



Homeostasis of Naive and Memory T Lymphocytes

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Conventional CD4⁺ and CD8⁺ T lymphocytes comprise a mixture of naive and memory cells. Generation and survival of these T-cell subsets is under strict homeostatic control and reflects contact with self-major histocompatibility complex (MHC) and certain cytokines. Naive T cells arise in the thymus via T-cell receptor (TCR)-dependent positive selection to self-peptide/MHC complexes and are then maintained in the periphery through self-MHC interaction plus stimulation via interleukin-7 (IL-7). By contrast, memory T cells are largely MHC-independent for their survival but depend strongly on stimulation via cytokines. Whereas typical memory T cells are generated in response to foreign antigens, some arise spontaneously through contact of naive precursors with self-MHC ligands; we refer to these cells as memory-phenotype (MP) T cells. In this review, we discuss the generation and homeostasis of naive T cells and these two types of memory T cells, focusing on their relative interaction with MHC ligands and cytokines.

T lymphocytes play a central role in adaptive immune responses. Conventional CD4⁺ and CD8⁺ T cells expressing $\alpha\beta$ T-cell receptors (TCRs) are generated in the thymus via a process of positive and negative selection to a range of self-peptides bound to major histocompatibility complex class I (pMHC I) and class II (pMHC II) molecules (Klein et al. 2014). In this process, CD4⁺ CD8⁺ precursor thymocytes are tested for their relative TCR reactivity to pMHC ligands. A small proportion of cells with high avidity for these ligands is deleted, while the majority of thymocytes fail to interact with pMHC ligands

and undergo “death by neglect.” The residual 1%–2% of double-positive (DP) thymocytes have moderate TCR affinity to self-antigens (Ags) and these cells are signaled to survive and migrate into the periphery as naive CD4⁺ and CD8⁺ T lymphocytes. In addition, another small subset of CD4⁺ CD8⁺ thymocytes with slightly higher affinity is signaled to differentiate into Foxp3⁺ CD4⁺ regulatory T cells (Tregs).

In the periphery, naive T lymphocytes have a resting CD44^{lo} CD62L^{hi} CCR7^{hi} phenotype and circulate in the lymphoid tissues as they screen antigen-presenting cells (APCs) for MHC

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ligands. When naive T cells recognize cognate foreign Ags, they proliferate extensively to give rise to CD44^{hi} Ag-specific effector cells that serve to eliminate the pathogen concerned. After pathogen clearance, most effector cells die while a small fraction of these cells survive to form long-lived Ag-specific memory cells (Sprent and Surh 2002). Mature memory T cells express a high density of CD44 and comprise a mixture of central memory (Tcm), effector memory (Tem), and resident memory (Trm) cells (Seder and Ahmed 2003; Sheridan and LeFrançois 2011). Tcm cells are marked by expression of lymph node homing receptors, CD62L and CCR7, and reside largely in the lymphoid tissues, whereas Tem cells lack these markers and circulate mostly in nonlymphoid tissues. For Trm cells, they reside in nonlymphoid organs such as the intestines and lungs and provide a first line of defense against secondary infection. All of these subsets are long-lived cells with a slow turnover.

In addition to memory cells for foreign Ags, there is accumulating evidence that some T cells with a memory phenotype (MP) arise from naive precursors responding to self rather than foreign Ags (Sprent and Surh 2011). Thus, in normal conditions, T lymphocytes consist of foreign Ag-specific memory and self-driven MP cells as well as naive cells (Table 1). These cell populations are tightly regulated by homeostatic mechanisms that keep total peripheral T-cell numbers relatively constant throughout adult life (Surh and Sprent 2008). During acute infection, T cells specific for pathogens proliferate extensively to form effector cells, leading to a temporary increase in peripheral T-cell numbers; however, most effector T cells are rapidly eliminated, causing T-cell numbers to fall to their original level. Conversely, conditions of

lymphopenia induce residual T cells to expand to restore normal T-cell numbers. In this review, we will discuss the various mechanisms that control such homeostasis of naive, foreign Ag-specific memory, and MP T-cell populations.

NAIVE CELLS

TCR Signaling

As the result of positive selection in the thymus, mature naive T lymphocytes have relatively low TCR affinity for self-ligands and high affinity for cognate foreign Ags. Some of the self-pMHC ligands that induce positive selection of CD8⁺ and CD4⁺ T cells have been defined (Santori et al. 2002; Ebert et al. 2009; Lo et al. 2009), and it is well established that post-thymic contact with self-pMHC I and pMHC II ligands is important for controlling optimal homeostasis of peripheral CD8⁺ and CD4⁺ T cells, respectively (Fig. 1; Takeda et al. 1996; Tanchot et al. 1997; Dorfman et al. 2000). For CD4⁺ T cells, the requirement for post-thymic contact with self-MHC ligands for survival is most apparent in mice with total rather than partial absence of MHC II (Martin et al. 2003). Indeed, half-lives of peripheral naive CD8⁺ and CD4⁺ T lymphocytes are known to be reduced in the absence of MHC contact, especially for CD8⁺ T cells (Labrecque et al. 2001; Polic et al. 2001; Seddon and Zamoyska 2002).

To ensure post-thymic survival of naive T cells, it has generally been assumed that the ligands controlling positive selection in the thymus and T-cell survival in the periphery are the same (Ernst et al. 1999; Goldrath and Bevan 1999). Although this is clearly the case in some situations, it is now known that the MHC-bound peptides in the thymic cortex

Table 1. Classification of T lymphocytes in steady state

	Phenotype	Turnover	Stimulus
Naive	CD44 ^{lo}	Slow	Self-Ag
Foreign Ag-specific memory	CD44 ^{hi}	Relatively slow	Foreign Ag
Memory-phenotype	CD44 ^{hi}	Relatively slow + intermittently fast	Self-Ag

CD4⁺ and CD8⁺ T cells in normal specific pathogen-free (SPF) mice consist of naive, foreign Ag-specific, and memory-phenotype cell subpopulations. Their typical phenotype, turnover, and stimuli for survival and turnover are shown.

and the peripheral lymphoid organs are essentially different. Thus, positive selection in the thymus is directed in part to private peptides generated in cortical epithelial cells by the thymoproteasome for pMHC I and cathepsin L and thymus-specific serine protease (TSSP) for pMHC II (Nakagawa et al. 1998; Murata et al. 2007; Gommeaux et al. 2009; Klein et al. 2014). At least for CD8⁺ T cells, selection of cells by private cortical peptides is especially important for cells with relatively low self-affinity (Nitta et al. 2010; King et al. 2013), that is, cells with a low surface density of CD5, CD5 expression being a reliable marker for TCR affinity to self as discussed below. For CD5^{lo} T cells, contact with self-ligands in the periphery presumably reflects cross-reactivity with the selecting peptides.

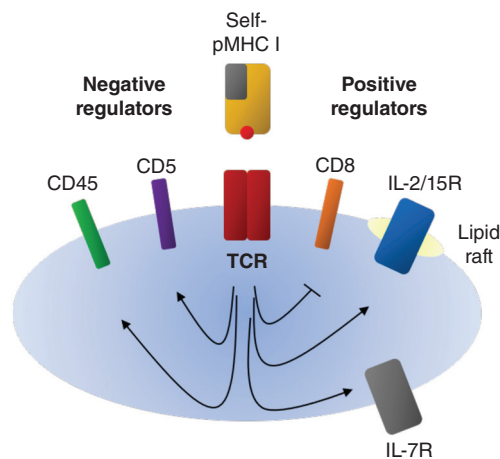


Figure 1. Self-Ag recognition modulates T-cell receptor (TCR) sensitivity and cytokine reactivity in CD8⁺ T cells. Self-antigen (Ag) recognition promotes CD8⁺ T-cell survival, partly by enhancing interleukin-7 receptor (IL-7R) expression levels. Also, CD8⁺ T cells with strong reactivity to self-Ags have high levels of inhibitory CD5 and CD45 molecules and reduced expression of CD8. Altered expression of these molecules causes a reduction in TCR sensitivity as defined by short-term assays *in vitro*. However, despite reduced TCR sensitivity, CD5^{hi} cells show enhanced expression of lipid rafts, thereby leading to augmented responsiveness to cytokines, notably IL-2 and IL-15. For this reason, CD5^{hi} cells give stronger responses to Ags *in vivo* than CD5^{lo} cells. This applies both to homeostatic proliferation to self-Ags in lymphopenic hosts and typical responses to foreign Ags.

The notion that peripheral naive T cells require sustained self-pMHC recognition for their survival suggests that the population size of each T-cell clone, or the “space” available for each cell, is determined by the expression level of its corresponding self-ligand. Whereas self-Ags as a whole are abundantly expressed in the periphery, it has been suggested that individual APCs may express not all but only a partial set of endogenous peptides (Paul et al. 2013; Grossman et al. 2020). If this is the case, the niche available for each clone could be quite small. Supporting this idea, monoclonal CD4⁺ T cells adoptively transferred in large numbers tend to disappear rapidly, whereas cells injected in small numbers persist (Hataye et al. 2006); in part, this finding may reflect the relative levels of homeostatic cytokines in the recipients. In the steady state, the number of naive CD4⁺ T cells specific for a given foreign epitope is generally very low, varying from 10 to several hundred cells/mouse (Moon et al. 2007).

Under normal conditions, post-thymic contact of naive T cells with self-pMHC ligands does not induce overt T-cell activation, even for cells with relatively high self-reactivity. Such tolerance is due in part to relatively low levels of costimulatory molecules on APCs and inhibition by Tregs (Paul et al. 2013). Thus, acute depletion of Tregs induces up-regulation of B7 molecules on dendritic cells (DCs) and leads to autoimmunity (Kim et al. 2007; Yi et al. 2019). Hence, the quiescent state of naive T cells is partly due to extrinsic mechanisms. In addition, a T-cell-intrinsic mechanism of partial TCR desensitization occurs after positive selection (Grossman and Paul 2015). Such TCR “tuning” limits overt responsiveness to self-ligands and focuses TCR reactivity to foreign Ags. TCR tuning is known to correlate with surface levels of CD5, expression of CD5 being up-regulated during positive selection and maintained in the periphery (Azzam et al. 1998; Smith et al. 2001; Stamou et al. 2003). Under *in vivo* conditions, T cells with high CD5 expression give strong responses (see below) (Smith et al. 2001; Takada and Jameson 2009). Under *in vitro* conditions, however, CD5 acts as a negative regulator of TCR signaling, possibly via SHP-1 phosphatase (Tarakhovskiy et al. 1995;

Perez-Villar et al. 1999), although recent studies are against this idea (Dong et al. 2016). In addition to CD5, TCR signaling is also negatively regulated by surface expression of CD45, a phosphatase that induces tyrosine dephosphorylation of p56^{lck} (Lck) (Cho et al. 2016).

Countering the negative activity of CD5 and CD45 on naive T cells, the presence of CD8 and CD4 molecules on these cells augments TCR signaling, largely by binding to nonpolymorphic epitopes on MHC I and MHC II molecules, respectively, thereby focusing Lck to the vicinity of the TCR (Sarmiento et al. 1980; Marrack et al. 1983; Gay et al. 1987; Rudd et al. 1988; Veillette et al. 1988; Turner et al. 1990; Park et al. 2007; Takada and Jameson 2009). In addition, certain microRNAs can augment TCR signaling. Thus, miR-181a increases TCR sensitivity for self-MHC during thymic selection and is then down-regulated in the periphery (Ebert et al. 2009). Together, these and the above positive and negative regulators cooperatively “fine-tune” the activation threshold for Ag-specific T-cell responses.

In addition to promoting survival, interaction of naive T cells with self-pMHC ligands can modulate TCR sensitivity (Fig. 1). For CD8⁺ T cells, depriving these cells from contact with pMHC I in a nonlymphopenic setting leads to a decline in CD5 levels but up-regulation of CD8 expression in vivo (Takada and Jameson 2009). These changes were found to improve responsiveness to foreign Ags, although only to weak Ags. Hence, although important for cell survival, T-cell contact with pMHC I can attenuate naive T-cell sensitivity by modulating CD8 levels. In addition, as mentioned above, TCR sensitivity in vitro can be reduced by high CD5 and CD45 expression. Thus, short-term extracellular signal-regulated kinase (ERK) phosphorylation and Ca²⁺ signaling induced by TCR ligation of CD8⁺ T cells is less marked for CD5^{hi} CD45^{hi} cells than for CD5^{lo} CD45^{lo} cells (Cho et al. 2016). However, reciprocal findings apply in vivo. Thus, CD5^{hi} CD8⁺ T cells generally give stronger responses to foreign Ags in vivo than CD5^{lo} cells (Cho et al. 2010). This discrepancy is explained by the finding that CD5^{hi} CD45^{hi} CD8⁺ T cells (i.e., cells with relatively strong

self-reactivity) show enhanced expression of cell surface lipid rafts and are thereby more sensitive to the stimulatory effect of cytokines than low-affinity cells (Cho et al. 2010). Under in vivo conditions, the increased sensitivity of the high-affinity cells to cytokines counters the small reduction in their TCR sensitivity. In addition to regulation by CD5 and CD45, the TCR sensitivity of CD8⁺ T cells is also influenced by their initial contact with the thymoproteasome during development. Thus, although the latter is especially important for selection of low-affinity CD8⁺ T cells, even high-affinity clones such as OT-I display reduced TCR sensitivity when generated in a thymus lacking the thymoproteasome (Takada et al. 2015).

For CD4⁺ T cells, studies with TCR-transgenic T cells have shown that short-term blockade of TCR/MHC II interaction reduces TCR sensitivity and responsiveness to foreign Ags (Stefanová et al. 2002). Conversely, exposing naive TCR-transgenic CD4⁺ T lymphocytes to increased concentrations of the self-peptides that induced positive selection heightens responses to their cognate foreign Ags (Ebert et al. 2009; Lo et al. 2009). These findings indicate that steady-state contact of CD4⁺ T cells with pMHC II ligands augments responses to foreign Ags. As for CD8⁺ T cells, CD4⁺ T cells with high self-reactivity show enhanced reactivity to foreign Ags. Thus, CD5^{hi} naive CD4⁺ T cells give stronger responses to pathogens in vivo than CD5^{lo} cells (Mandl et al. 2013). The authors of this study favor a model where thymic selection by strong pMHC ligands has a direct effect in enhancing TCR interaction with agonist pMHC epitopes. An alternative idea, also applicable to CD8⁺ T cells, is that corecognition of self-peptides during responses to foreign pMHC ligands augments the avidity of T/APC interaction and thereby amplifies the response (Sprent and Surh 2011). Direct evidence on this important issue is needed.

IL-7 Signaling

In addition to TCR signaling, naive T cells require interleukin-7 (IL-7) for their survival. IL-7 is produced by bone marrow- and thymus-derived stromal cells and promotes survival of

lymphoid progenitors (Namen et al. 1988; Sakata et al. 1990) as well as mature naive CD8⁺ and CD4⁺ T cells (Schluns et al. 2000; Tan et al. 2001). Thus, blocking IL-7 decreases the number of peripheral T cells in the absence of thymic output (Vivien et al. 2001; Kondrack et al. 2003), while IL-7-transgenic mice have increased naive cell counts without a major alteration in thymic cellularity (Mertsching et al. 1995; Kieper et al. 2002). The receptor for IL-7 (IL-7R) comprises heterodimers of IL-7R α and the common γ chain (γ c) (Kondo et al. 1994b) and is mainly expressed on T cells (Sudo et al. 1993; Kondo et al. 1994a). In T cells, IL-7 signaling augments expression of antiapoptotic factor Bcl-2, which counterbalances the effect of proapoptotic factors such as Bax and Bim (Akashi et al. 1997; Vivien et al. 2001; Khaled and Durum 2002; Khaled et al. 2002; Wojciechowski et al. 2007).

Because naive CD4⁺ and CD8⁺ T cells need to enter secondary lymphoid organs to receive survival signals from IL-7 (Link et al. 2007), this cytokine is thought to be largely cell membrane-bound and expressed in peripheral lymphoid organs. In support of this notion, IL-7 reporter mice show high IL-7 mRNA expression in fibroblastic reticular and lymphatic endothelial cells in peripheral lymph nodes (Alves et al. 2009; Mazzucchelli et al. 2009; Repass et al. 2009; Shalpour et al. 2010; Hara et al. 2012; Onder et al. 2012; Miller et al. 2013). IL-7 synthesis by these cells is limited and leads to strict competition between T cells and innate lymphoid cells for survival (Martin et al. 2017).

Ag-SPECIFIC MEMORY CELLS

Generation

It is well documented that naive T-cell responses to foreign Ags involve TCR recognition of pMHC ligands (signal 1) plus joint interaction with costimulatory ligands on mature APCs (signal 2) together with stimulation by cytokines, especially IL-2 (signal 3) (Sprent and Surh 2002; Kaech and Cui 2012; Ratajczak et al. 2018). These three signals induce the responding T cells to undergo marked expansion and differentiation into effector cells. After

pathogen clearance, >90% of the effector cells die rapidly, leading to a contraction phase where only a small fraction of cells survive as Ag-specific memory T cells (Fig. 2).

In the case of CD8⁺ memory T cells, the immune response generates a mixture of short-lived effector cells and memory precursor effector cells (SLECs and MPECs, respectively). Memory cell generation is complex and is driven in part by IL-7 via selective retention of IL-7R expression on MPECs (Joshi et al. 2007). However, many factors influence the fate of SLECs and MPECs, including TCR affinity, exposure time of contacting foreign Ags (Wherry et al. 1999; Prlic et al. 2006; Zehn et al. 2009), relative ligation of T-cell costimulatory molecules such as CD28, CD27, OX40, and 4-1BB (Hendriks et al. 2000, 2005; Mittrücker et al. 2001; Mousavi et al. 2008), and stimulation with cytokines such as IL-2, IL-21, interferon- γ (IFN- γ), and type I IFN (Marrack et al. 1999; Cheng et al. 2002; Blattman et al. 2003; Williams et al. 2006; Whitmire et al. 2007; Davis et al. 2008). Preferential generation of SLECs occurs with exposure to high concentrations of Ag (Wherry et al. 1999; Badovinac et al. 2004; Joshi et al. 2007; Zehn et al. 2009) and/or inflammatory cytokines such as IL-2, IFN- γ , and IL-12 (Badovinac et al. 2004; Cui et al. 2009; Kalia et al. 2010; Pipkin et al. 2010), whereas memory cells tend to be drawn from “latecomer” T cells that contact Ags during later stages of the immune response and thereby undergo relatively brief TCR ligation and exposure to inflammatory signals (Sprent 1994; D’Souza and Hedrick 2006; Sarkar et al. 2008).

The above findings apply largely to CD8⁺ T cells, and less is known about CD4⁺ memory T-cell generation (Marshall et al. 2011; Choi et al. 2013). As with CD8⁺ T cells, formation of memory CD4⁺ T cells is dependent on contact with various costimulatory molecules (Hendriks et al. 2000; Soroosh et al. 2007; Pagán et al. 2012). Likewise, very high concentrations of Ags favor terminal differentiation into Th1 effector cells rather than T follicular helper cells and memory cells. Thus, although CD4⁺ memory T-cell generation requires strong stimulation (Williams and Bevan 2004; Williams et al.

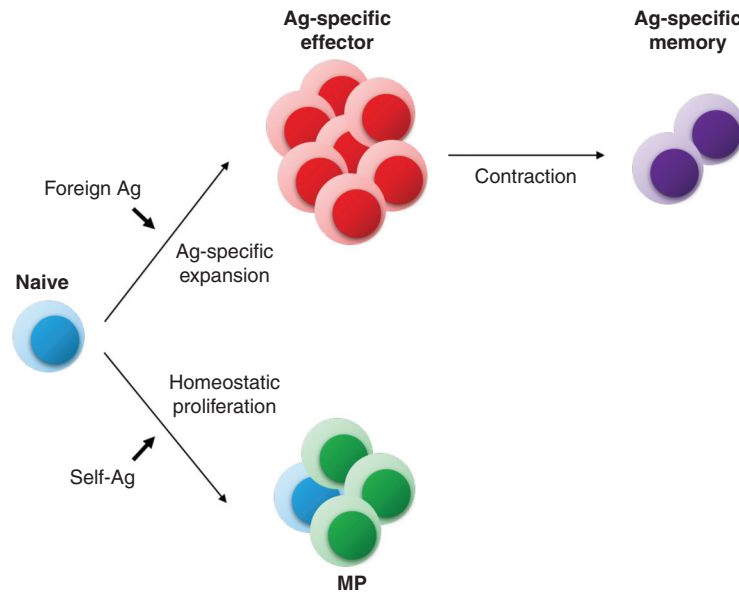


Figure 2. Recognition of foreign (strong) versus self (weak) antigens (Ags) generates different outcomes. When naive T cells recognize foreign Ags in the presence of sufficient levels of costimulation and cytokines, they proliferate extensively to give rise to Ag-specific effector T cells. After pathogen clearance, most effector T cells die, while a small fraction of cells survives as Ag-specific memory T cells. On the other hand, when naive T cells recognize self-Ags, they undergo a relatively mild proliferative response; such homeostatic proliferation generates a population of cells with a memory phenotype (MP).

2008), most of these cells are derived from effector cells expressing low rather than high levels of the activation marker CD25 (a component of the IL-2 receptor) (Snook et al. 2018). This finding implies that, as for CD8⁺ T cells, CD4⁺ memory T cells arise from precursors subjected to relatively weak TCR signaling and low stimulation by cytokines.

Maintenance

Both for CD8⁺ and CD4⁺ T cells, Ag-specific memory cells are largely maintained in the absence of MHC recognition (Murali-Krishna et al. 1999; Swain et al. 1999), although tonic TCR/MHC contact may be required for optimal recall responses (Kassiotis et al. 2002). For CD8⁺ memory cells, the transition to MHC independence correlates with a mild reduction in TCR sensitivity but increased sensitivity to cytokines, relative to naive cells (Cho et al. 2016).

It is now well established that the survival of Ag-specific memory T cells depends crucially on

contact with IL-7, both for CD8⁺ (Goldrath et al. 2002; Lenz et al. 2004) and CD4⁺ (Kondrack et al. 2003; Lenz et al. 2004; Purton et al. 2007) T cells. Thus, for CD8⁺ T cells, studies on the effects of IL-7 blockade and the use of genetically engineered mice with IL-7 signaling defects in mature T cells leads to progressive elimination of Ag-specific memory cells (Buentke et al. 2006; Carrio et al. 2007; Osborne et al. 2007). This finding reflects the capacity of IL-7 to up-regulate Bcl-2 (Kondrack et al. 2003; Carrio et al. 2007; Osborne et al. 2007) and thereby enables the cells to survive and undergo basal homeostatic proliferation (HP) (Goldrath et al. 2002; Lenz et al. 2004; Purton et al. 2007). Proliferation is slow and uniform, both for CD8⁺ and CD4⁺ memory T cells. For CD8⁺ memory cells, most of these cells have the CD62L^{hi} phenotype of Tcm, whereas typical memory CD4⁺ cells have a CD62L^{lo} Tem phenotype.

Like IL-7, IL-15 plays a key role in the survival of memory T cells. IL-15 shares many biological properties with IL-2 (Grabstein et al.

1994) and, like IL-2, IL-15 binds to a receptor composed of an IL-2 receptor β chain and γc (Giri et al. 1994; Grabstein et al. 1994). This $\beta\gamma c$ receptor, the low-affinity receptor for both IL-2 and IL-15 (IL-2/15R), is expressed at a high level on memory T cells, particularly on CD8⁺ T cells (Zhang et al. 1998; Becker et al. 2002; Purton et al. 2007). Contact with IL-15 is required for optimal maintenance of memory CD8⁺ and CD4⁺ T lymphocytes, both by inducing Bcl-2 expression and stimulating basal cell turnover (Becker et al. 2002; Goldrath et al. 2002; Lenz et al. 2004; Purton et al. 2007). Reflecting higher expression of the $\beta\gamma c$ receptor on CD8⁺ than CD4⁺ memory cells, IL-15 is especially important for memory CD8⁺ T-cell survival (Zhang et al. 1998; Lenz et al. 2004).

The main cellular source of IL-15 is still unclear. Unlike IL-2, IL-15 is uniquely anchored to the surface of presenting cells via an IL-15 receptor α chain (IL-15R α) to exert its biological function (Dubois et al. 2002; Sato et al. 2007). IL-15 and IL-15R α are broadly expressed in hematopoietic and nonhematopoietic cells (Burkett et al. 2003; Schluns et al. 2004), and in the case of hematopoietic cells, these two molecules need to be expressed by the same cell to maintain memory T-cell homeostasis (Burkett et al. 2004; Sandau et al. 2004). IL-15 reporter mice have revealed CD8 α ⁺ DCs as the main source of peripheral IL-15 mRNA (Colpitts et al. 2012; Sosinowski et al. 2013; Cui et al. 2014), which is consistent with the observation that DCs express IL-15R α (Dubois et al. 2005). In addition, one study using IL-15 knockin reporter mice suggested that fibroblastic reticular and blood endothelial cells are possible sources of IL-15 in peripheral lymph nodes (Cui et al. 2014). These stromal cell sources could be important because IL-15 production by radioresistant cells contributes to memory T-cell maintenance (Schluns et al. 2004).

MEMORY-PHENOTYPE CELLS

Definition

In normal unimmunized mice, mature T cells comprise a mixture of typical naive CD44^{lo} cells

plus a small proportion of cells with a CD44^{hi} MP. In young mice, the CD44^{hi} cell subpopulation accounts for approximately 10% of total CD4⁺ T cells and a slightly higher proportion of CD8⁺ cells. Because CD44^{hi} cells closely resemble Ag-specific memory cells, it has tacitly been assumed that these cells arise through contact with a spectrum of environmental Ags derived from commensal microbes and/or food. Supporting this notion, CD44^{hi} cells increase progressively with age and become the dominant population in old age (Dobber et al. 1992).

However, the simple idea that CD44^{hi} T cells are generated in response to diverse foreign Ags has been called into question by the finding that CD44^{hi} CD8⁺ and CD4⁺ T cells are present not only in specific pathogen-free (SPF) mice but also in germ-free (GF) and antigen-free (AF) animals, the latter lacking both commensal microflora and food Ags (Pereira et al. 1986; Dobber et al. 1992; Huang et al. 2005; Kim et al. 2016). Likewise, T cells with a similar phenotype exist in human cord blood and fetal spleen (Byrne et al. 1994; Szabolcs et al. 2003). These observations indicate that T cells with an MP can arise independently of foreign Ag stimulation, both in mice and humans. Notably, the MP CD8⁺ T cells, and to a lesser extent CD4⁺ cells, in unimmunized SPF mice have specificity for foreign Ags (as defined by specific tetramer staining) despite no previous contact with these Ags (Moon et al. 2007; Haluszczak et al. 2009); similar findings apply in humans (Su et al. 2013). These findings indicate that MP cells generated in the absence of foreign Ags have a broad TCR repertoire (Younes et al. 2011). In the case of CD8⁺ T cells, MP cells have also been termed “virtual” memory cells (Haluszczak et al. 2009).

With regard to turnover, typical MP T cells differ from Ag-specific memory T cells in comprising two broad subsets of cells proliferating at different rates (Tough and Sprent 1994; Younes et al. 2011). Thus, in normal unimmunized SPF mice, the majority of MP CD8⁺ T cells display a Tcm phenotype and have a slow turnover, but ~30% have an activated phenotype and proliferate rapidly (Boyman et al. 2006a). Likewise, a considerable proportion (18%–35%) of CD4⁺ MP cells are in cell cycle, which is substantially

higher than for Ag-specific memory cells (Younes et al. 2011). MP CD4⁺ T cells with a fast turnover also exist in human cord blood (Szabolcs et al. 2003). For both CD8⁺ and CD4⁺ MP cells, cells with a slow turnover closely resemble Ag-induced memory cells (see above). Also, MP cells are similar to Ag-induced memory cells in being responsive to pathogens, and thereby may provide an important “pre-immune” response to infection (see below). Nevertheless, these two memory subsets do display distinct differences, especially for CD8⁺ T cells. Thus, MP CD8⁺ T cells differ from Ag-induced memory cells in lacking expression of CD49d (integrin $\alpha 4$) and show only low synthesis of IFN- γ in response to TCR ligation (Haluszczak et al. 2009; Lee et al. 2013).

The above findings suggest that most of the MP T cells present in normal unimmunized SPF mice are generated in response to self-ligands rather than to foreign Ags (Fig. 2). Hence, MP T cells are a unique population distinct from conventional, foreign Ag-specific memory cells. In this respect, the frequency of MP (CD44^{hi}) cells in SPF, GF, and AF mice is quite similar, and, at least for CD8⁺ T cells, >90% of CD44^{hi} T cells in SPF mice lack CD49d expression (Kim et al. 2016; J Yi and T Kawabe, unpubl.). Nevertheless, it should be emphasized that, in marked contrast to mice raised in a clean environment, pet store mice and other mice housed in a “dirty” environment resemble adult humans in containing a high proportion of activated T cells with a Tem phenotype (Beura et al. 2016). The mechanisms controlling the homeostasis and function of the typical MP cells found in normal SPF mice are discussed below.

Generation

Information on the generation of MP cells has come largely from studies on the effects of transferring T cells to lymphopenic mice. Here, early studies documented that “homeostatic” proliferation of T cells following transfer to lymphopenic hosts applies not only to unseparated T cells but also to purified populations of naive T cells, both for CD8⁺ and CD4⁺ T cells (Ernst et al. 1999; Goldrath and Bevan 1999; Kieper

and Jameson 1999; Oehen and Brduscha-Riem 1999; Viret et al. 1999; Murali-Krishna and Ahmed 2000). Such HP also applies to TCR-transgenic T cells and is demonstrable not only in constitutively lymphopenic hosts such as RAG-deficient, severe combined immunodeficient (SCID), and CD3-deficient hosts but also in normal mice following acute host T-cell depletion. Under these conditions, the injected naive T cells expand considerably and thereby return total T-cell numbers toward normal levels.

Most studies on HP have been conducted by transferring carboxy-fluorescein succinimidyl ester (CFSE)-labeled total, naive, or MP CD8⁺ or CD4⁺ T cells into congenic lymphopenic mice and then analyzing the donor cells several days to weeks later. Based on CFSE dilution, such proliferation has two distinct components: slow (homeostatic) and fast (spontaneous) (Table 2; Kieper et al. 2005; Min et al. 2005). Proliferation of naive T cells in acutely lymphopenic hosts—also termed lymphopenia-induced proliferation—is typically quite slow with cells dividing up to 3–4 times by 1 week after transfer and gradually increasing their CD44 levels but without up-regulation of activation markers such as CD25 and CD69 (Min et al. 2005); for CD4⁺ T cells, whether all of the proliferating cells eventually become CD44^{hi} is still unclear (Kawabe et al. 2017; T Kawabe, unpubl.). This type of proliferation is generally stronger for CD8⁺ than CD4⁺ T cells. For both subsets, slow proliferation is mostly MHC-dependent, requiring contact with MHC I for CD8⁺ cells and MHC II for CD4⁺ cells (Ernst et al. 1999; Goldrath and Bevan 1999; Kieper and Jameson 1999; Viret et al. 1999; Tan et al. 2002). The rate of cell division by naive T cells is faster with CD5^{hi} than CD5^{lo} cells, both for polyclonal and TCR-transgenic cells (Kassiotis et al. 2003; Kieper et al. 2004). With regard to the stimulus for proliferation, early experiments established that slow HP can be induced by the ligands that induce initial positive selection in the thymus (Ernst et al. 1999; Goldrath and Bevan 1999). Also, it was shown that HP does not involve classic costimulation (Gudmundsdottir and Turka 2001; Prlc et al. 2001; Hagen et al. 2004; Kawabe et al. 2013), and that the rate of cell division is

Table 2. Homeostatic proliferation of naive T cells in lymphopenic specific pathogen-free (SPF) hosts

Tempo of proliferation	Stimuli required	Generation of effector cells	Proliferation in lymphopenic hosts	
			Acutely irradiated	RAG-deficient
Slow	Self-major histocompatibility complex (MHC) Interleukin-7 (IL-7)	-	++++	+++ ^a
Fast	Self-MHC Costimulation IL-2/15	+	+	++++ ^b

When transferred into lymphopenic recipient mice, naive CD4⁺ and CD8⁺ donor T cells proliferate slowly or rapidly, depending on the host used. Characteristics of these two kinds of proliferation are shown.

^aSlow proliferation is slightly less prominent in RAG-deficient than irradiated hosts, although only for polyclonal and not T-cell receptor (TCR)-transgenic T cells.

^bFast proliferation is much less in germ-free (GF) and antigen-free (AF) than SPF mice (see text for details).

reduced by competition from other T cells (Ernst et al. 1999).

The early finding that slow HP of naive T cells was inhibited by host T cells led to speculation that, in addition to MHC ligands, proliferation also involved in contact with cytokines. This notion led to the subsequent discovery that HP is controlled largely by the relative concentration of IL-7, the level of this cytokine being much higher in lymphopenic than normal hosts (Schluns et al. 2000; Tan et al. 2001, 2002; Min et al. 2005; Martin et al. 2017). Collectively, these findings support a model where, during continuous interaction of naive T cells with self-MHC ligands, contact with above normal levels of IL-7 signals the cells to enter cell cycle and begin to proliferate. After expansion, the cells also become dependent on IL-15 (see below).

The above data apply to the typical slow rate of intermittent proliferation seen following naive T-cell transfer to lymphopenic hosts. As mentioned above, however, a proportion of T cells can undergo rapid proliferation and acquire a CD44^{hi} phenotype in these hosts. Such fast proliferation is generally higher for CD4⁺ than CD8⁺ T cells and is more intense in mice that are constitutively lymphopenic such as RAG-deficient hosts than in mice made acutely lymphopenic (e.g., following irradiation) (Ernst et al. 1999). For CD4⁺ T cells, fast proliferation is abolished or greatly reduced in MHC II-deficient hosts (Ernst et al. 1999; Tan et al. 2002; Martin et al.

2003) and reflects contact with DCs (Do and Min 2009); likewise, fast proliferation of CD8⁺ T cells is MHC-dependent, although the data here are less clear-cut because of MHC I expression on the responding T cells (Cho et al. 2007) coupled with a small proportion of CD8⁺ T cells being MHC II-specific (Tyznik et al. 2004; Do and Min 2009). Unlike slow HP, fast proliferation requires costimulatory CD28 and OX40 signaling (Gudmundsdottir and Turka 2001; Hagen et al. 2004; Kawabe et al. 2013). Also, fast proliferation is considerably enhanced in hosts containing elevated levels of various γ c cytokines, including IL-2, IL-4, IL-7, and/or IL-15 (Boyman et al. 2006b; Cho et al. 2007, 2010; Ramsey et al. 2008); conversely, proliferation is inhibited by transforming growth factor- β (TGF- β) (Zhang and Bevan 2012).

For SPF hosts, a key issue is whether fast proliferation is directed to foreign or self-Ags. This question was initially addressed by examining the rate of naive CD4⁺ T-cell proliferation in RAG knockout hosts maintained in a GF versus SPF environment. Here, the important finding was that fast proliferation was considerably lower (but not abolished) in GF than SPF hosts, whereas slow HP was similar in both hosts (Kieper et al. 2005). This finding strongly suggests that, for CD4⁺ T cells, the bulk of the cells proliferating in RAG-deficient hosts are proliferating in response to foreign Ags, namely, to microbial Ags. Because fast proliferation is lim-

ited in acutely irradiated hosts, the intense proliferation seen in constitutively lymphopenic RAG-deficient hosts is thought to reflect a “leaky” gut as the result of the chronic absence of protective mucosal T cells in these mice (Sheridan and Lefrançois 2011). Though lower than in RAG knockout mice, commensal-dependent fast proliferation does occur in irradiated hosts and generates $\alpha 4\beta 7^+$ cells that synthesize IL-17 (Kawabe et al. 2013).

Although the residual fast proliferation of naive $CD4^+$ T cells in GF RAG-deficient hosts might be responding to food Ags, this possibility is unlikely because comparable proliferation is also seen in AF mice (Kieper et al. 2005; Yi et al. 2019). Moreover, the extent of fast proliferation can be augmented to high levels simply by increasing the number of T cells injected, thereby inducing strong activation of host APCs. These findings in GF and AF mice indicate that fast proliferation of $CD4^+$ T cells can be prominent in these hosts and is directed selectively to self-pMHC ligands. Based on parallel studies in Foxp3-DTR mice given diphtheria toxin (DT) to deplete Tregs (Yi et al. 2019), fast proliferation of naive $CD4^+$ T cells in hosts lacking Tregs, including RAG knockout hosts, is MHC II-restricted and directed to elevated levels of costimulation (CD80, CD86) on host APCs caused by the absence of Tregs. This finding follows previous evidence that fast proliferation in constitutively lymphopenic mice is inhibited by Tregs by IL-10-independent, CTLA4-dependent mechanisms (Bourgeois and Stockinger 2006; Winstead et al. 2008; Bolton et al. 2015; Yi et al. 2019, 2020). As for slow HP, fast proliferation is more prominent with $CD5^{hi}$ than $CD5^{lo}$ T cells, implying that the response is skewed to cells with the highest affinity for self-pMHC ligands (Kawabe et al. 2017; Yi et al. 2019). Based on studies with H2M knockout mice, proliferation includes reactivity to ubiquitous self-peptides.

In mice, fast proliferation is especially conspicuous in the neonatal period (Ichii et al. 2002; Min et al. 2003). Some of the proliferation during this period may be directed to foreign Ags, but rapid proliferation is also seen in AF neonates (K Frimpong-Boateng and CD Surh, pers. comm.). Because newborn mice are lympho-

penic, much of the fast proliferation in neonates could be driven by the combination of increased availability of Ags and cytokines and the paucity of Tregs at birth (Jiang et al. 2006). Another interesting possibility is that the rapid tempo of proliferation in neonates is due in part to a lack of feedback inhibition by a mature T-cell repertoire. In this respect, studies in adult SPF mice have shown that fast proliferation is enhanced if the TCR repertoire of preexisting T cells is curtailed (Min et al. 2004). The model here—which is also applicable in adult mice—is that a polyclonal TCR repertoire, especially of memory T cells, is necessary to block contact of naive T cells with self-ligands on APCs (Do et al. 2012). Studies on the rate of donor T-cell proliferation in GF or AF hosts coinjected with polyclonal versus pauciclonal T cells will be needed to assess this notion.

In addition to the proliferation seen in lymphopenic hosts, T cells undergoing fast proliferation are also generated under lymphoreplete physiological conditions (e.g., in the spleen of adult AF mice [Pereira et al. 1986] and cord blood of humans [Szabolcs et al. 2003]). Notably, significant fast proliferation of naive $CD4^+$ and $CD8^+$ T cells is apparent following transfer to normal adult hosts (White et al. 2016; Kawabe et al. 2017); the responding cells in these hosts are largely $CD5^{hi}$ cells, and their progeny differentiate into typical MP cells that provide protection against infection (see below) (Kawabe et al. 2017). These findings provide direct support for the view that the component of MP cells found in normal mice is a reflection of an ongoing response to self-ligands.

Maintenance

Although the initial response of naive T cells to self-MHC ligands is TCR-dependent, subsequent proliferation of the cells appears to switch progressively to stimulation largely via cytokines, especially IL-7. Once fully formed, the resultant population of MP cells divides intermittently in response to background levels of both IL-7 and IL-15 (Tan et al. 2002; Purton et al. 2007; Younes et al. 2011). As for Ag-induced memory T cells, dependency on these two

cytokines applies to both CD4⁺ and CD8⁺ MP cells, although IL-15 is more important for CD8⁺ cells than CD4⁺ cells, reflecting the higher density of IL-2R β on CD8⁺ cells. For CD8⁺ T cells, except for the minor MHC-dependent population of precursor cells, MP T-cell proliferation and survival are largely MHC-independent, which correlates with a mild reduction in their TCR sensitivity; whether this also applies to CD4⁺ T cells is less clear (Boyman et al. 2006a; Cho et al. 2016; Kawabe et al. 2017). For CD4⁺ MP cells, in addition to cytokines, their proliferation and survival require signaling via costimulatory molecules such as CD28 and OX40 (Yamaki et al. 2014; Kawabe et al. 2017).

Function

As mentioned earlier, MP cells resemble naive T cells in containing an extensive repertoire of cells reactive to foreign Ags and thus may be important in providing an accelerated Ag-specific response to pathogens relative to naive T cells (Fig. 3). This response might be conspicuous for cells in the early TCR-dependent phase of MP generation because fast-dividing T cells produce various types of effector cytokines/molecules such as IL-2, tumor necrosis factor- α (TNF- α), IFN- γ , IL-13, IL-17A, and Granzyme B in response to ex vivo TCR stimulation (Murali-Krishna and Ahmed 2000; Gudmundsdottir and Turka 2001; Min et al. 2003; Kieper et al. 2005; Min et al. 2005; Winstead et al. 2008; Kawabe et al. 2013; Yi et al. 2019). Indeed, TCR-transgenic MP CD8⁺ T lymphocytes generated in lymphopenic hosts showed better Ag-specific protection against pathogens than naive cells (Hamilton et al. 2006). Similarly, Ag-specific CD8⁺ MP cells defined by tetramer staining responded to their cognate Ags more rapidly than did their naive counterparts (Haluszczak et al. 2009). Whether CD4⁺ MP cells have this function in vivo is less clear (Kawabe et al. 2016).

In addition to adaptive memory function, MP cells also display innate immune function by several parameters (Fig. 3). Thus, for CD8⁺ T cells, MP cells produce only low amounts of IFN- γ after TCR ligation (Lee et al. 2013), but strong IFN- γ synthesis when stimulated with a

cocktail of cytokines (IL-2, IL-12, IL-18) without TCR ligation, presumably reflecting enhanced cytokine receptor expression on MP cells (Haluszczak et al. 2009). Furthermore, as for bystander immunity mediated by conventional memory CD8⁺ T cells (Berg et al. 2003; Soudja et al. 2012; Chu et al. 2013; Schenkel et al. 2014), MP CD8⁺ T cells can induce strong non-Ag-specific host defense against pathogen infection in vivo (White et al. 2016). Likewise, whereas the evidence for bystander immunity by conventional memory CD4⁺ T cells is limited (Guo et al. 2015), CD4⁺ MP cells can generate effective innate IFN- γ responses, both in vitro (Hu and August 2008) and in vivo (Kawabe et al. 2017). Under in vivo conditions, IFN- γ responses by CD4⁺ MP cells are mediated by a T-bet^{hi} subset induced by background levels of IL-12; these cells provide non-Ag-specific protection against *Toxoplasma gondii* infection (Kawabe et al. 2020). Collectively, these findings provide increasing evidence that, like other innate immune subsets, MP CD8⁺ and CD4⁺ T cells provide an important first-line defense against infectious agents (Kawabe et al. 2018; Kedl and White 2018).

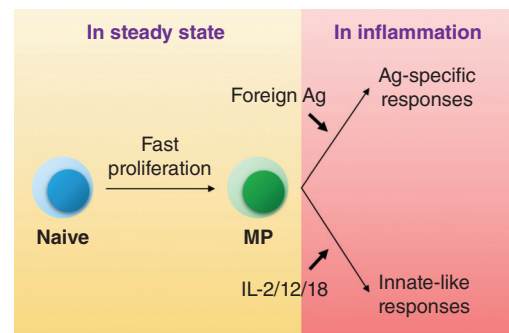


Figure 3. Steady-state generation of memory-phenotype (MP) cells and their immunological functions during infection. CD4⁺ and CD8⁺ MP cells are homeostatically generated from naive precursors. Once generated, they engage in typical antigen (Ag)-specific immune responses to pathogens. In addition, they exert innate-like effector activity by responding to cytokines such as interleukin (IL)-2, IL-12, and IL-18 and by producing effector cytokines in the absence of cognate Ag recognition.

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In addition to protection against pathogens, MP T cells may be important for maintaining self-tolerance. Thus, in parallel with typical CD4⁺ Tregs, a subset of MP CD8⁺ cells with an IL-2/15R^{hi} Ly49⁺ phenotype regulates the function of T follicular helper cells, thereby serving to prevent autoimmune disease (Kim et al. 2011).

Summary

To summarize the data on MP T cells, these cells arise continuously from naive precursors responding to self-pMHC ligands. Under steady-state conditions, small numbers of high-affinity CD4⁺ and CD8⁺ T cells are induced to proliferate transiently through contact with APCs and then differentiate into MP cells. This process is intensified when T-cell numbers are reduced, causing residual T cells to undergo compensatory, slow, MHC-restricted costimulation-independent expansion in response to increased host levels of stimulatory cytokines, mainly IL-7. In certain situations, altering the host environment can intensify T/APC interaction and drive fast proliferation. These conditions are extreme in T/B-cell-deficient RAG knockout and Treg-deficient, DT-treated Foxp3-DTR mice. Here, host APCs are activated to up-regulate costimulatory molecules by unregulated T cells, and together these cells stimulate production of very high levels of stimulatory cytokines such as IL-2 and IL-15. Under these conditions, the response to self-ligands can be as intense as the normal immune response to foreign Ags. Overall, the tempo of proliferation leading to MP cell formation is determined by the interplay of multiple factors, including relative TCR affinity of responding naive T cells, the influence of Tregs and possibly preexisting MP cells, the activation status of APCs, and the concentration of various cytokines and expression of receptors for these cytokines on T cells. Once formed, MP cells divide intermittently in response to cytokines and play an important role in providing rapid Ag-specific and -nonspecific responses to pathogens.

CONCLUDING REMARKS

As outlined in this review, CD8⁺ and CD4⁺ memory T cells comprise two broad subsets of

cells, each derived from naive precursor cells but generated via essentially different pathways of differentiation. In normal adult animals, exposure to a multiplicity of environmental Ags generates an extensive repertoire of foreign Ag-specific memory cells that provide strong protective immunity against pathogens previously encountered. However, in early neonatal life and adults maintained in an SPF environment, Ag-induced memory T cells are rare and most T cells with an MP are the progeny of naive T cells responding to self-MHC ligands. These MP cells have a broad TCR repertoire and complement the naive adaptive immune system in giving rapid responses to pathogens; in particular, together with natural killer (NK) and other innate lymphoid cells, they provide an intermediary layer of immune activity between myeloid cell-mediated innate and lymphocyte-mediated adaptive immunity. Conceivably, manipulating MP cell generation and/or function might be useful to augment pathogen-targeted immunity or regulate autoimmune disease. Future studies will be needed to assess this possibility.

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