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A spatial view of the CD8⁺ T-cell response: the case of HCV

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SUMMARY

In viral infections, a memory T-cell population comprises multiple subtypes of cells, distributed in diverse anatomic compartments and possibly re-circulating among them. Accordingly, memory T cells display distinct phenotypes and functions, depending on the nature of the infecting virus, the anatomic location of the infection, and the differences between the sites of active infection and T-cell collection. This paper explores the body compartments where virus-specific CD8⁺ T cells have been found during chronic hepatitis C virus infection, describes the cells’ memory qualities, and discusses how they are spatially regulated, in comparison with other human viral infections. Understanding the role of compartmentalization and diversity of HCV-specific memory T-cell subsets may be the key to developing effective immunotherapies.

TOWARDS A CELLULAR DEFINITION OF CD8⁺ T-CELL MEMORY

Leafing through the pages of most immunology textbooks, it is easy to run into the classic definition of immunological memory, which is the ability to respond faster and more effectively on a second encounter with the same pathogen. Conversely, it is not as easy to find a univocal definition of memory T cell. It is referred to as a cell capable of inducing long-term protection or defined as a cell having the capacity to survive after antigen elimination and still simply described as an antigen-experienced cell (i.e. a cell that has been primed and is no longer naïve). This confusion is mainly related to the effort of drawing semantic and kinetic generalizations from the paradigm of a typical CD8⁺ T-cell response to acute viruses. According to this model, when a naïve CD8⁺ T cell encounters its cognate antigen, it becomes activated, dividing and differentiating into many effector CD8⁺ T cells that are able to kill infected cells and/or secrete cytokines that inhibit viral replication. Once infection is cleared, most of the effector CD8⁺ T cells die, leaving a numerically stable long-lived population of virus-specific CD8⁺ T cells that can undergo rapid re-activation after re-infection[1]. In other words, CD8⁺ T cells are effectors when an antigen is present and become memory when the antigen is eliminated.

It is possible, however, that some effector functions persist after antigen elimination, whereas memory cells ensuring protection are maintained in the presence of an antigen. This is the case of latent infections, such as those caused by Epstein–Barr virus (EBV) or
cytomegalovirus (CMV), where permanent control of infection is achieved but low viral loads may intermittently be present. In this setting, antigen-specific CD8+ T cells might be better defined as “resting vigilant effector cells” because of their ability to continuously control viruses in latently infected cells [2]. It is also possible that when antigen persists, mixed antigen-experienced cell populations have the potential to achieve different levels of memory maturation without ever becoming “true” memory cells [3]. This is the case of chronic infections, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infections, where antigen-specific CD8+ T cells may be effector-type cells or may form a population comprising effector and memory T cells together, possibly with cells at intermediate states of differentiation.

The generation of memory is believed to occur via one of two pathways. There is a classic, linear pathway whereby memory populations emerge directly from effector precursors that have been initially primed. Alternatively, there is a branched pathway, in which memory cells are generated in parallel with effector populations during the initial priming, and both continue on to their predetermined fates after the primary immune response [4,5]. Several variations of these models have been proposed based on differential T-cell activation levels during priming [6,7].

In the last decade, several molecules have been proposed to classify antigen-experienced CD8+ T cells. These include human leukocyte antigens (HLA-DR), enzymes (e.g. CD38), receptors involved in T-cell activation (e.g. CD45RA, CD45RO), costimulation (e.g. CD27, CD28) and regulation (e.g. CD69, killer cell lectin-like receptor G1 (KLRG1), programmed death-1 (PD-1)), cytokines (e.g. IL-2, IFN-γ, TNF-α) and cytokine receptors (e.g. IL-7Rα also known as CD127), chemokine receptors (e.g. CCR7) and adhesion molecules (e.g. CD62L, CD57), cytolytic proteins (e.g. perforin, granzyme K and B), oncoproteins (e.g. Bcl2), chromosome regions (e.g. telomeres). Beyond the exact recognition of naïve T cells (CD45RA+ CCR7+ CD27+ CD28+), none of these molecules, alone or in combination, allows to identify cell categories that are common to every human infection. For instance, CD69, CD38, and HLA-DR, which are used to identify effector T cells, are often reexpressed by memory cells that have re-encountered antigen [8]. CCR7 and CD62L, which are widely used to subdivide CD45RA− cells into central memory (T_{CM}; CCR7+ CD62L^{high}) and effector memory (T_{EM}; CCR7− CD62L^{low}) [9], can only be applied to “resting” cells (i.e. cells that are not engaged in antigenic stimulation). CD27 and CD28, which are very useful to discriminate early (CD27+ CD28+), intermediate (CD27+ CD28−), and late (CD27− CD28−) antigen-experienced CD8+ T-cell subsets under persistent antigenemia [10], cannot easily distinguish among naïve, early differentiated, and true memory CD8+ T-cell populations because all of them co-express CD27 and CD28. IL-7Rα (CD127) and KLRG1, which are the most faithful indicators of the number of T-cell receptor (TCR) interactions, have not been found to parallel the duration of antigen exposure in some human viral infections. IL-7Rα, in particular, is retained on the surface of peripheral virus-specific CD8+ T cells during persistent HCV infection [11], somehow contradicting its meaning in mouse models as an early indicator of cells that are destined to cytokine-dependent long-term survival in the absence of antigen [12]. As for KLRG1, it is not expressed by peripheral virus-specific CD8+ T cells of persistently HCV-infected patients, despite its straight linkage with antigen-experienced CD8+ T cells that have undergone a large number of cell divisions, that have immediate effector functions and that cannot undergo further clonal expansions [13]. Granzyme K, granzyme B, IL-2, IFN-γ, and TNF-α are not necessarily expressed in a correlated fashion. Granzyme K production is a feature of CCR7+ CD27+ CD28+ CD8+ T cells, while granzyme B production is prevalent in CCR7− CD27− CD28− CD8+ T cells [14,15]. IFN-γ and TNF-α secretion, which was originally attributed to T_{EM} [9], has been found to be equally good in T_{CM}, upon re-
stimulation with cognate antigen [16,17]. IL-2 production remains a property of T<sub>CM</sub> but is the most sensitive to inactivation during chronically evolving viral infection, where a selected loss of specific functions occurs progressively and the boundary line between T<sub>CM</sub> and T<sub>EM</sub> vanishes [18]. Even CD45RA, which is the major marker of naïve cells, has been found on antigen-experienced cells. This phenotype “reversion” has been long associated with terminally differentiated memory CD8<sup>+</sup> T cells with limited proliferative potential and heightened sensitivity to apoptosis. Only recently, its finding on highly proliferating virus-specific CD8<sup>+</sup> T cells during the memory phase of vaccine-induced immune responses [19] has demonstrated that it may also represent a signal of highly functional memory CD8<sup>+</sup> T cells generated after acute viral infections.

Several other qualities have been proposed to delineate a memory T cell on a functional level, but additional layers of complexity have come into sight. High proliferative potential, multipotency (memory T cell can maintain identity and, at the same time, can rapidly re-activate antiviral effector functions upon re-infection), long-term survival, and self-renewal in the absence of antigen via IL-7-driven and IL-15-driven homeostatic turnover are certainly unique for memory cells in a resting state [6]. However, there is not a stereotype combination of cytokine profile, proliferative extent, and cytotoxic potential that allows to equate an antigen-experienced cell with an effector or a memory cell in a state of dynamic T-cell activation. In this context, the model proposed by Appay et al. (progressive decrease of proliferative capacity and progressive increase of cytotoxic potential moving from early to intermediate to late differentiation) [10] has provided an important conceptual advance, which, however, does not fully encompass the picture of antigen-experienced CD8<sup>+</sup> T cells in vivo. As we will discuss later, a broad range of tissue-specific variations exists, and HCV infection is its best example.

**MEMORY T-CELL FACETS: AS MANY AS T-CELL COMPARTMENTS**

In recent years, the development of techniques to directly study antigen-specific T cells has altered the dogma that memory T cells persist only in secondary lymphoid organs, such as spleen and lymph nodes. Non-lymphoid or tertiary tissues (liver, gut, lung, bone marrow, etc.) have been shown to hold large memory T-cell pools and to impart to them distinct tissue-specific properties [20,21]. The connection between memory T-cell homing and memory T-cell properties was evident since the original classification by Sallusto et al. [9]. T<sub>CM</sub> and T<sub>EM</sub> subsets were found to have distinct tissue distributions, with T<sub>CM</sub> residing primarily in lymphoid sites and in peripheral blood and with T<sub>EM</sub> predominating in non-lymphoid and mucosal compartments [20,22]. Further studies have demonstrated that the anatomic dichotomy between T<sub>CM</sub> and T<sub>EM</sub> does not apply to multiple antigen-specific models in vivo. For instance, CCR7<sup>+</sup> CD62L<sup>+</sup> T<sub>CM</sub>-phenotype cells can be found in non-lymphoid sites, a mixed CCR7<sup>-</sup> CD62L<sup>-</sup> phenotype may be expressed by memory CD8<sup>+</sup> T cells [23], and memory CD8<sup>+</sup> T cells in the gut may resemble neither T<sub>CM</sub> nor T<sub>EM</sub> CD8<sup>+</sup> Tcells isolated from spleen or blood [24]. In terms of function, it has been found that non-lymphoid-derived memory CD8<sup>+</sup> T cells possess highly constitutive cytotoxic potential, whereas splenic memory CD8<sup>+</sup> T cells, despite a CD62L<sup>-</sup> phenotype, are poorly lytic directly ex vivo [22], and that entry of blood-borne non-lytic memory cells into non-lymphoid tissues results in gaining of granzyme B contents and cytotoxicity and downregulation of CD27 [25]. Memory CD8<sup>+</sup> T cells may also convert from T<sub>EM</sub> to T<sub>CM</sub> in most tissues, including non-lymphoid tissues such as the liver [26]. The rate of this memory CD8<sup>+</sup> T-cell differentiation is different in different locations, although it is not currently known whether these differences represent biases in migration or tissue-specific influences on memory T-cell differentiation. Altogether, these observations support the idea that memory cell phenotype and function are plastic and are dynamically modulated as a result of migration to and location in different anatomic compartments. Unfortunately, much of
this information comes from murine models because accessing human tissues is not always possible and determining the time from acquisition of a human infection is rarely precise. In this respect, HCV infection is representative given that it is not reproducible in a small animal model and is clinically silent in the majority of individuals. Nevertheless, its impact on the anatomical distribution and quality of T cells is crucial. For example, while the frequency of HCV-specific T cells in the blood is low, liver inflammation persists; while hepatocytes are the major site of viral replication, a broad spectrum of immune-mediated extrahepatic diseases complicates chronic infection; and, while HCV-specific CD8+ T cells do develop, memory differentiation is disrupted and appropriate functions are not achieved [27].

The strong influence that the environment has on the differentiation of antigen-specific CD8+ T cells begins at the time of the primary T-cell response already. Whereas low levels of tissue inflammation, such as those that are present during dendritic cells vaccination, shift the balance toward enhanced generation of memory precursors, high levels of inflammation, such as during infections, lead to rapid and preferential expansion of terminally differentiated effector cells [28,29]. In particular, direct IL-12 signaling in CD8+ T cells is critical for the generation of KLRG1+ IL-7Rαlow effector subpopulations but not for memory precursors [30,31]. Thus, it has been suggested that excessive and prolonged exposure to IL-12 promotes the formation of short-lived effector cells that will die after infection, whereas limited and short exposure induces effector cells that have memory T-cell potential [6]. Although it has not yet been studied in the context of human viral infections, this process may play a major role during HCV infection, given that a genetic background of enhanced IL-12 production is associated with apparent resistance to virus [32].

A SPATIAL VIEW OF THE CD8+ T-CELL RESPONSE TO CHRONIC HCV

HCV-specific CD8+ T cells are detectable in the blood of acutely infected patients regardless of virological outcome [33]. They appear stunned, with impaired proliferation, IFN-γ production, and cytotoxicity [34–36] and increased levels of PD-1 [37]. Whether the stunned phenotype is induced by a viral factor or whether it represents a natural step in the maturation and/or migration of HCV-specific CD8+ T cells is still unknown. Antiviral therapy in this phase of the infection results in a rapid decay of CD8+ T-cell responses [38], which suggests that most HCV-specific CD8+ T cells are short-lived, antigen-dependent effector cells rather than self-sustaining memory T cells. In patients who are able to finally control infection, the dysfunction of HCV-specific CD8+ T cells resolves, and IL-7Rα-positive CD8+ T cells become detectable as soon as HCV-specific CD4+ T-cell responses develop and the HCV titer decreases [34,35,39,40]. In contrast, when HCV infection persists, an exhausted CD8 T-cell phenotype with increased PD-1 expression is maintained [41]. The virus-specific T-cell compartments that have so far been studied in chronically HCV-infected patients are liver, blood, bone marrow, and lymph nodes; in these compartments, virus-specific T-cell function (and dysfunction) varies substantially. The CD8+ T-cell response to chronic HCV infection in these different compartments is described in the following sections.

LIVER

At the site of active infection and virus replication, the majority of HCV-specific CD8+ T cells is characterized by low or absent IL-7Rα expression [11,42,43] and low CCR7 expression [43–45]. These cells also overexpress the early activation marker CD69 [43,46] and contain perforin after in vitro stimulation [44]. However, they retain expression of CD27 and CD28. Thus, the phenotype of intrahepatic HCV-specific CD8+ T cells resembles that of “TEM-like cells that are not fully differentiated.” Contrary to expectations, however, they do
not express (or express only weakly) KLRG1 [42], indicating that they may not be completely reverted toward an effector phenotype and therefore not fully capable of immediate effector function. In fact, it has been shown that only a few of them have prompt IFN-γ production capacity after cognate peptide stimulation in vitro [47]. This dysfunction (i.e. differentiated but exhausted) is directly associated with expression of PD-1 [11,41,43,48,49] and with upregulation of the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) [50] and the T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) [51].

Within the population of CCR7− intrahepatic virus-specific CD8+ T cells, Accapezzato et al. [44] found cells that do not produce IFN-γ do produce considerable amounts of IL-10 and exert strong suppressor functions. Of note, whereas the frequency of IFN-γ-producing CCR7− HCV-specific CD8+ T cells is directly correlated with the total histological activity index (HAI) score, that of IL-10-producing CCR7− HCV-specific CD8+ T cells is not. Rather, it is inversely correlated, suggesting a major role for IL-10-producing cells in controlling chronic liver immunopathology mediated by the cells producing IFN-γ. These observations provide further support for the hypothesis that there may be two distinct HCV-specific CD8+ cell populations present in the liver of chronically infected individuals and that these populations have distinct effects on viral clearance and tissue damage. However, it should also be noted that the HAI does not necessarily equate to the number of antigen-specific T cells. The HAI score is only partially based on the extent of the intrahepatic lymphomononuclear infiltrate and only a small fraction consists of HCV-specific T cells.

The tissue-specific phenotype of the antigen-experienced T-cell population in HCV is the opposite of that observed for T cells at the infection sites of other viruses. In acute resolving infections such as influenza (FLU) or respiratory syncytial virus (RSV) infection, the lung harbors antigen-specific CD8+ T cells that retain high IL-7Rα expression. FLU-specific and RSV-specific T cells also display a well-differentiated memory phenotype reflected by loss of CCR7 and low expression of CD27 and CD28 [52]. This apparent inconsistency (high expression of IL-7Rα, loss of CCR7, and low expression of CD27 and CD28) suggests that these lung-residing cells are activated, although not continuously, by their cognate antigen and may rely on IL-7 for maintenance in the absence of antigen. Interestingly, whereas FLU-specific cells have low CD28 expression only, RSV-specific cells have low levels of both CD27 and CD28 [52]. Because differentiation of virus-specific CD8+ T cells is associated first with loss of surface CD28 and then with loss of CD27 [53], one explanation for these observations is that humans are less frequently exposed to FLU and more frequently (but intermittently) exposed to RSV, thereby facilitating the development of RSV-specific memory cells. In contrast, intrahepatic HCV-specific T cells not only lose CD127 but retain CD27 and CD28, reflecting their failure to fully differentiate.

BLOOD

HCV-specific CD8+ T cells in the blood are about 30 to 300 times less frequent than in liver. Additionally, the HCV antigen to which circulating CD8+ T cells respond can differ from that recognized by CD8+ T in the liver [46]. Nearly all circulating HCV-specific CD8+ T cells express IL-7Rα [11]. They are largely CCR7+ [44,45,54,55] and express both CD27 (90%) and CD28 (90%) [10,56]. They do not have the activation marker CD38 [39]. Like their liver counterparts, HCV-specific CD8+ T cells in the blood have weak or absent KLRG1 expression. This phenotype is also seen in almost all (about 100%) FLU-specific and RSV-specific CD8+ T cells in the blood, which are positive for IL-7Rα [52,57,58], maintain CCR7 (although at low level) [45,59], express high levels of CD27 and CD28, do not contain cytotoxic molecules [57,59], and may also be considered “early memory T cells.” FLU-specific CD8+ T cells, in particular, express very low levels of KLRG1 [13],
indicating that they have no immediate effector function. On the contrary, following antigen stimulation \textit{in vitro}, they expand and express both perforin and granzyme B efficiently [43].

The peripheral blood HCV-specific CD8$^+$ T-cell population seems to be “T$_{CM}$-like or in an early differentiation state,” similar to that seen in acute infections such as FLU and RSV. Altogether, data on the expression of CD27, CD28, CD127, CCR7, and KLRG1 by HCV-specific CD8$^+$ T cells suggest that a large fraction has a phenotype resembling that of memory T cells, which develop following an acute resolving infection such as FLU and RSV. Because KLRG1 and CD127 are directly influenced by ongoing TCR interactions, these results suggest that these HCV-specific CD8$^+$ T cells are not repetitively triggered by viral antigen despite persistence of viremia. In fact, recent data indicate that most CD8$^+$ T-cell responses in chronic HCV infection do not target the circulating virus and that the appearance of HCV-specific CD127$^+$ T cells is driven by virus sequence variations [60]. This means that, according to the infection duration, CD127-highly positive CD8$^+$ T cells, which target original, escaped epitopes, live together with CD127-low or intermediately positive CD8$^+$ T cells, which recognize current, nonescaped epitopes. These cells are distinguished for their limited proliferative capacity and co-expression of the inhibitory receptors 2B4, CD160, and KLRG1 next to PD-1 [61]. Indeed, expression of this last molecule during chronic infection seems to require preservation of cognate antigen and to contribute to virus persistence and immune evasion only when the virus fails to escape the T-cell response via epitope mutation [62].

The phenotypes of circulating CD8$^+$ T cells in other chronic viral infections are not the same. For example, HIV-1-specific CD8$^+$ T cells display very low levels of IL-7Ra [63,64]. The majority of these cells has no evidence for activation (minimal CD38 expression) or proliferation (minimal Ki67 expression) and has up-regulated the survival factor Bcl-2. They express the “CCR7$^-$ T$_{EM}$ phenotype” but have relatively high levels of CD27 expression (80%) and very low levels of CD28 expression (10%) [10]. Thus, these cells may be considered to have an \textit{intermediate differentiation} status. Furthermore, over 90% of the cells express KLRG1 [13,65]. Upon stimulation \textit{in vitro}, these CCR7$^-$ CD27$^+$ CD28$^-$ cells do not show clear evidence of proliferation [10]. \textit{Ex vivo}, they present lytic activity [66]. Interestingly, this peripheral blood (CCR7$^-$) T$_{EM}$ phenotype is also seen in HIV-specific CD8$^+$ T cells in the rectum, although these latter cells express minimal amounts of the cytolytic protein perforin, unlike in the peripheral blood where 23% of Gag-specific CD8$^+$ T cells express perforin [67]. Nevertheless, these cells do express other proteins associated with granule-mediated cytotoxicity, such as granzyme A, and are also capable of releasing IFN-\gamma upon stimulation with cognate peptide. These observations in HCV and HIV infections suggest that expression of cytotoxic effector proteins is regulated in a tissue-specific manner and that expression and accumulation of perforin may be subject to stringent regulatory control in mucosal tissues.

Circulating CD8$^+$ T cells specific to HCV also differ from those specific to latent viruses. In latent CMV infection, virus-specific CD8$^+$ T cells in blood (as well as in lung, tonsils, and liver) have a \textit{late differentiation (CCR7$^+$) phenotype} biased toward a CCR7$^-$ CD45RA$^-$ T$_{EM}$ and a CCR7$^+$ CD45RA$^+$ terminally differentiated phenotype. Moreover, CD27 and CD28 expressions are down-regulated, Bcl-2 expression is up-regulated, Ki-67 expression is minimal and CD38 is for the most part absent. The majority of cells (>92%) expresses KLRG1 and CD57 [13,65]. In latent EBV infection, a good part (70%–90%) of virus-specific CD8$^+$ T cells in blood as well as in tonsils, bone marrow, lung, and liver [10,13,43,52,58,65,68,69] expresses IL-7Ra [52,58]. However, whereas memory CD8$^+$ T cells responsive to peptides encoded during latent infection tend to include both \textit{central memory (CCR7$^+$) and effector memory (CCR7$^-$) subsets, memory CD8$^+$ T cells specific for peptides derived from proteins encoded during lytic replication of the virus are shifted}
toward an effector phenotype, with a few cells even acquiring a terminally differentiated status (CD45RA+ revertant memory cells). Apart from their peptide specificity, the majority (90%) of EBV-specific CD8+ T cells expresses CD27, and 60% expresses CD28 [10]. They show minimal CD38 and Ki67 expression and overexpress Bcl-2. The majority (>90%) expresses KLRG1 (and CD57) [13,42].

LYMPH NODES AND BONE MARROW

HCV-specific CD8+ T cells have been detected in perihepatic lymph nodes [70] and bone marrow [45] of patients with persistent HCV infection. Data on this matter come from only two papers and therefore are too scanty to draw definitive conclusions. Nevertheless, they offer the cue for intriguing speculations. Perihepatic lymph nodes, the likely site of HCV-specific T cell priming, hold a large number of HCV-specific T cells that are CD57− and produce more IFN-γ than their blood and liver equivalents upon in vitro stimulation with HCV peptide pools. These qualities are consistent with a “T_CMs-like” phenotype, which is typical of secondary lymphoid organ [70]. Bone marrow, which is neither a site of HCV infection nor a conventional secondary lymphoid organ, has been found to contain an HCV-specific CD8+ T-cell population 25 times greater than that in the blood and two times greater than that in the liver. This population, however, does include not only cells directed against viral antigens of the ongoing infection but also cells directed against historical HCV antigens from prior infections (true memory cells) or minor viral strains of the ongoing infection. Of note, bone marrow resident CD8+ T cells display the same “T_EMs-like” phenotype as liver-derived equivalents but have greater antiviral effector functions such as antigen-specific cytotoxicity and IFN-γ production [45]. This bone marrow picture is not replicated in latent infections and consequently seems to be exclusive of HCV. Studying a single CMV epitope, Letsch et al. [71] found that, despite similar frequencies of CMV-pp65-specific CD8+ T cells in blood and bone marrow, T_CMs (CCR7+) is the primary T-cell population in the bone marrow, whereas T_EMs predominates in the blood. Moreover, CMV-pp65-specific CD8+ T cells are more efficiently expanded from bone marrow than from blood. Accordingly, in bone marrow, few CMV-specific CD8+ T cells display prompt IFN-γ production after short stimulation. EBV lytic-specific CD8+ T cells are three to five times more frequent in the bone marrow than cells of similar specificity in the blood, whereas EBV latent-specific CD8+ T cells do not show preferential accumulation in either compartment. Moreover, bone marrow memory CD8+ T cells have a unique CCR5+ CXCR3− CXCR6+ homing phenotype different from that of CD8+ T cells in secondary lymphoid organs, for example, gut, skin, and inflamed tissues [69].

CONCLUSIONS

The memory CD8+ T-cell response to most viruses is surprisingly diverse in phenotype and function and undergoes dynamic changes during its development and maintenance in vivo. This heterogeneity is related to the nature of the infecting virus, to its cellular tropism and to the location of the CD8+ T cells. As the amount of antigen available to T cells in different anatomic compartments varies, different memory cell types can be identified over the course of a viral infection (Table 1).

In resolved acute infections, the presence of memory CD8+ T cells at the sites of the original virus entry and replication is crucial for a rapid response to a secondary infection. For instance, lung-resident memory CD8+ T cells may promote effective protective responses to FLU and RSV challenges in situ. In latent infections, the presence of memory CD8+ T cells at sites of virus persistence is important for immune surveillance of virus reactivation. For example, asymptomatic seropositive carriers of CMV harbor latent virus in multiple cell types and in various organs including liver, kidney, spleen, pancreas, and smooth muscle of
the arterial wall [72]. In the same way, about one of every million B cells of asymptomatic EBV carriers is latently infected and this reservoir of latently infected B cells preferentially populates the mucosal lymphoid tissues, the bone marrow, and the peripheral blood. Therefore, dissemination of memory T cells specific for these viruses is sustained by the co-presence of antigen in all these sites. In some chronic infections, such as that caused by HIV, the presence of memory CD8\(^+\) T cells in the genitourinary and gastrointestinal tracts (particularly the rectum) may be justified by the presence of infected lymphocytes in submucosal lymphoid follicles [73]. Of note, circulating and mucosal HIV-specific CD8\(^+\) T cells have similar HIV-1 epitope specificities [74], suggesting that they may have a common origin and can traffic between anatomically distinct compartments.

Chronic HCV infection represents a special case. Antigen-specific T cells persist at the site of virus infection as well as in other tissues. In the liver, where antigen is concentrated (continuous antigen presentation), virus-specific CD8\(^+\) T cells do not express IL-7R\(\alpha\) and maintain a not fully developed effector-like phenotype. Indeed, these cells are anergic and lack cytotoxic function and the ability to produce antiviral cytokines. In the blood, where exposure to antigen is more limited, virus-specific CD8\(^+\) T cells exhibit features characteristic of early stages: they may retain IL-7R\(\alpha\) expression and moderate effector function and develop a T\(\text{CM}\) phenotype. Alternatively, there may be a selective recruitment of specific T-cell populations to the site of infection by specific inflammatory and/or homeostatic expression of chemokine and chemokine ligands that results in the migration of T cells to the site of infection. For example, CCR5 expressing CD8\(^+\) T cells are particularly enriched in the liver [75]. Interestingly, CXCR6 is expressed on effector CD8\(^+\) T cells both in the blood and in the liver, and recently, Northfield et al. [76] have identified antigen-specific subset of these intrahepatic CXCR6\(^+\) T cells that express CD161 and secrete IFN-\(\gamma\) and IL-17. Importantly, the functional impairment of HCV-specific CD8\(^+\) T cells has been directly associated with the expression of PD-1 [11,41,48–50]. The level of PD-1 expression defines a hierarchy of HCV-specific CD8\(^+\) T-cell function based on the extent of active antigenic exposure in different compartments in vivo. The more intrahepatic CD8\(^+\) T cells express PD-1, the more they are exhausted and increase the expression of cytotoxic T lymphocyte antigen 4 (CTLA-4) while reducing that of CD28 and IL-7R\(\alpha\) [50]. Moreover, the liver may act as a specific site where activated T cells die [77,78].

These findings, together with the observation that one ligand for PD-1, PD-L1, is highly expressed on (mouse) liver sinusoidal endothelial cells and Kupffer cells, suggest that the PD-1/PD-L1 pathway may be involved in downregulating or impairing the function of activated intrahepatic CD8\(^+\) T cells. Expression of PD-L1 in the liver is crucial for shielding this organ from immune-mediated tissue damage: the PD-1/PD-L1 pathway limits the damage caused by overaggressive T cells but negatively influences antiviral immunity [11,43]. These results suggest a model of chronic infection where a nodal HCV-specific CCR7\(^+\) T\(\text{CM}\) population provides a source of peripheral CCR7\(^+\) T\(\text{CM}\) cells that become CCR7\(^-\) T\(\text{EM}\) cells upon entry into the liver. Here, virus-specific CD8\(^+\) CCR7\(^-\) T\(\text{EM}\) cells are capable of controlling the infection but not clearing it, resulting in chronic hepatitis. The pathway is two-way with cells circulating back to the blood either directly or through the lymph. These circulating virus-specific CD8\(^+\) CCR7\(^-\) T\(\text{EM}\) cells emigrants from the liver gain access to the bone marrow where they complete their differentiation process and become fully differentiated T\(\text{EM}\) cells (Figure 1). Because of its abundance of cytokines and growth factors, the bone marrow provides an environment supportive of prolonged survival of virus-specific T cells that find refuge from the immunosuppressive influence of the liver.

Understanding the spatial and temporal priorities of the HCV-specific T-cell response will be the key to developing effective immunotherapies.
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Abbreviations used

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<th>Abbreviation</th>
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<tr>
<td>HCV</td>
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<td>HIV</td>
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<td>T-cell receptor</td>
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References


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Figure 1.
Localization and potential trafficking of antigen-experienced CD8+ T cells in the chronic phase of hepatitis C virus infection.
Table 1
Viruses causing resolving, latent, or chronic infections in humans, and phenotypes of the antigen-specific CD8+ T cells during the memory phase of the infection, in selected compartments

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus tropism (organ or cell)</th>
<th>Infection course</th>
<th>Phenotype of virus-specific CD8+ T cells</th>
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