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Tumor Necrosis Factor Receptor/Tumor Necrosis Factor Family Members in Antiviral CD8 T-Cell Immunity

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CD8 memory T cells can play a critical role in protection against repeated exposure to infectious agents such as viruses, yet can also contribute to the immunopathology associated with these pathogens. Understanding the mechanisms that control effective memory responses has important ramifications for vaccine design and in the management of adverse immune reactions. Recent studies have implicated several members of the tumor necrosis factor receptor (TNFR) family as key stimulatory and inhibitory molecules involved in the regulation of CD8 T cells. In this review, we discuss their control of the generation, persistence, and reactivation of CD8 T cells during virus infection.

Introduction

The outcome of a virus infection in a previously unexposed individual is the result of a race between the ability of a virus to replicate inside the host and the capacity of the host to mount and maintain an effective immune response (Wong and Pamer 2003; Davenport and others 2009; Kohlmeier and Woodland 2009). An effective immune response to viruses relies on the ability of a subset of T cells ($CD8^+$) to quickly generate an expanded population of effector cells or cytotoxic T lymphocytes (CTL) (Wong and Pamer 2003; Davenport and others 2009; Kohlmeier and Woodland 2009). For long-term protection, part of the antigen-specific T-cell pool must be retained as memory cells (Seder and others 2008). Effector and memory CD8 T cells mediate defense against viruses by direct cytolysis of infected cells, which is generally mediated through perforin and granzyme release and Fas/FasL interactions (Harty and others 2000). Another important feature of CD8 T cells in antiviral defense is their capacity to secrete cytokines such as tumor necrosis factor (TNF), interferon-gamma (IFN-γ), and interleukin-17 (IL-17) (Harty and others 2000; Hamada and others 2009).

Recently, it has emerged that effector and memory CD8 T cells are broadly heterogeneous in terms of their antigenic specificity, migratory capacity, anatomical locations, protective capacity, and longevity (Seder and others 2008; Jameson and Masopust 2009; Woodland and Kohlmeier 2009). Importantly, there are still many unanswered questions with regards to the source and nature of specific signals required for development, maintenance, and recall responses of each memory population, or how these cells can be effectively generated by vaccination strategies. Answering these key questions has important implications for vaccine design and in the management of adverse immune reactions associated with many infections.

Among factors influencing the fate of T cells after antigen encounter, co-stimulatory and co-inhibitory receptors on the surface of T cells play an important role after interacting with their soluble or membrane-bound ligands expressed on antigen-presenting cells (APCs) or other tissue cells. These molecules largely fall into 4 main groups, namely cytokines (Haring and others 2006), pattern recognition receptors (PRRs) (Asprodites and others 2008; Cottalorda and others 2009; McCarron and Reen 2009; Mercier and others 2009), Ig superfamily members, and TNFR/TNF superfamily members (Croft 2003a). Interleukin-12 (IL-12) and interferon-alpha (IFN-α) typify stimulatory cytokines; Toll-like receptor-2 (TLR2) and TLR5 are examples of PRRs that can be co-stimulatory for CD8 cells; CD28-B7, ICOS-ICOSL, and CD2-LFA-3 typify co-stimulatory molecules of the Ig superfamily, whereas stimulatory TNFR/TNF family members include the interactions of OX40 (CD134) with OX40L, 4–1BB (CD137) with 4–1BBL, CD27 with CD70, GITR with GITRL, CD30 with CD30L, CD40 with CD40L, HVEM with LIGHT, and LTα with LTβR and TNFR. Inhibitory molecules in the TNFR superfamily include the interactions of Fas (CD95) and FasL, and DR4/5 with TRAIL that can result in apoptosis and death of T cells. Why there are so many molecules

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that can regulate CD8 T-cell responsiveness to antigen has been the subject of speculation over the past decade.

In this regard, it is important to bear in mind that antiviral CD8 T-cell responses are likely generated in varying inflammatory milieu upon pathogen encounter, dictated by several factors (Wong and Pamer 2003; Harty and Badovinac 2008). The site of initial infection, virulence and immune modulatory mechanisms, antigenic load, cell tropism, and the transduction of signals by PRRs are factors that, in combination, create the inflammatory environment (Wong and Pamer 2003). This in turn may regulate the availability of stimulatory and inhibitory receptors or ligands and then lead to a degree of flexibility in use. In this review, we will discuss the use and role of select TNFR/TNF family members in initiating and sustaining the CD8 T-cell response in promoting long-lived protective immunity to viral infections.

Development of Memory CD8 T Cells

The generation of memory CD8 T cells can be broadly divided into 2 phases after infection or vaccination (Kaech and Wherry 2007; Williams and Bevan 2007; Jameson and Masopust 2009). The first phase begins when circulating peripheral naïve CD8 T cells recognize, via their T-cell receptor (TCR), antigenic peptides bound to major histocompatibility complex (MHC) class I complexes on the surface of mature dendritic cells (DCs). Studies using a number of different experimental approaches calculated that the precursor frequencies of naive virus-specific CD8 T cells range from 1 to 5 in 100,000 (Blattman and others 2002), but in some cases can reach as high as 1 in 1,444 for certain viruses such as vaccinia virus (VACV) and 1 in 2,985 for lymphocytic choriomeningitis virus (LCMV) (Seedhom and others 2009). After antigen recognition, these small numbers of antigen-specific precursor CD8 T cells undergo about 11 to 15 divisions to become a large population of cells found at the peak of the primary response, which is typically between Days 6 and 7 postinfection (Butz and Bevan 1998; Doherty 1998; Flynn and others 1998; Murali-Krishna and others 1998). During this expansion phase, CD8 T cells also differentiate into effector cells that can be subdivided into functionally distinct populations based on their cytokine secretion profiles. These include T cytotoxic 1 (TC1), TC2, and TC17 cell populations, which parallel the $CD4^+$ Th1, Th2, and Th17 cell populations. Optimal clonal expansion of effector CD8 T cells is achieved when antigenic stimulation is prolonged for at least 2–4 days (van Stipdonk and others 2001; Prlic and others 2006) and if antigenic encounter is shorter than 20 h the proliferative response is aborted (van Stipdonk and others 2001, 2003). Depending on the CD8 T-cell precursor frequency, and on the abundance of a given antigenic peptide, effector CD8 T cells with various specificities for a given virus are induced and dominant or subdominant CD8 T-cell populations can be distinguished based on their relative numbers (Yewdell and Bennink 1999; Crowe and others 2003).

After antigen clearance, a second phase of memory T-cell development ensues, whereby most of the antigen-specific effector CD8 T cells die and the surviving effectors go on to persist as long-lived memory T cells (Kaech and Wherry 2007; Harty and Badovinac 2008). The effectiveness of the memory response when antigen is re-encountered is dictated by the overall size of the antigen-specific CD8 memory T-cell pool that is generated after the contraction phase of the primary immune response, as well as by their anatomical location, functional avidity, and the particular array of cytokines that these cells are programmed to secrete (Kaech and Wherry 2007). Identification of naïve, effector, and memory cells has been based on a combination of phenotypic, biochemical, and functional changes that occur upon activation (Kaech and Wherry 2007). Phenotypically, naïve CD8 T cells express high levels of the lymph node homing receptor molecule CD62L (MEL-14, L-selectin), and the CD45 high-molecular-weight isoform (CD45RB/A), CD127 (IL-7Rα), and low CD44 expression. Effector CD8 T cells express several activation markers including CD69, IL-2 receptor a (CD25), and are CD44 high, and have down-regulated the expression of IL-7Rα. Memory cells can be heterogeneous with respect to CD62L expression (so-called central and effector memory subsets), although it can be argued that true memory cells are CD62L high (central memory). Memory cells are generally CD45RB/A low, CD44 high, have regained IL-7Rα expression but have lost the expression of activation markers such as CD25 and CD69.

TNFR Involvement in Effector and Memory CD8 T-Cell Responses

OX40 and OX40L

OX40, in the TNFR family, represents a major co-stimulatory receptor for CD4 T cells (Croft 2003a, 2009; Salek-Ardakani and Croft 2006) but it is only recently that its control of CD8 T cells has been appreciated. OX40 is not constitutively expressed on naïve T cells but is induced at varying times after antigen encounter (Croft 2003b, 2009). Although TCR signals are sufficient for inducing OX40, CD28-B7 interactions augment and sustain its expression and T-cell- and APC-derived cytokines like IL-1, IL-2, and TNF may further modulate the extent and length of expression (Croft 2003a). Similarly, OX40 ligand (OX40L), in the TNF family, is also induced on APC such as B cells, macrophages, and DCs after activation, with ligation of other TNFR receptors like CD40, or PPRs like TLR4, or the receptors for innate cytokines like IL-18, and thymic stromal lymphopoietin (TSLP) leading to its expression (Croft 2003a, 2009). Information regarding the expression of OX40 and its ligand during acute and persistent/latent human viral infections is limited however, and represents an important area for future studies.

Insight into the role that OX40 plays in immunity to viral infections has been primarily acquired using gene-deficient mice. Much of the early data were negative with respect to CD8 T cells. $OX40^{-/-}$ mice had defective effector $CD4^+$ T-cell responses following intranasal (i.n.) infection with influenza (PR8; A/Puerto Rico8/34, H1N1 subtype) or intravenous (i.v.) infection with LCMV (isolate WE) (Kopf and others 1999). Despite impaired T-cell help, effector CD8 T-cell and antibody responses were comparable with wild-type levels. Furthermore, humoral responses elicited by either vesicular stomatitis virus (VSV) (Kopf and others 1999) or Theiler's murine encephalomyelitis virus (TEMV) (Pippig and others 1999), were also unaltered in $OX40^{-/-}$ mice. Critically, in all these systems, control of viral replication was not affected by the absence of OX40 signaling. Both the primary expansion and functionality of $NP_{366–374}$-specific CD8 T cells in the spleen were unaffected in OX40L-deficient mice infected i.p. with influenza A HKx31 strain (Dawicki and others 2004).

Moreover, OX40L had no detectable effect on the capacity of virus-specific CD8 T cells to respond to i.p. challenge with a serologically distinct influenza A PR8 strain (Dawicki and others 2004).

OX40L deficiency also did not affect the initial priming of $NP_{366–374}$-specific effector T cells in either lung, lung draining LN, or spleen after intranasal (i.n.) challenge with another strain of influenza virus (A/NT/60/68) that does not induce extensive lung inflammation (Hendriks and others 2005). However, 6 weeks after primary infection OX40L-deficient mice exhibited reduced formation of virus-specific memory CD8 T cells in the blood and spleen, but interestingly not in the lung (Hendriks and others 2005). After secondary challenge with the same virus strain, the absence of OX40L during priming led to reduced accumulation of secondary memory effector CD8 T cells in the lung but not DLN (Hendriks and others 2005). Therefore, OX40 has apparently a quite selective effect only on some aspect of memory to several viruses without impacting effector generation.

In contrast to LCMV, VSV, and influenza virus infection, OX40 strongly controlled the magnitude of the primary effector CD8 T-cell population after infection with a highly virulent mouse-adapted VACV, Western Reserve (VACV-WR) strain, regardless of whether the immunodominant or subdominant populations were examined (Salek-Ardakani and others 2008). Significantly, high frequencies of CD8 memory T cells that can persist in the lung and protect against lethal respiratory VACV challenge also did not develop in mice deficient in OX40. Notably, the hierarchy of epitopes recognized did not change in the presence or absence of OX40 co-stimulation (Salek-Ardakani and others 2008). Using OX40-deficient CD8 T cells from TCR transgenic mice responding to antigen expressed in recombinant VACV, it was found that direct OX40 signaling in CD8 T cells was not essential for induction of early cell division but was crucial for T-cell survival during the later stages of VACV infection (Salek-Ardakani and others 2008).

Vaccinia virus-WR strain can replicate very rapidly to high titers in multiple tissues (Reading and Smith 2003; Salek-Ardakani and others 2008), causes strong and sustained inflammation, and in many respects elicits CD8 T-cell responses that are highly analogous to LCMV and influenza (Salek-Ardakani and others 2008). This then raises the question of why OX40 has varying roles in CD8 T-cell responses during different infections. In this regard, recent analysis of LCMV revealed a differential requirement for IFN-I in controlling the initial expansion and generation of memory CD8 T cells not seen with VACV (Thompson and others 2006). With VACV infection, the predominant cytokine that supports memory is IL-12 (Xiao and others 2009). Although each of these pathogens can induce the expression of type I IFNs at least by certain cell types, the magnitude and kinetics of the response are dependent on the pathogenic organism (Thompson and others 2006). Thus the alternate use of OX40 might depend on the amount and type of innate cytokines induced, which may be a direct or indirect consequence of the virus and the specific virulence mechanisms they poses. These might simply mimic and hence replace the need for the OX40 signal. Additionally, as clearly revealed by the studies in different influenza infection models the time and use of OX40 might vary depending on the stage of the CD8 response examined and the specific tissues in which the responses are initially generated and maintained.

In addition to acute viral infections, CD8 T cells play a central role in the control of a number of clinically important chronic viral infections such as EBV, CMV, and HIV-1. In these infections, CD4 T cells can play a crucial role in the maintenance of an effective ongoing CD8 T-cell response. Very high OX40 expression was detected on activated peripheral human CD4 T cells isolated from HIV-1-seropositive individuals and to a lesser extent on HIV- and EBV-specific CD8 T cells (Sousa and others 1999; Takasawa and others 2001; Yu and others 2006). Using an *in vitro* culture system in which peptide-pulsed monocyte-derived DCs and T cells are co-cultured in the absence of exogenous cytokines to expand memory CTL responses, Yu and others (2006) reported that ligation of OX40 on CD4 T cells, with a human OX40L-IgG1Fc, enhanced their ability to help virus-specific CTL responses directed against HIV-1 and EBV. Relatively small enhancement of CTL responses was also observed in PBMCs from HIV-1-infected individuals, in the absence of CD4 T-cell help, suggesting that OX40 may have a direct effect on activated CD8 T cells as well. However in another study, co-culture of human EBV-specific memory T cells with syngeneic adherent monocytes infected with replication-defective recombinant adenoviruses expressing OX40L resulted in significantly enhanced antiviral memory CTL responses only in the presence of CD4 T cells (Serghides and others 2005), implying that the co-stimulatory effects of OX40 may sometimes be mediated through its capacity to stimulate CD4 T cells. Consistent with this, OX40 promoted virus-specific CD8 T-cell responses during late stages of MCMV infection, in a manner dependent upon CD4 T-cell help (Humphreys and others 2007). In contrast to VACV infection (Salek-Ardakani and others 2008), the requirement for OX40 by CMV-specific CD8 T cells appeared to be epitope-specific (Humphreys and others 2007).

Given its role in CD8 T-cell memory development highlighted in these virus studies, augmenting OX40 signaling has the potential to enhance protection afforded by vaccination. Co-administration of a eukaryotic DNA vaccine construct encoding OX40L, and foot-and-mouth disease (FMD) virus VP1, resulted in enhanced humoral and CD4 and CD8 T-cell responses and correlated with significantly improved protective efficacy of the vaccine against FMDV infection (Xiao and others 2007). Stimulation of OX40, in cooperation with 4–1BB, during vaccination with an OVA-expressing poxvirus vector also enhanced OVA-specific memory responses (Munks and others 2004). Recombinant poxvirus expressing OX40L in combination with B7–1, ICAM-1, and LFA-3 also enhanced CD4 and CD8 T-cell memory development (Grosenbach and others 2003). Furthermore, targeting OX40 with an agonistic mAb enhanced expansion of protective CD8 T cells during MCMV infection (Humphreys and others 2007). Taken together, these data highlight the potentially exciting approach of utilizing recombinant viral vectors expressing the vaccinating antigen of choice in combination with a ligand such as OX40L.

Although CD8 T cells are critical for protection from infection, exaggerated responses induced by certain viruses can actually be harmful. This is particularly relevant in the lung where inflammation in response to infection can result in airway occlusion and clinical disease and is a feature of viruses such as influenza (Wissinger and others 2008). Following inhibition of OX40/OX40L interactions with an OX40:Ig fusion protein, influenza-induced T-cell

inflammation and associated weight loss and cachexia were reduced, even when treatment was initiated after the onset of disease (Humphreys and others 2003; Hussell and others 2004). Importantly, OX40:Ig did not alter immune control of influenza replication (Humphreys and others 2003), suggesting that this anti-inflammatory approach may be applicable to a variety of viral infections where immunopathology is a causative factor in clinical disease.

4–1BB and 4–1BBL

4–1BB, another TNFR family member, is also induced on CD8 T cells after activation (Watts 2005; Croft 2009). Similar to OX40L, 4–1BB-ligand (4–1BBL) can be expressed on DCs, B cells, and macrophages, and again is induced by triggering TLR4, Ig, or CD40 signals (Watts 2005; Croft 2009). The *in vivo* role of 4–1BBL/4–1BB in antiviral T-cell immunity has been addressed in 4–1BB$^{-/-}$ and 4–1BBL$^{-/-}$ mice. After LCMV-Armstrong infection, 4–1BBL$^{-/-}$ mice generated 2- to 3-fold fewer effector/memory CD8 T cells compared with wild-type mice but were able to clear the virus by Day 8 post-infection (Tan and others 1999). Similar to OX40, after i.p. influenza A HKx31 (H3N2) infection, 4–1BBL$^{-/-}$ mice showed no defect in primary $NP_{366–374}$-specific CD8 T-cell expansion. However, they did exhibit a defect in the numbers and functionality of antigen-specific CD8 T cells late in the primary response, which then led to a decrease in the secondary response on re-challenge (Bertram and others 2002). Adoptive transfer experiments ruled out a programming model in which 4–1BB/4–1BBL is required during initial priming to allow T cells to re-expand during the secondary response (Bertram and others 2002). Hendricks and others (2005), however, used an i.n. model of influenza virus (A/NT/60/68) infection and although they found that memory cell reactivity was similarly dependent on 4–1BBL, they concluded the requirement for 4–1BBL was during the early response and not later in the response. In contrast to acute influenza infection, 4–1BBL deficiency did not affect the numbers of MHV-68-specific CD8 T cells that formed and persisted over time, but significantly affected their cytolytic function and the ability to control latent infection (Fuse and others 2007). In other studies, 4–1BB$^{-/-}$ mice had normal antibody responses to VSV, but showed defects in the recall cytotoxic CD8 T-cell response (Seo and others 2003). Together these studies suggest that in different models 4–1BB and 4–1BBL may have differing abilities to modulate primary versus secondary CD8 T-cell responses to virus.

Related to viral immunopathology, clinical symptoms of herpetic stromal keratitis (HSK), induced by the RE strain of HSV-1, were significantly abrogated in 4–1BB-deficient mice or after treatment with a blocking antibody against 4–1BBL (Seo and others 2003). The absence of 4–1BB signals was associated with reduced T-cell migration from the regional lymph nodes to the corneal stroma and the inhibition of inflammatory cytokine and chemokine responses in the corneal stroma (Seo and others 2003). This again suggests that targeting an interaction like 4–1BB/4–1BBL could additionally have therapeutic uses in situations where an overactive immune response to a virus leads to detrimental host effects.

Similar to OX40L, 4–1BBL expressing monocytes or fibroblasts were found to be extremely effective at augmenting the expansion, cytokine production, and cytolytic effector function of human memory influenza-, EBV-, HIV-(Bukczynski and others 2003, 2004), and HCMV-specific CD8 T cells (Waller and others 2007). Compared with B7.1, B7.2, ICOSL, or OX40L, 4–1BBL was the most effective at stimulating antiviral CD8 T-cell activation with limiting doses of antigen (Serghides and others 2005; Waller and others 2007). Synergistic or additive effects were observed when 4–1BBL co-stimulation was combined with OX40L or B7.1 co-stimulation (Serghides and others 2005). Interestingly, CD70, the ligand for another TNFR, CD27, on its own was found to have marginal activity in stimulating HIV-specific CD8 T cells. However, the combination of 4–1BBL with CD70 was better than 4–1BBL in generating HIV-specific CD8 T cells capable of producing multiple cytokines (Wang and others 2007).

Further evidence that targeting 4–1BB might be useful as an adjuvant for boosting antiviral T cells comes from animal studies. When mice were vaccinated with a fowlpox virus-VACV prime boost strategy, inclusion of 4–1BBL in the boost, but not the priming resulted in the enhancement of the anti-HIV T-cell response generated in response to this vaccination (Harrison and others 2006). Systemic administration of an agonistic 4–1BB mAb also enhanced cytotoxic and T-helper cell responses elicited by a recombinant adenovirus encoding hepatitis C virus (HCV)-NS3 (rAdNS3) and augmented the ability of rAdNS3 to induce protective immunity against challenge with a recombinant VACV expressing HCV proteins (Arribillaga and others 2005). Anti-4–1BB agonistic treatment in macaques immunized with a DNA vaccine encoding SIV Gag antigen (pSIVgag) furthermore led to enhanced and long-lasting SIV Gag-specific IFN-γ, granzyme B, and perforin responses (Calarota and others 2008). Agonistic 4–1BB treatment also enhanced HSV-1 (Kim and others 2009)- and influenza (Halstead and others 2002)-specific CD8 T-cell responses. Taken together, these studies show that in addition to OX40, 4–1BB represents a promising new target for enhancing therapeutic vaccination against both acute and chronic viruses.

CD27 and CD70

Unlike OX40 and 4–1BB, which are not present on naïve CD8 T cells, CD27 is constitutively expressed on most, if not all, T cells (Croft 2003b; Borst and others 2005; Nolte and others 2009). After T-cell activation, the expression of CD27 is increased, but it can be down-regulated when T cells differentiate toward effector cells. Indeed, analysis during various persistent infections such as with CMV, EBV, HIV, HCV, and LCMV revealed that effector CD8 T cells can have varying levels of CD27 expression (high, intermediate, and low) and that CD27 negative CD8 T cells are closely associated with poor control of viral infection (Nolte and others 2009). In contrast, memory T cells that reside in secondary lymphoid organs do express high levels of CD27, which correlates with their more resting phenotype (Nolte and others 2009). Antigen receptor, CD40, and TLR stimulation on T cells, B cells, and DCs has been shown to induce CD70, the ligand for CD27 (Nolte and others 2009). During influenza infection, CD70, like 4–1BBL and OX40L, can be detected on lung resident $CD11c^+$ DC and B cells as well as on lung infiltrating-activated T cells (Tesselaar and others 1997, 2003; Hendriks and others 2005).

Mice lacking CD27 had decreased numbers of virus-specific T cells in the lung, lung draining LNs, and the spleen,

during primary and secondary responses to i.n. infection with influenza (A/NT/60/68) (Hendriks and others 2000, 2005). In this model, CD27 acted independent of and complementary to CD28 (Hendriks and others 2003). Effects of CD27 deficiency appeared to be greater in the lung compared with other organs with more pronounced defects seen in the secondary response (Hendriks and others 2005). To assess a possible redundancy among CD27, 4–1BB, and/or OX40 in establishing the size of the virus-specific CD8 T-cell pool, 4–1BBL/CD27$^{-/-}$ and OX40L/CD27$^{-/-}$ double-deficient mice were assessed. In DLN and lung, the additional 4–1BBL or OX40L deficiency did not exacerbate the phenotype seen in CD27-deficient mice during the effector phase of the response (Hendriks and others 2005). In contrast, in the secondary response, the accumulation of virus-specific CD8 T cells was further reduced in the double-deficient mice (Hendriks and others 2005). These data demonstrate that the memory CD8 T-cell response to intranasal infection with influenza A virus is critically dependent on the collective contributions of CD27, 4–1BBL, and OX40L. Like data from separate studies of OX40 and 4–1BB, CD27 did not appear to be required for early T-cell division or differentiation toward CTL (Hendriks and others 2000, 2003). Instead, CD27 promoted survival after initial activation and thereby contributed to the accumulation of effector cells in the lung, perhaps in part by inducing autocrine IL-2 production (Hendriks and others 2000; Peperzak and others 2010).

A more recent study in the i.n. influenza A/HKX31 (HKX31, H3N2) infection model further revealed roles for CD27/CD70. Blocking CD27 signaling during the primary response decreased the frequency of $NP_{366–374}$-specific effector CD8 T cells found in the lung (Dolfi and others 2008). This was suggested to be due to enhanced apoptosis through Fas/FasL interactions. In contrast to previous studies, CD27 was not required for the generation of virus-specific memory CD8 T cells, but its activity in the priming phase led to reduced secondary expansion and reduced functionality (Dolfi and others 2008). In other studies, treatment with anti-CD70 blocking mAb inhibited priming of splenic effector CD8$^+$ T-cell responses after infection with VACV-WR and VSV (Schildknecht and others 2007). In contrast, CD27 was not required for primary LCMV-specific effector cell formation and viral clearance (Matter and others 2005; Schildknecht and others 2007). However, ligation of CD27 on LCMV-specific memory CTLs during re-stimulation strongly enhanced autocrine IL-2 production and thereby promoted secondary expansion and protection from re-infection (Matter and others 2005, 2008). Thus, CD27 co-stimulation can also play critical roles at several phases during the generation of virus-specific effector and memory CD8 T cells, but again the time and type of activity differs somewhat depending on the infection studied.

LTα and LTβR

Lymphotoxin (LT) is present in 2 forms: soluble LT-α_3 homotrimers and membrane-associated LT-$\alpha_1\beta_2$ heterotrimers (Schneider and others 2004; Ware 2005). Soluble LT-α_3 is structurally related to TNF and can bind to both TNF receptors, TNFR1 and TNFR2 (Schneider and others 2004; Ware 2005). In contrast, membrane LT-$\alpha_1\beta_2$ heterotrimers have high-affinity binding to the LT-β receptor (LT-βR), without binding to TNFR1 and TNFR2 (Schneider and others 2004; Ware 2005).

LT-$\alpha^{-/-}$ and LT-$\beta R^{-/-}$ mice lack lymph nodes and Peyer's patches and have severely disrupted splenic architecture and the loss of follicular dendritic cells (FDCs), germinal centers, and significantly diminished numbers of DCs (Schneider and others 2004; Ware 2005; De Trez and Ware 2008). LT-$\alpha^{-/-}$ and LT-$\beta R^{-/-}$ mice, but not TNF/TNFR1$^{-/-}$ mice, displayed impaired TMEV-specific CTL activity in the CNS of a normally resistant C57BL/6 mouse strain (Lin and others 2009). This defect resulted in persistent virus infection and immune-mediated demyelination (Lin and others 2009). Thus, membrane LT-$\alpha_1\beta_2$, but not soluble LT-α_3 or TNF, played a critical role in mediating host resistance to TMEV infection and disease (Lin and others 2009). After LCMV infection, LT-$\alpha^{-/-}$ mice showed substantially lower numbers of functionally competent virus-specific CD8 T cells in the spleen and non-lymphoid organs (Suresh and others 2002), which again correlated with defective clearance of the virus from these tissues. LT-$\alpha^{-/-}$ T cells did not exhibit any defect in activation, expansion, or effector function upon adoptive transfer into WT mice, which were then infected with LCMV (Suresh and others 2002). In contrast, WT CD8 T cells failed to expand after transfer into LTα-deficient host (Suresh and others 2002), implying that impaired T-cell activation in LT-$\alpha^{-/-}$ mice was most likely due to abnormal lymphoid architecture and not to an intrinsic defect in T cells *per se* (Suresh and others 2002). Interestingly, LT-$\alpha^{-/-}$ mice developed CD8 T cells at normal frequencies when infected with HSV but they failed to differentiate into CTLs and produced minimal IFN-γ. Consequently, the mice failed to clear the virus, resulting in HSV-induced encephalitis and eventual death (Kumaraguru and others 2001).

Despite lacking most LNs and not being able to generate normal germinal centers in the spleen, LT-$\alpha^{-/-}$ mice were found to clear respiratory low-dose infection with MHV-68 (Lee and others 2000) and influenza (Lund and others 2002) and to produce high virus-specific IgG and IgA antibody titers, albeit with delayed kinetics (Lee and others 2000, Lund and others 2002). However, when LT-$\alpha^{-/-}$ mice were infected with higher titers of influenza, they succumbed to infection before the adaptive immune response could be initiated (Lund and others 2002). Together, these studies demonstrate that LT signaling, the presence of LNs, or organized splenic architecture are not always essential for the development of an effective immune response and clearance of certain viruses. However, an intact LT signaling pathway and the presence of lymphoid organs may be important to facilitate the rapid induction of protective immunity against highly virulent viruses or when infectious dose is high.

CD40 and CD40L

CD40 is constitutively expressed on many cell types, including B cells, DCs, follicular DCs, monocytes, macrophages, epithelial cells, and endothelial cells (Ma and Clark 2009). In contrast, CD40L is predominantly expressed on activated CD4 T cells, but expression on mast cells, basophils, eosinophils, natural killer cells, activated B cells, and DCs has been reported (Ma and Clark 2009). Weak expression of CD40L has also been detected on some CD8 T cells.

The interaction of CD40L on activated CD4 T cells with CD40 on APC can enhance the expression of several

co-stimulatory ligands on APC, and this "licensing" of APC can promote effective CD8 T-cell immunity (Bevan 2004; Khanolkar and others 2007; Ma and Clark 2009). For many CD8 T-cell responses, this is manifest as a loss of CTL activity upon exposure to antigen in the absence of CD4 T cells (Bevan 2004; Khanolkar and others 2007). However, CD40L-deficient mice acutely infected with LCMV-Armstrong, Pichinde virus, HSV, West Nile virus (WNV), or VSV generated strong primary effector CTL and cleared these viruses (Borrow and others 1996; Whitmire and others 1996; Thomsen and others 1998; Whitmire and others 1999; Edelmann and Wilson 2001; Sitati and others 2007), suggesting that CD40L is not required for all antiviral CTL or antibody responses. $CD40L^{-/-}$ mice infected with a rapidly replicating strain of LCMV (Traub strain), vaccinia virus (VACV-WR) (Fang and Sigal 2005), or mouse pox (ectromelia virus) (Fang and Sigal 2005) also showed efficient activation, expansion, and differentiation of virus-specific CD8 T cells during the early stages of infection, consistent with reports showing that primary CTL induction to these viruses can occur in the absence of CD4 T-cell help (Bevan 2004; Khanolkar 2007; Fuse and others 2009; Salek-Ardakani and others 2009). Interestingly, however, their ability to control virus replication, to maintain virus-specific CTL memory, and to respond to secondary infection diminished over time (Borrow and others 1996; Thomsen and others 1998; Andreasen and others 2000). This indicates that maintenance of effective antiviral immune surveillance can be critically dependent upon interactions between CD40 and CD40L. These data are again consistent with reports describing reduced antiviral CTL memory responses in virus-infected CD4 T-cell-deficient or CD4-depleted mice (Bevan 2004; Khanolkar and others 2007). One hypothesis for the intact primary CD8 T-cell responses in the absence of CD40/CD40L is that certain viruses are able to directly infect professional APC and induce their maturation, and/or they can elicit proinflammatory cytokines such as IFN-I (Le Bon and others 2003), which then independently induce co-stimulatory activity on APCs and by doing so circumvent the need for CD4 T cells and CD40-mediated APC activation. Interestingly, one recent study indicated that CD70 expression by DCs can mediate effective CD40-independent activation of CD8 T cells (Van Deusen and others 2010). Thus, like other members of the TNFR superfamily, CD8 T-cell responses to viruses can be either CD40-dependent or -independent, and in some cases this alternate dependency can manifest at different times in response to the same virus.

CD30 and CD30L

CD30L has been reported to be present on activated T cells, and many APCs such as B cells and DCs (Watts 2005). Similarly, CD30 expression on T cells is activation-dependent. Although it has been suggested that CD30L/CD30 interactions have an important function in regulating the division or survival of CTLs (Podack and others 2002; Nishimura and others 2005), the precise role of CD30/CD30L interactions in antiviral CD8 T-cell responses remains poorly characterized at present. An early report demonstrated that, similar to OX40, primary CD8 T-cell responses to VSV as well as anti-VSV antibody production were unaffected by a CD30 deficiency (Amakawa and others 1996). The finding that OX40 and CD30 have in common the expression of their ligands on certain accessory cells at sites of T cells/B cells interaction (Kim and others 2003) raised the issue of whether they might cooperate in priming CD8 T cells. Bekiaris and others (2009) recently provided evidence that OX40 and CD30 integrate synergistic signals during the expansion of CD8 effectors. A double deficiency in OX40 and CD30 led to markedly fewer CD8 T cells expanding and being maintained after infection with MCMV, although there were alternate effects on persisting dominant versus subdominant populations. Previous reports have clearly demonstrated that during MCMV infection different epitopes are associated with distinct CD8 responses and it is possible that OX40- and CD30-specific effects are seen only for some epitopes as was shown before using $OX40^{-/-}$ mice (Humphreys and others 2007).

HVEM and LIGHT

The TNF family molecule LIGHT (Lymphotoxins, Inducible, competes with HSV Glycoprotein D for HVEM, expressed by T cells) primarily binds both HVEM (herpesvirus-entry mediator) and the LTβR (Croft 2005; Ware 2005, 2008). HVEM can additionally bind the ITIM-containing co-inhibitory molecule BTLA, which belongs to the Ig-like CD28-B7 family (Croft 2005; Sedý and others 2008). Since LTβR is not expressed on T cells, this points toward the LIGHT-HVEM system as likely the primary co-signaling pathway in T cells, although LIGHT on a T cell interacting with LTβR on an APC or other cell could also control T-cell priming. To further add to the complexity of this system, CD160, a glycosphingolipid-linked Ig domain protein, was recently identified as yet another HVEM ligand (Cai and others 2008).

Under experimental conditions that were previously employed to demonstrate a role for 4–1BBL and CD27 in CD8 T-cell memory to influenza A virus, neither primary expansion nor memory and recall responses of $NP_{366–374}$-specific CD8 T-cell responses were affected by the absence of LIGHT (Sedgmen and others 2006). Similarly, influenza-specific CD4 T-cell and antibody responses detected in infected $LIGHT^{-/-}$ mice were comparable with those observed in WT mice (Sedgmen and others 2006). Thus, although similar to 4–1BBL, OX40L, and CD27 in certain situations, LIGHT is clearly dispensable for the T-dependent immune response to influenza virus in mice with no effect on the induction, maintenance, or reactivation of CD8 T-cell memory. In contrast, recent work has established a strong role for HVEM/LIGHT/BTLA interactions in driving the CD8 T-cell response to VACV-WR (Salek-Ardakani, unpublished data) further adding to the idea that alternate viruses and/or infection conditions will reveal differential requirements for members of this superfamily.

GITR and GITRL

GITRL is expressed largely on APCs, including DCs and B cells. TLR stimulation transiently up-regulates GITRL expression on APCs (Watts 2005). GITR is expressed on resting CD4 and CD8 T cells but can be up-regulated after activation (Watts 2005). There are currently no studies of virus responses in the absence of GITR or GITRL, but *in vivo* administration of an agonistic antibody to GITR showed that more robust protective immunity could be generated against a persistent retrovirus infection (Dittmer and

others 2004, 2008). In a model of corneal blindness caused by ocular infection with HSV, anti-GITR mAb treatment also enhanced HSV-specific CD8$^+$ T-cell effector function but decreased HSV-induced corneal lesion severity (La and others 2005; Suvas and others 2005). The diminished keratitis was attributed to the effects of the treatment on the reduced influx of CD4 T cells into the infected corneas and decreased levels of ocular matrix metalloproteinase-9 (MMP-9), a molecule involved in ocular angiogenesis, an important step in the influx of inflammatory cells and pathogenesis of herpetic ocular lesions (Suvas and others 2005). In another study, multimeric soluble GITRL augmented the CD8 T-cell, CD4 T-cell, and antibody responses to HIV DNA vaccination (Stone and others 2006). Together, these studies suggest that targeting GITR/GITRL might again be a promising new target as a vaccine adjuvant.

Fas and FasL

Interactions between the death receptor Fas (CD95) and FasL (CD95L) can lead to cytolysis via the activation of a death domain and a caspase apoptotic pathway (Shresta and others 1998). This can be a mechanism by which FasL-positive CD8 effector T cells kill targets (Lowin and others 1994; Doherty and others 1997). Alternatively, engagement of Fas on T cells may have a detrimental effect on the host immune response to infection by eliminating these effector cells (Mueller and others 2001; Petrovas and others 2004). Thus, Fas-mediated apoptosis may have pleiotropic functions in host antiviral defense, which may vary depending on the virus.

Both *lpr* (Fas-deficient) and *gld* (FasL-deficient) mice were highly susceptible to infection with virulent HSV-2 strain 186 and showed significantly increased viral titers in the spinal cord compared with WT mice (Ishikawa and others 2009). In this model migration of HSV-2-specific CD4 and CD8 T cells into the spinal cord and their capacity to produce IFN-γ was unaffected in the absence of Fas/FasL signaling (Ishikawa and others 2009). The target was not fully elucidated, but FasL expressed on CD4 T cells was found critical for the protection against lethal infection with HSV-2 (Ishikawa and others 2009). Similarly, *gld* mice were highly susceptible to lethal WNV infection (Shrestha and Diamond 2007) that, similar to HSV, causes meningitis and encephalitis (Wang and others 2004). However, in this model the results of cell transfer experiments indicated that FasL expression on CD8 T cells was essential for host defense against WNV infection (Ishikawa and others 2009). Interestingly, Fas/FasL signaling was not important in protection against a less virulent strain of WNV (Wang and others 2004) or attenuated HSV-2 (Milligan and others 2004; Dobbs and others 2005). These results imply that the importance of Fas-mediated protection in antiviral immunity may be critically determined by the virulence of the virus.

Influenza-specific CD8 T cells were found to use FasL-mediated mechanisms to eliminate Fas$^+$ virus-infected cells (Lowin and others 1994; Doherty and others 1997). However, the activated T cells themselves can also be Fas$^+$ and therefore susceptible to FasL-mediated killing (Wolfe and others 2002). Recent studies by Legge and Braciale (2005) indicated that the generation of a robust influenza-specific effector CD8 T-cell response was dependent upon the down-regulation of FasL expression on DC. High-dose infection with influenza virus resulted in sustained production of IL-12p40, which maintained elevated FasL on DC (Legge and Braciale 2005). This then contributed to increased apoptosis of activated proliferating CD8 T cells and decreased effector CD8 T-cell generation (Legge and Braciale 2005). Consistent with this, FasL-deficient mice had markedly increased numbers of effector CD8 T cells and showed enhanced resistance to high-dose influenza infection (Legge and Braciale 2005). These results establish an inverse relationship between virus inoculum size and the magnitude of the subsequent CD8 T-cell response through IL-12p40-dependent FasL expression.

Induction of FasL on APC and subsequent elimination of Fas$^+$ T cells has also been proposed as a potential contributing factor to the gradual immune suppression seen in individuals infected with HIV (Petrovas and others 2004; Poonia and others 2009a). Accordingly, HIV-specific CD8 T cells are much more prone to undergo Fas-induced apoptosis compared with CMV- or EBV-specific CD8 T cells, and HIV-infected FasL$^+$ macrophages or DC can induce apoptosis of HIV-specific CD8 T cells (Mueller and others 2001; Quaranta and others 2004). Additionally, CD4 T lymphocytes can be primed to express FasL and contribute to CD8 T-cell apoptosis (Quaranta and others 2004), thus resulting in escape of virally infected cells from the CTL response. In line with this, blocking Fas/FasL interactions allows the preservation of SIV-specific CD8 T cells and virus-specific cellular immunity, suggesting an active role of FasL in deleting SIV- and possibly HIV-specific CD8 T cells (Poonia and others 2009a, 2009b).

TRAIL and DR4/5

TRAIL (also known as Apo2L) is a type II transmembrane protein belonging to the TNF superfamily (Cummins and Badley 2009). It binds to 2 death receptors, DR4 (TRAIL-R1) and DR5 (TRAIL-R2) in humans, and DR5 in mice (Cummins and Badley 2009), that are similar to Fas. Only a few studies have focused on the contribution of TRAIL to the adaptive immune response following acute virus infection. One investigated a role for TRAIL during LCMV infection in the presence (termed "helped") and absence (termed "helpless") of CD4 T cells (Janssen and others 2005). It was found that helpless but not helped memory CD8 T cells selectively up-regulated TRAIL upon re-stimulation *in vitro*, whereas both helped and helpless populations had similar levels of DR5. The increased ability of helpless memory CD8 T cells to produce TRAIL led to increased cell death upon secondary Ag challenge, leading to an abortive response. This prompted the proposal of a programming model in which CD4 T-cell help might be imprinted early after naive CD8 T-cell activation (Khanolkar and others 2007). However, in a subsequent study Badovinac and others (2006) found that TRAIL deficiency only delays but does not prevent erosion in the quality of helpless virus-specific memory CD8 T cells, implying that CD4 help consists of both TRAIL-dependent and -independent mechanisms. Specific pathogenic organisms may critically dictate the involvement of TRAIL. Consistent with this, using an intranasal infectious model of VACV-WR, Fuse and others (2009) showed that the defective recall response by helpless virus-specific memory cells was not associated with increased cell death and was independent of TRAIL. Instead, they observed excessive up-regulation of the inhibitory

receptor programmed cell death 1 (PD1), and blocking PD1 resulted in an enhanced recall response of the memory T cells.

The expression of mRNA for TRAIL and its mouse receptor DR5 was increased in the lungs during influenza virus infection (Ishikawa and others 2005; Brincks and others 2008), and influenza-specific memory CD8 T cells were found to utilize TRAIL to kill virus-infected cells and control primary infection (Brincks and others 2008). Thus, in addition to Fas/FasL and perforin/granzyme pathways, virus-specific CD8 T cells may use TRAIL to drive recovery from primary infection. Expression of TRAIL by CD8 T cells has also been associated with the transient lymphopenia seen after infection with RSV (Roe and others 2004). More recently, Bucks and others (2009) found that chronic live influenza A virus infection resulted in the exhaustion of virus-specific CD8 T cells as indicated by their failure to re-expand or produce IFN-γ upon viral re-challenge, and that inhibition of signals delivered by TRAIL prevented this phenomenon. Contrary to other models of chronic infection such as HIV or LCMV (clone 13), exhaustion of influenza-specific CD8 T cells was completely independent of another inhibitory receptor, PD1, despite the fact that it was dramatically up-regulated on these cells (Bucks and others 2009).

TNFR Family and Requirement for Reactivation of Memory CD8 T Cells

In contrast to the extensive literature on the generation of memory T cells (reviewed in part earlier), there is a surprising lack of understanding of what are the critical requirements for memory T cells to undergo effective recall responses and to persist as functional populations after repeated antigenic encounters. In particular, other than the data on TRAIL discussed earlier (Janssen and others 2005), the role of co-stimulatory and co-inhibitory molecules in the TNFR/TNF superfamily in modulating memory T-cell responsiveness to antigen has substantially lagged behind our understanding of how memory is generated.

Studies carried out in a number of experimental systems suggest that memory CD8 T-cell populations can be extremely heterogeneous, containing subsets that reside or migrate through lymph nodes ($CD62L^+CCR7^+$; "central" memory T cells: T_{CM}) and those that preferentially reside in non-lymphoid organs such as the lung ($CD62L^-CCR7^-$; "effector" memory T cells: T_{EM}) (Woodland and Dutton 2003; Sallusto and others 2004). The precise relationship between T_{EM} and T_{CM} subsets remains to be elucidated especially, with regards to their ability to confer protective immunity against different viruses (Hikono and others 2006; Woodland and Kohlmeier 2009). There is good animal and clinical evidence to show that the degree of protection against highly virulent (rapidly replicating) or certain persistent viruses critically depends on the magnitude and quality of long-living T_{EM} subsets residing at the specific entry site of the virus (Roberts and Woodland 2004; Bachmann and others 2005; Hikono and others 2007; Salek-Ardakani and others 2008; Li and others 2009). In contrast, protection against slowly replicating viruses can also be mediated by T_{CM} subsets (Wherry and others 2003; Roberts and others 2005).

The existence of anatomically and phenotypically distinct memory subsets raises an interesting question as to whether various memory subsets have different requirements for co-stimulatory signals with respect to their survival and functionality. In this regard, a recent study demonstrated that splenic memory CD8 T cells generated by i.n. or i.p. Sendai virus or influenza virus infection can be divided into 3 distinct subpopulations based on their expression of CD27 and CD43: $CD27^{hi}/CD43^{hi}$, $CD27^{hi}/CD43^{lo}$, and $CD27^{lo}/CD43^{lo}$ (Hikono and others 2007). The $CD27^{hi}$ memory subpopulations were found to be 2–10-fold more efficient than their counterparts at contributing to the recall responses in the lung and airways (Hikono and others 2007). Whether CD27 is just a marker for these cells or it is directly linked to their ability to mount a robust recall response is not clear at present. Some negative data on CD27 being required for memory recall responses has been published previously, as briefly mentioned earlier (Dolfi and others 2008; Boesteanu and Katsikis 2009). But in other studies, Belz and others found that influenza-specific memory CD8 T cells required antigen presentation by lymph node resident $CD8\alpha^+$ DCs for activation in response to secondary influenza challenge and this involved the interaction of CD27 with CD70 (Belz and others 2007). In support of this, Ballesteros-Tato and others (2010) recently showed that migratory lung $CD103/CD11b^{hi}$ DCs were the only DC subset that up-regulated CD70 after influenza infection and that CD70 expression was again functionally important for CD8 T-cell recall responses to influenza. Whether these DC subsets were the same is not clear, nor whether CD70 was required in the LN or tissue. Generation of a protective virus-specific CD8 T-cell response also requires effector CD8 T cells to interact with antigen-presenting pulmonary DCs once they enter the lungs (McGill and others 2008; McGill and Legge 2009), and therefore a $CD27^+$ subset of memory CD8 T cells might receive reactivation signals in several locations for effective recall response.

Further studies will be required to understand whether there is truly a differential requirement for co-stimulatory signals by alternate memory T-cell subsets and to what extent the microenvironment that these cells reside in dictates the involvement of particular sets of signals in their recall responses. Demonstrating that the requirement for co-stimulatory signals is not confined to influenza-specific memory CD8 T cells, nor CD27/CD70, we have also found that blocking OX40/OX40L interaction during secondary respiratory challenge with VACV-WR markedly impaired the capacity of VACV-specific memory CD8 T cells to confer protection against disease and death (unpublished data). The finding that reactivation of some types of memory CD8 T cells require co-stimulation through TNFR family members could have important ramifications for designing more efficient vaccines to combat infections and should be further explored.

TNFR Members and Persistence of CD8 Memory T Cells

Memory CD8 T cells can survive for a long time at least as a population without a secondary contact with the infectious organism concerned. Memory appears to be maintained at least in part through periodic slow cell division and continuous replacement of cells, a process termed homeostatic or basal proliferation. After clearance of acute infection, virus-specific memory CD8 T-cell maintenance depends on IL-7 and IL-15 (Gaspal and others 2005; Haring and others 2006;

Harty and Badovinac 2008; Surh and Sprent 2008). Several studies in non-infectious and viral (VSV and LCMV) models have shown a role for IL-15 in controlling both proliferation and survival of memory CD8 T cells (Haring and others 2006; Harty and Badovinac 2008; Surh and Sprent 2008). In contrast, IL-7 may be important for survival rather than proliferation (Haring and others 2006; Harty and Badovinac 2008; Surh and Sprent 2008).

In acute viral infection models, there is some indirect evidence to suggest that the ability of IL-15 and IL-7 to maintain memory T cells or allow longer survival of effector T cells could be in part related to the TNFR family. IL-15 treatment of spleen or bone marrow cells resulted in increased cell surface expression of 4–1BB, CD27, TNFR2, and GITR, but not OX40 or CD30, on $CD8^{+}CD44^{hi}$ "memory phenotype" cells (Pulle and others 2006), implying that in certain situations TNFR family members might be available and hence active downstream of this cytokine (Sabbagh and others 2007). In line with this, adoptive transfer of central memory ($CD62L^{hi}$ $CD44^{hi}$ $IL\text{-}7R^{+}CCR7^{+}$ CD69) phenotype CD8 T cells into 4–1BBL-deficient mice resulted in a 2–3-fold defect in recovery compared with wild-type hosts 3 weeks post-transfer (Pulle and others 2006). IL-15 was found to induce expression of 4–1BB on CD8 but not on CD4 memory T cells (Pulle and others 2006). On the other hand, IL-7 was found to induce OX40 on memory CD4 T cells (Gaspal and others 2005) but failed to induce 4–1BB on CD4 or CD8 T cells (Gaspal and others 2005). Additionally, there may be an interesting feedback loop where 4–1BB and OX40 might increase the expression of IL-7R and possibly IL-15R further assisting T-cell survival (Lee and others 2007). Analysis of memory T-cell subsets at various times after influenza infection further revealed that the $CD27^{hi}/CD43^{lo}$ subpopulation progressively dominated the memory T-cell pool, representing ~90% of the antigen-specific T cells detected at 2 years after infection, in contrast to the $CD27^{lo}/CD43^{lo}$ subpopulation that progressively declined (Hikono and others 2007). This implies a possible role of CD27/CD70 interactions in maintaining these memory T cells. In another study, it was found that many of the phenotypic and functional characteristics of virus-specific CD8 memory T cells are maintained by signaling via CD27 and 4–1BB, but not via OX40 (Allam and others 2009). Together, these studies suggest that virus-specific memory T-cell number/survival and/or functionality might be maintained over time through periodic interaction between certain TNFR/TNF family members.

Chronic infections can markedly alter T-cell survival requirements. Memory CD8 T-cell maintenance during persistent infection is associated with extensive and rapid proliferation of these cells, rather than the slow memory turnover observed after acute infection. Results related to the inhibitory molecule PD1 have shown that it can become highly active with certain strains of LCMV (Barber and others 2006), and with HIV (Day and others 2006), that are persistent and infect chronically. Whether molecules such as OX40, 4–1BB, CD27, or their related family members limit or oppose the activity of suppressive or co-inhibitory receptor/ligands pairs during chronic viral infections is not known.

Conclusions and Future Directions

Understanding the regulatory mechanisms involved in the development of virus-specific effector and memory CD8 T cells has important implications with regards to vaccine design. Successful vaccines induce strong and long-term humoral responses. Similarly, an important component of an effective T-cell vaccine must be the capacity to induce strong initial clonal expansion of virus-specific precursor cells. Further success in vaccine design might rely on developing strategies that reduce cell death at the end of the primary immune response, thereby augmenting the entry of effector cells into the long-lived memory cell pool. In this review, we have discussed experimental evidence demonstrating that signals delivered through a group of molecules that belong to the TNFR/TNF superfamily can both enhance and oppose the survival and functionality of effector and memory CD8 T cells during virus infections. These results strongly imply that targeting TNFR/TNF family members could represent a promising avenue for manipulating CD8 memory cell generation, reactivation, and persistence. An emerging theme in the field is that we cannot apply one rule with regard to the use and functional requirement for these molecules. How much plasticity and redundancy in use of TNFR family receptors is influenced by the virus strain, dose of infection, route of infection, and expression of viral immune evasion strategies remains to be determined. Therefore, which receptor/ligand pairs are the relevant molecular interactions to target in terms of providing therapeutic benefit in vaccination strategies is not clear.

In view of recent findings demonstrating that great specificity, migratory, and functional heterogeneity exists among antiviral CD8 memory T cells, it will be important to examine whether TNFR family signals are preferentially required by different memory subsets (eg, central versus effector). In this regard, further characterization of lymphoid and non-lymphoid memory subsets in different organs and systems is needed to dissect the effects of location and specificity on memory T-cell survival/function and use of stimulatory and inhibitory interactions. Furthermore, an important but relatively unexplored area of research is the question of whether long-term survival and functionality of memory CD8 T cells requires continuous or periodic signals from these molecules.

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