCMV disease. Those patients that developed hCMV disease had $CD8^+$ T cell responses only to pp65 and sometimes lacked $CD4^+$ T cell responses to pp65, IE-1, or both. The question of diversity of the response was addressed as a side issue. Although $CD4^+$ T cell responses tended to be more diverse in patients that did not develop disease, the difference was not statistically significant.

Diversity of the hCMV-specific $CD8^+$ T cell response

In this issue of the *JEM,* Sacre et al. (2) examined hCMV responses in several groups of patients infected with both HIV and hCMV in which the critical distinction was whether or not the patients had active hCMV infection. Group I consisted of HIV^+ patients with quiescent hCMV; group II were patients being treated for hCMV infection who either responded (group IIA) or required continued treatment for greater than 5 years (group IIB), and group III were patients with active hCMV infection. The numbers of epitopes recognized by $CD8^+$ T cells in Elispot assays, using different pools of test epitopes, were greater in those patients that controlled hCMV infection: groups I and II. This observation held true for both pp65 and IE-1 $CD8^+$ T cell responses. Group IIA had greater IE-1–specific $CD8^+$ responses than group IIB, consistent with the data from Bunde et al. (1), suggesting that IE-1–specific responses were protective.

Diversity of $CD8^+$ T cells in other infections

Previous reports have indicated that narrow $CD8^+$ T cell responses correlate with viral persistence and that broad responses correspond to control and resolution of viral infection. For instance, in hepatitis C virus (HCV) infection, broad and persistent $CD8^+$ T cell responses were associated with resolution of viral infection, whereas weak and

narrowly focused responses were seen in patients with persistent infection (8). CD4 T cell responses to several HCV proteins were focused on an average of 10 epitopes in subjects with resolved infection compared with <1 epitope in those with persistent infection (9).

In primary HIV infection, it has been known for some time that clonotypically diverse $CD8^+$ T cell responses directed at many, rather than few, epitopes are associated with a lower set point of viral load and higher $CD4^+$ T cell counts during the early phase of chronic infection, and therefore correlate with slow disease progression (10–12).

Clonally restricted $CD8^+$ T cell expansions are frequently seen in chronic persistent viral infections. Are they of any utility to the host? In AIDS patients, they appear to be ineffectual in controlling virus. Successful antiviral therapy is associated with resolution of these expanded clonotypes, suggesting that the continued presence of replicating virus was driving the clonal expansions (13). Similarly, massive expansions of individual $CD8^+$ T cell clones specific for hCMV have been observed in elderly patients with hCMV infection where they appear to be ineffectual and are associated with poor immune function and possibly decreased survival (4).

Different levels of T cell diversity

Studies that use only peptide pools to quantify numbers of recognized epitopes, such as the papers discussed above (1, 2), fail to assay for clonal T cell receptor (TCR) diversity among T cells that react to the same peptide–MHC (pMHC) complex. Whether this type of diversity is also important for control of virus is not yet clear, although analysis of CDR3 lengths in TCRs from HIV-specific T cells does support this conclusion (10).

Aged individuals have a more restricted T cell repertoire, due to expansion of memory clones and perhaps diminished thymic output of naive T cells. Their immune responses may be less heterogeneous on two levels: fewer numbers of epitopes recognized and less diversity of TCRs that recognize individual pMHC complexes. Aged individuals have a decreased ability to fend off infections, and one of several proposed reasons may be this decrease in T cell diversity.

Children with congenital hCMV infection also have hCMV-specific $CD8^+$ T cell responses targeted to pp65 and IE-1 (14). However, it is not yet clear whether diversity of the T cell response at the level of recognition of larger numbers of epitopes is more typical of neonatal as opposed to adult $CD8^+$ T cells. At the level of TCR diversity to a single epitope, the neonatal response to an immunodominant matrix peptide of influenza is very diverse using many different TCRs. However, with aging (and repeated exposures to influenza), the response to the same pMHC is dominated by TCR $V\beta17$ $CD8^+$ T cells, such that depletion of this subset abrogates the in vitro response to the influenza peptide (15). It is unclear how this observation relates to the ability to deal with influenza infection.

Is diverse better?

Does heterogeneity at the level of T cell clones defined by TCR diversity really matter? This question has been addressed by Nikolich-Zugich and coworkers (16). In mice, greater than 90% of $CD8^+$ T cell responses specific for the immunodominant epitope glycoprotein B (gB498-505) of herpes simplex virus (HSV)-1 use $V\beta10$ or $V\beta8$ TCRs. The response is less vigorous and less diverse in C57BL/6 (B6) mice than in coisogenic bm-8 (B6.C-H-2^{bm8}) mice, which differ only by a few amino acid residues in the peptide binding groove of the MHC-I K^b molecule. B6 mice succumb to viral infection at 25-fold lower doses of virus than bm-8 mice (16). When diversity was experimentally reduced, by eliminating $V\beta8$ cells with an antibody, resistance to infection was further reduced. Interestingly, the less diverse $CD8^+$ T cell response in B6 mice was also characterized by lower avidity in MHC tetramer decay assays, which measure the off-rate of the TCR–pMHC interaction. The authors suggested that a more diverse T cell response provides a broader pool of clones from which higher avidity CTLs are more likely to be recruited. In old mice that had a diminished repertoire among $CD8^+$ T cells, particularly within the $V\beta8$ family due to spontaneous $CD8^+$ T cell clonal expansions, there were reduced numbers of MHC $tetramer^+$ cells, undetectable antiviral lytic function, and lowered resistance to challenge with HSV-1 (17).

The clinical data discussed above have only provided associations, and it is conceivable that lack of a diverse response is not the cause but rather the result of uncontrolled viral infection. Indeed, over time persistent viral antigens are likely to cause an increasingly focused T cell response dominated by only a few clones. However, the experiments of Nikolich-Zugich and colleagues (16) clearly show that in their system lack of a diverse response can result in decreased protection against a lethal viral infection.

Relevance to tumor immunology

The diversity of the $CD8^+$ T cell response is also relevant to cancer immunity. Tumor-infiltrating antigen-specific $CD8^+$ T cells that arise with tumor progression and T cells that are stimulated after vaccination with MAGE or Melan-A antigens are diverse. However, it has been assumed that high avidity T cells (for example, those that stain brightly with MHC tetramers), which have superior antitumor activity when tested in vitro (18), are sufficient for tumor immunity. The diversity of the $CD8^+$ response to an artificial tumor antigen was decreased in old compared with young mice (19), but a careful study correlating the degree of diversity of the $CD8^+$ response and the efficiency of in vivo tumor killing is still needed.

Promoting diversity

The arguments in favor of a diverse T cell response have been made in previous papers (20) and are listed in Table I. Side stepping the effects of the escape

Table I. Arguments in favor of a multipronged attack

	Reference
1. Prevents escape mutants in immunodominant epitopes of SIV and HIV (and other viruses)	25–28
2. Creates choices for the host to select the highest avidity TCRs	20
3. Avoids holes in the repertoire	20
4. Provides backup in case of clonal exhaustion or clonal anergy	29

mutations in immunodominant epitope, a favored trick of rapidly mutating viruses such as HIV or SIV, is one argument. Others pertain to avoiding holes in the T cell repertoire that arise due to ageing or to clonal anergy or exhaustion. As discussed above, a broader repertoire may provide choices that allow for the selection of higher avidity clones.

What are the mechanisms that promote a diverse response? Allison and colleagues have suggested that the inhibitory receptor CLTA-4 serves to attenuate extensive expansion of individual dominant clones, thereby allowing other clones to expand and participate in the response. This attenuation mechanism may ensure a polyclonal response (21). In support of this model, CTLA-4 expression has been shown to increase as a function of the number of cell divisions and high expression levels of CTLA-4 correlate with inhibition of clonal expansion. Cytokines might also broaden the $CD8^+$ T response. Although the mechanism is unknown, exogenous IL-7 administered after immunization boosted the response to a subdominant epitope of the experimental antigen H-Y (22). If diversity is truly important, there must be several physiological mechanisms to promote such a response. These mechanisms remain to be fully explored.

Practical applications

Assuming that a multipronged, diverse $CD4^+$ and $CD8^+$ T cell response is a good thing, how might this notion affect future strategies for immunizations? HIV vaccines are already being designed that include many epitopes from several genes and many peptide variants (to cover all possible viral clades). But this approach remains educated guesswork, since the exact epitopes presented in a given patient, and therefore the breadth of the immunization, are unknown. To obtain a broad immune response, additional strategies have been proposed. Cytokines are being evaluated as adjuvants (22). Engineered immunogens incorporating multiple heteroclitic peptides that cover an array of immunodominant and subdominant epitopes are being used successfully in vaccine models. Alternatively, tumor-derived RNA transcribed to cDNA can be used for immunization, providing a source of multiple antigens (23), and α virus replicase can be used to amplify multiple RNA species in "replicon" vectors (23, 24).

We conclude that the concept of the phalanx as a mode of attack for the immune system is outdated. Studies of the natural immune response to common viruses like hCMV (1, 2) can teach us all some important lessons regarding the advantages of diverse immune responses. They provide a rational basis for vaccines that produce broad immune responses. This has special relevance for the elderly, who need vaccines most urgently and are least likely to respond with an efficient and broad response.

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