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# CD8 T cell memory development: CD4 T cell help is appreciated

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**Abstract** An important goal of vaccination strategies is to elicit long term, effective immunity. Therefore it is imperative to define the parameters that regulate the development and preservation of the numbers and functional quality of cells that confer this property to the host. CD8 T cells are a key component of the host adaptive immune response that helps eradicate invading viruses and other cell-associated pathogens. Once the primary infection is controlled, the CD8 T cells transition from being effector cells into memory cells that act as sentinels of the immune system capable of rapidly purging the host of recurrent infections by the same pathogen. The factors that regulate and orchestrate this transition from effector CD8 T cells into functionally robust memory CD8 T cells are poorly understood. In recent years it has been determined that CD4 T cells play a vital role in the survival and functional responsiveness of memory CD8 T cells. However, the mechanism(s) of this interaction are still unclear.

**Keywords** Memory · Helped · Unhelped · Rechallenge · Cytokines · Immunization · Proliferation · Adoptive transfer

## Introduction

CD8 T cells play a crucial role in combating infections caused by intracellular pathogens such as viruses and certain bacteria [1]. Following initial encounter with the pathogen, CD8 T cells embark on a program of differentiation that is marked by distinct phases of

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activation, intense proliferation, effector function followed by a significant reduction in total numbers, and eventually stable maintenance for the life of the host [2–7] (Fig. 1). The prolonged maintenance of this pool of antigen-specific CD8 T cells is what accounts for immunological memory. It is this subset that confers protective immunity to the host against subsequent challenge by the same pathogen. Secondary exposure to the pathogen is marked by a vigorous and accelerated response by the memory CD8 T cells that rapidly and efficiently rid the host of the invading microbe thereby minimizing the effects of the pathogenic insult [2–7].

Characteristics of memory CD8 T cells:

(1) Can persist long term at higher numbers compared to naïve CD8 T cells: One property of bone marrow derived cells is a finite lifespan. The majority of these cells are rather short-lived. Memory CD8 T cell populations, however, are an exception to this rule as they can potentially last for the life of the host. CD8 T cells that develop into memory cells survive the contraction phase that eliminates 90–95% of the effector population and this memory pool is stably maintained at numbers significantly above the naïve precursor population. However, the reason for this extreme population longevity is unclear. Potential contributing factors may include, but are not limited to, higher expression of Bcl2, IL7 receptor (IL7R) on memory CD8 T cells vis a vis effector CD8 T cells [8–10]. However, naïve CD8 T cells also express high levels of Bcl2 and IL7R but lack the lifespan of memory CD8 T cells [9]. Some reports also suggest that upregulation of IL15R might promote survival but this molecule is not uniformly upregulated on all long-lived memory CD8 T cells [11, 12]. Another feature of memory CD8 T cells that could account for their survival advantage is a more well-organized signal transduction machinery. Memory CD8 T

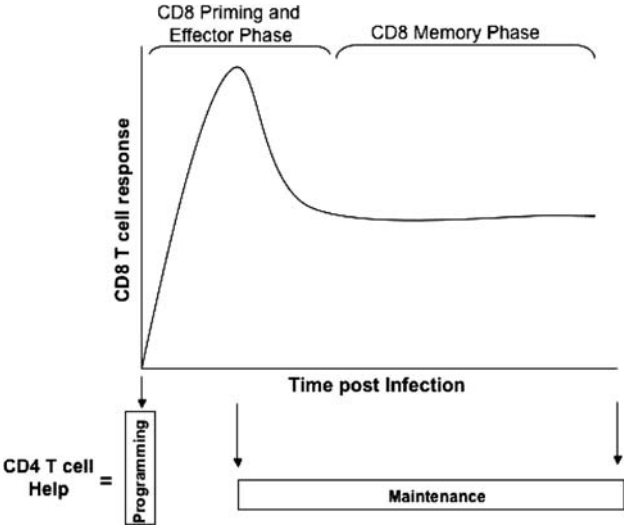


**Fig. 1** CD4 T cell help is required for the optimal development and survival of memory CD8 T cells. The figure depicted above describes the two proposed models of how CD4 T cell help influences CD8 T cell memory development. CD4 help delivered at the time of priming can ‘‘program’’ the responding CD8 T cells to differentiate into functionally robust memory CD8 T cells. The continued interactions between the two subsets following priming can promote the long-term ‘‘maintenance’’ of fully functional CD8 T cells

cells have constitutive phosphorylation of ζ-associated protein (ZAP-70) and CD3ε, a dense network of lipid rafts, and can activate the MAP-kinase signaling pathway more efficiently [13, 14].

(2) Rapid response to re-infection and protective immunity: Following stimulation memory CD8 T cells can elaborate effector functions (cytolysis and cytokine production) very rapidly and efficiently. In contrast to naïve CD8 T cells they require only a brief (5–6 h) period of stimulation in vitro with the appropriate MHC-peptide complex to elaborate effector cytokines such as IFNγ. Although effector CD8 T cells respond similarly following in vitro restimulation, memory CD8 T cells are unique in that an overwhelming majority of cells co-produce IFNγ and TNFα, and a subset is also efficient at IL2 production [15–17]. This rapid upregulation in cytokine producing capability is brought about by permanent and heritable DNA modifications that occur following priming, e.g., demethylation of IFNγ locus, which allows for easier access to the gene transcription machinery [18]. This enhanced responsiveness is also evident in secondary responses in vivo following rechallenge during which memory CD8 T cells produce copious amounts of effector cytokines and lyse infected cells to rapidly eliminate the invading pathogen.

(3) Vigorous proliferation during recall responses: This is a key property of memory CD8 T cells that accounts for the robust secondary response following rechallenge. Memory CD8 T cells have a much abbreviated lag phase between priming and entry into cell-cycle in comparison to naïve T cells. This is because unlike naïve CD8 T cells that are in G0 phase of the cell cycle, memory CD8 T cells exist in late G1 phase [19]. This is made possible by accumulation of a set of molecular complexes in their cytoplasm that regulate the cell cycle machinery. Specifically they contain preactivated cyclin D3/CDK6 complexes and lower levels of p27kip inhibitors that enable them to exist in late G1 phase [20]. But what really enables memory CD8 T cells to ramp up their numbers during a recall response is their ability to survive during this process of cellular expansion. Normally, during the course of a primary immune response, as naïve CD8 T cells get activated and begin to divide, the expansion phase is also marked by a significant amount of cell death brought about by cell division associated DNA duplication errors [21–23]. What factors are responsible for minimizing this cell division associated cell death in memory CD8 T cells remain to be clearly defined. Importantly, the endproduct of this vigorous secondary proliferative response of memory CD8 T cells is a higher setpoint for secondary memory cell numbers. This ''booster'' response is the basis for amplifying protective immunity by sequential immunizations.

CD4 T cells constitute another component of the cell-mediated adaptive immune response. These cells have been demonstrated to perform an equally vital but distinct function of cellular assistance and are therefore referred to as ''helper'' T cells. The helper function of the CD4 T cells is well documented but not completely understood. Their interaction with B cells is critical for the process of antibody isotype switching, and the cross-talk between CD4 T cells and dendritic cells (DCs) results in qualitative changes that enhance antigen presenting capabilities of the DCs [24–27]. CD4 help is believed to be essential for priming CD8 T cell responses to cell-associated, non-inflammatory antigens while being dispensable for responses generated against infectious agents. The help rendered in a non-inflammatory milieu to ''helper-dependent'' antigens is believed to involve the appropriate conditioning of professional antigen-presenting cells which then become licensed to effectively stimulate naïve CD8 T cells [26–28]. On the other hand

optimal priming of the CD8 T cell response to a variety of pathogens does not appear to be reliant on CD4 help. Hence these antigens are referred to as being ''helper-independent''. The ability of the pathogen to independently license or activate the professional antigen presenting cells (APCs) via pattern recognition receptors (PRR) expressed by the APCs is thought to circumvent the necessity for CD4 help [29–31].

It is also a well established fact that CD4 T cell help is critical for maintaining functionally competent CD8 T cells in the setting of chronic infections [32–35], but less is understood about the role of CD4 help involved in the maturation of CD8 T cells in the setting of an acute infection. In recent years the role that the CD4 T cells play in the peripheral development and differentiation of CD8 T cells directed against pathogens that are rapidly cleared has been the subject of intense investigation in the field of immunologic memory. A number of recently published studies have investigated this phenomenon in the context of both helper-dependent and helper-independent antigens and the emerging data seem to suggest that although CD4 help may not always be required for priming a CD8 T cell response, the lack of it invariably adversely affects the quality of the memory CD8 T cell pool that subsequently develops. The communication between the helper T cells and CD8 T cells appears to be essential to equip the CD8 T cells with appropriate and full complement of anti-microbial functions [36–41]. In other words, the most effective CD8 T cell memory appears to be dependent on CD4 help for both ''helper-dependent'' and ''helper-independent'' antigens. A careful understanding of this phenomenon is essential for the development of better vaccine strategies.

Investigators have also attempted to address critical aspects regarding the nature of CD4 T cell help, such as in programming or maintenance of the CD8 T cell memory pool, and sought to identify the specific CD4 T cell-associated molecules/factors that participate in this process. The remainder of this review will discuss the evolution of this concept, as well as ideas that are currently prevalent in this field of research.

## CD8 T cell memory development requires CD4 T cell help

In one of the initial studies, Bourgeois et al. utilized a male/female mixed chimera system in which they adoptively transferred HY(male)-Ag specific transgenic (Tg) CD8 T cells into irradiated female RAG−/− mice reconstituted with a mixture of male/female bone marrow derived cells [36]. Following priming, the Tg T cells were activated and capable of eliminating male cells, antigen-specific proliferation and secretion of IL2 and IFN$\gamma$ provided CD4 help was present at the time of priming. The absence of CD4 help induced a state of ''lethargy'' characterized by impaired proliferation and cytokine secretion, but these cells could still eliminate male APCs indicating that absence of CD4 help did not induce tolerance. Lethargic cells responded poorly to rechallenge, but this state of lethargy could be reversed if CD4 help was given during secondary responses.

Analogous findings were published in a study that compared helped versus unhelped CD8 T cell responses to influenza virus [37]. While the effector CD8 T cell responses were unaffected by the lack of concurrent CD4 help, the authors observed that the numbers of Ag-specific CD8 T cells participating in the recall response were severely compromised in the spleens and mediastinal lymph nodes of the rechallenged mice. This impairment correlated directly with delayed viral clearance from the lungs of challenged mice.

A later study published by Janssen et al. also showed that CD8 T cells specific for the adenoviral E1B (192–200) epitope cross-primed following depletion of CD4 T cells

displayed a diminution in the peak burst size as well as level of IFN$\gamma$ production on a per cell basis, which was not attributable to lowered functional avidity for their peptide ligands [38]. The unhelped CD8 T cells also displayed significant deficits in Ag-specific cytotoxic function as determined by in vivo and in vitro cytotoxicity assays. The abrogation of CD4 help at the time of immunization severely impaired the secondary expansion of E1b-specific CD8 T cells but the administration of exogenous IL2 in in vitro cultures at the time of restimulation appeared to restore proliferative and cytotoxic capacities of these CD8 T cells. The authors also analyzed the requirement of CD4 help for CD8 T cells in the context of a Th-independent antigen using lymphocytic choriomeningitis virus (LCMV) infection of both wildtype (WT), CD4 depleted, and MHC class-II knockout (I-Ab−/−) mice. CD8 T cell responses to the immunodominant GP33 epitope of LCMV were similar at day 7 in the three groups of mice, however a marked attrition in their numbers was observed at day 28 post infection (p.i.) in mice where CD4 T cells were lacking. In addition, as observed with the Th-dependent cross-primed responses, in vitro restimulation of GP33-specific CD8 T cells primed by viral infection in mice deficient in CD4 help induced a much weaker secondary CD8 T cell response in comparison to CD8 T cells primed in the presence of CD4 T cells.

The role of CD4 help in promoting memory CD8 T cell development was also examined by other groups that employed additional models of infection to prime CD8 T cell responses. Sun et al. [39] infected mice with the bacterial pathogen, recombinant *Listeria monocytogenes* expressing chicken ovalbumin protein (rLm-Ova) and observed that the kinetics of bacterial clearance, magnitude of the primary antigen-specific CD8 T cell responses, and killing ability in MHC class II deficient mice were similar to wildtype (WT) controls. The kinetics of contraction and the numbers of memory CD8 T cells were also similar between the two groups when examined up until 60 days post infection, but a decline in numbers of unhelped memory CD8 T cells was evident at later time points. When the mice were rechallenged with rLm-Ova 2 months following the primary infection, mice lacking CD4 cells displayed a severe deficiency in their ability to rapidly eliminate the bacteria and this corresponded directly with their inability to vigorously mount a secondary response and kill infected cells. Adoptive transfer experiments provided additional evidence that memory CD8 T cells generated in the absence of CD4 help display defects in proliferative function. Another interesting piece of data to emerge from this study was that CD4 help appeared to be dispensable for the secondary response to *Listeria monocytogenes* as depleting WT mice of CD4 T cells only at the time of rechallenge did not hinder the secondary expansion of memory CD8 T cells.

Similar observations were made by Shedlock et al. utilizing a regimen involving recombinant vaccinia virus expressing the LCMV-gp33 epitope (rVV-gp33) to prime a GP33-specific response followed by a rLm-gp33 boost to induce secondary expansion of GP33-specific CD8 T cells [40]. This report showed that mice in which the *Cd4* gene has been deleted (CD4 knockout mice), displayed markedly diminished gp33-specific recall responses despite generating adequate numbers of Ag-specific CD8 T cells following priming that cleared the primary infection. Similar to Sun et al. [39] they showed that CD4 help is crucial at the time of primary immunization as depleting CD4 T cells at the time of the rLm-gp33 boost did not have any effect on the ability of the memory CD8 T cells to mount a vigorous anamnestic response. Additional proof of the impaired recall responsiveness of the unhelped memory CD8 was also demonstrated by adoptively transferring purified CD8 T cells from CD4+/+ and CD4−/− mice into congenic hosts followed by rLm-gp33 infection.

CD4 T cell dependent CD8 T cell differentiation was also investigated by Khanolkar et al. who showed that the absence of CD4 help prevented the generation of central memory phenotype T cells (Tcm) following LCMV infection [41]. These atypical CD8 T cells displayed a phenotype that was CD44intermediate, CD122lo, and remained CD62Llo up to 1-year post infection. Their capacity to produce TNF$\alpha$ and IL2 was also diminished. CD4 deficiency was also accompanied by a gradual loss in the number of GP33-specific CD8 T cells, but curiously the numbers of NP396 and GP276-specific CD8 T cells were stably maintained. Upon rechallenge, these unhelped CD8 T cells did mount a secondary response but were slightly less effective in controlling the infection, and displayed a marked impairment in proliferative capacity. Although these data overall support previous results, some of the findings of this study, such as the absence of attrition in numbers of certain specificities of CD8 T cells could indicate that perhaps not all constituents of the heterogeneous memory CD8 T cell pool are equally dependent on CD4 help. Another caveat that merits a careful interpretation of these observations is the use of CD4 knockout mice in this and other studies because the peripheral T cells population in these mice includes CD8 T cells that are MHC class II restricted and a smaller proportion of double negative (CD4−CD8−) T cells that might respond to MHC class II presented antigen and provide some help [42, 43].

## Programming versus maintenance

An important aspect of this phenomenon is the determination of when CD4 T cell help is required. Do the CD8 T cells receive this blueprint for optimal differentiation and survival from the CD4 cells en masse in a single hit, such as at the time of immunization and then develop into functionally robust memory cells in the absence of additional CD4 help (''programming'') or does this process require these subsets to co-exist and engage in extended interactions that provide critical cues for development of long-term functional memory (''maintenance'') (Fig. 1)? The experimental evidence up to this point suggested that the absence of CD4 help at the time of immunization led to the development an aberrant population of memory CD8 T cells that were deficient in conferring optimal protective immunity [36–41]. The data also suggested that CD4 help was dispensable at the time of rechallenge to elicit a robust secondary CD8 T cell response to a pathogen [39, 40]. The issue of programming versus maintenance was elegantly addressed in a study published by Sun et al. in which they examined CD8 T cell responses in MHC class II deficient mice infected with rLm-gp33 or LCMV [44]. They reported that GP33-specific CD8 T cells gradually decline over time in the absence of CD4 help and a majority these cells fail to reacquire CD127 on their surface. Adoptive transfer of effector phase T cells specific for GP33 from a CD4 sufficient host into mice lacking CD4 T cells resulted in a gradual loss in their numbers of over time. These cells were also defective in their capacity to produce IFN$\gamma$ and IL2 and conferring protective immunity. Conversely, when GP33-specific CD8 effector cells were transferred from a CD4 deficient mouse into a normal mouse their numbers were stably maintained post contraction and remained functionally fully capable. Similar trends were observed in adoptive transfer experiments using memory phase CD8 T cells. This suggested a role for CD4 T cells in the stable maintenance and differentiation of CD8 memory after, but not during, the initial programming phase.

Further support for this concept was provided in a separate study that demonstrated that CD4 T cell help for CD8 memory development varied depending on the infecting pathogen. *Listeria monocytogenes* specific CD8 T cell responses suffered in the absence of

CD4 T cell help while those directed against vesicular stomatitis virus (VSV) were unaffected in this situation. This report concluded that CD4 T cell help contributed more toward promoting survival and augmenting proliferation during recall responses (maintenance) rather than ''imprinting'' memory CD8 T cell function (programming) [45].

## Mechanisms of CD4 T cell help in the development of memory CD8 T cells

### Role of TRAIL in promoting CD8 T cell memory

TNF-related apoptosis-inducing ligand (TRAIL) has been implicated in apoptosis of tumor cells but recent studies showed that TRAIL is also involved in the death of other nontransformed cell types [46–49]. Janssen et al. showed that mRNA for TRAIL was selectively upregulated in CD8 T cells primed in the absence of CD4 T cell help (unhelped CD8 T cells) compared with the antigen-specific CD8 T cells primed in CD4 competent environment (helped CD8 T cells) [50]. Using cross-priming against cell-associated antigen as well as LCMV infection models, they showed that, despite similar expression of TRAIL receptor DR5 (death receptor 5) on helped and unhelped CD8 T cells, the increased ability of unhelped CD8 T cells to produce TRAIL correlated with the increased activation induced cell death (AICD) upon repeated antigen encounters. Treatment with recombinant TRAIL of 'helped' CD8 T cells inhibited their secondary expansion, suggesting that CD8 T cells primed in the presence of CD4 T cell help are also susceptible to TRAIL-mediated death. The authors suggested that CD4 T cell help might be imprinted early after activation of naïve CD8 T cells and that signal(s) delivered by CD4 T cells might be remembered (downregulation of TRAIL expression) as CD8 T cells progress and differentiate into memory. Recently, it has been shown by Hamilton et al. that TRAIL-deficient unhelped homeostatic memory CD8 T cells were able to provide substantial anti-listerial immunity and decreased AICD compared to TRAIL sufficient unhelped homeostatic memory CD8 T cells suggesting that CD4 T cells might influence the antigen-independent, lymphopenia-induced CD8 T cell responses as well [51].

The role for TRAIL in CD8 T cell homeostasis after viral (LCMV) infection was recently investigated [52]. TRAIL-deficiency did not influence the overall kinetics of antigen-specific CD8 T cell responses, and TRAIL-deficient mice were able to generate functional memory CD8 T cells that were indistinguishable from TRAIL-sufficient wild-type CD8 T cells. Interestingly, this study also showed that the ability of unhelped CD8 T cells to retain their memory phenotypic and functional (expansion after secondary antigen encounter) characteristics was prolonged in CD4-depleted TRAIL-deficient mice compared to WT CD4-depleted controls. However, TRAIL deficiency only delayed, but did not prevent, the eventual erosion in quality of unhelped memory CD8 T cells, and that correlated with their inability to respond to second round of antigen-driven proliferation. Taken together, these data suggested that the protective CD4 T cell help for memory CD8 T cell differentiation and maintenance has both TRAIL-dependent (programming) and -independent (maintenance) components.

### Contribution of CD40–CD40L interactions in promoting CD8 T cell memory

CD40 is a molecule that belongs to the TNF receptor superfamily and is abundantly expressed on the surface of APCs such as B cells and DCs [53]. Its interaction with its

ligand (CD40L) is known to play a crucial role in the cross talk between CD4 T cells and APCs, [26–28, 54]. This communication between CD40 and CD40L has also been reported to be critical for CD8 T cells as its absence can impair CD8 T cell responses [53, 55–58]. And in the absence of CD4 T cells providing a surrogate CD40 derived signal with an agonist CD40 antibody can circumvent the delivery of CD4 help to CD8 T cells [26–28].

However the contribution of this signal in promoting CD8 T cell memory is still a matter of debate. Bourgeois et al. reported that activated CD4 and CD8 T cells can also express CD40 albeit at much lower levels than APCs [59]. And the direct interaction between CD40 on CD8 T cells and CD40L on CD4 T cells, independent of CD40 expression on APCs was suggested to promote the development of fully functional memory CD8 T cells. They arrived at this conclusion after comparing responses of CD40−/− and CD40+/+ H-Y (male) Ag-specific Tg CD8 T cells adoptively transferred into congenic hosts and primed in the presence of CD4 T cells. Although CD40 deficient transgenic T cells behaved similar to wild-type cells during the primary phase of the response, they displayed significant deficits in their capacity to proliferate and secrete cytokines following rechallenge.

The findings of this study were challenged in other reports that utilized bacteria (rLm-Ova, rLm-GP) and viruses (LCMV) to generate CD8 memory responses. Following infection CD40 and CD40L deficient mice generated primary responses that were comparable to WT counterparts and also proliferated rapidly and provided adequate protection upon rechallenge [60]. Treatment of mice lacking CD4 help with agonistic anti-CD40 Ab was unable to boost the recall response suggesting that signaling through CD40 on the surface of CD8 T cells was not sufficient to generate functionally competent memory T cells. Similar results were obtained after infection of bone marrow chimeric mice in which WT and CD40−/− CD8 T cells develop and encounter Ag in the same environment. This study concluded that expression of CD40 on CD8 T cells does not play a role in the generation of functionally robust memory CD8 T cells in the context of acute bacterial and viral infections. The results of this study were also supported by other reports citing the lack of a role for CD40 signaling in the development of influenza-specific CD8 T cell memory [61], and elaboration of primary and secondary CD8 T cell responses targeting secreted and non-secreted bacterial antigens [62].

## Influence of IL2 on CD8 memory development

In addition to signaling via surface molecules expressed on CD4 and CD8 T cells, it is quite likely that CD4 help may be delivered to CD8 T cells through soluble factors either produced directly by the CD4 T cells or indirectly through stimulation of growth and survival factor production by neighboring accessory cells. One such soluble factor that is abundantly expressed by CD4 T cells is interleukin-2 (IL2). A recent study by Williams et al. examined the contribution of IL2 in the development of functional memory CD8 T cells [63]. Using mixed chimera mice populated with both WT and IL2Rα (CD25)−/− cells the study showed that following infection with LCMV and rLm-OVA antigen-specific primary CD8 T cell responses were similar between the two groups. In the absence of IL2 signaling CD8 T cells cells converted to a central memory phenotype (CD62Lhi, CD127hi, and increased IL2 production) faster, and displayed no deficiency in homeostatic and antigen-specific proliferation. However these cells were unable to mount robust secondary responses following rechallenge. Interestingly, the provision of an IL2 signal at the time of primary immunization restored the ability of the memory CD8 T cells to mount a vigorous

recall response. In the experiments described this IL2 signal was delivered in the form of exogenously provided IL2 and anti-IL2 antibody complex that bypassed the need for surface expression of CD25 on the surface of the CD8 T cells. Data were also presented to show that secondary responsiveness could not be restored in cells that received the IL2 signal only at the time of the secondary response but not the primary response. Lastly, the study also showed that IL2 derived from a paracrine source, most likely CD4 T cells or activated DCs, is sufficient for the development of fully functional memory CD8 T cells.

In addition to IL2, preliminary reports also indicate that CD4 T cells could promote stable maintenance of memory CD8 populations by regulating the expression of and hence signaling through the IL7 and IL15 receptors on CD8 T cells. IL7 is required for the long-term survival of CD8 memory cells, while IL15 supports basal homeostatic proliferation. In the absence of CD4 help, memory CD8 T cells fail to express IL7R$\alpha$ (CD127) and also downregulate IL15R$\beta$ (CD122), thus adversely impacting both survival and turnover [64].

## Conclusion

The role of CD4 help in regulating CD8 T cell memory responses that develop following acute infections is a new twist in the concept of CD4 T cell help. Immunization strategies that focus solely on CD8 T cell immunity might prove to be shortsighted, because even though they might stimulate vigorous early responses, they will be unable to confer long-term protective immunity. Defining the factors(s) that are involved in this complex interaction between these cellular subsets will greatly enhance our knowledge about the generation, function, and stable maintenance of memory CD8 T cells, as well as impairment of immune function following defects in CD4 T cell responses in certain diseases. A better understanding of this phenomenon will also be informative for translational approaches aimed at infection control, autoimmune diseases, and vaccine design.

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