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Editorial

Editorial: (CD)40 winks to prevent CD8$^+$ T cell lethargy

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The development of vaccines against many pathogens was one of the most successful public health stories of the 20th century. The effectiveness of these vaccines is highly dependent on the generation of neutralizing antibody responses. Much less is known about how to develop effective memory CD8$^+$ T responses, which may be key to the treatment of many chronic infections that afflict the 21st century. The elaboration of adaptive CD8$^+$ T cell responses can be categorized as either CD4$^+$ Th-dependent or -independent, depending on the nature of the immunogen. CD4$^+$ T cells play two major roles in assisting the development of CD8$^+$ T cell responses: the activation of naive CD8$^+$ T cell particularly in response to tissue-associated antigens; and the differentiation/survival of memory CD8$^+$ T cells. CD4$^+$ T cell support for the activation of naïve CD8$^+$ T cells is generally thought to be a consequence of CD4$^+$ T cell-mediated licensing of DCs. This helper-dependent licensing is efficiently [1], but not exclusively [2, 3], induced by the stimulation of CD40 on DCs by CD40L-expressing CD4$^+$ T cells, and the absence of CD4$^+$ T cells can often be overcome by stimulating DCs with agonistic antibodies specific for CD40. Whether the ability of CD4$^+$ T cells to promote CD8$^+$ T cell memory is also dependent on stimulation of CD40 on DCs is more controversial. On the one hand, CD8$^+$ T cells that respond to CD40-stimulated DCs have been shown to be able to differentiate into competent memory CD8$^+$ T cells. On the other hand, studies of the role of CD4$^+$ T cells in generating CD8$^+$ T cell responses to the HY minor histocompatibility antigen by Tanchot and co-workers suggested that CD40 stimulation of CD8$^+$ T cells, but not APCs, was necessary to permit the development of fully functional memory CD8$^+$ T cells [4–6]. In the absence of this interaction, "lethargic" memory CD8$^+$ T cells developed, which exhibited transcriptional patterns associated with naïve CD8$^+$ T cells and were hyporesponsive to restimulation.

The impact of CD40 stimulation on CD8$^+$ T cell function has been questioned by other studies using infectious agents, which determined that CD40 expression on CD8$^+$ T cells was dispensable for the activation and differentiation of CD8$^+$ T cells [7, 8]. These data suggest that the relevance of CD40 expression by CD8$^+$ T cells may be limited to responses to tissue antigens. However, although the expression of CD40 on CD8$^+$ T cells was initially controversial and characterized incompletely, subsequent studies have found functional evidence of a role for direct CD40 signaling in CD8$^+$ T cells responses: CD40-deficient CD8$^+$ T cells are reticent to anti-PDL1-mediated rescue from exhaustion after *Toxoplasma gondii* infection [9]; CD40$^+$ CD8$^+$ T cells have been reported to play a role in restraining Tregs in *Leishmania donovani* infections [10]; and CD40 expression by CD8$^+$ T cells was found to be necessary for primary CD8$^+$ T cell responses initiated by TLR-mediated, induced expression of CD40L on DCs [11]. Thus, the expression and function of CD40 on CD8$^+$ T cells have gained support, and its role has expanded to pathogen-associated immune responses. Given these data, it remained important to clarify whether CD4$^+$ T cell support for the initial activation of naïve CD8$^+$ T cells and their subsequent development into memory CD8$^+$ T cells are two separate events involving CD40 stimulation on DCs or CD8$^+$ T cells, respectively. Alternatively, is CD40 stimulation of CD8$^+$ T cells sufficient for both stages of the CD8$^+$ T cell response? As there is considerable therapeutic potential for targeting CD40, a premium is placed on understanding the location and nature of its impact on cellular processes.

In this issue of the *Journal of Leukocyte Biology*, using an elegant model system in which CD40 is expressed selectively on APCs or CD8$^+$ T cells by chimera generation, Meunier et al. [12] have sought to further define the contribution of CD40 expressed by CD8$^+$ T cells or APCs in the activation and differentiation of primary and memory CD8$^+$ T cells. CD40-intact or -deficient, T cell-deficient (CD3ϵ-knockout) female mice received male bone marrow cells as a source of antigen and TCR-transgenic CD8$^+$ T cells, which are specific for the HY-antigen, as a responder population. Help was provided in the form of HY-specific TCR transgenic CD4$^+$ T cells or agonistic anti-CD40 antibody. This model is notable for two aspects. First,

Abbreviations: CD40L=CD40 ligand, PD1=programmed death 1, PDL1=programmed death ligand 1, Treg=regulatory T cell

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in most instances, the transferred T cells used to reconstitute sublethally irradiated animals would undergo extensive homeostatic proliferation, making it difficult to discern the impact of antigen-induced proliferation. However, in the model used here, the proliferation of the donor HY-specific $CD8^+$ T cells is entirely antigen-driven. Second, the ability of the HY-$CD8^+$ T cells to respond to the male antigen is dependent on $CD4^+$ T cells or anti-CD40, even at high HY-$CD8^+$ T cell transfer frequencies. Together, these two nuances allow for a careful dissection of the role of APC- or $CD8^+$ T cell-expressed CD40 in aspects of the primary and memory $CD8^+$ T cell responses to antigen. In this model, whenever APCs did not express CD40, deficits occurred in the expansion of the primary $CD8^+$ T cell response when help was provided in the form of anti-CD40 administration; this deficit was notably greater in situations where neither APCs nor $CD8^+$ T cells expressed CD40 but was limited when CD40 deficiency was restricted to $CD8^+$ T cells. These data indicate a role for CD40 on APCs and $CD8^+$ T cells for optimal primary responses but that CD40 expression on APCs is dominant for the elaboration and differentiation of the primary $CD8^+$ T cell response. Interestingly, and in support of previous observations from this group and others [2, 3, 5, 13], help in the form of $CD4^+$ T cells was not dependent on APC-expressing CD40. One might have expected that stimulation of CD40 on $CD8^+$ T cells would therefore be sufficient to overcome the absence of CD40 on DCs. However, this was not so, indicating that CD40-licensed DCs provide additional, critical costimulatory signals to naïve $CD8^+$ T cells beyond CD40 stimulation. Quite remarkably, however, despite relatively small reductions in the number of memory $CD8^+$ T cells that formed in the absence of CD40 expression on the $CD8^+$ T cells, these CD40-deficient $CD8^+$ T cells demonstrated little capacity to expand after re-exposure to antigen. In contrast, memory $CD8^+$ T cells that are generated in the absence of CD40 on APCs were limited in number but had normal re-expansion kinetics. Thus, in this model system at least, signals delivered by CD40 stimulation to $CD8^+$ T cells play a critical role not so much in the development or survival of memory $CD8^+$ T cells but in their ability to respond to antigen (**Fig. 1**). Further credence of the importance of direct CD40 signaling in $CD8^+$ T cell responses to at least tissue antigens may be achieved by assessing the importance of CD40 stimulation in the generation and memory differentiation of tumor antigen-specific $CD8^+$ T cells.

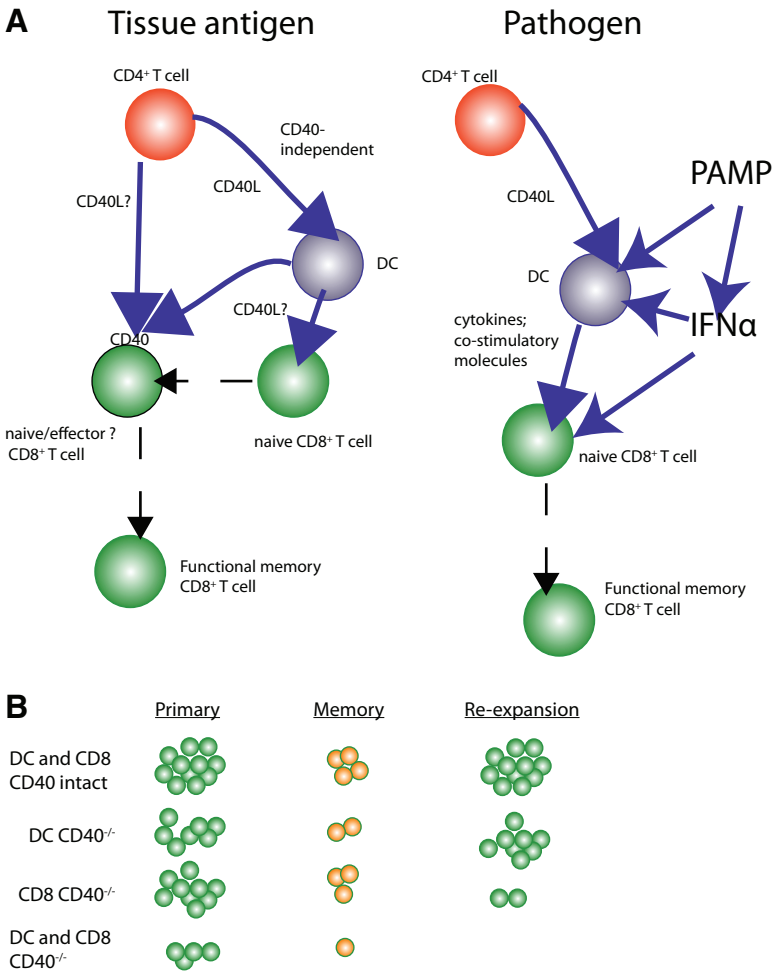

Figure 1. The impact of CD40 signaling on $CD8^+$ T cell responses. (A) $CD4^+$ T cells can license DCs to promote $CD8^+$ T cell responses to tissue antigens using CD40-dependent or -independent pathways. Functional $CD8^+$ T cell memory is dependent on further stimulation of CD40 expressed by $CD8^+$ T cells. With pathogen infection, CD40 stimulation of DCs is not always necessary, and the primary $CD8^+$ T cell response may instead be supported by type-1 IFN stimulation of DCs or $CD8^+$ T cells. The source of CD40L and timing of CD40 stimulation are unclear. (B) In the absence of CD40 on DCs, the primary $CD8^+$ T cell response is predominantly affected; in the absence of CD40 on $CD8^+$ T cells, the capacity of memory $CD8^+$ T cells to re-expand is compromised.

The results presented by Meunier et al. [12] in this issue of the *Journal of Leukocyte Biology* contribute to our understanding about the relationship between CD40 and $CD8^+$ T cell memory and provide a possible explanation for the much-heralded defect in $CD8^+$ T cell memory that occurs in the absence of $CD4^+$ T cells in several model systems. However, these studies also raise some notable questions. Paramount among them and as yet unanswered is why studies using some infectious agents found little importance for CD40 expression by $CD8^+$ T cells. Is this reflective of differential expression of CD40 on $CD8^+$ T cells, and if so, what factors regulate CD40 expression? More likely, this discrepancy reflects a difference in the necessity of having additional sig-

nals to promote full memory cell differentiation after encounter with antigen in the relatively noninflammatory environment of the bone marrow transfer. One strong possibility lies in the differences in the immunization approaches, with supportive cytokines such as IL-12 and type-1 IFNs, which are produced during pathogen infections, substituting for the signals provided by CD40. Indeed, studies have demonstrated recently that provision of IFN-α can overcome the requirement for CD4$^+$ T cells in response to helper-dependent antigens [14, 15], although it has yet to be ascertained whether IFN-α works independently from CD40 stimulation or supports it. These notions are experimentally testable by providing exogenous cytokines or even concurrent infections in the system used by Meunier et al. [12] and determining whether the CD40 independence of primary responses to lymphocytic choriomeningitis virus, influenza, and *Listeria monocytogenes* is dependent on IFN-$\alpha\beta$R expression by CD8$^+$ T cells. A molecular assessment that indicates convergent transcriptional outcomes after IFN-α or CD40 stimulation would suggest that these pathways can substitute for each other. Alternatively, PAMPs are known activators of DCs and can influence the function of CD4$^+$ T cells. Therefore, CD8$^+$ T cell differentiation in response to tissue antigens may be more dependent on direct CD40 signals as a result of the lack of cytokines, such as IL-21, or costimulatory molecules, such as 4-1BB or CD70, which could be elicited more efficiently in response to pathogens.

In the absence of information delineating the source of CD40L or the timing of CD40L interactions with CD8$^+$ T cells, it is difficult to generate a model that accounts for the outcomes presented by Meunier et al. [12]. Johnson et al. [11] determined recently that helper-independent CD8$^+$ T cell responses remained dependent on TLR-mediated induction of CD40L expression on DCs. However, the tissue antigen system used by Meunier et al. [12] is not likely to induce stimulation of TLR, leaving activated CD4$^+$ T cells or NKT cells [16] as the strongest candidate source of CD40L. Without knowing the source of CD40L responsible for CD8$^+$ T cell stimulation, it is hard to envisage where and when this interaction is occurring, although it is reasonable to speculate that co-dwelling on DCs could account for the proximity necessary to allow DC- or CD4$^+$ T cell-derived, CD40L-mediated stimulation. The timing of CD40 stimulation is also of interest. Fuse and colleagues [17] demonstrated recently that unhelped CD8$^+$ T cells generated in response to vaccinia overexpress PD1 on re-exposure to antigen. Although PD1 expression on memory CD8$^+$ T cells was not assessed by Meunier et al. [12], CD40 expression on CD8$^+$ T cells has been shown to be vital for anti-PDL1-mediated rescue of CD8$^+$ T cell exhaustion in response to *T. gondii* infection [9]. This raises the question as to when the CD40 signals are necessary to rescue memory CD8$^+$ T cell function: during the primary response or during the challenge?

As the predominant impact of CD40 signaling on CD8$^+$ T cells is in the functional capacity of the memory CD8$^+$ T cells that are generated, it is of considerable interest to define how CD40 signals influence CD8$^+$ T cell memory differentiation. Munroe and Bishop [18] demonstrated that CD40 stimulation, concomitant with CD3 and CD28 stimulation, could augment IL-2 production and AP-1, NF of activated T cell (NFAT), and NFκB activation in CD4$^+$ T cells. Whether these pathways contribute to the phenotypical observations with CD8$^+$ T cells provided by Meunier et al. [12] is uncertain, as is whether they are unique to CD40 or can be engaged by stimulating different members of the TNF superfamily, such as CD27 and 4-1BB. However, CD40 stimulation might provide a link to studies by Schoenberger and colleagues [19], which recently identified autologous IL-2 production as a critical factor in the development of memory CD8$^+$ T cells.

In the absence of a mechanistic understanding that accounts for the differences in the importance of CD40 signaling in CD8$^+$ T cells in the different models and the source and timing of CD40L stimulation of CD8$^+$ T cells, it is difficult to ascertain how important this stimulatory pathway might be. In the absence of a functional requirement for CD40 signaling during pathogen infections, opportunities to exploit this pathway are less obvious. Many of the practical applications of CD40 stimulation and blockade (i.e., for enhancing vaccine efficacy or inducing tolerance to transplant, respectively) have been anticipated, although at the level of DC stimulation. However, the studies presented by Meunier et al. [12] would suggest that CD8$^+$ T cells as well as DCs should be monitored to assess the impact of CD40-mediated immunotherapies and that CD8$^+$ T cells expanded in vitro for adoptive transfer therapy may have better long-term survival if CD40 stimulation is included during expansion. Furthermore, if CD40 stimulation of CD8$^+$ T cells turns out to be temporarily or spatially separated from the initial priming of the CD8$^+$ T cells (i.e., during contraction or during re-exposure to antigen), then the efficacy of CD40-based in vivo interventions could be increased by incorporating this knowledge.

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