

Heterogeneous Memory T Cells in Antiviral Immunity and Immunopathology

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ABSTRACT

Memory T cells are generated following an initial viral infection, and have the potential for mediating robust protective immunity to viral re-challenge due to their rapid and enhanced functional responses. In recent years, it has become clear that the memory T cell response to most viruses is remarkably diverse in phenotype, function, and tissue distribution, and can undergo dynamic changes during its long-term maintenance *in vivo*. However, the role of this variegation and compartmentalization of memory T cells in protective immunity to viruses remains unclear. In this review, we discuss the diverse features of memory T cells that can delineate different subsets, the characteristics of memory T cells thus far identified to promote protective immune responses, and how the heterogeneous nature of memory T cells may also promote immunopathology during antiviral responses. We propose that given the profound heterogeneity of memory T cells, regulation of memory T cells during secondary responses could focus the response to participation of specific subsets, and/or inhibit memory T-cell subsets and functions that can lead to immunopathology.

INTRODUCTION

THE PROMISE THAT MANIPULATION OF MEMORY T CELLS HOLDS for providing long-lasting protective immunity against viral infections is matched by the challenge of understanding their complex and heterogeneous properties. In primary responses to newly encountered viruses, naive T cells become activated in lymphoid tissue and differentiate into effector T cells, which then migrate to peripheral sites and coordinate viral clearance. Most of these effector cells die after virus is cleared, although a subset of primed, virus-specific T cells develops into long-lived memory. These memory T cells can mediate rapid and effective recall immune responses conferring protective immunity to viral challenge. There are two main features of antigen-specific memory T cells that distinguish them from naive T cells and enable them to

coordinate efficient secondary responses. One feature is the ability of memory T cells to mediate rapid effector responses upon antigenic recall, compared to naive T cells that lack immediate effector function. The other feature is the remarkable heterogeneity of memory T cells in homing capacities, function, and tissue distribution in lymphoid and non-lymphoid sites—starkly contrasting the homogenous phenotype and exclusive lymphoid residence of naive T cells. This memory heterogeneity imparts a functional and spatial diversity to the recall response; however, the role of heterogeneous memory T cells in secondary responses and protective immunity to viral challenge remains poorly understood. In particular, it is not known whether maximizing memory heterogeneity or focusing a memory T-cell response is more beneficial to protective immunity. In this review, we will discuss heterogeneous properties of memory T cells, how

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specific memory T-cell subsets differ in their antiviral efficacy, and how functional diversity may lead to both protection and immunopathology in antiviral responses. We also propose that optimizing protective immunity by virus-specific memory T cells can be achieved by regulating the heterogeneity of existing memory T-cell populations.

Memory T cells: Basic properties

Memory T cells exhibit enhanced functional properties and distinct phenotypic features, compared to naive T cells (60,132). Functionally, memory T cells exhibit rapid effector cytokine production within hours of stimulation, whereas naive T cells require days of sustained activation to differentiate into effector cytokine producers (26,60). This “rapid recall” response is the defining feature of memory CD4 and CD8 T cells, and includes production of Th-1-type effector cytokines IFN- γ , IL-2, and TNF- α ; Th-2-like cytokines IL-4, IL-5, and IL-10 (93); or the Th-17-type cytokine IL-17 (148,149). Memory T cells also have less stringent activation requirements compared to naive T cells, including a reduced activation threshold for low antigen doses (114). Memory T cells can be fully activated by many antigen-presenting cell (APC) types such as resting B cells, macrophages, and endothelial cells (31,37), as well as dendritic cells

(DCs) (166), which are the primary APCs for activating naive T cells. Phenotypically, memory T cells differ from naive T cells in their elevated expression of adhesion markers CD44 (22) and CD11a (147) or CD45RO in humans. The CD45RB isoform was originally found to be differentially expressed on naive versus memory CD4 T cells (19,73); however, CD45RB expression occurs on a subset of memory CD4 T cells (16) (Table 1), and the human counterpart, CD45RA, can also be expressed on subpopulations of memory T cells (131). Thus, the two invariant functional and phenotypic features that define memory T cells are rapid effector function, and increased CD44 expression in mice or CD45RO expression in humans.

MEMORY T-CELL HETEROGENEITY

The profound heterogeneity of memory T cells first became apparent 8 years ago in studies by several groups (5,6,119,120,141). This heterogeneity was found in both mouse and human CD4 and CD8 memory T cells, and was defined by variations in expression of the lymph node homing receptors CD62L and/or CCR7 (6,120,141), and diverse distribution in lymphoid and non-lymphoid tissue sites (86). Lanzavecchia and colleagues defined two subsets of memory T cells in human peripheral blood

TABLE 1. PHENOTYPIC VARIATIONS THAT DEFINE FUNCTIONAL SUBSETS OF MEMORY T CELLS

<i>Phenotypic markers</i>	<i>Memory subsets</i>	<i>Functions</i>	<i>References</i>
CCR7	Effector-memory	Effector cytokine production	6,118–120,141
CD62L	CD62L ^{lo} /CCR7 ⁻	IL-2, high proliferation	
	Central-memory		
	CD62L ^{hi} /CCR7 ⁺		
CD45RB	CD45RB ^{hi}	Low effector responses	16
	CD45RB ^{lo}	Effector cytokines	
VLA1	CD49b ⁺	TNF- α , protective	48, 62
VLA-2 (CD49b)	CD49b ⁻	IL-10	
	VLA-1 ⁺	Th-1 functions	
CD27	Effector memory	Effector cytokine production	18, 46, 53
	CD27 ^{lo}		
	Central memory	IL-2, high proliferation	
	CD27 ^{hi}		
CD28	CD28 ⁺	All resting memory	98, 106, 150
	CD28 ⁻	Newly activated memory/CMV downmodulation	
CD43	CD43 ^{lo}	Effector cytokines	53, 71, 100
	CD43 ^{hi}	Naive	
CCR6, CCR4	CCR6 ⁺ CCR4 ⁺	IL-17	2, 9
CXCR3	CXCR3 ⁺	Th-1 responses	68, 131

based on CD62L/CCR7 expression and functional capacity, with the CCR7⁺CD62L^{hi} population producing predominantly IL-2 designated as “central -memory” (T_{CM}), and the CCR7⁻CD62L^{lo} subset producing effector cytokines defined as “effector-memory” (T_{EM}) (118–120). Central and effector memory subsets also were found to delineate memory subsets with distinct tissue distribution, with T_{CM} residing primarily in lymphoid sites and peripheral blood, and T_{EM} predominating in non-lymphoid sites and mucosal compartments (75,86,118). Further *in vitro* analysis of human T_{CM} and T_{EM} subsets led to a model in which the T_{CM} subset served as a continuously renewing “memory stem cell” which also replenished the T_{EM} pool (72).

Although the T_{EM}/T_{CM} concept and nomenclature has been widely adopted, there is accumulating *in vivo* evidence that the function, heterogeneity, and lineage relationship of memory T-cell subsets do not follow the central/effector memory paradigm. First, the original functional dichotomy between T_{CM} and T_{EM} subsets does not apply to multiple antigen-specific models. Equivalent effector function was found to be produced by mouse LCMV-specific T_{CM} and T_{EM} CD8 T cells (139,155), CD62L^{lo} subsets of mouse memory CD4 T cells (16), and similar effector cytokine functions in human virus and antigen-specific T_{EM} and T_{CM} subsets (25,36, 118,134,135), indicating that antigen-specific memory subsets may not have intrinsic differences in cytokine production. Second, phenotypic heterogeneity of memory T cells is not limited to CD62L/CCR7 expression, and the expression of activation markers, adhesion molecules, homing receptors, co-stimulatory receptors, and chemokine receptors has been shown to delineate memory subsets with distinct functions (Table 1). For example, differences in the expression of integrins CD49b (VLA-2) (62) and VLA-1 (48) define functional subsets of memory CD4 T cells in mice and humans, respectively, with VLA⁺ memory T cells exhibiting more Th-1-like functions (Table 1). The co-stimulatory molecules CD28 and CD27 are differentially expressed by human and/or mouse memory subsets (46,115). While CD28 is expressed by most resting effector and central memory T cells (17,44,98), a proportion of human CD28⁻ memory T cells has been detected in the periphery and is associated with aging or chronic infection (109,150). Variations in CD27 expression also occur on human and mouse memory T cells, with the CD27^{lo} phenotype on memory CD8 T cells in mucosal sites denoting lytic capacity (18,53,84). In addition, coordinate expression of CD27 and the adhesion molecule CD43 together define functional subsets of mouse memory CD8 T cells with different recall and proliferative capacities (53). Human memory T cell subsets can also be distinguished by variations in CD43 expression that likewise correlate to dif-

ferent functional capacities (71,100), independent of CD62L expression (Table 1). Therefore, phenotypic classification into T_{EM} and T_{CM} subsets, which is based on CD62L or CCR7 homing receptor expression, does not fully describe the phenotypic and functional complexities of a given memory T-cell population.

The expression of chemokine receptors, which control leukocyte migration to tissue sites, inflammation, and interactions with immune accessory cells (70), also exhibit considerable variation on memory T-cell populations and can define functional subsets (Table 1). The original T_{CM} and T_{EM} subsets were defined based on expression of the CCR7 chemokine receptor (119,120), that mediates lymphoid homing similar to CD62L. However, the coordinate expression of CCR7 and CD62L on T_{CM} and their downregulation on T_{EM} does not occur on virus-specific memory CD8 T cells in mice (110,140), and in subsequent studies, CCR7 did not distinguish functional subsets of virus-specific memory T cells in humans and mice (25,35,139). The expression of other types of chemokine receptors can delineate subsets of memory T cells with distinct cytokine profiles and replicative history, as defined by telomere length. Thus, expression of CXCR3 and/or CCR4 can define subsets of memory CD4 T cells having the capacity to produce Th-1 or Th-2 cytokines, and also delineate subsets with different *in vivo* turnover (68,111,131). CCR8 expression on memory T cells can indicate the potential for production of Th-2 cytokines (130), and recently, expression of both CCR6 and CCR4 has been shown to mark a population of memory CD4 T cells that produce the proinflammatory cytokine IL-17 (2,9). It is not known whether expression of chemokine receptors directly controls functional capacity, or rather reflects stimulation, trafficking, or replicative history of memory T cells that has more directly biased their cytokine profile. These broad variations in surface marker expression on memory T cells suggest a diverse usage of trafficking markers that may alter during subsequent recall to antigen challenge.

The compartmentalization of memory T cells in diverse tissue sites adds yet another layer to the phenotypic and functional complexity of memory T cells described above. While the majority of memory T cells in non-lymphoid tissue bear a predominant CD62L^{lo} profile (16,85–87), there is increasing evidence that tissue-resident memory T cells exhibit compartment-specific phenotypic, functional, and homing properties. For example, mouse bone marrow (BM)-resident memory CD8 T cells exhibit enhanced effector and proliferative capacities (14,105) as we also found for human BM memory T cells (169). Lung memory T cells, by contrast, exhibit reduced proliferation yet highly activated phenotypes and effector responses (24,41,42,113,159). Gut-resident memory T cells exhibit further variations in phenotypes, homing,

and increased apoptosis (76,88). In addition, we have found that lung and spleen memory CD4 T cells exhibit tissue-specific homing tropism (16), indicating that certain tissue compartments can impart specific properties on their indigenous memory T cells. In addition, CCR7⁺/CD62L⁺ T_{CM}-phenotype cells can be found in non-lymphoid sites including lung (16,101), CNS (65), and gut (145,146), and these non-lymphoid CD62L^{hi} cells are not functionally equivalent to lymphoid CD62L^{hi} subsets (16,146). When taken together, these results indicate that the memory subsets in spleen and peripheral blood are not equivalent to memory T cells of comparable CD62L phenotype resident in peripheral tissue parenchyma. A given memory T-cell population therefore consists of multiple functional subtypes in diverse tissue sites that are maintained by homeostasis, and may recirculate between tissue sites or turnover within each compartment (Fig. 1A). The functional properties of individual memory T cells are influenced by a combination of surface marker expression and tissue compartment, although the precise contributions of these factors in directing memory T-cell responses *in vivo* are unknown.

The lineage relationship between CD62L memory T-cell subsets and lymphoid and non-lymphoid memory T cells also remains an unresolved issue. In particular, it is not known whether memory subset delineation is established during priming, and/or varies over time during memory maintenance (95). The CD62L profile of memory T cells can be affected by initial priming conditions, including the extent of antigenic stimulation during priming and the antigen-specific precursor frequency (74,95). For CD4 T cells, strong or sustained antigenic stimulation during priming yielded memory T cells with increased effector function (80) and increased CD62L heterogeneity (94), whereas for CD8 T cells, limiting the extent of antigen exposure during infection resulted in more CD62L^{hi} lymphoid memory CD8 T cells (143,156). The precursor frequency of antigen-specific CD8 T cells also affects the development of heterogeneous memory cells. A low precursor frequency responding to vesicular stomatitis virus (VSV) infection gave rise to primarily CD62L^{lo} cells, whereas higher T-cell precursors generated more CD62L^{hi} T_{CM} cells (13,83). The role of CD4 T-cell precursors in heterogeneous memory generation during viral infection is not yet defined, although quantitation of peptide-specific CD4 T-cell precursors based on new MHC class II tetramer reagents revealed correlations between initial naive precursor and memory cell frequency (92). CD62L expression can also vary over time during memory persistence in the periphery (112,155), or upon homing to lymphoid or non-lymphoid tissue sites (16,84). At present, it remains unknown whether the tissue-specific influences on resident memory T cells are reversible or inducible upon exit or entry

into a specific compartment, respectively. The factors that regulate CD62L expression and homing capacity of memory T cells *in vivo* remain unresolved issues and are important parameters for understanding the lineage relationship of CD62L memory subsets.

In summary, although the use of T_{EM} or T_{CM} predominates in the literature, this classification does not account for the multiple variations in phenotype, function, and homing of peripheral memory T cells, as well as tissue-specific variations that exist in non-lymphoid memory populations. In addition, designating memory subsets as T_{CM} or T_{EM} implies a lineage relationship that is not yet established. Thus, we propose that a more accurate designation of memory subsets would be to indicate both the tissue of residence and homing receptor phenotype, such as LN-CD62L^{hi}, lung-CD62L^{lo}, or spleen-CD62L^{lo} memory T cells. This designation uses the CD62L phenotype to indicate lymphoid homing capacity, and the tissue or origin to indicate potential tissue-specific influences, and makes no assumptions regarding lineage relationships.

MEMORY T-CELL HETEROGENEITY AND PROTECTIVE IMMUNITY

The heterogeneous nature of memory T cells raises important questions concerning the role of this memory diversity in antiviral protective immunity. In recall responses to viral infection, multiple types of memory subtypes can participate—including memory T cells at the site of infection, lymphoid memory T cells that become activated and migrate to peripheral sites, and memory T cells circulating from other non-lymphoid compartments, with each of these memory subtypes exhibiting specific functional and proliferative capacities (Fig. 1B). The different types of memory T cells required for recall responses to specific viruses are important issues that need to be resolved in order to promote long-term T-cell immunity. While heterogeneous memory T cells may diversify the recall response, leading to more effective protective immunity, it is possible that only specific subpopulations of memory T cells within the heterogeneous pool can mediate protective responses. However, the existence of heterogeneous memory T-cell subsets may also result in certain subsets of memory T cells being ineffective or potentially detrimental to antiviral protective immunity.

Adoptive transfer approaches have been informative in assessing the *in vivo* protective capacities of specific memory subsets, and have revealed that certain memory subsets may be more effective than others in mediating viral clearance. The protective capacities of central and effector memory subsets differing in CD62L expression

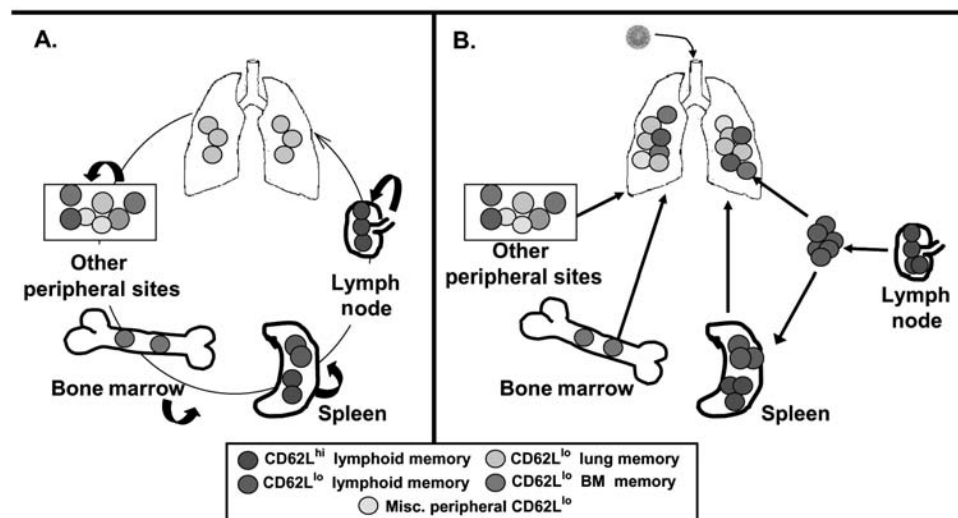


FIG. 1. Schematic model of heterogeneous memory T cells during steady-state conditions and in response to a respiratory infection. **(A)** During steady-state conditions, memory T cells in different tissue sites are maintained by homeostasis, with some replenishment from lymphoid sites, and possible trafficking between distinct non-lymphoid sites. **(B)** During a site-specific infection, as in the lung, memory T cells from multiple lymphoid and non-lymphoid sites can potentially become activated and migrate to the infectious site, resulting in a rapid and heterogeneous response. The participation of multiple subsets and their potential for mediating protective immunity and/or immunopathology are discussed in the text.

vary depending on the viral infection system. In LCMV infection, purified splenic CD8 T_{CM} cells transferred into adoptive mouse hosts mediated more effective viral clearance than purified T_{EM} (155), whereas for Sendai virus infection, the protective capacity of memory CD8 T-cell subsets varied over time and did not depend on CD62L profile (53,112). By contrast, protection from vaccinia virus infection was restricted to the T_{EM} subset of CD8 T cells (12). For CD4 T-cell subsets, less is known concerning their protective capacity in viral systems. CD4 T cells have been shown to provide protection against a number of different viruses, including respiratory viruses (57), rotavirus (89), gamma-herpes viruses (29), and picornaviruses (99). The subset specificity of protective memory CD4 T cells for most of these viruses remains undefined, although CD62L^{lo} CD4 T cells have been shown to be protective in responses to rotavirus (128), and vaccinia virus (1). Further refinements in the phenotypic and functional characterization of memory T cells should lead to insights into the type of memory T cells necessary for optimal protective immunity to viral infections.

The efficacy of a secondary T-cell response to viral infections may ultimately reside within memory T cells in specific anatomical compartments, given that viruses enter and disseminate in different tissue sites. Recent studies provide evidence that the tissue-resident memory T cells rather than the peripheral homing subtype, drive the recall response and mediate protection (63,66,157). How-

ever, the requirements for lymphoid and/or non-lymphoid memory T cells can depend on the viral system, and lymphoid memory T cells can serve important protective roles, particularly for systemic viral infection. In response to ectromelia virus (mousepox) infection, memory CD8 T cells in lymph nodes were found to be important “gatekeepers” in pathogen clearance (161). Lymphoid-derived central memory CD4 T cells have also been associated with protective responses to HIV/SIV and EBV infection. In primates vaccinated with simian immunodeficiency virus (SIV), animals that preserve their central memory pools and have strong IL-2 recall responses have lower levels of initial viral replication and prolonged survival (136). Moreover central memory CD4 T cells in blood comprise half of the responding EBV-specific memory CD4 T cells and directly replenish the effector-memory pool (52). Lymphoid memory can also predominate in response to the respiratory virus SARS, where the majority of the blood virus-specific memory CD4 T cells bear a CD62L^{hi} phenotype (164). However, for influenza virus infection that is restricted to the lung, memory CD8 T cells can mediate recall responses to viral challenge in mice congenitally devoid of secondary lymphoid tissue (96), suggesting that lymphoid memory T cells are dispensable for protection to respiratory pathogens. While it is likely that secondary responses to viruses at specific tissue sites involve both tissue-resident memory T cells as well as an influx of memory T cells from lymphoid and other non-lymphoid sites (Fig. 1B),

whether lymphoid memory T cells mediate significant protective responses in site-specific infections remains to be determined.

Although memory T cells are found in multiple peripheral sites, those resident in the lung and lamina propria of the gut have been the focus of a number of studies due to their abundance and importance in viral infections at these sites. Lung memory CD4 and CD8 T cells persist in humans and mice following respiratory virus infection with influenza, parainfluenza, and respiratory syncytial virus (RSV) (34,45,56,102). These lung-resident memory T cells have been shown to be replenished from the peripheral pool and maintained by continuous turnover (55,84,159). Lung memory CD4 and CD8 T cells produce higher levels of IFN- γ compared to counterparts in peripheral blood or secondary lymphoid organs (16,86), and may be particularly adapted to mediate effective protective responses *in situ*. Hogan and colleagues demonstrated *in situ* rapid viral clearance when virus-specific memory CD4 T cells were administered directly to the lungs of recipient mice (57), indicating that localization of memory T cells at the site of infection is sufficient to provide protection. In the natural infection, however, influenza-specific CD8 T cells are widely dispersed, present in spleen, lung, bone marrow, and other tissue sites (82), and memory CD4 T cells specific for influenza HA are also heterogeneous in CD62L expression and tissue distribution (6,16,137). These findings indicate that multiple memory T cell subtypes could respond and home to the lung during secondary influenza challenge (Fig. 1B). Several groups have found that influenza antigens persist long after virus is cleared in the lung (59,167), and may maintain effector memory populations. Understanding the exact interplay of multiple memory subsets as well as the pathogenesis of the infecting virus would contribute significantly to targeting protective subsets during vaccination.

In the lamina propria (LP), memory T cells bear a predominant CD62L^{lo}/CD27^{lo} effector-memory-like phenotype, yet also exhibit distinct characteristics including up-regulated expression of the early activation marker CD69, indicating a semi-activated state, and expression of the $\alpha 4\beta 7$ integrin, reflecting unique homing properties (64,67,76). These LP or mucosal memory T cells are associated with protection against intestinal viruses such as rotavirus (116). By contrast, in HIV/SIV infection, LP memory CD4 T cells are impaired in protective capacity and facilitate viral dissemination. The semi-activated state of LP memory CD4 T cells renders them highly permissive for HIV/SIV infection and severe depletion through direct infection or bystander activation with Fas-mediated apoptosis (8,20,32,76). In addition, CD4^{hi}CD8^{lo} double positive T cells make up a significant proportion of resident CD4 T cells in the intestines

(5–20% in primates and humans), produce high levels of effector cytokines, and are highly susceptible to HIV infection (49,103,144). While dendritic cells and gut resident macrophages tend to promote an immunosuppressive state (61,129), LP memory CD4 T cells have heightened levels of CD2 and associated LIGHT expression, indicating heightened activation (30,40). Thus, the mucosal immune system, with its direct interaction with pathogens, can mediate responses highly distinct from those observed in peripheral blood or spleen. This mucosal population of memory T cells may make important contributions to antiviral immunity and may also mediate immunopathology.

FUNCTIONAL HETEROGENEITY IN MEMORY RESPONSES

In addition to heterogeneity in homing and tissue distribution, memory T cells also exhibit diverse capacities to produce cytokines and mediate cytolytic functions on a cellular level. Individual memory T cells can produce multiple types of cytokines rapidly following antigenic recall. Both memory CD8 and CD4 T cells have been shown to simultaneously produce IFN- γ , TNF- α , and/or IL-2 (127,154), and memory CD8 T cells also vary in expression of lytic molecules including granzyme B and perforin (138). Memory CD4 and CD8 T cells producing IL-2, IFN- γ , and/or TNF- α are referred to as “polyfunctional” memory T cells (23,33,81). Polyfunctional memory T cells can be potent antiviral T cells in that they are highly proliferative and severely limit viral replication in infected cells (50). Not all memory T cells are polyfunctional, and within a given population of virus-specific memory T cells, individual cells vary in their capacity for production of cytokines. For example, human antiviral memory CD8 T cells comprise a mixed population with most cells secreting IFN- γ alone, and a smaller number secreting both IL-2 and IFN- γ , whereas memory CD4 T-cell responses contain fairly even distributions of cells producing IL-2 or IFN- γ alone, or IL-2 and IFN- γ in combination (50). Polyfunctional responses involving IL-17 have not yet been identified, although IL-17-producing cells are exclusive of IFN- γ production *in vivo* (162). It has been proposed that targeting the generation of specific types of multi-functional memory T-cell clones in vaccines may be particularly advantageous for protective immunity (104). Given the complex properties of memory T cells described above, the most effective protective response will likely depend on generating the appropriate functional subtype at the appropriate tissue locale.

Functionally heterogeneous memory T cells are also endowed with plasticity in the type of recall cytokines

they produce. We originally demonstrated that a population of antigen-specific memory CD4 T cells can alter the cytokine profile, depending on the nature and avidity of the recall stimulus (4,106). Similar plasticity in cytokine production has been demonstrated among human memory CD4 T cells (90), and most recently in mouse memory CD4 T cells responding to bacterial challenge *in vivo* (69). These findings suggest that the functional fate of memory T-cell populations is not fixed, and can be altered in different infectious environments. Inherent plasticity in memory responses to viruses has been proposed to account for adaptability in the immune responses to diverse viral antigens (124). Plasticity within memory populations can also alter productive recall responses, and therefore understanding how memory plasticity is regulated is essential to preserve the efficacious features of the anamnestic T-cell response.

MEMORY T CELLS AND IMMUNOPATHOLOGY

The rapid effector responses of memory T cells, their diverse distribution in peripheral tissue sites, and their ability to interact with tissue macrophages and endothelial cells (28,108,125) enables them to coordinate recall responses at the site of pathogen entry, but also predisposes them to be involved in immunopathology and local tissue destruction during an antiviral response. Memory CD8 T cells can mediate lethal immunopathology in response to LCMV infection (107), and destruction of lung epithelia in influenza virus infection through direct cytotoxicity and the production of TNF- α (21,160). Memory CD8 T cells have also been shown to cause severe immunopathology in responses to vaccinia virus, RSV, and LCMV (10,78,117,126). Despite their low cytotoxic potential, memory CD4 T cells can also direct responses that lead to immunopathology. Memory CD4 T cells were found to direct potent immune-mediated meningitis in LCMV-immune β_2 -microglobulin-deficient mice lacking CD8 T cells (54), and to promote demyelination and immunopathology during neurotropic mouse hepatitis virus infection (133). Memory CD4 T cells secreting TNF- α were also shown to promote severe tissue inflammation in LCMV, influenza, and secondary dengue virus infections (11,47,62,153). The generation of TNF- α -secreting memory T cells is therefore common in many viral diseases for which immunopathology plays a large role in tissue destruction.

The identification of memory T-cell subtypes that promote effective viral clearance with minimal immunopathology would be beneficial for optimizing memory T-cell responses during vaccinations. It will be necessary to precisely define memory T cell functions and/or

subsets that lead to immunopathology. For example, memory T cells that rapidly produce high levels of IFN- γ with limited TNF- α may prove more beneficial to eliminating virus with minimal tissue damage, whereas a high level of TNF- α relative to IFN- γ production may predispose a memory T cell to mediate tissue destruction. In addition, the contribution of lymphoid versus non-lymphoid memory T cells to immunopathology may differ in responses to certain viruses. While lung resident memory T cells may promote effective protective responses to respiratory virus challenge *in situ*, the involvement of lymphoid memory T cells with enhanced proliferative capacities may cause increased recruitment and inflammation in the lung. By contrast, lymphoid memory T cells that are recalled in lymphoid tissue or peripheral blood may not lead to local tissue destruction. Dissecting the beneficial and detrimental functions of memory T cells and mechanisms for their actions is necessary to ensure the generation of protective T-cell vaccines.

Memory T cells generated from exposure to a pathogen can also cross-react with antigens present in an unrelated pathogen, a phenomenon termed “heterologous immunity” (122,124), which is thought to predominate in adult immune processes (27,121,151,152). Heterologous memory CD8 T cells specific for one virus can mediate immunopathology in response to an unrelated virus (27,123), suggesting that the presence of *any* memory T cells creates the potential for deleterious immune reactions. Thus, the enhanced and beneficial immune clearance properties of memory T cells includes the potential for increased tissue damage and immunopathology in response to viral challenge.

This dual nature of the memory T-cell response suggests that the presence of virus-specific memory T cells may not always yield a productive type of protective response, and that a given population of heterogeneous memory T cells may contain subsets with propensities for mediating immunopathology. In the case of influenza virus infection, memory T cells specific for influenza have been shown to persist in the lungs of previously infected mice and humans (34,45,56,57,158), and to be present in the peripheral blood of most older children and adults (38,43,51). However, these memory T cells are not known to provide protection in the form of sterilizing immunity to influenza. It is possible that flu-specific memory T cells participate in influenza immunity, enabling effective viral clearance; however, the immunopathology triggered by memory T cells and resultant illness masks their role. It is interesting to note that a population profoundly affected by immunopathology in the 1918 flu was adults in the 20- to 40-year-old range (39), and recent avian flu cases have had their most pathological impact in adults, with milder clinical signs in children under 5 year of age (165). While the unusual deaths of

younger adults in the 1918 pandemic may be due to their naiveté to related flu strains at that period as was recently suggested (7), it is also possible that a deleterious immune reaction was triggered by memory T cells in this adult population. Further elucidation of the memory T-cell functions and subsets that promote immunopathology during viral infection is required to understand the nature of the antiviral memory T-cell response.

OPTIMIZATION OF MEMORY HETEROGENEITY IN ANTIVIRAL IMMUNITY

The functional, phenotypic, and spatial diversity of memory T cells and their potential for mediating immunopathology suggests that modulating memory T-cell heterogeneity could be an effective strategy for optimizing secondary responses. Targeting memory heterogeneity could be accomplished by strategies which regulate and focus memory T-cell responses to optimize their capacity for protective immunity. In general, memory T cells are believed to be resistant to regulation, as strategies that inhibit naive T-cell activation and effector generation are ineffective in the presence of memory T cells (97). For example, blockade of the CD40L/CD40 pathway, which effectively hinders primary T-cell activation, does not inhibit memory T-cell responses *in vivo* (3,142,168). In addition, regulatory T cells that suppress naive T-cell activation are ineffective in curtailing memory T cell-mediated rejection of allografts (163). However, there is increasing evidence that inhibition of certain co-stimulatory pathways can modulate specific memory T-cell functions and/or homing potentials. The CD28 co-stimulatory pathway was initially believed to be dispensable for memory T-cell activation based on *in vitro* studies (31,79); however, we and others have recently reported that CD28 signaling is required for optimal memory CD4 and CD8 T-cell secondary responses to antigenic peptides and influenza (17,98). Using an *in vivo* system to follow early and late recall of memory CD4 T cells *in vivo*, we found that inhibiting CD28 signaling on memory CD4 T cells preferentially limited antigen-driven expansion and IL-2 production, and reduced the tissue homing capacity and CD62L downregulation of memory T cells *in vivo* (98). CD28 has also been shown to direct homing capacity on human memory T cells (91), and we have also found that a similar change in homing capacity occurs on memory CD4 T cells responding to influenza virus (unpublished data), suggesting that CD28 co-stimulation can drive the migration of memory T cells to non-lymphoid sites. CD28 is also known to be downregu-

lated on human memory T cells in response to viruses such as CMV (109), although the protective role of these CD28-deficient memory T cells is not yet defined. These newer findings on the role of CD28 co-stimulation in memory T-cell responses *in vivo* suggest that CD28 inhibition using approved biologicals such as CTLA4Ig (77) could regulate deleterious memory T-cell responses to viral infection. Other co-stimulatory pathways such as ICOS and OX40 likewise show promise in regulating primary and/or secondary antiviral responses (15,58), although their specific effects on memory T-cell functions, heterogeneity, and homing remain to be determined. Identifying additional pathways involved in memory T-cell responses will be particularly important for immunotherapeutic modulation and optimization of protective recall immunity.

CONCLUDING REMARKS

Cellular and functional heterogeneity is a universal feature of virus-specific memory T cells. Summarized below are the key features of memory heterogeneity reviewed here, their implications for antiviral protective immunity and immunopathology, and how regulation of memory T-cell heterogeneity during recall responses *in vivo* may be a promising strategy for optimization of protective responses.

1. Identification of phenotypic markers for memory T cells is constantly evolving. Although expression of certain phenotypes may be associated with specific functions, whether these phenotypes direct functional capacity remains unknown.
2. Memory T cells in lymphoid and non-lymphoid tissues exhibit compartment-specific features that are likely important to secondary recall to specific virus infections.
3. Specific memory subtypes may promote immunopathology during secondary responses, and the degree of immunopathology may depend on the tissue site, homing capacity, and functional profile of the participating memory T cells.
4. Modulating memory populations may be a way of directing more effective protective immunity without immunopathology.

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