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Citation for published version (APA):
vан Aалдерен, M. C. (2016). The individuality of (virus-specific) CD8 T cells

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CHAPTER ONE

BLOOD AND BEYOND: PROPERTIES OF CIRCULATING AND TISSUE-RESIDENT HUMAN VIRUS-SPECIFIC αβ CD8+ T CELLS

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ABSTRACT
CD8+ αβ T cell responses form an essential line of defence against viral infections. An important part of the mechanisms that control the generation and maintenance of these responses have been elucidated in experimental mouse models. In recent years it has become clear that CD8+ T cell responses in humans not only show similarities, but also display differences to those occurring in mice. Furthermore, while several viral infections occur primarily in specialised organ systems, for obvious reasons, most human CD8+ T cell investigations were performed on cells deriving from the circulation. Indeed, several lines of evidence now point to essential functional differences between virus-specific CD8+ memory T cells found in the circulation and those providing protection in organ systems, such as the lungs. In this review, we will focus on summarising recent insights into human CD8+ T cell differentiation in response to several viruses and emphasise that for a complete understanding of anti-viral immunity, it is pivotal to scrutinise such responses in both blood and tissue.
INTRODUCTION
Recent work has defined the details of anti-viral responses in mice, identifying key transcription factors regulating these responses \(^1\). However, direct translation to the human setting is difficult because the human immune system has coevolved with species-specific pathogens. For instance, mouse and human CMV (hCMV) are genetically distinct, which may well reflect the adaptation of the viruses to the specific hosts \(^2\). Also, the murine and human immune system use different receptor families on NK cells that counteract the action of hCMV's immune evading genes \(^3\). Additionally, the life expectancy of mice and humans is entirely different. As a consequence, immunological memory in targeting human viral infections, or in the case of persistent infections maintenance of latency, has to be sustained for decades rather than months (as it is in mice). This would be expected to impose specific constraints on the homeostatic mechanisms of immune cells. Indeed mouse and man use different ways to maintain pools of naïve T cells \(^4\). Furthermore, while most of the research on virus-specific CD8\(^+\) T cells in humans has focused on those found in the circulation, it has become clear that circulating CD8\(^+\) T cells targeting typical respiratory viruses, such as influenza, differ entirely with regard to phenotype and function from their counterparts found in the lungs, where the antigenic and inflammatory pressure is likely much higher than in the circulation. Also, because each organ system is targeted by distinct sets of viruses, it seems logical that each site requires its own specialised CD8\(^+\) T cell memory populations. Below, we review the literature pertaining to the CD8\(^+\) αβ T cell response in humans, highlighting the differences in differentiation processes between circulating and tissue-retained resident T cell populations.

SPECIFIC ATTRIBUTES OF CIRCULATING, ACUTE-PHASE HUMAN VIRUS-SPECIFIC αβ EFFECTOR CD8\(^+\) T CELLS
When virus-specific CD8\(^+\) T cells are activated during primary infection, they undergo pronounced changes in metabolism, phenotype and function. Naïve T cells are able to persist in an antigen- and inflammation-independent fashion via homeostatic proliferation, involving intermittent stimulation with self-antigen and cytokines, such as IL-7; however, human CD8\(^+\) T cells in the acute phase of infection lose this dependence \(^5,6\). In these acute phase activated cells, CD127 (the IL-7R\(\alpha\) chain) undergoes a rapid down-regulation \(^6,7\). Both IL-7 and TCR/anti-CD28 stimulation can induce IL-7R\(\alpha\) down-regulation in vitro. However, whereas TCR/anti-CD28 induces prolonged or permanent down-regulation, IL-7 alone induces a reversible decrease in IL-7R\(\alpha\) expression \(^7\). Transcriptome analyses of acutely in vivo primed, hCMV-specific human CD8\(^+\) T cells have confirmed this down-regulation of IL-7R\(\alpha\) at the level of mRNA transcripts, and revealed that this coincides with an up-regulation of transcripts coding for components of other common gamma chain cytokine receptors, such as IL-2 and IL-15 \(^8\). Concomitantly, a marked increase in enzyme transcripts involved in maintaining the supply of structural components for DNA synthesis and repair, in histone deacetylation, and in regulating cell cycle progression was noted, reflecting vigorous proliferation of the primed cells \(^8\). Murine models have revealed that naïve T cells
utilise a catabolic metabolism that relies strongly on lipid oxidation and autophagy for their maintenance, whereas activated T cells undergo a significant metabolic reprogramming towards an anabolic metabolism that involves macromolecular synthesis, glutaminolysis, Warburg metabolism, and the pentose phosphate pathway. Finally, activated murine T cells strongly up-regulate the expression of amino acid transporters, glucose transporters and transferrin transporters to promote the supply of essential components. Interestingly, the switch back to a catabolic metabolism, artificially induced with rapamycin or metformin, significantly enhanced memory cell formation in mice and macaques.

The receptor-type protein tyrosine phosphatase CD45 isoforms are among the most frequently used surface markers for distinguishing human CD8+ T cell subsets. Although they are involved in regulating the phosphorylation state of activating and inhibitory tyrosine residues on essential signalling molecules, little is understood of their specific role in regulating human CD8+ T cell activation and differentiation. The switch from CD45RA to CD45R0 expression is one of the first observable changes after T cell priming. Early in the acute phase of infection, human antiviral CD8+ T cells with different specificities (hCMV, EBV, HCV and HIV) are remarkably similar, displaying a CD45RA−CCR7−IL-7Rα−CD28+CD27+ phenotype. This is suggestive of a common activation pathway of CD8+ T cells regardless of the type of infection. The co-stimulatory receptors CD28 and CD27 are essential for the generation of primary and secondary antiviral T cell responses, and for the build-up of a diverse repertoire of virus-specific T cells. Not only are the signalling cascades initiated by these receptors crucial to the afore mentioned metabolic switch, they also ensure the survival of activated clones via autocrine IL-2 production and the induction of anti-apoptotic members of the B-cell lymphoma 2 family, such as B-cell lymphoma extra large and myeloid leukaemia 1. Furthermore, recently activated virus-specific human CD8+ T cells undergo a change in homing potential that is demarcated by the loss of the lymphoid tissue-homing molecules CCR7, CXCR4 (where CXCR is C-X-C motif chemokine receptor) and CD62L, and an increase in various chemokine receptors, such as CCR1, CCR5, CXCR3, CXCR6 and CX₃CR1, that mediate T cell trafficking to and past cellular barriers, such as epithelial and endothelial linings, towards sites of inflammation. In a similar fashion, murine T cells have also been described to lose the expression of CCR7 and CD62L upon activation. Also, it was recently shown that CXCR3 is highly expressed on murine influenza-specific T cells, seemingly recruited early during the primary response.

Acute phase human T cells possess various effector functions and highly express cytotoxic effector molecules, such as perforin and granzyme B, which translates into cytotoxic potential. In addition, these cells also produce ample amounts of IFN-γ, the capacity of which seems to increase after control of the virus in the memory or latency stage. In fact, polyfunctionality, or the capacity to produce combinations of IL-2, IFN-γ, TNF-α, MIP-1β and CD107a, was found to increase significantly for circulating HCV-specific T cells that had progressed to the memory or chronic phase of infection when compared to those circulating during the acute phase of infection.

During convalescence, when the viral loads start to decline and inflammation wanes, the circulating CD8+ T cell population reaches a peak with regard to numbers. Such large
populations of cells with high cytotoxic capacity are not only potentially harmful to tissue integrity and the health of the organism, ongoing proliferation would also constitute a tremendous metabolic demand. Several levels of control that govern population growth and T cell contraction following infection have been identified. Among these is the up-regulation of various co-inhibitory molecules, such as CTLA-4, which is recruited into the immunological synapse upon T cell activation, where it competes with CD28 for the shared ligands CD80, CD86 and CD275 expressed on APCs, to diminish CD28-mediated signalling \(^8,^{29,30}\). Furthermore, CTLA-4 is able to trigger the release of the potent T cell inhibitor IDO from dendritic cells (DCs), thus acting as an independent mechanism to control T cell activation and proliferation \(^31\). Other inhibitory molecules expressed by activated virus-specific CD8\(^+\) T cells are programmed cell death 1, killer cell lectin-like receptor G1, T cell Ig and mucin protein 3 and lymphocyte-activation gene 3 \(^8,^{16,32}\). Together with secondary co-stimulatory receptors, such as inducible T cell costimulator, their combined interactions are thought to be important regulators of T cell activity.

A second level of control is exerted by apoptosis, a process that is especially relevant during the contraction phase when T cell numbers decline. The intrinsic apoptosis pathway revolves around the balance between pro- and anti-apoptotic molecules from the B-cell lymphoma 2 family. As an example, cells deficient for the pro-apoptotic Noxa or B-cell lymphoma 2 interacting mediator of cell death (BIM) display a survival benefit over cells with a high or wild-type expression of these molecules during nutrient deprivation \(^33,34\). As mentioned above, IL-2 is known to be important for the sustenance of activated human CD8\(^+\) T cells by maintaining the expression of anti-apoptotic myeloid cell leukaemia-1, and indeed, IL-2 deprivation resulted in BIM-dependent cell death \(^35\). Furthermore, circulating hCMV-specific CD8\(^+\) T cells during the acute phase of primary infection highly up-regulate expression of CD95, CD95 ligand, and TRAIL, another death receptor ligand, indicating a role for the extrinsic pathway in regulating T cell pool size as well \(^8\).

**PHENOTYPE AND FUNCTION OF CIRCULATING HUMAN VIRUS-SPECIFIC αβ CD8\(^+\) T CELLS AFTER THE ACUTE PHASE**

A number of different cell surface molecules can be used to separate functionally distinct human CD8\(^+\) T cell subsets \(^36,37\). Although acute phase antiviral CD8\(^+\) T cell phenotypes are strikingly similar, regardless of their specificity, memory (or latent) populations show differences with respect to phenotype and function according to the virus they target. A rough segregation between antigen-primed CD8\(^+\) T cell subsets can be made based on CD28 and CD27 expression \(^14\). While CD28\(^+\)CD27\(^+\) primed cells express low levels of CD95 ligand, perforin and granzyme B, this is markedly increased in CD28\(^-\)CD27\(^-\) cells, which translates into superior cytotoxic capacity \(^38,39\). CD28 and CD27 expression appears to be lost in an orderly fashion by virus-primed CD8\(^+\) T cells in vivo (Figure 1). Given the presumed temporal context of these changes, where the expression of CD28 is generally lost before that of CD27, Appay et al. defined CD28\(^-\)CD27\(^+\) as ‘early’ differentiated CD8\(^+\) T cells, while CD28\(^-\)CD27\(^+\) T cells exist in an ‘intermediate’ state and CD28\(^-\)CD27\(^-\) T cells represent a ‘late’ differentiation state \([18]\).
Further segregation within the late differentiated population can be made based on the (re)expression of CD45RA by CD27⁻ non-cycling cytolytic CD8⁺ T cells (Figure 1)³⁸,³⁹. Because of this cytotoxic capacity, and the entire functional profile of these cells, we define CD28⁺CD27⁻ cells as ‘effector-type’ cells.³⁸,⁴⁰ In addition to this established hierarchy of CD28 and CD27 expression, a minute CD95⁺LFA-1⁺CXCR3⁺ population (where LFA-1 is lymphocyte function-associated antigen 1) among CD45RA⁺CD28⁺CD27⁺CCR7⁺ cells (normally considered to be naïve) has been identified as putative long-lived memory stem cells.⁴¹,⁴² Furthermore, Romero et al. more recently defined a CD28⁺CD27⁻ memory CD8⁺ T cell subset with similar attributes to CD28⁺CD27⁺ early cells, for example concerning high proportions of mRNA transcripts coding for IL-7Rα, while granzyme B transcripts were absent in both populations. This suggests an additional differentiation pathway affecting a minor population of CD8⁺ T cells that lose CD27 prior to CD28 ³⁹.

Circulating virus-specific CD8⁺ T cells are found to preferentially exist in one of these states. For example, influenza-, RSV- and BKV-specific (where BKV is polyomavirus BK) memory T cells, exist mainly in the CD45RA⁻CD28⁺CD27⁺ early memory subset, and express no, or negligible, amounts of perforin and granzyme B.⁴³–⁴⁶ CD8⁺ T cells specific for epitopes of latent EBV proteins also exist primarily in the early subset, but cells targeting epitopes of lytic EBV proteins mostly display a further differentiated phenotype.⁴⁷ Circulating hCMV-specific CD8⁺ T cells often exist in the CD45RA⁺/⁻CD28⁻CD27⁻ effector-type subsets (Figure 1)¹⁴,⁴⁸. It must be noted that these correlations between virus specificity and CD8⁺ T cell properties are generalisations and may occasionally vary between donors.¹⁷

Although the early memory populations resemble effector T cells in the acute phase of infection with regard to their expression of CD45RA, CD28 and CD27, they are distinct with regard to other phenotypic and functional aspects. First, in contrast to the acute phase T cells, the vast majority of the CD45RA⁻CD28⁺CD27⁺ early cells express IL-7Rα, which also extends to early differentiated virus-specific CD8⁺ memory T cells, with its expression declining among the further differentiated populations.⁶,³⁹,⁴⁶. While most CD45RA⁺CD28⁺CD27⁺ acute phase CD8⁺ T cells carry perforin and granzyme B, and have potent cytotoxic capability, this is not the case for CD45RA⁺CD28⁺CD27⁺ early differentiated memory cells.³⁸,³⁹. As mentioned above, among the memory subsets, cytotoxic capacity is particularly pronounced in the CD45RA⁺/⁻CD28⁻CD27⁻ effector-type subsets. Thus, there seems to be an inverse correlation between IL-7Rα expression and cytotoxic potential.

Other differences between the early and late memory subsets involve expression of granzyme A, K and M, serine proteases that have also been implicated in mediating cell death.⁴⁹,⁵⁰ Additional functions comprise non-cytotoxic control of viral replication and reactivation via direct cleavage of host and viral proteins necessary for the viral life cycle.⁵¹,⁵² Human granzyme A and murine granzyme K may also trigger cytokine release from neighbouring immune cells.⁵³,⁵⁴ While granzyme A is expressed by all non-naïve (virus-specific) CD8⁺ T cell subsets, granzyme M is also expressed by a significant proportion of naïve CD8⁺ T cells. The expression of both molecules increases as T cells advance through the differentiation states, reaching a peak frequency of expression among
CD45RA+CD28−CD27− effector-type cells. In contrast, the expression of granzyme K is restricted to the early and intermediately differentiated subsets, and decreases as differentiation progresses being nearly absent among the late differentiated populations. As such, latent EBV protein-specific cells frequently express granzyme K, and little granzyme B, while late hCMV-specific cells frequently express granzyme B and far less often granzyme K.

Figure 1. Human CD8+ (memory) αβ T-cell differentiation states. A. Upon activation, naive CD8+ T cells give rise to virus-specific CD8+ effector T cells that universally express CD28 and CD27, regardless of the type of viral infection. B. Early differentiated ‘central-memory’ cells approximate naïve T cells with regard to phenotype, but not to function, especially concerning cytokine production capacity: central memory cells produce much more IL-2, but also IFN-γ and TNF-α. CCR7 expression would enable homing to secondary lymphoid tissues. C. Early differentiated CCR7+ memory cells that are otherwise functionally approximating the central-memory population. D. An alternative early CD28−CD27− population described by Romero et al. appears more similar in function to CD28−CD27− (C) cells than to other CD27− cells that generally carry granzyme B. E. Intermediately differentiated memory cells (only HIV-specific cells have been reported in this class to date). F. CD45RA− late differentiated effector-type cells. G. CD45RA+ late differentiated effector-type cells. Signs indicate a degree of heterogeneity with regard to the expression of the respective molecule.

Human CD8+ memory T cell subsets also differ with regard to their cytokine production profiles. While early cells frequently produce IL-2 upon in vitro stimulation, this function diminishes as they enter more advanced differentiation states, until it is nearly absent in the CD45RA−CD28−CD27− effector-type subset. In line with this, substantial numbers of
circulating influenza, BKV- and latent EBV protein epitope-specific CD8+ T cells produce IL-2, whereas this is less frequently observed for hCMV-specific CD8+ T cells \(^{46,58}\). In contrast, IFN-\(\gamma\) and TNF-\(\alpha\) are produced by the majority of total non-naïve, as well as influenza-, BKV-, EBV- and hCMV-specific cells, apparently regardless of their differentiation state \(^{38,46,58,59}\). Finally, when compared with hCMV-specific CD8+ T cells, influenza- and BKV-specific cells infrequently produce the chemokine MIP-1\(\beta\), or express the marker of degranulation, CD107a upon stimulation \(^{46,58,59}\).

Other functional differences between the circulating human T cell populations concern migratory potential. Although CCR7 and CD62L expression is lost early after T cell priming, CD8+ T cells can re-express these molecules upon antigenic recall in the presence of IL-2, IL-15 or IL-21, thereby restoring their ability to migrate to lymphoid tissues \(^{60,61}\). Yet, when examining the circulating memory CD8+ T cell pool, only a subset of antigen-experienced CD8+ T cells are CCR7\(^+\), virtually all being CD45RA\(^-\)CD28\(^-\)CD27\(^+\) early cells that have been denominated as ‘central-memory’ T cells \(^{36,39,46,62}\). It is not clear if these cells differ functionally from CCR7\(^-\)CD28\(^-\)CD27\(^+\) cells, apart from being able to migrate to lymphoid tissues. Also for virus-specific cells, CCR7 is expressed only by a minor proportion of mainly early differentiated populations, such as RSV-, BKV- and influenza-specific cells, and rarely by further differentiated EBV lytic protein- and hCMV-specific cells \(^{43,46}\). Another lymphoid tissue-homing receptor, CXCR4, which is involved in T cell migration to bone marrow, is also frequently expressed by naïve cells, and its expression is progressively lost as cells advance in their differentiation state \(^{63}\). Furthermore, we have shown that influenza- and BKV-specific cells display a particularly high expression of CXCR3, which was less often observed for lytic EBV protein epitope-specific cells, and rarely for hCMV-specific cells \(^{46}\). This suggests that the CXCR3\(^{hi}\) expression state is a trait of early differentiated memory cells \(^{46}\). In mice, CXCR3 also mediates T cell migration towards reactive LNs as well as sites of inflammation in a general sense. At the reactive LN, the migrating T cells were found to control further T cell expansion by eliminating locally active APCs \(^{64}\). In contrast to the early memory T cells, late effector-type human memory cells far more often display a high expression of CXCR1, mediating homing to the potent inflammatory chemokine IL-8 \(^{63,65}\). CX3CR1 is also mainly expressed by late total- and hCMV-specific CD8+ T cells and binds to fractalkine expressed on inflamed endothelium. Interestingly, CX3CR1\(^{hi}\) hCMV-specific cells may mediate endothelial cell damage and have been implicated in the process of plaque formation and atherosclerosis \(^{26}\). Altogether, the CD45RA\(^+\)/CD28\(^-\)/CD27\(^-\) effector-type cells are very capable of homing to sites of active inflammation, while the naïve and CD45RA\(^-\)/CD28\(^-\)/CD27\(^+\) early subsets more often express markers involved in homing to lymphoid tissues.

**GENERATION OF DISTINCT VIRUS-SPECIFIC CD8\(^+\) MEMORY T CELL SUBSETS**

In contrast to the vast majority of acute phase effector cells, memory cells have an extended life span. Studies with in vivo labelling of cells with deuterated glucose have shown that hCMV-specific (CD45RA\(^-\)/CD28\(^-\)/CD27\(^-\) effector-type) CD8+ memory T cells have
a much slower uptake of glucose than the general population of CD8+ T cells, suggesting a low turnover rate that is supported by only very few cells expressing HLA-DR, CD38 and Ki-67, indicating that they are not activated or proliferating. Furthermore, hCMV-specific clonotype populations may remain stable for periods spanning more than 5 years. CD45RA+CD28−CD27− effector-type cells seem to have undergone a further selection process, as suggested by the finding that these cells are highly restricted in their TCR Vβ repertoire, which was found to narrow down with age (or as time elapsed since primary infection). An important determinant of this process seems to involve the avidity of T cells for their peptide-MHC counterparts, because the CD45RA+CD28−CD27− effector-type T cells circulating in elderly individuals display a skewing towards decreased-avidity antigen binding.

As stated above, circulating hCMV-specific CD8+ memory T cells far more often undergo the differentiation process toward the late CD45RA+/−CD28−CD27− states than most of the CD8+ T cells targeting other viruses. An explanation for this phenomenon may be found in the fact that hCMV uniquely uses the endothelial and smooth muscle cells of the blood vessels as a primary target of latent infection, a niche from which it frequently reactivates even in healthy individuals. Further evidence comes from hCMV reactivations in kidney transplant recipients with a predominantly CD28+CD27+ early hCMV-specific CD8+ T cell phenotype prior to transplantation, where hCMV reactivation resulted in the accumulation of CD28−CD27− T cells, as well as in dropping numbers of CD28+CD27+ cells. In fact, hCMV infection has such a great impact that seropositive individuals have significantly more total CD8+ T cells, as well as CD45RA+/−CD28−CD27− effector-type cells, than uninfected individuals. Similarly, EBV-specific CD8+ T cell populations targeting lytic protein epitopes are larger in size and, as mentioned before, exist more frequently in a CD27− state than populations specific for EBV latent protein epitopes, further supporting a role for greater antigenic exposure, and likely also a more intense inflammatory milieu for the generation of these subsets. Indeed, specific environmental cues that are able to induce the loss of CD27 expression have been identified. For example, the binding to its ligand CD70, which is expressed by APCs and activated T cells, results in the removal of this co-stimulatory molecule from the membrane. Furthermore, prolonged antigenic stimulation alone, or in the presence of IFN-α, induces the loss of expression of both CD28 and CD27 in vitro. Other environmental cues, such as IL-15, have been identified to trigger the conversion from a CD45RA−CD27− late to a CD45RA+CD27− differentiation state.

HCV- and HIV-specific CD8+ memory T cells predominantly display a CD45RA+CD28−CD27+(early) and, respectively, CD45RA+CD28−CD27+(intermediate) phenotype. Both viruses are known to replicate at such a high rate that they rapidly generate mutant offspring that are not (yet) recognised by the prevailing CD8+ T cell population. Thus, T cells that do recognise viral epitopes will control only non-escaped viruses, but will never be able to efficiently control viral infection its entirety. Furthermore, both viruses utilise strategies to disrupt CD4+ T cell and APC-CD8+ T cell interactions at various levels, ultimately resulting in diminished or impaired CD8+ T cell responses that may be reflected in these less-
advanced differentiation states and their absence among the late differentiated memory subsets, which would require ongoing T cell stimulation of previously recruited cells. Yellow fever and smallpox vaccinations using a live attenuated strain 17D (yellow fever live attenuated strain 17D) and Dryvax, respectively, induce memory cells with rather unexpected phenotypes, whereas the acute phase cells resemble the other virus-specific cells, the memory stage cells adopted a CD45RA+CD28+, not truly CD27-negative but rather ‘dim’ state, while strikingly carrying granzyme B and being CCR7-. Also, these cells are highly polyfunctional with regard to cytokine production. Possibly, vaccination-induced CD8+ T cell phenotypes may progress along a specific differentiation pathway. It should be stressed here, however, that it is unresolved if the putative progression from the early to the late differentiation state is a truly ordered linear differentiation program or, alternatively, depending on the environmental cues (e.g. a combination of antigenic load, co-stimulatory receptor ligands [CD70], cytokines, and in the case of yellow fever live attenuated strain 17D and Dryvax, likely also vaccination adjuvants), specific phenotypic and functional changes may be induced. In this regard, the clear relation between phenotypic markers and function found in circulating primed CD8+ T cells is lost when analysing virus-specific T cells in tissues, which will be discussed in the next section.

TISSUE-RESIDENT αβ CD8+ T CELLS: PHENotypically AND FUNCTIONALLY DIFFERENT FROM CIRCULATING T CELLS

In comparison to the circulating pool, human LNs contain substantially more CD27+ early T cells-, as well as CCR7-expressing memory T cells, while they hardly contain any CD27-granzyme B+ late memory cells. Furthermore, in the LN hCMV-specific T cell numbers are significantly lower than in the circulation, and also when compared with EBV-specific cell numbers (targeting both latent and lytic protein epitopes). This may be attributed to the fact that the majority of the hCMV-, but less so EBV-specific cells in the circulation, exist in the late differentiation states, and express homing receptors that mainly target non-lymphoid tissues, such as the aforementioned CX3CR1, but not CCR7 or CD62L. Interestingly, those relatively few hCMV-specific CD8+ T cells in the LNs express CCR7 and CD27 significantly more often when compared with their circulating counterparts (Figure 2). The LNs also contain higher frequencies of polyfunctional, multi-cytokine-producing cells than the peripheral blood. Both lytic and latent epitope-specific CD8+ T cell frequencies during the acute phase of infection in patients suffering from acute infectious mononucleosis are generally lower in the tonsils when compared with that in the circulation, and also more often comprise CD45RA+CD28+CD27+ cells. However, during latency, cell frequencies are higher in the tonsils and these cells are significantly more often activated as judged by their expression of CD38. A possible explanation is that during latency, EBV reactivates from the oropharynx of otherwise healthy carriers, as such contributing to the accumulation of EBV-specific memory cells in the tonsils over time. Lastly, although there is some controversy with regard to CCR7 expression, hCMV-specific CD8+ T cells in human bone marrow were reported to be quite similar to their circulating counter parts.
CD8+ T cells found in non-lymphoid tissues differ substantially from circulating virus-specific CD8+ T cells. For example, CD8+ T cells residing at the healthy human dermal–epidermal junction often exist in a CD45RA+CCR7− differentiation state, as such resembling the late effector-type subset found in the circulation, but remarkably do not express perforin. Nevertheless, the bulk of these cells produce IL-2, IFN-γ and TNF-α upon stimulation. Furthermore, these skin T cells are expressing specific homing receptors, such as CLA, CCR8, CCR4, CXCR6 and CCR6. Similar CD8+ T cell phenotypes have been observed in the perivascular spaces in the human brain, and specifically in the corpus callosum. While these cells also predominantly display CD45RA+/−CD27−CCR7− late phenotypes, they too express neither perforin nor granzyme B. Moreover, the majority of these brain CD8+ T cells express IL-7Rα, suggesting an IL-7 dependence, which sharply contrasts with late differentiated circulating T cells. High frequencies of late differentiated cells have also been observed in healthy human livers, however, while these cells express CXCR4, data on effector molecule- or IL-7Rα expression are lacking. In line with the circulation phenotypes, CD8+ T cells isolated from human lung specimens predominantly exist in a CD45RA−CD28−CD27−CCR7− late differentiation state, and also carry granzyme B. Nevertheless, the lung-derived cells were found to contain this cytotoxic effector molecule significantly less often than their counterparts in the circulation. Human lung CD8+ T cells were also found to frequently express CCR5, CXCR3, and similar to the liver cells, also CXCR4. Less extensive phenotypic analyses are available on human intestinal CD8+ T cells found in the jejunum, ileum and colon tissue samples, which were described to predominantly display a CD45R0−CCR7− phenotype, regardless of whether they derived from small bowel or colon. Furthermore, the small bowel cells express CCR9 and the α4β7 integrin. With regard to the latter, it must be noted that this may not be intestine-specific since it is expressed by all the circulating non-naïve CD8+ T cell subsets, while it is also involved in mediating T cell migration into various other tissues, among which the CNS. Taken together, these data suggest that in tissue, the hierarchy of CD8+ T cell differentiation appears to differ from that observed in the circulation.

While the specific tissue T cell phenotypes argue against contamination from non-tissue-resident circulating cells present in the microcirculation of these organs, the distinction between true tissue-resident T cells, that is those cells that reside in the organ or tissue for a prolonged, if not indefinite period of time, and cells that are simply passing through, is important. Therefore, specification of T cell localisation by immunohistochemistry, as well as characterisation of the expression of specific T cell adhesion and retention markers is required. In this regard, lung and intestinal CD8+ T cells are frequently located intra-epithelially and express CD103, the αE chain of the integrin αEβ7 complex known to mediate T cell tethering to E-cadherin expressed on epithelial cells.

The unexpected phenotypic and functional differences also extend to virus-specific CD8+ T cells residing in the tissues. In line with a more intense antigenic exposure of lung T cells to typical respiratory viruses, CD8+ T cells specific for influenza and RSV were found in significantly increased frequencies in this site when compared with their frequencies in the circulation that are generally low. While the influenza- and RSV-
specific CD8+ T cells in the circulation predominantly exist in a CD45RA−CD28+CD27+ early differentiation state, their epitope-specific counterparts in the lung display a more advanced CD45RA−CD28−CD27+/− phenotype (Figure 2). In contrast, EBV- and hCMV-specific cells in the lungs have a phenotype similar to their circulating counterparts 91. Although a proportion of the overall late CD8+ T cell subsets in the lungs carry granzyme B, this is not the case for the influenza and RSV-specific memory cells. However, for influenza-specific cells, the expression of granzyme B is rapidly induced upon stimulation with their cognate peptide, inducing a potent cytotoxic capacity 91,96. A similar mechanism of controlled cytotoxic potential may also affect T cells residing in the skin and brain, which also rarely carry cytotoxic effector molecules despite their seemingly late phenotypes. Indeed, the tight regulation of cytotoxicity may serve to prevent T cell-mediated damage that would disrupt tissue integrity.

Figure 2. Differences between human virus-specific CD8+ T cells in the circulation and tissues. Influenza-specific CD8+ T cells in the circulation are virtually all displaying an early differentiated phenotype. In contrast, their counterparts in the lung, which reside intra-epithelially and are CD103+, exist in a seemingly more advanced phenotype. Yet, they were not readily carrying granzyme B. However, this is rapidly up-regulated upon stimulation (top). In contrast to the circulation, LNs barely contained late differentiated, granzyme B-expressing memory cells. Also, in contrast to what is seen in the circulation, only small populations of hCMV-specific cells are found in this compartment. Interestingly, these cells were far more often displaying an early phenotype in comparison to what is seen in the circulation (bottom).
TRANSCRIPTION FACTORS: CONTROLLING HUMAN CD8+ αβ T CELL DIFFERENTIATION

The search for transcription factors involved in CD4+ T_h 1-cell differentiation, similar to the T_h2 fate-determining transcription factor GATA3, led to the discovery of the T-box transcription factor expressed in T cells (T-bet) in mouse and human 97. The expression of T-bet in both species is restricted to CD4+ T_h 1 cells, NK cells and CD8+ T cells, in which it induces the expression of IFN-γ by directly binding to the Ifng promoter through its T-box DNA-binding domain 97,98. In contrast to what is observed in murine CD4+ T cells, IFN-γ production is not completely abrogated in T-bet-deficient murine CD8+ T cells, a finding that preceded the discovery of another T-box family member, eomesodermin (Eomes), which is highly homologous to T-bet and is also involved in regulating type 1 T cell responses 98,99. T-bet and Eomes show a high degree of functional redundancy with regard to the induction of type 1 responses. However, while T-bet seems to be particularly important for the generation of effector cells during the acute phase of infection, Eomes is important for the formation of memory cells and secondary responses in mice 100,101.

Human circulating CD8+ T cell memory subsets display different T-bet/Eomes expression patterns, whereas few naïve T cells express these transcription factors, T-bet is abundantly expressed by the CD45RA+ effector-type cells. These latter cells also highly express Eomes; however, the less differentiated memory subsets express proportionally more Eomes than T-bet 102.

Human virus-specific CD8+ T cells in the circulation of healthy people have distinct patterns of T-bet/Eomes expression, with hCMV-specific memory CD8+ T cells predominantly displaying a T-bet_hi/Eomes_hi/lo phenotype. EBV lytic protein-specific cells also often expressed a T-bet_hi/Eomes_hi phenotype, however, at lower frequencies than hCMV cells and predominantly displayed a T-bet_int/Eomes_hi phenotype. Furthermore, influenza- and BKV-specific CD8+ T cells, virtually all existing in an early memory cell differentiation state, were found to uniformly display a T-bet_lo/int/Eomes_lo phenotype, suggesting that while Eomes is needed for memory cell generation and expansion in mice, it may not be required for the persistence of these human cells in this compartment after viral control (Figure 3) 46. Furthermore, T-bet MFI correlates positively with multimer (fluorescent HLA class I complexes carrying specific viral peptides) frequency, supporting a role for repetitive stimulation in generating memory cells with a type 1 functional profile 103. Taken together, the different memory subsets each display highly specific T-bet/Eomes expression patterns, which are likely involved in sustaining their specialised functional profiles.

Studies on murine T-bet and Eomes have revealed that they are integrated into an intricate network of other transcription factors where the balance of their combined (inter) actions ultimately determines CD8+ T cell lineage differentiation decisions, as well as the CD8+ T cell memory- or effector-type memory differentiation state 1. Transcriptome analysis of hCMV-specific CD8+ T cells progressing from acute effector cells during primary infection to effector-type cells after control of the virus confirmed that both T-bet and Eomes were strongly up-regulated during the acute phase. T-bet and Eomes expression continued to remain high after convalescence 8. Also, these cells up-regulated the expression of B lymphocyte-induced maturation protein 1 (BLIMP-1), a transcription
factor that is induced by IL-2 stimulation in mouse CD8+ T cells. Because BLIMP-1 in turn inhibits IL-2 production by the respective cells in a feed backward manner, it may be important for terminating T cell responses. Interestingly, hCMV-specific CD8+ T cells were noted to also strongly up-regulate mRNA transcripts coding for a previously unknown homolog of BLIMP-1, Znf683, or homolog of BLIMP-1 in T cells (Hobit), during the acute phase of infection and after convalescence, particularly within effector-type memory CD8+ T cell populations. However, while the functions of human Hobit in CD8+ T cells are currently being elucidated, the expression of this transcription factor in mice was found to be restricted to NKTs. Here, it seems to have an inhibitory effect on IFN-γ production, and functions to induce granzyme B expression upon antigen-independent stimulation of these cells. Curiously, Hobit is not found in conventional murine CD8+ T cells, thereby nicely illustrating how specific evolutionarily conserved transcription factors may differentially affect the T cell response between mouse and human species.

IN SUMMARY

Studies comparing different virus-specific CD8+ T cells have provided important clues as to the distinct alterations that human T cells undergo in response to exposure to various viruses. Although initial studies have suggested a linear type of differentiation model in which phenotypic and functional changes are co-regulated in a seemingly linear, controlled fashion, analysis of tissue-resident T cells show that the linear differentiation model may be a simplification. Adaption to particular tissues may not only lead to specific mechanisms for homeostatic maintenance, but also to transcriptional and/or translational repression of effector molecules, thereby preventing immunopathology but warranting immediate responses in case of re-infection. Finally, certain transcription factor expression patterns

Figure 3. Distinct expression patterns of T-bet and Eomes per virus-specific memory population. Simplification of the positions of the different virus-specific populations (coloured spots) in a dot plot displaying T-bet fluorescence intensity plotted on the X-axis against Eomes fluorescence intensity on the Y-axis. In line with the substantial functional differences between influenza MP1/NP- and BKV-specific CD8+ T cells (mainly early differentiated) depicted in green, EBV lytic protein-specific CD8+ T cells (more differentiated) depicted in blue, and hCMV pp65/IE-specific CD8+ T cells (predominantly late differentiated) CD8+ T cells depicted in red, these cells also display distinct T-bet/Eomes expression patterns.
uniquely vary between mouse and human species, implicating that the human immune system has indeed developed its particular means to provide immunity and maintain latency to human-specific pathogens.

ACKNOWLEDGEMENTS

Our research is in part supported by the Dutch Kidney Foundation (IP11.32).
REFERENCES


BLOOD AND BEYOND: PROPERTIES OF CIRCULATING AND TISSUE-RESIDENT HUMAN VIRUS-SPECIFIC αβ CD8+ T CELLS


