

CENTRAL MEMORY AND EFFECTOR MEMORY T CELL SUBSETS: Function, Generation, and Maintenance

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Key Words T_H1/T_H2, T lymphocyte activation, chemokine receptors, lymphocyte migration, effector function

■ **Abstract** The memory T cell pool functions as a dynamic repository of antigen-experienced T lymphocytes that accumulate over the lifetime of the individual. Recent studies indicate that memory T lymphocytes contain distinct populations of central memory (T_{CM}) and effector memory (T_{EM}) cells characterized by distinct homing capacity and effector function. This review addresses the heterogeneity of T_{CM} and T_{EM}, their differentiation stages, and the current models for their generation and maintenance in humans and mice.

THE CELLULAR BASIS OF IMMUNOLOGICAL MEMORY

Memory is the hallmark of the acquired immune system. It results from the clonal expansion and differentiation of antigen-specific lymphocytes that ultimately persist for a lifetime. Memory lymphocytes confer immediate protection in peripheral tissues and mount recall responses to antigens in secondary lymphoid organs. In the B cell system these functions are carried out by distinct cell types. Protective memory is mediated by plasma cells that secrete antibodies, whereas reactive memory is mediated by memory B cells that proliferate and differentiate to plasma cells in response to secondary antigenic stimulation (1–3). A similar division of labor has recently emerged in the T cell system (4–6). According to the model proposed (7), protective memory is mediated by effector memory T cells (T_{EM}) that migrate to inflamed peripheral tissues and display immediate effector function, whereas reactive memory is mediated by central memory T cells (T_{CM}) that home to T cell areas of secondary lymphoid organs, have little or no effector function, but readily proliferate and differentiate to effector cells in response to antigenic stimulation. In this review we first describe the properties of T_{CM} and T_{EM} in terms of their heterogeneity, effector functions, and responsiveness to antigen or cytokines.

0732-0582/04/0423-0745\$14.00

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We then discuss a pathway of progressive T cell differentiation along which T_{CM} are arrested at an intermediate stage preceding that of T_{EM} . Finally, we consider current models for generation and maintenance of memory T cell subsets.

PHENOTYPIC AND FUNCTIONAL PROPERTIES OF T_{CM} AND T_{EM}

Definition of Human T_{CM} and T_{EM}

T_{CM} and T_{EM} were initially defined in the human system based on two distinct criteria: (a) the absence or presence of immediate effector function and (b) the expression of homing receptors that allow cells to migrate to secondary lymphoid organs versus nonlymphoid tissues (4). Human T_{CM} are CD45R0+ memory cells that constitutively express CCR7 and CD62L, two receptors that are also characteristic of naïve T cells, which are required for cell extravasation through high endothelial venules (HEV) and migration to T cell areas of secondary lymphoid organs (8, 9). When compared with naïve T cells, T_{CM} have higher sensitivity to antigenic stimulation, are less dependent on costimulation, and upregulate CD40L to a greater extent, thus providing more effective stimulatory feedback to dendritic cells (DC) and B cells. Following TCR triggering, T_{CM} produce mainly IL-2, but after proliferation they efficiently differentiate to effector cells and produce large amounts of IFN- γ or IL-4.

Human T_{EM} are memory cells that have lost the constitutive expression of CCR7, are heterogeneous for CD62L expression, and display characteristic sets of chemokine receptors and adhesion molecules that are required for homing to inflamed tissues. When compared with T_{CM} , T_{EM} are characterized by rapid effector function. CD8 T_{EM} carry large amounts of perforin, and both CD4 and CD8 produce IFN- γ , IL-4, and IL-5 within hours following antigenic stimulation. Some CD8 T_{EM} express CD45RA (here defined as T_{EMRA}) and carry the largest amount of perforin. Thus in humans, the T_{EM} pool contains bona fide T_H1 , T_H2 , and CTL.

The relative proportions of T_{CM} and T_{EM} in blood vary in the CD4 and CD8 compartments; T_{CM} is predominant in CD4 and T_{EM} in CD8. Within the tissues, however, T_{CM} and T_{EM} show characteristic patterns of distributions. T_{CM} are enriched in lymph nodes and tonsils, whereas lung, liver, and gut contain greater proportions of T_{EM} (10). In antigen-primed individuals, tetanus toxoid-specific CD4 T cells can be detected in circulating T_{CM} and T_{EM} up to 10 years after antigenic stimulation, and their frequencies increase in both subsets following booster immunization (4). The same is true for the CD8 compartment: Antigen-specific T cells, detected by HLA/peptide multimer staining, can be found in T_{CM} and T_{EM} subsets, although the relative proportion can be highly variable. For example, HIV-specific T cells are largely T_{EM} (CD45RA-CCR7-), whereas CMV-specific T cells are predominantly T_{EMRA} (CCR7-CD45RA+) (11). In some melanoma patients, melan-A tetramer+ T cells are mainly CCR7+CD45RA+ and do not respond to

antigenic stimulation *ex vivo*, whereas in other patients melan-A tetramer+ cells have a T_{EM} or T_{EMRA} phenotype ($CCR7^- CD45RO^+$ or $CCR7^- CD45RA^+$) and responses to melan-A peptide can be readily demonstrated (12, 13). Interestingly, a longitudinal study of EBV-infected patients revealed differences in lytic versus latent epitope-specific composition of T cell populations in the chronic carrier stage of infection (14). Thus while tetramer-positive cells in the infectious stage have a $CCR7^- CD45RA^-$ phenotype, cells specific for lytic but not latent epitopes acquire CD45RA in the chronic carrier stage.

A detailed TCR repertoire analysis was performed by spectra-typing in CD45RO+ memory CD8 T cells using CD62L to discriminate between T_{CM} and T_{EM} (15). The analysis of six influenza-specific T cell clones showed that two clonotypes were shared between T_{CM} and T_{EM} , whereas four were detected only in T_{CM} . A similar picture was obtained after a nine-month period. These results indicate that the same expanded clone can be present in both T_{CM} and T_{EM} subsets and that, within the influenza-specific CD8 memory T cell pool, the clonotype distribution is remarkably stable with no evidence of conversion from CD62L+ and CD62L- memory subsets or vice versa.

The presence of memory T cells with different migratory capacity and effector function was also documented in mice. Two populations of memory CD4 T cells survive for months after immunization with antigen in adjuvant: One, found primarily in the lymph nodes, produces IL-2; the other larger population found in nonlymphoid tissues produces IFN- γ (5). Similarly, two populations of antigen-specific memory CD8 T cells are present following viral or bacterial infection (6). Whereas CD8 memory T cells isolated from nonlymphoid tissues exhibit lytic activity directly *ex vivo*, their splenic counterparts do not. These results extended the T_{CM}/T_{EM} paradigms to the mouse system.

In summary, there is now convincing evidence that antigen-specific CD4 and CD8 memory T cells persist as T_{CM} and T_{EM} populations. However, since the first description of T_{CM} and T_{EM} , it became evident that these two broad subsets were heterogeneous in expression of chemokine receptors, adhesion, and costimulatory molecules (4).

Heterogeneity of T_{CM} and T_{EM}

Subsets of T_{CM} and T_{EM} with distinct functional programs can be identified according to the expression of surface molecules. Costimulatory molecules have been the first markers used to dissect the heterogeneity of memory T cells. CD27 and CD28, which are expressed on naïve T cells, are also expressed on some memory T cells but are absent in a subset of CD8 memory T cells characterized by high effector function and expression of CD45RA (16, 17). The CD27-CD45RA+ CD8 T cell population largely overlaps with T_{EMRA} . However, some cells within T_{EMRA} express CD27 and display phenotypic and functional features that are intermediate between naïve and effector T cells (18).

A sizable proportion of circulating T_{CM} expresses CXCR5, the receptor for CXCL13, a chemokine produced in B cell follicles (19, 20). These CXCR5+ T

cells, which have been defined as follicular helper T cells (T_{FH}), are nonpolarized and upon activation produce IL-2 and some IL-10. They are also present in tonsils where they express CD40L and ICOS and provide spontaneous help to B cells (21–23).

The T_{EM} pool can be subdivided according to the expression of chemokine receptors characteristic of T_H1 or T_H2 cells (24). Thus within T_{EM} , CCR5 and CXCR6 discriminate T_H1 and CTL, whereas CCR3 and CRTh2 identify T_H2 cells (25–28). CXCR3 and CCR4 are expressed on T_H1 and T_H2 (29, 30), respectively, but are also expressed on distinct subsets of T_{CM} . As is discussed below, CXCR3+ and CCR4+ T_{CM} subsets represent pre-effector cells (pre- T_H1 and pre- T_H2 , respectively).

The combinatorial expression of adhesion molecules and chemokine receptors allows tissue-specific targeting of T cell and leukocyte subsets (31, 32). Thus the simultaneous expression of CLA and CCR4 identifies skin homing T cells (33), whereas the expression of $\alpha 4\beta 7$ and CCR9 is characteristic of gut-homing T cells (34). Some skin-homing and gut-homing T cells express CCR7 (10), suggesting that they may be capable of homing to lymphoid as well as nonlymphoid tissues. In addition, while most T_{CM} simultaneously express CCR7 and CD62L, there are several T_{EM} , especially within CD4, that lack CCR7 but express CD62L (4). This finding is consistent with the ability of CD62L+ T_{EM} to enter lymph nodes through HEV using other receptors that bind to chemokines capable of mediating arrest under flow (35). This may be particularly relevant in inflammatory conditions when chemokines produced in peripheral tissues may be transported and displayed on the luminal face of endothelial cells (36, 37).

In summary, functional subsets can be discriminated within T_{CM} and T_{EM} using chemokine receptors and other markers (Figure 1). It should be noted, however, that some of these markers are rapidly and transiently modulated upon cell activation. For instance, following antigenic stimulation T_{EM} transiently upregulate CCR7 and CXCR5 while downregulating CCR5 (38). Furthermore, CD62L is rapidly shed after TCR triggering or following lymph node immigration (39, 40). Thus the phenotypic characterization of T_{CM} and T_{EM} applies to only resting cells, i.e., those that are not engaged in an antigen-driven response.

Response of T_{CM} and T_{EM} to Antigenic Stimulation

When stimulated in vitro, memory T cells show low-activation threshold and vigorous proliferation. Although both T_{CM} and T_{EM} have a high responsiveness to antigenic stimulation, the expansion potential decreases from T_{CM} to T_{EM} and is very low in CD8 T_{EMRA} (4, 41). The reduced proliferative capacity correlates with a decrease in telomere length and with an increased propensity to undergo apoptosis. These intrinsic constraints can be overridden by costimulation, which induces telomerase activity and upregulation of antiapoptotic molecules (42, 43). Indeed, whereas memory T cells (especially T_{EM}) have short telomeres (4), a small subset

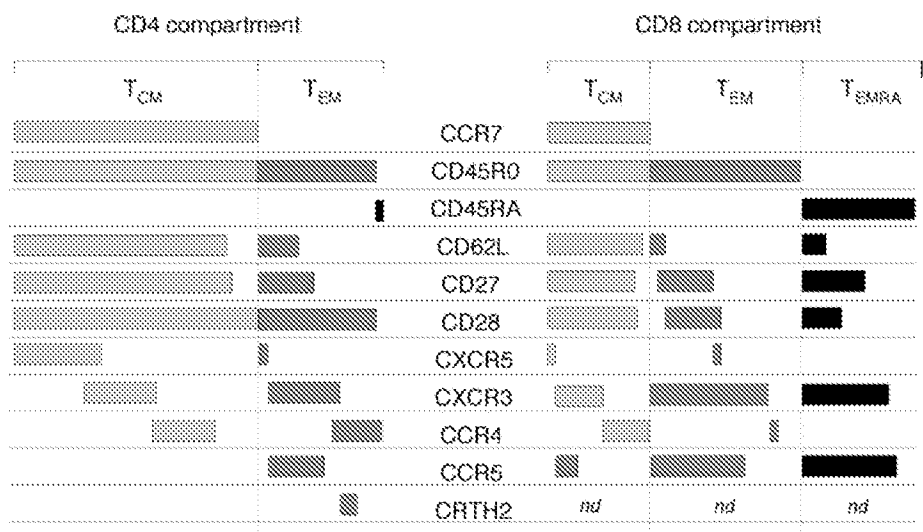


Figure 1 Phenotypic heterogeneity of human memory T cells. Note that the percentages are only indicative of those found in a healthy human adult because there is considerable individual variability.

of CD8 memory T cells, which have undergone several rounds of cell division in vivo (as detected by low levels of T cell receptor excision circles), has long telomeres, possibly owing to induction of telomerase activity (18).

When analyzed at the clonal level, T_{CM} stimulated in vitro appear to be heterogeneous in their ability to differentiate (44). Some cells can be propagated in a noneffector state by stimulation under neutral conditions and can be induced to differentiate to T_H1 and T_H2 upon stimulation in the presence of IL-12 or IL-4, respectively. Others, however, spontaneously differentiate to IFN- γ - or IL-4-producing cells, even if stimulated in the absence of polarizing cytokines. Therefore, these cells represent pre-T_H1 and pre-T_H2 and can be identified according to the expression of CXCR3 and CCR4, respectively (41; J. Geginat, unpublished data).

When stimulated under neutral conditions, T_{EM} retain their T_H1 or T_H2 phenotype, demonstrating that the pattern of cytokine gene expression that is imprinted at priming can be stably maintained (44). However, when stimulated under opposite polarizing conditions, most T_{EM} can acquire the ability to express the alternative cytokine. Thus T_H1 maintain the IFN- γ -producing ability but are also able to produce IL-4. Similarly, most T_H2 maintain IL-4 production and acquire IFN- γ -producing ability. An exception is represented by CRTh2+ T cells, which are irreversibly committed to the T_H2-lineage and are unable to acquire IFN- γ -producing ability when stimulated with IL-12. The ability of most human T_{EM} to undergo divergent

T_H1 and T_H2 differentiation is a property that is not shared by mouse T cells, which become rapidly committed following stimulation (45). Considering that in the human system a substantial number of T_{CM} are uncommitted and most memory T cells maintain cytokine flexibility, it is possible that expression of the opposite cytokine can be enforced by delivering recall antigens together with the appropriate polarizing signals.

In summary, T_{CM} are heterogeneous and contain both uncommitted and pre-committed noneffector cells. Importantly, the CCR7+ phenotype is rapidly lost upon antigenic stimulation concomitant with the differentiation of T_{CM} to effector cells. In addition, with the exception of a transient CCR7 upregulation induced by TCR stimulation, T_{EM} remain CCR7— and maintain both memory and flexibility of cytokine gene expression. These results are consistent with the notion that CCR7 expression is irreversibly lost upon differentiation and marks the T_{CM} to T_{EM} transition.

How do T_{CM} and T_{EM} achieve antigenic stimulation *in vivo*? T_{CM} , with access to secondary lymphoid organs, can be stimulated by antigen presented by mature DC, whereas T_{EM} , which are largely excluded from these areas, can be stimulated by antigen presented by nonprofessional APC in a milieu that does not favor stable cell-cell interactions (46, 47). How then can the quality of the primary response be perpetuated in the secondary response? We consider three possibilities. First, the T cell stimulatory conditions provided by the innate immunity through DC activation may be similar in both primary and secondary responses and therefore generate the same type of effector cells from uncommitted precursors. Second, precommitted T cells may have been generated in the primary response, and upon subsequent antigen exposure, the cells rapidly differentiate to T_H1 or T_H2 . A third possibility is that T_{EM} may condition DC in the peripheral tissues or may reach the antigen-stimulated lymph nodes by upregulating CCR7 or through alternative pathways. In both of these cases the local production of IFN- γ and IL-4 by T_{EM} may further direct a T_H1 or T_H2 response.

It is interesting to consider the differentiation stage of memory T cells within the framework of the hygiene hypothesis (48). Individuals that have undergone multiple T_H1 responses may be protected from allergic diseases not only by memory T_H1 cells present in nonlymphoid tissues but also by the large repertoire of potentially cross-reactive pre- T_H1 cells present in secondary lymphoid organs.

Response of T_{CM} and T_{EM} to Homeostatic Cytokines

Proliferation of memory T cells can be driven not only by antigenic stimulation but also by cytokines. Indeed, under steady-state conditions, memory T cells slowly turn over in the absence of antigen (49, 50). *In vivo* studies using gene-targeted mice demonstrated that IL-7 and IL-15, which are constitutively produced by a variety of cells, play an essential role for maintenance of both CD4 (51) and CD8 T cells (52–54). Early studies in the human system showed that T cells proliferate in response to γ -common dependent cytokines in a TCR-independent

fashion (55). More recent studies demonstrated that T_{CM} not only proliferate in response to homeostatic cytokines but also differentiate to effector cells expressing receptors for inflammatory chemokines and producing large amounts of effector cytokines (41, 56). In response to IL-7 and IL-15, $CXCR3+ T_{CM}$ differentiate to T_{H1} , whereas most $CCR4+ T_{CM}$ differentiate to T_{H2} , consistent with the notion that these subsets are precommitted. Under the same culture conditions, $CD8 T_{CM}$ generate a variety of effector cells that include $CD45RA+$ and $CD45RA-$ CTL (41, 57). The findings that T_{EMRA} appear only late during the immune response (14) and that $CD45RA+$ can be upregulated on memory T cells following cytokine-driven but not antigen-driven proliferation (41) suggest that the T_{EMRA} subset is generated primarily through homeostatic rather than antigen-dependent pathways.

T CELL DIFFERENTIATION: EFFECTOR CELLS AND INTERMEDIATES

The molecular definition of T_{H1} and T_{H2} differentiation provides a powerful paradigm to understand where, along the T cell differentiation pathway, T_{CM} and T_{EM} are positioned and to identify the signals involved in their generation. The evidence that we discuss below indicates that T_{CM} are cells arrested at intermediate stages of differentiation preceding T_{EM} and suggests that T_{CM} are generated by subthreshold stimulation.

Imprinting of T_{H1} and T_{H2} Differentiation

Upon antigenic stimulation, naïve T cells can enter a T_{H1} or T_{H2} differentiation program, which involves the coordinated expression of genes controlling tissue homing and effector function. Signals emanating from the TCR and from the IL-12 or the IL-4 receptor act in synergy to induce specific transcription factors (58, 59) that mediate chromatin remodeling events at target genes (60–64). In particular in differentiating T_{H1} and T_{H2} cells, T-bet and GATA-3 induce histone modifications and DNA methylation of the IFN- γ and IL-4 genes, respectively, increasing their accessibility to the transcriptional machinery (65). Other genes characteristic of T_{EM} , such as receptors for inflammatory chemokines and perforin, appear to be controlled by similar chromatin remodeling events (66, 67). Histone acetylation at a locus can be inherited through mitosis and can therefore contribute to the maintenance of specific states of gene activity from one generation to the next. Circulating $CCR5+$ and $CRTh2+ T_{EM}$ show patterns and levels of histone acetylation at cytokine genes characteristic of in vitro cultured T_{H1} and T_{H2} cells, whereas T_{CM} have an acetylation state similar to that of naïve T cells (44).

The cytokine gene flexibility characteristic of human T_{EM} described above can be explained by the capacity of these cells to upregulate the relevant transcription factor. Indeed, upon stimulation under T_{H2} conditions, $CCR5+ T_{H1}$ cells upregulate GATA-3 and acquire *Ii4* acetylation and expression. In contrast, when

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stimulated in the presence of IL-12, CRTh2+ T_H2 cells are unable to upregulate T-bet and therefore fail to acquire IFN- γ -producing capacity (44). These results are in agreement with transfection experiments demonstrating that T-bet and GATA-3 are necessary and sufficient to induce IFN- γ and IL-4 gene expression (58, 68). Recent data on the expression of T-bet and GATA-3 in T_{CM} and T_{EM} suggest a progressive loss of flexibility as T helper cells proceed from naïve to the effector memory stages of differentiation (69).

Chromatin remodeling at the *Ifng* and *Il4* loci is acquired progressively as a function of TCR and cytokine stimulation (70). Regulation of cytokine gene accessibility allows for a wide range of expression levels in response to antigenic stimulation. For instance, IFN- γ and IL-4 are produced in very small amounts by naïve T cells (45) and at increased levels in some T_{CM}, whereas a rapid and greater production is characteristic of highly differentiated T_H1 and T_H2 present within T_{EM} (44). The levels and kinetics of cytokine gene expression are obviously relevant parameters of effector function and, in this context, it is worth considering that in vitro and in vivo read outs can differ significantly and that the nature of the stimulus (i.e., antigenic versus pharmacological stimulation) may lead to divergent results (68, 71).

T cell differentiation to T_H1 or T_H2 can be inhibited either by neutralizing IL-12 and IL-4 or by addition of TGF- β (30, 72). This results in the generation of nonpolarized T cells that retain constitutive CCR7 expression, have low levels of GATA-3 and T-bet, and carry hypoacetylated IFN- γ and IL-4 genes (44, 73, 74). These cells have all the characteristics of T_{CM}, i.e., being either uncommitted or precommitted to T_H1 or T_H2.

Signal Strength and Progressive T Cell Differentiation

In addition to the role cytokines play, it is worth emphasizing that of TCR signal strength as a major factor in determining T cell differentiation. The strength of signaling that T cells receive can vary widely depending not only on the concentration of antigen and costimulatory molecules (determining the rate of TCR triggering and signal amplification) (75, 76) but also on the duration of the interaction between T cells and APC (determining the duration of signaling) (77). Recent studies revealed that DC/T cell interactions are highly dynamic and can last from a few minutes to several hours (46, 78). Importantly, a prolonged stimulation can compensate for a lower level of antigen or costimulatory molecules, whereas an increase in antigen or costimulatory molecules can compensate for a shorter stimulation.

With use of an in vitro priming system in which the strength of antigenic stimulation is tightly controlled, naïve CD4 T cells primed by a weak stimulus proliferate but do not develop effector function (79). Upon in vivo transfer these cells migrate to lymph nodes where they rapidly proliferate and differentiate in response to antigen. In contrast, T cells primed by a strong stimulus in the presence of IL-12 or IL-4 differentiate to T_H1 or T_H2 that, upon in vivo transfer, are excluded from lymph nodes and migrate to inflamed peripheral tissues. After an initial

stimulation, CD8 T cell differentiation to peripheral tissue homing effector cells is promoted by IL-2, whereas IL-15 expands nonpolarized T cells that, upon *in vivo* transfer, home to both lymphoid and nonlymphoid tissues (80, 81). Signal strength can also be modulated *in vitro* by the number and type of DC used (82). Whereas low DC/T cell ratio preferentially generate T_{CM} -like cells, higher ratios favor priming of effector cells and eventually lead to T cell deletion by activation-induced cell death. The expression of costimulatory molecules, such as ICOS-L and 4-1BBL, plays a role in reinforcing the signals by APC and is required for effective T cell responses (83–86).

Initial studies on the requirements for CD8 T cell priming suggested that their differentiation follows an autonomous program after brief stimulation with highly stimulatory engineered APC (87). However, further studies showed that signal strength also plays a key role in CD8 T cell differentiation (88, 89). Priming by a signal of suboptimal strength, such as that provided by immature DC, induces CD8 T cell proliferation, but the cells fail to upregulate antiapoptotic molecules and receptors for homeostatic cytokines (88). Upon *in vivo* transfer these cells, defined as unfit, die by neglect. Thus both CD4 and CD8 T cells show a signal strength-dependent differentiation despite the more readily inducible proliferation and differentiation of CD8 T cells.

Taken together the available data are consistent with the concept that the strength of the signals delivered by TCR and cytokine receptors drives T cells through hierarchical thresholds of differentiation (90). The progression follows a sequence of proliferation, preceding the acquisition of fitness, effector function and, eventually, death (Figure 2). Because TCR and cytokine stimulation are stochastic events, not all proliferating T cells receive the same strength of signal. Consequently, primed T cells reach a variety of differentiation stages that contain effector cells as well as cells that have been arrested at intermediate levels of differentiation. These intermediates retain lymph node homing receptors and have initiated, but not completed, the remodeling events of genes involved in effector functions. This spectrum can be simply resolved into distinct subsets of T_{CM} and T_{EM} surviving after antigen clearance.

The question remains—how can different levels of signal strength be delivered in the course of the same immune response? We can envision two possibilities. The first is that in highly stimulatory conditions some activated T cells may prematurely detach from DC and fail to receive further stimulation consistent with observations of dynamic DC/T cell interactions *in vivo* (78). Second, different levels of stimulation may be delivered at different times. At early stages, large numbers of mature DC carrying high doses of antigen and secreting large amounts of polarizing cytokines would promote a massive proliferation of antigen-specific T cells and drive them into effector cells, some of which will persist as T_{EM} . At later time points, the stimulatory conditions may change substantially with arrival of fewer DC, which furthermore carry small amounts of antigen and have exhausted their cytokine-producing capacity (91). Such conditions may lead to the expansion of noneffector cells, of which the fit ones will persist as T_{CM} . According

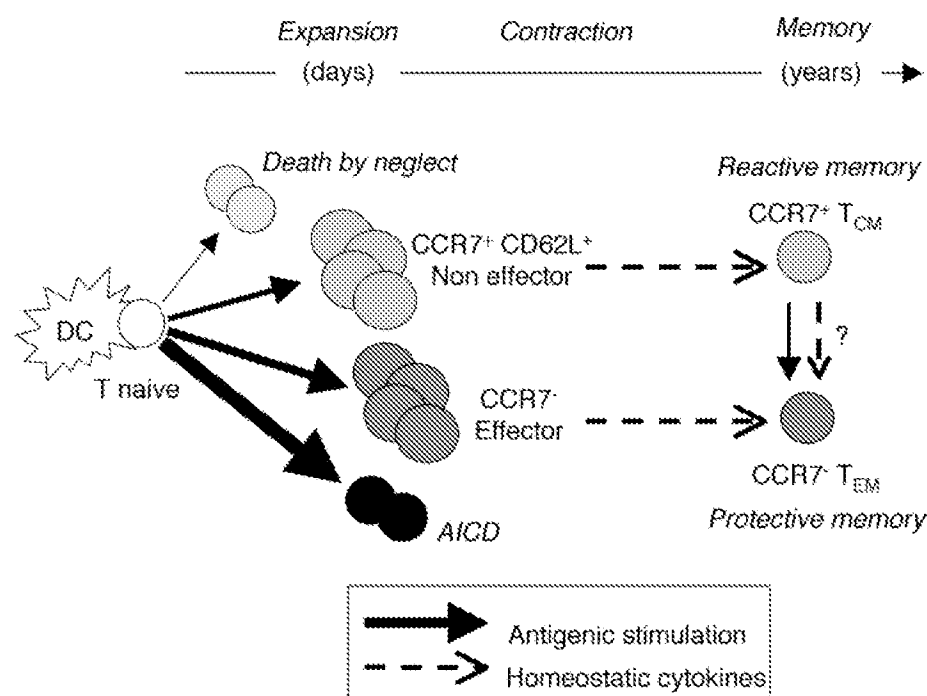


Figure 2 A signal strength model for T cell differentiation and memory T cell generation. The duration and intensity of antigenic stimulation is indicated by the length and thickness of the solid arrows. Antigen-independent events leading to T cell proliferation and differentiation are indicated by the dotted lines. AICD, activation-induced cell death.

to this model (7), precursors of T_{EM} would be preferentially generated early on, whereas precursors of T_{CM} would be preferentially generated at late stages of the immune response. The model proposed above is consistent with the observation that memory precursors are not present at the peak of the immune response but progressively appear during the contraction phase coincident with global changes in transcriptional profiling (92).

MONITORING THE GENERATION OF T_{CM} AND T_{EM}

The signal strength model discussed above is consistent with several studies that established a relationship between the nature of antigenic stimulation in vivo and the fate of T cells generated. Strong stimuli, such as live bacteria or antigen formulated with microbial products, induce fully differentiated CTL and T_H1 cells; killed bacteria or antigen associated with the bacterial cell wall-derived Ribi adjuvant induce the expansion of noneffector CD8 or CD4 T cells (93, 94). Furthermore,

antigens cross-presented by immature DC induce abortive proliferation or expansion of noneffector cells depending on the level of antigen expression (95–99). In these conditions, *in vivo* administration of IL-12 or T cell help increases the generation of effector cells. Reintroduction of antigen, but not inflammatory stimuli, during primary infection reinforces the generation of memory T cells, indicating that a sustained antigen presentation is critical for effective memory T cell generation (100).

Several studies examined the heterogeneity of responding T cells in the course of antigen-driven immune responses. Following immunization with a protein antigen, two types of responding T cells can be identified as early as day five: B helper T cells expressing the follicular homing receptor CXCR5+ and tissue homing inflammatory T cells expressing CXCR3 (101). CD4 T cells responding to influenza virus were found to be a heterogeneous continuum of cells in terms of number of cell divisions, phenotype, and function (102). Interestingly, those migrating to the lung are characterized by loss of CD62L, downregulation of CCR7, secretion of large amounts of IFN- γ , and reduced IL-2 levels relative to those in the secondary lymphoid organs. While the response declines with viral clearance, a range of resting cell subsets reflecting the pattern at the peak of response is retained, suggesting that heterogeneous effector populations may give rise to corresponding memory populations (102). Furthermore, the same CD8 T cell clonotypes responding to influenza are present in the lymphoid tissue and in the virus-infected lung (103), demonstrating that the heterogeneity in tissue localization can be generated within the same T cell clone.

In the mouse system it has been possible to directly address the relationship between population of primed T cells and memory subsets. A study on CD4 T cells showed that cytokine-secreting cells fail to generate memory, whereas nonpolarized cells, with the characteristic of pre-T_H1, have the ability to transfer strong secondary T_H1 responses (104). In a recent study the precursor-product relationship between T_{CM} and T_{EM} has been analyzed in the CD8 system by adoptive transfer experiments (105). The results point to three main conclusions. First, T_{EM} are present only transiently, and, upon transfer, they all convert to T_{CM} by reacquiring CCR7 and CD62L expression. Second, the rate of conversion from T_{EM} to T_{CM} is inversely proportional to the strength of stimulation. Third, T_{CM} convert to T_{EM} upon antigenic restimulation but not under steady-state conditions. These findings led to the proposition that in mice T_{CM} and T_{EM} do not necessarily represent distinct subsets, but are part of a continuum in a linear nave $\rightarrow$ effector $\rightarrow$ T_{EM} $\rightarrow$ T_{CM} differentiation pathway (106). These results are in apparent contrast with the persistence and stability of both T_{CM} and T_{EM} subsets in humans (15) and with the failure of T_{EM} to reacquire the constitutive CCR7 expression *in vitro* (107), suggesting a relevant difference between these two species.

Recent findings indicate that T cell help is an absolute requirement for generation of functional CD8 T cell memory. Indeed, animals primed in the absence of T cell help mount normal primary CTL responses but fail to mount secondary responses to the same antigen in spite of normal numbers of memory T cells

(108–110). The nature of the T cell defect it is not clear, but it is tempting to speculate that it may be due to a qualitative or quantitative defect of T_{CM} . The mechanism of T cell help is also unclear. Helper T cells can boost CTL responses not only through DC activation (111–113) and provision of IL-2 but also through direct T-T interactions mediated by CD40L (114). Positive signals controlling the generation of memory T cells are provided by other members of the TNF receptor family such as OX40 and CD27 (115–117).

OPEN QUESTIONS AND PERSPECTIVES

T_{CM} and memory B cells represent memory stem cells because they respond to chronic or repeated antigenic stimulation by self-renewing and generation of effector T cells and plasma cells (118). Memory cells divide constitutively, even in the absence of antigen (50), and in response to cytokines proliferate and differentiate to effector T cells. We therefore propose that T_{CM} and memory B cells may also function as stem cells under homeostatic conditions. Accordingly, T_{CM} may divide asymmetrically in response to cytokines, maintaining their number and, at the same time, differentiating and replenishing T_{EM} that are lost in peripheral tissues, such as lung or gut. A similar mechanism was recently proposed for human memory B cells, which maintain serological memory through continuous polyclonal activation (119). Cell tracking experiments and detailed kinetics and repertoire analysis of memory T cell subsets should be performed in order to address this important issue in humans.

Mouse experiments showed that T_{CM} have much higher capacity to reconstitute the memory T cell pool than effector cells (104, 105). These observations are highly relevant for immunotherapy. Injection of large numbers of effector cells may provide immediate protection but fail to reconstitute long-term memory (120). It would be important to find out the optimal conditions to prime and expand T_{CM} -like cells in humans.

Although the existence of T_{CM} and T_{EM} is now well documented, there are significant differences between mouse and human that need to be clarified. One aspect relates to the extent of differentiation of the T_{CM} subset and the mechanisms responsible for cytokine memory and flexibility in both CD4 and CD8 compartments. In addition, human T_{CM} and T_{EM} populations appear to be stable with no evidence of interconversion, whereas they appear to be dynamic and plastic in the mouse system (15, 105). This difference may be related to the experimental systems or may reflect differences between the species. A particularly striking difference between experimental animals and humans relates to number of specificities that need to be accommodated and maintained in the memory pool. Since the total pool does not substantially increase with aging, new specificities that progressively accumulate lead to dilution of pre-existing memory T cells. The same mechanism will favor the expansion of cross-reacting T cells, which become enriched in the memory pool. Both attrition and cross-reactivity have been documented in mice (121) and may

play an even more important role in humans because of the much longer lifespan and antigenic exposure. The prevalence of cross-reactive memory T cells in elderly should be tested experimentally. Solving any of these questions will have not only theoretical but also practical implications for vaccination and immunotherapy.

ACKNOWLEDGMENTS

We thank Amanda Gett for critical reading and comments. The Institute for Research in Biomedicine is supported by the Helmut Horten Foundation. The work on immunological memory in the authors' laboratories is supported by grants from the European Community (QLK2-CT-2001-01250) and the Swiss National Funds (3100-63885 and 3100-101962).

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