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CD8 T Cell Clonal Expansions & Aging: A Heterogeneous Phenomenon with a Common Outcome

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Abstract

A highly diverse CD8 T cell repertoire is thought to be critical for maintaining appropriate immune defenses against a variety of pathogens. However, in many aged individuals, the diversity of T cell receptors is significantly reduced by the presence of large, monoclonal expansions of CD8 memory T cells. While ongoing research is focused on understanding the molecular alterations in these expansions, one major hurdle to this goal is the apparent heterogeneity of CD8 clonal expansions, which is apparent even in reductionist systems. In this review, we discuss current evidence that CD8 clonal expansions are a heterogeneous phenomenon, and our evolving understanding of what this heterogeneity tells us about CD8 memory T cell homeostasis and how it is altered in aged individuals.

1. The phenomenon of CD8 clonal expansions

In healthy, young individuals, the repertoire of antigen receptors (T cell receptors, TCRs) on CD8 T cells is highly diverse, with no single TCR dominating the repertoire. With increasing age, however, one common change to the TCR repertoire is the development of CD8 T cell expansions, in which a single clone of CD8 T cells, bearing a single TCR, occupies a significant proportion of the total CD8 T cell pool.

CD8 T cell clonal expansions are a common age-dependent alteration, affecting about one-third of humans over the age of 65, and almost 60% of mice over the age of 2 years (Ku et al., 1997; Ricalton et al., 1998). While this phenomenon is common, the magnitude of CD8 clonal expansions can vary widely. In the most dramatic cases found in inbred mice, a CD8 clonal expansion (i.e. a single CD8 T cell specificity) can occupy 90% of the CD8 T cell repertoire (unpublished data), whereas in humans, CD8 clonal expansions can occupy up to 50% of the CD8 T cell repertoire.

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CD8 clonal expansions were originally identified by monoclonal antibodies that recognize different T cell receptor (TCR) variable-alpha ($V\alpha$) and -beta ($V\beta$) chains (Callahan et al., 1993; Posnett et al., 1994). In young individuals, different $V\alpha$ s and $V\beta$ s are used on approximately the same proportion of T cells in different individuals. However, aged individuals have marked inter-individual skewing in TCR- $V\alpha$ and - $V\beta$ usage, with some individuals having a significant increase in the representation of a particular TCR- $V\beta$ chain relative to young individuals (greater than 2-3 standard deviations above the mean TCR- $V\beta$ usage in young individuals, Figure 1). This phenomenon is caused by the expansion of a clone of CD8 T cells bearing the $V\beta$ in question, such that the affected $V\beta$ is proportionally enriched within the total CD8 T cell pool.

Given the large number of monoclonal antibodies available to detect TCR- $V\beta$ s, CD8 clonal expansions are defined operationally by a bias in $V\beta$ usage. Nonetheless, CD8 clonal expansions are truly clonal as revealed by molecular analysis of the TCR (Ku et al., 2001; LeMaoult et al., 2000). These analyses have included sequencing of the TCR $\alpha\beta$ genes, as well as analysis of the TCR- $V\beta$ chain complementarity determining region 3 (CDR3) length by spectratyping (Cochet et al., 1992; Pannetier et al., 1993). On a population basis, over-representation of a particular TCR- $V\beta$ chain on the CD8 T cells of an individual is the best indicator that that individual has a CD8 clonal expansion.

2. Impact of CD8 clonal expansions on immune function and health

By using the above criteria, CD8 clonal expansions are large, monoclonal outgrowths of memory CD8 T cells in aged, healthy individuals. Although CD8 clonal expansions meet some criteria that are common to tumors (e.g. clonality & increased competitive advantage relative to their neighbors), CD8 clonal expansions are clearly not tumors. First, individuals with expansions have no change in the total number of CD8 T cells (Messaoudi et al., 2004). Second, individuals (both humans and mice) live healthy lives with clonal expansions (Chamberlain et al., 2000), and the incidence of CD8 memory T cell tumors in humans is exceedingly rare. Thus, despite their competitive advantage, CD8 clonal expansions are still subject to growth regulatory conditions in vivo.

With the presence of such a large number of T cells bearing a single TCR specificity, it is interesting to note that individuals with CD8 clonal expansions are relatively healthy. In fact, analysis of individuals with CD8 clonal expansions has only recently revealed that these individuals may have impaired immune function in very specific contexts. First, in humans, there are correlative data indicating that individuals with clonal expansions are less likely to respond successfully to an influenza vaccination (Saurwein-Teissl et al., 2002). Second, in mice, there are data that the presence of CD8 clonal expansions may result in very narrow holes in the T cell repertoire, which in some circumstances may impair immune responses to pathogens (Messaoudi et al., 2004). While these data indicate that clonal expansions may contribute to age-dependent immune compromise, it is important to note that affected individuals are not generally immune-suppressed.

3. Factors that promote the development of CD8 clonal expansions

There are three factors that have been associated with an increased risk for the development of CD8 clonal expansions: age, lymphopenic conditions, and infection with cytomegalovirus. The best defined of these criteria is age. There is a clear age-dependent increase in the incidence of CD8 clonal expansions, and in general, young, healthy individuals with CD8 clonal expansions are rare (Callahan et al., 1993; Ricalton et al., 1998). While chronological age clearly increases the risk of developing a clonal expansion, at this time, it is unclear mechanistically how aging promotes CD8 clonal expansions. Curiously, age is not an absolute requirement for the development of expansions. Recent analysis of a variety of lymphopenic

mice revealed that these mice tend to have: 1) more CD8 clonal expansions, 2) that occur at a younger age (Messaoudi et al., 2006a). Lymphopenia may promote CD8 clonal expansion development by increasing the number of CD8 memory T cells that are actively proliferating (Messaoudi et al., 2006a). While it is clear that clonal expansions can be accentuated under lymphopenic conditions, it is unknown at this time whether these clonal expansions are identical to those that spontaneously arise in aged, immunocompetent individuals. Finally, infection of humans with cytomegalovirus (CMV) has also been linked to the development of CMV-specific CD8 clonal expansions (Khan et al., 2002; Ouyang et al., 2003). While CMV has the ability to elicit such responses, it remains undetermined at this time what proportion of human clonal expansions result from this stimulus.

4. Altered properties of CD8 clonal expansions

Given the unusual accumulation of CD8 T cells in clonal expansions, research has focused on the biological properties of, and molecular alterations within, CD8 clonal expansions. In particular, studies of mice have provided new insight into unusual properties of CD8 clonal expansions. First, many CD8 clonal expansions have an increased rate of basal proliferation compared to normal CD8 memory T cells, with CD8 clonal expansions dividing once every 15 days in contrast to normal CD8 memory T cells which divide once every 22 days (Ku et al., 2001). Second, based on antibody blocking experiments, CD8 clonal expansions are still responsive to the cytokines IL-15 and IL-2 (Ku et al., 2001). Despite these initial data, recent work has indicated that clonal expansions do have perturbations in their response to cytokines. For example, CD8 clonal expansions do not alter their rate of proliferation when put into either a lymphopenic or a “full” lymphoid compartment (Messaoudi et al., 2006b). This suggests that CD8 clonal expansions are relatively insensitive to environmental cues that typically regulate CD8 memory T cell proliferation. Whether there are other environmental cues that CD8 clonal expansions have an altered response to remains to be addressed.

5. Heterogeneity among CD8 clonal expansions

Although CD8 clonal expansions clearly have altered biological properties, the molecular basis of these changes remain poorly defined. One significant challenge to elucidating the molecular basis of clonal expansions is the apparent heterogeneity of this phenomenon. CD8 clonal expansions are heterogeneous by at least three criteria.

i) CD8 clonal expansions appear to have different requirements for antigen. On one hand, some expansions appear to be relatively antigen-independent. This is best revealed in studies of inbred mice in which genetically identical littermates are housed together. In such studies, mice develop CD8 clonal expansions with a random TCR usage among their CD8 clonal expansions (Callahan et al., 1993; LeMaout et al., 2000). If these expansions were actively driven to become expansions by a common antigenic source (e.g. an infectious organism), some bias in the TCR usage of CD8 clonal expansions would be predicted.

While the above data indicate that, at least in mice, there may not be a common antigen driving these expansions, these data do not address whether antigen is required for continued growth of clonal expansions. However, adoptive transfer experiments of clonal expansions in mice have revealed that clonal expansions still have increased proliferation upon transfer into beta-2 microglobulin-deficient mice (which have significantly reduced major histocompatibility complex (MHC) class I ligands) (Ku et al., 2001). These data clearly demonstrate that many clonal expansions continue to have increased growth capacity even in the absence of cognate interactions with MHC class I.

In contrast to expansions that appear to be antigen-independent, there are also CD8 clonal expansions whose growth may be actively driven by antigen. The best data for these types of

clones is the presence of CMV-specific CD8 clonal expansions in some CMV seropositive individuals (Khan et al., 2002; Ouyang et al., 2003). At this time, it is unclear whether these clones are simply CMV-specific, if they actively require CMV antigens to maintain their enhanced growth, or if the inflammatory environment elicited by CMV infection promotes the growth of clonal expansions. Nonetheless, it should be noted that CD8 clonal expansions routinely have a non-activated phenotype, with no detectable expression of recent activation markers (e.g. CD69, CD25) (Ku et al., 2001).

ii) Another criterion in which CD8 clonal expansions are heterogeneous is their stability in vivo. In longitudinal analyses of mice with CD8 clonal expansions, some clones are very transient, disappearing within a 2-month interval (Callahan et al., 1993; LeMaout et al., 2000). In contrast, other clones are extremely stable, and are capable of being maintained through serial adoptive transfers over a 4-year period (C-C. Ku, unpublished data).

iii) Finally, CD8 clonal expansions have variable responses to in vitro stimulation. This parameter has been defined by how CD8 clonal expansions proliferate and survive in response to polyclonal stimulation, including the stimulatory lectin, concanavalin A, or the chemicals phorbol 12-myristate 13-acetate (PMA) and ionomycin (Callahan et al., 1993). On one hand, some CD8 clonal expansions have no selective advantage or disadvantage to stimulation and remain equally represented after in vitro stimulation. In contrast, other expansions have poor proliferation and/or survival post-stimulation (Callahan et al., 1993). As a result, these later expansions become under-represented following stimulation.

These diverse biological criteria indicate that CD8 clonal expansions are a heterogeneous phenomenon with the same common outcome: a selective advantage relative to their neighbors that results in their clonal outgrowth. While some of this variability may be due to differences in antigen dependence, the molecular basis of this variability and whether there are criteria to refine our definition of CD8 clonal expansions remain unresolved questions.

6. New insights into the heterogeneity of CD8 clonal expansions

To elucidate the molecular alterations in CD8 clonal expansions, we previously performed microarray analysis of CD8 clonal expansions (manuscript in preparation). In this analysis, we purified both CD8 clonal expansions as well as age-matched polyclonal, CD8 memory T cells from the same mice. After performing microarray analysis, we identified a number of genes whose expression was different between CD8 clonal expansions and polyclonal CD8 memory T cells. One of these alterations was in integrin $\alpha 4$ ($\alpha 4$), a cell-surface molecule involved in cell-cell adhesion and trafficking to many sites within the body (Rose et al., 2002). Microarray analysis demonstrated that many CD8 clonal expansions have reduced mRNA expression for integrin $\alpha 4$ (manuscript in preparation).

Based on this microarray analysis, we analyzed integrin $\alpha 4$ expression in a large cohort of CD8 clonal expansions from aged mice (ranging from 16-35 months of age). While many clonal expansions had a clear reduction in integrin $\alpha 4$ expression relative to young CD8 T cells (integrin $\alpha 4$ low expansions), there were also many CD8 clonal expansions in which integrin $\alpha 4$ expression was increased relative to young CD8 T cells (integrin $\alpha 4$ high expansions). Based on this analysis, there are two primary types of CD8 clonal expansions defined by their relative expression levels of integrin $\alpha 4$ (either integrin $\alpha 4$ low or integrin $\alpha 4$ high) (manuscript in preparation).

Further analysis of these two major types of CD8 clonal expansions revealed a number of interesting differences. First, while integrin $\alpha 4$ low clonal expansions have normal levels of CD8 α on their cell surface, the vast majority of integrin $\alpha 4$ high expansions have a CD8 α low phenotype, with a 2-3 fold reduction in CD8 α . Second, there is a clear age-bias in terms of the

integrin $\alpha 4$ phenotype of CD8 clonal expansions. While mice 16-20 months old have predominantly integrin $\alpha 4$ high, CD8 low clonal expansions, mice older than 20 months have CD8 clonal expansions that are almost exclusively integrin $\alpha 4$ low. This age-dependent alteration in integrin $\alpha 4$ phenotype suggests one of two possibilities: either integrin $\alpha 4$ high clones convert into integrin $\alpha 4$ low clones or integrin $\alpha 4$ high clones are inherently stable.

To assess the relative stability of these two types of clones, we performed a longitudinal analysis of CD8 clonal expansions. This analysis clearly revealed that the integrin $\alpha 4$ phenotype of an individual expansion remained constant (e.g. an integrin $\alpha 4$ low clone remained integrin $\alpha 4$ low). In contrast, when we analyzed the relative growth and stability of these two types of expansions in vivo, we discovered that integrin $\alpha 4$ high clones are frequently unstable in vivo (i.e. decreasing in size over a two-month interval).

Based on these initial data, CD8 clonal expansions are heterogeneous as revealed by integrin $\alpha 4$ expression. Moreover, integrin $\alpha 4$ expression appears to be related to the biological properties of expansions by multiple criteria including age at which expansions are present and stability in vivo. We are actively investigating whether these two types of CD8 clonal expansions have alterations in their trafficking and localization in vivo and whether these clones have altered responsiveness to proliferative stimuli. Based on these studies, however, we hypothesize that there are multiple, independent mechanisms that contribute to age-related alterations in CD8 T cell homeostasis. Moreover, these studies help us to further refine the current definition of CD8 clonal expansions, thereby facilitating further elucidation of the molecular mechanisms behind CD8 clonal expansions and their altered homeostasis.

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Figure 1.

Identification of CD8 clonal expansions in aged mice. CD8 clonal expansions are identified by an altered distribution of TCR-V β usage among CD8 T cells in an aged mouse (open bars) relative to that in a young mouse (black bars). In this hypothetical example, an aged mouse has a CD8 clonal expansion, defined by an increased percentage of CD8 T cells using TCR-V β 9.

Table 1

Characteristics of CD8 clonal expansions in humans and mice. Characteristics for which only a portion of CD8 clonal expansions have the listed properties are denoted by the italicized annotation (*some*). References are discussed in text, except those noted below.

	Human	Mouse
Size (within CD8 T cell pool)	Up to 50%	Up to 90%
Factors associated with development of expansions	Age, infection with CMV	Age, lymphopenic conditions
Impact on immune competence	Associated with impaired response to influenza vaccination.	May create holes in immune repertoire (e.g. T cell responses using limited TCR-V β diversity).
Rate of proliferation	Increased ^a	Increased
TCR specificity	Virus-specific (<i>some</i>)	Undefined specificity
Antigen requirement for continued growth	Undefined	Antigen not required (growth in absence of MHC class I)
Stability in vivo	Stable ^b	Stable, > 4 yrs (<i>some</i>) Unstable, < 2 months (<i>some</i>)
Response to stimulation	Intact (<i>some</i>) ^b Impaired (<i>some</i>) ^b	Intact (<i>some</i>) Impaired (<i>some</i>)

^a Increased proliferation relative to other CD45RA+ CD8 T cells, in a single CD8 T cell clonal expansion. Wallace, D.L., Zhang, Y., Ghattas, H., Worth, A., Irvine, A., Bennett, A.R., Griffin, G.E., Beverley, P.C., Tough, D.F., Macallan, D.C., 2004. Direct measurement of T cell subset kinetics in vivo in elderly men and women. J Immunol 173, 1787-1794.

^b Chamberlain et al, 2000.