Published in final edited form as:

Semin Immunol. 2009 April ; 21(2): 69–77. doi:10.1016/j.smim.2009.02.005.

Memory T cells need CD28 costimulation to remember

Alina C. Boesteanu and Peter D. Katsikis*

Drexel University College of Medicine, Department of Microbiology and Immunology, 2900 Queen Lane, Philadelphia, PA 19129, United States

Abstract

The activation and expansion of naïve T cells require costimulatory signals provided by CD28 and TNF family members. In contrast, for many years it was believed that memory T cells do not require CD28 costimulation for expansion during secondary responses. This was based on *in vitro* experiments that suggested the re-activation of memory T cells is somewhat independent of costimulation. Recent *in vivo* evidence, however, has challenged this and shown that both CD4+ and CD8+ memory T cells require CD28 costimulation for maximal expansion and pathogen clearance. This requirement has important implications for host immunity, vaccine development and immunotherapeutics.

Keywords

Costimulation; Memory; CD4+ T cells; CD8+ T cells; CD28

1. The evolution of the two signal theory for effective T cell activation

Ensuring that the immune system efficiently mounts immune responses against pathogens, yet fails to attack self-antigens, is critical for the survival of the host. For this purpose, the task of initiating immune responses is primarily assigned to professional antigen presenting cells (APCs). These professional APCs present both antigen to T cells and important co-activating and growth factors that have been termed as costimulation. Originally, costimulation was presented in the context of the two signal theory which requires two signals for full activation of T cells and the generation of optimal immune responses. Signal 1 is generated by TCR stimulation while signal 2 is generated by CD28, a receptor for CD80 (B7.1) and CD86 (B7.2). In recent years, the notion of costimulation has been expanded to include many other factors that can provide synergistic signals to TCR-induced T cell activation. Therefore, signal 2 does not consist of a single signal but of multiple signals mediated by CD28 family members, TNF family members and other cytokines.

The theory stipulating that activation of an immune cell requires two signals was first published in 1970 [1] and was developed based on the immune response of B cells to antigens that can induce either antibody production or failed to produce antibodies. Bretscher and Cohn postulated that lack of antibody production is induced when an antigen receptor on the surface of the immune cells interacts with only one antigenic determinant, whereas activation (antibody production) is the result of recognition of two determinants on the same antigen, by two receptors. This concept was further developed when scientists realized that there are two classes of lymphocytes that are involved in the immune response: one class was associated with bursa of Fabricius in chickens (in mammals this role is taken

*Corresponding author. Tel.: +1 215 991 8380; fax: +1 215 848 2271., Alina.Boesteanu@Drexelmed.edu (A.C. Boesteanu), Peter.Katsikis@Drexelmed.edu (P.D. Katsikis).

by the bone-marrow) [2] and was producing antibodies, and the other class was associated with the thymus and was recognizing histocompatibility antigens [3,4]. In the late 1960s, while studying the nature of the allogeneic immune responses, Lafferty proposed that antigen alone is not sufficient to stimulate an allogeneic response and in fact lymphocytes required a second signal that he called “allogeneic stimulus” [5]. Lafferty et al. also determined that cells of the hematopoietic system were the ones delivering the “antigenic stimulus” and they needed to be metabolically active in order to stimulate lymphocytes [6,5]. At this point it became evident that the “secondary signal” from Bretscher and Cohn theory is actually delivered by a “stimulatory cell” which binds to the responder cell through an “antigen bridge” [7], yet they did not make any distinction between the immune activation of T and B cells. Once Zinkernagel and Doherty made the discovery of MHC restriction [8], Lafferty and Cunningham modified their theory to explain activation of T cells, in that binding of the MHC molecule presenting antigen by a specific T cell receptor, was the trigger for delivery of the second signal [9,10]. In the situation when only signal one was present, for example by killing or fixing the antigen presenting cells, T cells became anergic [11]. In trying to explain how tolerance towards self- and immune responses to pathogens work together in the same organism, Janeway proposed that the second signal is not triggered by the TCR engaging the MHC/antigen complex but rather by microbial products binding receptors on or inside APC [12]. In contrast to Janeway’s theory about the mechanism of induction of the second signal, Matzinger proposed that activation of the APC and the provision of costimulation depend on host-cell damage and release of “danger signals”, such as DNA, RNA, heat-shock proteins, and interferons [13]. Overall, these theories put forth the idea of costimulation playing a critical role for activation of T cells, either in response to pathogens or in alloreactive immune responses, but also in the maintenance of tolerance. Research at the molecular level allowed identification of many costimulatory molecules, which belong to the CD28 and the TNF/TNFR superfamilies.

2. The CD28 and TNF/TNFR family members as costimulatory molecules

The identification of CD28 [14,15] led to the discovery of a series of other costimulatory molecules. To date, there are five confirmed members of the classical CD28 family which share structural homology (a variable Ig like extracellular domain and a short cytoplasmic tail): CD28, CTLA-4, ICOS, PD-1 [16] and BTLA [17]. These costimulatory molecules interact with their respective ligands on APC surface: CD28:B7-1 or B7-2, CTLA-4:B7-1 or B7-2 [18], ICOS:B7h [19,20], PD-1:B7H-1 or B7-DC [21,22] and BTLA:HVEM [17]. Two additional molecules which are believed to be CD28 superfamily ligands, but are as yet unpaired with receptors, are B7H3 (also known as B7x) [23] and B7H4 (also known as B7S1) [24,25]. A second group of costimulatory molecules, the TNF/TNFR superfamily [26] can be distinguished from CD28 members by the presence of a more complex cytoplasmic tail. TNF/TNFR receptor:ligand pairs which have known costimulatory functions include OX-40:OX40L, CD27:CD70, 4-1BB:4-1BBL, CD30:CD30L, GITR:GITRL and HVEM:Light [27,28]. Members of the TNF/TNFR family can be subdivided into three groups; those containing cytoplasmic death domains, those lacking a death domain but containing decoy receptors and those which lack a death domain but contain a TRAF motif [29].

The emphasis of this review is on the role played by the costimulatory molecule CD28 in primary and secondary immune responses mediated by T cells. However, we will include in our discussion a brief overview of the expression pattern and function of the other members of the CD28 family, as the function of CD28 can be largely impacted by the presence or absence of the other family members. CTLA-4 and ICOS are both structural homologs of CD28, yet they exhibit unique, non-redundant functions upon stimulation [30]. CTLA-4 competes with CD28 for binding to same ligands (B7-1 and B7-2), and it is not expressed on

resting or newly activated T cells. Rather, expression is largely restricted to fully activated T cells and regulatory cells [31]. The affinity of CTLA4 for B7-1 and B7-2 was estimated to be 10–20 times greater than the affinity of CD28 for the same ligands [32]. Binding of CTLA-4 by B7-1 or B7-2 inhibits T cell proliferation [33] by disruption of lipid rafts [34] and interruption of TCR signaling [35]. In contrast to CTLA-4, the inducible costimulator ICOS, does not share ligands with CD28 but rather binds its own ligand, B7-h [36]. Signaling through ICOS augments proliferation, antibody response and cytokine production [37–40]. With regard to viral infection, *in vivo* studies indicate a particularly important role for ICOS signaling during development of primary antibody responses and maintenance of late stage primary CD8+ T cell responses during LCMV, VSV and influenza [41].

The more recently identified members of the CD28 family of costimulatory molecules, Programmed Death-1 (PD-1) and B and T cell lymphocyte attenuator (BTLA), both exhibit inhibitory activity. PD-1 has at least two known ligands PD-L1 (B7H-1) and B7-DC, and is expressed on both T and B cells [42]. Signaling through PD-1 has been shown to be involved in peripheral tolerance [43] and in the regulation of anti-viral CD8+ T cell responses during chronic infection [44–48]. The newest member of the CD28 family, BTLA, has not been extensively characterized yet [49]. BTLA is constitutively expressed at low levels on T cells and can be up-regulated on activated B and T cells [50,51]. It has been suggested that BTLA plays an inhibitory role in the development of adaptive immune responses by inhibiting CTL maturation and memory generation [52].

3. CD28 costimulation in primary T cell responses

The importance of CD8+ T cells in the resolution of viral infection is widely accepted. Activation of naïve CD8+ T cells during virus infection occurs in local draining lymph nodes, where dendritic cells present viral antigens to CD8+ T cells [53,54]. During naïve CD8+ T cell–dendritic cell interactions, costimulatory signals delivered by molecules such as CD28 (signal two) determine whether CD8+ T cells will become activated and expand, or they will be suboptimally activated. Studies examining primary infection of mice with viruses such as VSV [55], MHV-68 [56] and influenza type A virus [57,58], indicated that CD28 was required for primary expansion of antiviral CD8+ T cells. In one of the earliest CTLA4-Ig blocking studies, using influenza virus, Lumsden et al. identified that the loss of CD28 signaling negatively impacted both CD4+ and CD8+ T cells [59]. In this study, there was a significant decrease in the production of antiviral antibodies, decreased expansion of virus specific CTLs and a loss of IFN- γ and cytotoxic function by those cells which did expand. Ultimately these CTLA4-Ig blocked mice resolved the infection, yet it was delayed in comparison to controls. In a complimentary study by Bertram et al., influenza virus infected CD28 $^{-/-}$ mice exhibited substantially decreased expansion of virus specific CD8+ T cells at the peak of the primary response whether virus was delivered intraperitoneally or intranasally [57]. Halstead et al. also showed that both dominant and subdominant primary CD8+ T cell responses against influenza virus are greatly reduced in CD28 knockout mice [58]. In contrast, studies using lymphochoriomeningitis virus (LCMV) infection of mice, initially showed that an efficient primary CD8+ T cell response could be generated in the absence of CD28 costimulation. CD28 knockout mice (CD28 $^{-/-}$) were infected with LCMV, and despite the absence of CD28 signaling, virus CD8+ T cells expanded and viral burden was eliminated at levels comparable to wild type controls. Similar CD8+ T cell expansion was observed against all measured epitopes of LCMV, including subdominant epitopes [60,61]. The reason for this discrepancy in the lack of requirement for a CD28 mediated signal in LCMV infection became apparent from studies that showed that if sufficiently high levels of TCR stimulation were obtained, the need for costimulation could be overcome [62,63]. Viola and Lanzavecchia elegantly showed in *in vitro* studies, that independent of the nature of the TCR stimuli, TCR stimulation must exceed a minimum

threshold in order to achieve complete activation of a T cell clone. However, in the presence of CD28 costimulation, that threshold is significantly lowered [63]. Kundig et al. utilized LCMV infection and showed that the disparity in requirement for CD28 in primary LCMV infection versus VSV infection was due to differences in TCR signal duration [62]. Indeed, of all the viruses examined, LCMV is the only virus whose natural host is the mouse and therefore it replicates much more rapidly and extensively than any of the other viruses examined. As a result, antigen presentation persists for a longer period of time and at higher levels, providing a strong and sustained TCR signal which overcomes the need for CD28 costimulation [62].

When viral infection is cleared the effector T cell population undergoes a steady contraction until a small stable memory pool is formed. Memory cells respond faster and more effectively in the event of secondary insult to the host [64]. One of the major contributing factors to the rapidity of memory T cell responses is their potentially higher affinity for antigen that leads to a lower threshold of activation [65,66]. Given that the strength of TCR signaling and predetermined threshold of activation can affect the need for costimulation, it is reasonable to question whether or not memory T cells have a requirement for CD28 costimulation during re-activation.

4. The role of CD28 costimulation in memory immune responses: early studies

CD28 signaling requirements in memory CD4⁺ and CD8⁺ T cell responses have been much less well studied than those for primary response generation. In fact the widely held belief that memory responses are CD28 costimulation independent, is based on very few studies which predominantly utilized *in vitro* systems of restimulation [67,68] or CD28 deficient mice [69,61]. These studies used a variety of different priming and re-challenge systems but the majority shared the common finding that both CD4⁺ and CD8⁺ memory T cell responses were independent of CD28 costimulation [67–70].

Some of the early information on the role of CD28 signaling in memory CD8⁺ T cell function, *in vivo*, was gained in studies which addressed the question whether memory CD8⁺ T cells can exist outside the lymphoid organs [69]. Here, OVA-specific OT-I TCR transgenic CD8⁺ T cells were transferred to wild type C57Bl/6 mice which were CD28 sufficient. Recipient mice were then infected with VSV-OVA virus and orally re-challenged by OVA feeding in the presence or absence of CTLA4-Ig. Examination of memory CD8⁺ T cells after oral re-challenge indicated that memory CD8⁺ T cells functioned independently of CD28 costimulation. This conclusion was reached based on the findings that both the degree of blastogenesis (size increase), and *ex vivo* cytolytic function were comparable in CTLA4-Ig treated and control mice. These findings are in agreement with previously published *in vitro* studies [67,70,68,71]. Although this study concluded that memory CD8⁺ T cell responses occurred independently of CD28 signaling, this was not proven in a comprehensive way. The conclusions were based on basic blastogenesis analysis of the antigen specific population. As we shall see below, the size of memory CD8⁺ T cells is not affected when cells fail to receive CD28 costimulation, because of an accompanying cell cycle arrest [72]. The *ex vivo* cytolytic assay on which the conclusion was also based, had the number of OT-I cells adjusted [69]. Therefore it did not demonstrate that there were fewer expanded secondary OT-I cells, only that on a per cell basis, killing is not affected by blocking CD28 costimulation. Additionally, this study and the others discussed, failed to examine the requirement for CD28 signaling in an *in vivo* secondary infection which would have provided the full array of costimulation [67–70].

As we have seen in the primary response, the requirement for CD28 signaling in the activation of CD8⁺ T cells can be largely impacted by the type of viral system studied. Similar to the primary CD8⁺ T cell response, memory LCMV specific CD8⁺ T cell responses in CD28 deficient mice also seem to be re-activated independently of CD28 costimulation [61]. This report by Suresh et al. [61] showed that, consistent with previous studies [60] in LCMV infected CD28^{-/-} mice, the primary CD8⁺ T cell responses exhibited a strong activation profile and primary expansion. Although the CD28^{-/-} population was reduced in size in comparison to controls, it still represented a sizeable population and exhibited cytotoxic functions. The LCMV specific CD8⁺ T cell memory population was examined more than 250 days after primary infection, and observed to be only slightly decreased in size relative to wild type controls. However, when wild type or CD28^{-/-} memory mice were re-challenged with a lethal dose of LCMV, all mice survived infection while all naïve controls in the study died. One interpretation of this data is that, during LCMV infection, memory CD8⁺ T cells, just like naïve CD8⁺ T cells, are capable of functioning independently of CD28 costimulation.

The studies discussed above presented convincing evidence indicating that CD28 costimulation is dispensable for CD4⁺ and CD8⁺ T cell memory responses, and in fact have constituted a generally accepted paradigm in immunology. However we should stress that all of these previously discussed studies have examined CD28 costimulation requirements under conditions where the T cell stimulus was not equivalent to the stimulus received under physiological conditions. For example, in the *in vitro* studies [67,68,63], the requirement for costimulation may have been overcome due to the strength of TCR signaling. In the previously described studies peptide was exogenously loaded onto cultured antigen presenting cells, effectively overriding normal antigen processing and presentation MHC class I pathway. In the report by Suresh et al. [61] using LCMV infection, the data require more careful examination since they utilized the most physiologically relevant antigen presentation system and functional readout of memory. While these studies indicate that memory CD8⁺ T cells re-activate independently of CD28 costimulation, we stress several caveats which may alter the interpretation of the data. First, the CD8⁺ T cell memory is generated in CD28^{-/-} mice. Costimulatory molecules such as CD28 have been reported to affect positive selection of T cells [73–76]. While CD28 knockout mice appear to have normal peripheral populations of CD4⁺ and CD8⁺ T cells [60,77], with the exception of regulatory T cells [78,79] we cannot exclude the possibility that the absence of CD28 during thymic development has negatively impacted the T cell function. The second important point is that, as we discussed above, during the primary response the requirement for CD28 costimulation can be impacted by both the duration and strength of TCR stimulation [62,63] and therefore, since LCMV infection circumvents the need for costimulation in the primary immune response, it is reasonable to believe that it may also do that in the secondary immune response. Finally, another piece of evidence that has to be discussed is that the presence of dendritic cells has been shown to be required for secondary immune responses to VSV and LCMV [80,81]. Depletion of CD11c⁺ expressing cells, including dendritic cells, was found to reduce the expansion of CD62L^{low} memory VSV-specific CD8⁺ T cell population about 60–80%, whereas the expansion of CD62L^{hi} memory CD8⁺ T cell population was reduced by 90%, suggesting that central memory CD8⁺ T cells are more dependent on the presence of dendritic cells for reactivation upon re-challenge [81]. In an experimental system employing bone-marrow chimeras, lack of bone-marrow-derived cells capable of presenting antigen (such as dendritic cells) reduced the expansion of memory LCMV-specific CD8⁺ T cells in the spleen (central memory) by more than 85% and of memory CD8⁺ T cells in the bronchiolar lavage by more than 55% [80]. Since dendritic cells provide efficient costimulation to CD8⁺ T cells during immune responses, this requirement for dendritic cells during secondary responses raised the question whether CD8⁺ T cell memory responses can indeed occur independently of CD28 costimulation.

5. The role of CD28 costimulation in memory immune responses: recent *in vivo* studies

Recent studies have challenged the long standing notion that CD28 costimulation is not required by memory T cells to mount an optimal secondary T cell response. A number of publications have demonstrated that CD28 is critical for secondary T cell responses [72,82,83]. To avoid generating virus specific memory cells in CD28 deficient mice, in the most current studies, including our own, memory CD4+ or CD8+ T cells, were generated in intact mice by acute *in vivo* viral infections. Following the development of intact primary T cell responses, the requirement of the resultant memory population for CD28 costimulation during a secondary response was examined in different experimental setups that used treatment with either CTLA4-Ig, anti-B7 or anti-CD28 monoclonal antibodies or transfer of memory cells to CD80/CD86 double deficient mice (B7.1 and B7.2 knockouts) [72,82,83].

In our studies, virus specific memory CD8+ T cells were generated through primary infection of C57Bl/6 mice with influenza type A virus or with herpes simplex virus (HSV-1) [72]. This allowed the primary immune response and the resulting memory population to develop in conditions of unimpaired costimulation. Since memory was generated in wild type C57Bl/6 mice, CD28 costimulation blockade was achieved by treating these mice with a non-depleting, blocking anti-CD28 monoclonal antibody [72,84] or with an isotype control antibody, only during secondary infection. Alternatively, influenza virus-specific memory CD8+ T cells generated in wild type C57Bl/6 mice were adoptively transferred into CD80/CD86 double deficient mice which were then challenged with influenza virus. The choice of using CD80/CD86 double deficient mice as hosts for virus specific CD8+ T memory cells had the additional advantage of excluding an enhanced inhibitory CTLA4 signaling, due to increased ligand availability when CD28 was blocked by anti-CD28 mAb treatment. This experimental design allowed us to overcome some of the confounding factors present in previously published studies and to focus on the role of CD28 costimulation exclusively during *in vivo* memory T cell reactivation. In each strategy that we employed, we measured the re-expansion of virus specific CD8+ T cells, the functional properties of those cells and the resultant viral loads. When CD28 costimulation was blocked, we observed a significant reduction in the re-expansion of memory CD8+ T cells and in the magnitude of the secondary immune response generated against influenza or HSV. At the peak of the secondary response of influenza challenged mice, we observed a three fold reduction in the absolute number of pulmonary virus-specific CD8+ T cells in anti-CD28 treated mice, when compared to untreated or isotype control treated mice, and a nine fold reduction when memory cells were transferred into CD80/CD86 deficient mice [72]. In addition to absolute numbers, a significant reduction in the cytolytic function was observed. To ensure that we were not merely observing a delay in kinetics of expansion in anti-CD28 treated mice, the virus specific CD8+ T cell population was measured at days 3, 5, 6, 7, 10 and 60 post-secondary challenge [72]. Interestingly, despite the reduction in secondary expansion, the resting secondary memory population on day 60 in anti-CD28 treated mice was equivalent to isotype control treated animals. Future studies will be required to determine whether or not the quality of the secondary memory population is affected in the absence of CD28 costimulation. The requirement for CD28 costimulation of CD8+ T cell memory was not limited to influenza viral infection alone. When HSV-1 specific memory CD8+ T cells were transferred to CD80/CD86 deficient mice and mice were challenged with HSV-1 in the foot pad, a significant 5 fold reduction in the absolute number of virus-specific CD8+ T cells found in the local draining lymph node was observed, in comparison to controls [72]. Beyond reduced virus specific CD8+ T expansion and function, we also observed a concurrent decrease in viral clearance. In influenza and in HSV infected mice that were treated with anti-CD28 blocking antibody, we determined significantly increased and

sustained peak viral loads when compared to their control or isotype treated counterparts. The finding of increased viral load reminds us of the critical role that CD8⁺ T cells play in the elimination of viral infection, and thus the significance of developing efficient CD8⁺ T cell secondary responses.

Further supporting evidence for the role of costimulation for optimal recall CD8⁺ T cell responses was recently presented by Fuse et al. [82]. The authors examined the role of the CD28 costimulation in the differentiation of memory CD8⁺ T cells by infecting C57Bl/6 or CD28 deficient mice with vaccinia virus and found that at the peak of the primary immune response (10 days post-infection) the numbers of virus-specific CD8⁺ T cells was reduced in the lungs and spleens of CD28 deficient mice compared to C57Bl/6 controls. However, 66 days post-infection, the numbers of virus-specific memory CD8⁺ T cells in the lungs or spleens of CD28 deficient mice were not different from the numbers of virus specific memory CD8⁺ T cells in the lungs or spleens of control C57Bl/6 mice. Although CD28 seemed not to play a role in generation of the memory pool, in fact the virus-specific memory cells generated in CD28 deficient mice presented an altered phenotype (lower levels of CD122 and CD27) and produced less IL-2 upon restimulation with peptide. In order to examine the correlation between CD28 costimulation and expansion of the virus-specific memory CD8⁺ T cell population upon re-challenge with virus, purified memory CD8⁺ T cells from either C57Bl/6 or CD28 deficient mice were transferred into naïve congenic recipients that were then infected with virus. Interestingly, virus-specific memory CD8⁺ T cells that were generated in the absence of CD28 costimulation expanded approximately 9 times, whereas virus-specific memory CD8⁺ T cells that were generated in C57Bl/6 mice expanded more than 40 times. These findings were further substantiated when virus-specific CD8⁺ memory T cells were generated in C57Bl/6 mice and transferred into CD80/CD86 deficient mice or wild-type control mice, challenged and analyzed 5 days later for expansion of the virus-specific memory population. Similar to results published by our group [72], in the work published by Fuse et al., the expansion of memory CD8⁺ T cells upon re-challenge was reduced in CD80/CD86 mice the absence of CD28 costimulation compared to wild-type mice, thus demonstrating that CD28 costimulation maximizes the secondary immune responses [82]. Moreover, Fuse and collaborators also demonstrated that CD28 costimulation is required for secondary immune responses during persistent low level infections such as murine gammaherpesvirus 68.

To examine the mechanism behind this loss of expansion of memory CD8⁺ T cells when CD28 costimulation was absent, we examined cellular markers of proliferation and apoptosis. In agreement with previous reports [85,86], we found Bcl-xL to be significantly decreased in CD28 blocked memory CD8⁺ T cells compared to controls. Surprisingly, a second anti-apoptotic molecule, Bcl-2, which is rapidly downregulated in activated naïve CD8⁺ T cells [87] fails to downregulate in CD28 blocked CD8⁺ memory T cells when compared to controls. In fact, during a normal activation cycle of a cell, this molecule is downregulated and the cell proceeds into cell cycle [87]. However, if Bcl-2 fails to downregulate, cell cycle is arrested and the cell fails to proliferate [88–91]. Indeed, in our experiments, cell cycle analysis of memory virus-specific CD8⁺ T cells in challenged CD80/CD86 deficient mice showed that these cells are selectively arrested in the G1/S phase of the cell cycle. Blastogenesis of these cells was not affected, and this is in agreement with earlier studies [69]. Although these findings do not illustrate a direct interaction, our data do suggest a previously unappreciated relationship between signaling through CD28 and downregulation of Bcl-2.

Similar to *in vitro* studies with memory CD8⁺ T cells, the memory CD4⁺ T cell population, *in vitro*, has also been suggested to function in a CD28 independent manner [70,71]. However more recently, Ndejemi et al. have provided *in vivo* data which challenges this

position [83]. Using influenza virus-specific memory CD4⁺ T cells, they showed that reactivation of memory requires CD28 costimulation. Following *in vitro* generation of transgenic memory CD4⁺ T cell or *in vivo* generation of polyclonal influenza specific memory CD4⁺ T cells, the capacity for reactivation was examined. *Ex vivo* restimulation of memory cells, in the presence or absence of CTLA4-Ig, led to no discernable differences in the amount of IFN- γ produced, yet IL-2 production by CTLA4-Ig treated memory CD4⁺ T cells was significantly decreased [83]. Most importantly, studies examining *in vivo* the reactivation of memory CD4⁺ T cells showed that the peak of the secondary immune response is significantly decreased when recipient mice were treated with CTLA-4 Ig during influenza virus re-challenge [83]. Taken together, this data strongly indicates that *in vivo*, during acute viral infection, CD28 costimulation is also required for secondary expansion and cytokine production by antigen specific memory CD4⁺ T cells.

The above carefully designed and controlled experiments that used costimulation competent animals for priming of the immune response and impaired costimulation exclusively during secondary immune responses, challenge the paradigm that memory immune responses occur independently of costimulatory signals. CD28 signaling during primary response may be affecting the quality of memory CD8⁺ T cells generated [82] while the expansion of memory T cells clearly requires CD28 costimulation for optimal secondary responses and pathogen clearance [72] (illustrated in Fig. 1). This CD28 signaling requirement for memory T cells raises important questions about their function in the induction of protective immune responses: if a pathogen or tumor downregulates costimulatory signals, then infection of a vaccinated host would result in blunted T cell responses and lack of protection.

6. Implications for memory immune responses to pathogens and tumors

The finding that CD28 costimulation is critical for secondary T cell responses has important implications for immunity against pathogens and tumors. T cell responses directly, but also secondary B cell responses indirectly, may ultimately be affected by the absence or by reduced CD28 costimulation of memory T cells. One of the mechanisms used by viruses, such as measles, Varicellazoster, and HIV-1, to evade immune responses, is the suppression of DC maturation and inhibition of CD28 costimulatory ligands, B7-1 and B7-2 upregulation [92–95] (illustrated in Fig. 2). Infection with these viruses may lead to reduced quality of memory T cells generated, and to ineffective secondary responses upon re-exposure to the pathogens (illustrated in Fig. 2). Furthermore, as these viruses inhibit expression of costimulatory molecules, vaccines that are designed to target such pathogens by eliciting T cell memory, may not be effective. Based on the previously presented studies [72,82,83], one could even predict that the absence of costimulation during reactivation of memory may even tolerize the responding memory T cell.

Similar to viruses, T cell immunity is important for tumor immune surveillance. Tumors utilize different mechanisms to evade CD8⁺ T cell responses, such as: the release of anti-inflammatory cytokines, exhaustion of tumor associated antigen specific CD8⁺ T cells by chronic stimulation, inhibition of costimulatory molecules that activate the immune response or upregulation of molecules with inhibitory function [96]. Specifically, expression of the PD-1 ligand B7-H1, have been found to be increased in a wide variety tumor cell lines derived from cancerous tissues, ranging from ovarian cancer to lung cancer, but not on normal tissues [38]. Also many studies suggested that DCs may be retained in an immature state, thus not express enough CD80 and CD86 to stimulate the CD28 receptor on intra-tumor T cells [97,98] (illustrated in Fig. 2). Furthermore, animal studies have demonstrated that induced expression of B7-1 and B7-2 on carcinoma tumor cells rendered tumors susceptible to rejection [99]. The same approach was used to show that expression of B7 h also provided costimulation and promoted the rejection of fibrosarcomas and

plasmacytomas by CD8⁺ T cells [100,101]. However, another set of studies showed that when tumors were poorly immunogenic, induced expression of B7-1 or B7-2 alone was not sufficient to augment the immune response against the tumors [102]. B7-1-transfected autologous tumor cells were tested in phase I trials of metastatic renal cell carcinoma [103], adenocarcinoma [104] and acute myeloid leukemia [105]. In some patients, tumor vaccination stabilized the disease or considerably reduced the metastatic lesions. In designing efficient tumor vaccines, one has to keep in mind that overexpression of B7 molecules on tumor cells may promote more interactions with CTLA-4 which has a higher affinity for B7 than CD28, and therefore inhibition rather than activation of T cells may result. The recent data on the effect the lack of costimulation during primary immune responses has on the quality of memory T cells, may also explain why providing costimulation fails in patients with preexisting tumor loads.

Specific blockade of CTLA-4 signals, while leaving TCR and CD28 signals intact, is a very attractive approach for tumor immunotherapy. In animal studies, administration of antibodies that block CTLA-4 interactions with B7-1 and B7-2 resulted in the rejection of colon carcinoma and fibrosarcoma, including preestablished tumors [106]. Furthermore, this rejection was accompanied by a long-lasting immunity to a secondary exposure to tumor cells in the absence of additional treatment [106]. This work proved in principle that CTLA-4 blockade could enhance tumor-elicited weak immune responses to a level that could mediate tumor rejection. Subsequent work showed that anti-CTLA-4 monotherapy could also promote the rejection of other transplantable tumors, including prostatic carcinoma, lymphoma, renal cell carcinoma, and colon carcinoma [107]. However, this approach does not seem to be effective for poorly immunogenic tumors, indicating that the threshold of TCR activation is a critical parameter for immunotherapy to be effective. Recurrent tumors following therapy would entail memory tumor-specific T cells being reactivated. In this case, immature dendritic cells would fail to induce-maximal T cell responses against tumors. Although the role of CD28 costimulation in secondary T cell responses against tumors is currently speculative, its potential is more obvious in the case where tumor specific vaccines are employed. In this scenario, the lack of CD80/CD86 expressing intra-tumor dendritic cells, would affect the T cell recall response against the tumor and reduce the efficiency of potential vaccines.

7. The CD28 requirement of memory T cell and ageing

The decreased capacity of elderly to mount efficient primary immune responses has been extensively documented [108–110]. However, little is known about the impact of ageing on established memory T cell pools. Animal studies on memory CD8⁺ T cell response to respiratory virus infections in aged mice revealed that there is a significant reduction of effector memory cells over time, which may reduce the immediate response of memory T cells to secondary challenge [111]. In humans, reduced antibody recall responses to influenza virus have been demonstrated in vaccinated elderly [112,113]. One potential reason for decreasing efficacy of the vaccination is accumulation of senescent CD28^{null} T cells [114]. Increased numbers of CD28^{null} T cells inversely correlate with the number of functionally active (granzyme positive) CD8⁺ central memory T cells in vaccinated aged individuals [115]. This T cell senescence may affect recall responses to pathogens after vaccination.

More relevant to our discussion here is the potential dysfunction of DC with ageing. T regulatory cells (Tregs) have been described to downregulate costimulatory molecules such as CD40 and CD86 on the surface of dendritic cells [116,117]. The accumulation in the lymphoid organs of old mice of Tregs expressing an effector/memory phenotype, induces the downregulation of expression of CD40 and CD86 costimulatory molecules by dendritic

cells, and this can be another mechanism of reducing immune responses during ageing [118]. It has been recently reported that bone-marrow-derived dendritic cells from aged mice are less effective than their young counterparts in inducing the regression of B16-ovalbumin melanomas [119]. Aged DC had an impaired ability to stimulate CD8⁺ T-cell proliferation and migrate to the lymph nodes and when tested *in vivo*, DC from aged animals did not induce tumor reduction [119]. Overall, these studies suggest that during ageing there is a decrease in costimulatory signaling by DC. Since memory T cells require CD28 costimulation such an impairment of DC in ageing would affect recall responses and reduce the efficacy of vaccinations as well as immune responses to tumors.

8. Implication of costimulation blockade in autoimmune diseases and transplantation

In contrast to vaccine development or tumor therapeutic, where the enhancement of CD28 costimulation or blockade of co-inhibitory molecules could prove beneficial to the host, in autoimmunity and transplantation the presence of costimulation may be responsible for inducing undesired immune responses. Therefore, in both autoimmune disease and transplantation, the goal is to dampen reactive immune responses against either self- or allograft tissue, respectively. Some successes in autoimmune disease have been observed with treatment using CTLA4-Ig which binds B7-1/B7-2 and prevents CD28 costimulation (illustrated in Fig. 2). Studies in which patients with *Psoriasis vulgaris* [120] and rheumatoid arthritis [121] were treated with the soluble fusion protein CTLA4-Ig, showed evidence of efficacy. Blocking costimulation has also been employed in transplantation. Organ rejection after transplantation is mediated by activation of host immune cells against grafted tissues. Inhibition of costimulation using CTLA4-Ig (Abatacept) [122,123] or a variant of it with higher affinity for CD80/CD86 (Belatacept) [124] has demonstrated some efficiency in prolonging transplant rejection [125]. However, blocking B7-1 and B7-2 with CTLA4-Ig may impair CD28 signaling, which we believe is essential for the reactivation of memory T cells [72,82,83], leaving open the possibility for more infection or vaccine induced memory responses failure. Patients undergoing transplantation are also treated with immunosuppressants and their immune system is overall suppressed. Therefore, it would be hard to discern whether blocking costimulation affects memory immune responses in immunosuppressed patients that become infected with pathogens. In the light of the latest findings [72,82,83], recall responses to pathogens would be diminished in the absence of costimulation, and this potential side effect we believe deserves further investigation in autoimmunity and transplantation costimulation blockade therapies.

The observation that memory T cell responses require CD28 costimulation to elicit optimal secondary responses raises important questions on how one can circumvent this potential problem. Some studies have suggested that TNF family members can replace CD28 costimulation in primary responses [126,58,127] and this may be also true for memory T cell responses. Indeed, CD137 (4-1BB) has been proposed to replace CD28 signaling [128] and other members of the TNF receptor superfamily (CD27, OX40) have already been described to play a role in the generation of T cells memory and in the secondary immune responses [126,129,130]. Furthermore it may be possible to devise strategies that elicit CD28 independent memory T cells. Understanding the requirement of CD28 signaling and devising strategies to overcome this problem *in vivo*, may lead to enhanced immunity against immune evading pathogens and tumors as well as to increased efficacy of vaccines in the elderly.

Acknowledgments

This work was supported by NIH R01 Grant AI 66215 to P.D.K.

References

1. Bretscher P, Cohn M. A theory of self-nonsel self discrimination. *Science* 1970;169:1042–9. [PubMed: 4194660]
2. Glick B, Whatley S. The presence of immunoglobulin in the bursa of Fabricius. *Poult Sci* 1967;46:1587–9. [PubMed: 4170311]
3. Mitchell GF, Miller JF. Immunological activity of thymus and thoracic-duct lymphocytes. *Proc Natl Acad Sci U S A* 1968;59:296–303. [PubMed: 4873344]
4. Warner NL, Szenberg A. Effect of neonatal thymectomy on the immune response in the chicken. *Nature* 1962;196:784–5. [PubMed: 13998954]
5. Lafferty KJ, Misko IS, Cooley MA. Allogeneic stimulation modulates the in vitro response of T cells to transplantation antigen. *Nature* 1974;249:275–6. [PubMed: 4275319]
6. Lafferty KJ, Jones MA. Reactions of the graft versus host (GVH) type. *Aust J Exp Biol Med Sci* 1969;47:17–54. [PubMed: 4890818]
7. Lafferty KJ, Cunningham AJ. A new analysis of allogeneic interactions. *Aust J Exp Biol Med Sci* 1975;53:27–42. [PubMed: 238498]
8. Zinkernagel RM, Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* 1974;248:701–2. [PubMed: 4133807]
9. Cunningham AJ, Lafferty KJ. A simple conservative explanation of the H-2 restriction of interactions between lymphocytes. *Scand J Immunol* 1977;6:1–6.
10. Lafferty KJ, Andrus L, Prowse SJ. Role of lymphokine and antigen in the control of specific T cell responses. *Immunol Rev* 1980;51:279–314. [PubMed: 7000674]
11. Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med* 1987;165:302–19. [PubMed: 3029267]
12. Jenkins MK, Pardoll DM, Mizuguchi J, Quill H, Schwartz RH. T-cell unresponsiveness in vivo and in vitro: fine specificity of induction and molecular characterization of the unresponsive state. *Immunol Rev* 1987;95:113–35. [PubMed: 2437012]
13. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994;12:991–1045. [PubMed: 8011301]
14. Aruffo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. *Proc Natl Acad Sci U S A* 1987;84:8573–7. [PubMed: 2825196]
15. Gmunder H, Lesslauer W. A 45-kDa human T-cell membrane glycoprotein functions in the regulation of cell proliferative responses. *Eur J Biochem* 1984;142:153–60. [PubMed: 6235110]
16. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002;2:116–26. [PubMed: 11910893]
17. Sedy JR, Gavrieli M, Potter KG, Hurchla MA, Lindsley RC, Hildner K, et al. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat Immunol* 2005;6:90–8. [PubMed: 15568026]
18. Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. *Annu Rev Immunol* 2001;19:225–52. [PubMed: 11244036]
19. Wang S, Zhu G, Chapoval AI, Dong H, Tamada K, Ni J, et al. Costimulation of T cells by B7-H2, a B7-like molecule that binds ICOS. *Blood* 2000;96:2808–13. [PubMed: 11023515]
20. Yoshinaga SK, Whoriskey JS, Khare SD, Sarmiento U, Guo J, Horan T, et al. T-cell co-stimulation through B7RP-1 and ICOS. *Nature* 1999;402:827–32. [PubMed: 10617205]
21. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027–34. [PubMed: 11015443]
22. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001;2:261–8. [PubMed: 11224527]

23. Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, et al. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. *Nat Immunol* 2001;2:269–74. [PubMed: 11224528]
24. Prasad DV, Richards S, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. *Immunity* 2003;18:863–73. [PubMed: 12818166]
25. Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, et al. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity* 2003;18:849–61. [PubMed: 12818165]
26. Gruss HJ, Dower SK. Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. *Blood* 1995;85:3378–404. [PubMed: 7780126]
27. Armitage RJ. Tumor necrosis factor receptor superfamily members and their ligands. *Curr Opin Immunol* 1994;6:407–13. [PubMed: 7917108]
28. Gaur U, Aggarwal BB. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochem Pharmacol* 2003;66:1403–8. [PubMed: 14555214]
29. Gravestein LA, Borst J. Tumor necrosis factor receptor family members in the immune system. *Semin Immunol* 1998;10:423–34. [PubMed: 9826575]
30. Rudd CE, Schneider H. Unifying concepts in CD28, ICOS and CTLA4 co-receptor signalling. *Nat Rev Immunol* 2003;3:544–56. [PubMed: 12876557]
31. Linsley PS, Greene JL, Tan P, Bradshaw J, Ledbetter JA, Anasetti C, et al. Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. *J Exp Med* 1992;176:1595–604. [PubMed: 1334116]
32. Stamper CC, Zhang Y, Tobin JF, Erbe DV, Ikemizu S, Davis SJ, et al. Crystal structure of the B7-1/CTLA-4 complex that inhibits human immune responses. *Nature* 2001;410:608–11. [PubMed: 11279502]
33. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994;1:405–13. [PubMed: 7882171]
34. Martin M, Schneider H, Azouz A, Rudd CE. Cytotoxic T lymphocyte antigen 4 and CD28 modulate cell surface raft expression in their regulation of T cell function. *J Exp Med* 2001;194:1675–81. [PubMed: 11733581]
35. Walunas TL, Bakker CY, Bluestone JA. CTLA-4 ligation blocks CD28-dependent T cell activation. *J Exp Med* 1996;183:2541–50. [PubMed: 8676075]
36. Ling V, Wu PW, Finnerty HF, Bean KM, Spaulding V, Fouser LA, et al. Cutting edge: identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor. *J Immunol* 2000;164:1653–7. [PubMed: 10657606]
37. Dong C, Temann UA, Flavell RA. Cutting edge: critical role of inducible costimulator in germinal center reactions. *J Immunol* 2001;166:3659–62. [PubMed: 11238604]
38. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002;8:793–800. [PubMed: 12091876]
39. Hutloff A, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, Anagnostopoulos I, et al. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999;397:263–6. [PubMed: 9930702]
40. McAdam AJ, Greenwald RJ, Levin MA, Chernova T, Malenkovich N, Ling V, et al. ICOS is critical for CD40-mediated antibody class switching. *Nature* 2001;409:102–5. [PubMed: 11343122]
41. Bertram EM, Tafuri A, Shahinian A, Chan VS, Hunziker L, Recher M, et al. Role of ICOS versus CD28 in antiviral immunity. *Eur J Immunol* 2002;32:3376–85. [PubMed: 12432568]
42. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat Rev Immunol* 2004;4:336–47. [PubMed: 15122199]
43. Keir ME, Francisco LM, Sharpe AH. PD-1 and its ligands in T-cell immunity. *Curr Opin Immunol* 2007;19:309–14. [PubMed: 17433872]
44. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;439:682–7. [PubMed: 16382236]

45. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 2006;443:350–4. [PubMed: 16921384]
46. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med* 2006;203:2281–92. [PubMed: 16954372]
47. Petrovas C, Price DA, Mattapallil J, Ambrozak DR, Geldmacher C, Cecchinato V, et al. SIV-specific CD8+ T cells express high levels of PD1 and cytokines but have impaired proliferative capacity in acute and chronic SIVmac251 infection. *Blood* 2007;110:928–36. [PubMed: 17440051]
48. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med* 2006;12:1198–202. [PubMed: 16917489]
49. Zeng C, Wu T, Zhen Y, Xia XP, Zhao Y. BTLA, a new inhibitory B7 family receptor with a TNFR family ligand. *Cell Mol Immunol* 2005;2:427–32. [PubMed: 16426492]
50. Han P, Goularte OD, Rufner K, Wilkinson B, Kaye J. An inhibitory Ig superfamily protein expressed by lymphocytes and APCs is also an early marker of thymocyte positive selection. *J Immunol* 2004;172:5931–9. [PubMed: 15128774]
51. Krieg C, Han P, Stone R, Goularte OD, Kaye J. Functional analysis of B and T lymphocyte attenuator engagement on CD4+ and CD8+ T cells. *J Immunol* 2005;175:6420–7. [PubMed: 16272294]
52. Krieg C, Boyman O, Fu YX, Kaye J. B and T lymphocyte attenuator regulates CD8+ T cell-intrinsic homeostasis and memory cell generation. *Nat Immunol* 2007;8:162–71. [PubMed: 17206146]
53. Belz GT, Smith CM, Kleinert L, Reading P, Brooks A, Shortman K, et al. Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus. *Proc Natl Acad Sci U S A* 2004;101:8670–5. [PubMed: 15163797]
54. Legge KL, Braciale TJ. Accelerated migration of respiratory dendritic cells to the regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity* 2003;18:265–77. [PubMed: 12594953]
55. McAdam AJ, Farkash EA, Gewurz BE, Sharpe AH. B7 costimulation is critical for antibody class switching and CD8(+) cytotoxic T-lymphocyte generation in the host response to vesicular stomatitis virus. *J Virol* 2000;74:203–8. [PubMed: 10590107]
56. Fuse S, Obar JJ, Bellfy S, Leung EK, Zhang W, Usherwood EJ. CD80 and CD86 control antiviral CD8+ T-cell function and immune surveillance of murine gammaherpesvirus 68. *J Virol* 2006;80:9159–70. [PubMed: 16940527]
57. Bertram EM, Lau P, Watts TH. Temporal segregation of 4-1BB versus CD28-mediated costimulation: 4-1BB ligand influences T cell numbers late in the primary response and regulates the size of the T cell memory response following influenza infection. *J Immunol* 2002;168:3777–85. [PubMed: 11937529]
58. Halstead ES, Mueller YM, Altman JD, Katsikis PD. In vivo stimulation of CD137 broadens primary antiviral CD8+ T cell responses. *Nat Immunol* 2002;3:536–41. [PubMed: 12021777]
59. Lumsden JM, Roberts JM, Harris NL, Peach RJ, Ronchese F. Differential requirement for CD80 and CD80/CD86-dependent costimulation in the lung immune response to an influenza virus infection. *J Immunol* 2000;164:79–85. [PubMed: 10604996]
60. Shahinian A, Pfeffer K, Lee KP, Kundig TM, Kishihara K, Wakeham A, et al. Differential T cell costimulatory requirements in CD28-deficient mice. *Science* 1993;261:609–12. [PubMed: 7688139]
61. Suresh M, Whitmire JK, Harrington LE, Larsen CP, Pearson TC, Altman JD, et al. Role of CD28-B7 interactions in generation and maintenance of CD8 T cell memory. *J Immunol* 2001;167:5565–73. [PubMed: 11698427]

62. Kundig TM, Shahinian A, Kawai K, Mittrucker HW, Sebzda E, Bachmann MF, et al. Duration of TCR stimulation determines costimulatory requirement of T cells. *Immunity* 1996;5:41–52. [PubMed: 8758893]
63. Viola A, Lanzavecchia A. T cell activation determined by T cell receptor number and tunable thresholds. *Science* 1996;273:104–6. [PubMed: 8658175]
64. Sprent J, Surh CD. T cell memory. *Annu Rev Immunol* 2002;20:551–79. [PubMed: 11861612]
65. Busch DH, Pamer EG. T cell affinity maturation by selective expansion during infection. *J Exp Med* 1999;189:701–10. [PubMed: 9989985]
66. Savage PA, Boniface JJ, Davis MM. A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity* 1999;10:485–92. [PubMed: 10229191]
67. Bachmann MF, Gallimore A, Linkert S, Cerundolo V, Lanzavecchia A, Kopf M, et al. Developmental regulation of Lck targeting to the CD8 coreceptor controls signaling in naive and memory T cells. *J Exp Med* 1999;189:1521–30. [PubMed: 10330431]
68. Flynn K, Mullbacher A. Memory alloreactive cytotoxic T cells do not require costimulation for activation in vitro. *Immunol Cell Biol* 1996;74:413–20. [PubMed: 8912004]
69. Kim SK, Schluns KS, Lefrancois L. Induction and visualization of mucosal memory CD8 T cells following systemic virus infection. *J Immunol* 1999;163:4125–32. [PubMed: 10510347]
70. Croft M, Bradley LM, Swain SL. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J Immunol* 1994;152:2675–85. [PubMed: 7908301]
71. London CA, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4+ T cells. *J Immunol* 2000;164:265–72. [PubMed: 10605020]
72. Borowski AB, Boesteanu AC, Mueller YM, Carafides C, Topham DJ, Altman JD, et al. Memory CD8+ T cells require CD28 costimulation. *J Immunol* 2007;179:6494–503. [PubMed: 17982038]
73. Degermann S, Surh CD, Glimcher LH, Sprent J, Lo D. B7 expression on thymic medullary epithelium correlates with epithelium-mediated deletion of V beta 5+ thymocytes. *J Immunol* 1994;152:3254–63. [PubMed: 7511640]
74. Gross JA, Callas E, Allison JP. Identification and distribution of the costimulatory receptor CD28 in the mouse. *J Immunol* 1992;149:380–8. [PubMed: 1320641]
75. Nelson AJ, Hosier S, Brady W, Linsley PS, Farr AG. Medullary thymic epithelium expresses a ligand for CTLA4 in situ and in vitro. *J Immunol* 1993;151:2453–61. [PubMed: 8395547]
76. Yang SY, Denning SM, Mizuno S, Dupont B, Haynes BF. A novel activation pathway for mature thymocytes. Costimulation of CD2 (T, p50) and CD28 (T, p44) induces autocrine interleukin 2/interleukin 2 receptor-mediated cell proliferation. *J Exp Med* 1988;168:1457–68. [PubMed: 3049912]
77. Walunas TL, Sperling AI, Khattri R, Thompson CB, Bluestone JA. CD28 expression is not essential for positive and negative selection of thymocytes or peripheral T cell tolerance. *J Immunol* 1996;156:1006–13. [PubMed: 8557973]
78. Tai X, Cowan M, Feigenbaum L, Singer A. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. *Nat Immunol* 2005;6:152–62. [PubMed: 15640801]
79. Tang Q, Henriksen KJ, Boden EK, Tooley AJ, Ye J, Subudhi SK, et al. Cutting edge: CD28 controls peripheral homeostasis of CD4+ CD25+ regulatory T cells. *J Immunol* 2003;171:3348–52. [PubMed: 14500627]
80. Belz GT, Wilson NS, Smith CM, Mount AM, Carbone FR, Heath WR. Bone marrow-derived cells expand memory CD8+ T cells in response to viral infections of the lung and skin. *Eur J Immunol* 2006;36:327–35. [PubMed: 16402408]
81. Zammit DJ, Cauley LS, Pham QM, Lefrancois L. Dendritic cells maximize the memory CD8 T cell response to infection. *Immunity* 2005;22:561–70. [PubMed: 15894274]
82. Fuse S, Zhang W, Usherwood EJ. Control of memory CD8+ T cell differentiation by CD80/CD86-CD28 costimulation and restoration by IL-2 during the recall response. *J Immunol* 2008;180:1148–57. [PubMed: 18178855]

83. Ndejemi MP, Teijaro JR, Patke DS, Bingaman AW, Chandok MR, Azimzadeh A, et al. Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. *J Immunol* 2006;177:7698–706. [PubMed: 17114440]
84. Prilliman KR, Lemmens EE, Palioungas G, Wolfe TG, Allison JP, Sharpe AH, et al. Cutting edge: a crucial role for B7-CD28 in transmitting T help from APC to CTL. *J Immunol* 2002;169:4094–7. [PubMed: 12370335]
85. Boise LH, Minn AJ, Noel PJ, June CH, Accavitti MA, Lindsten T, et al. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity* 1995;3:87–98. [PubMed: 7621080]
86. Wu LX, La Rose J, Chen L, Neale C, Mak T, Okkenhaug K, et al. CD28 regulates the translation of Bcl-xL via the phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway. *J Immunol* 2005;174:180–94. [PubMed: 15611240]
87. Grayson JM, Zajac AJ, Altman JD, Ahmed R. Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8+ T cells. *J Immunol* 2000;164:3950–4. [PubMed: 10754284]
88. Chen QM, Liu J, Merrett JB. Apoptosis or senescence-like growth arrest: influence of cell-cycle position, p53, p21 and bax in H2O2 response of normal human fibroblasts. *Biochem J* 2000;347:543–51. [PubMed: 10749685]
89. Kim R. Unknotting the roles of Bcl-2 and Bcl-xL in cell death. *Biochem Biophys Res Commun* 2005;333:336–43. [PubMed: 15922292]
90. Sasaki M, Kumazaki T, Takano H, Nishiyama M, Mitsui Y. Senescent cells are resistant to death despite low Bcl-2 level. *Mech Ageing Dev* 2001;122:1695–706. [PubMed: 11557274]
91. Schmitt CA. Senescence, apoptosis and therapy—cutting the lifelines of cancer. *Nat Rev Cancer* 2003;3:286–95. [PubMed: 12671667]
92. Chaudhry A, Das SR, Hussain A, Mayor S, George A, Bal V, et al. The Nef protein of HIV-1 induces loss of cell surface costimulatory molecules CD80 and CD86 in APCs. *J Immunol* 2005;175:4566–74. [PubMed: 16177101]
93. Majumder B, Janket ML, Schafer EA, Schaubert K, Huang XL, Kan-Mitchell J, et al. Human immunodeficiency virus type 1 Vpr impairs dendritic cell maturation and T-cell activation: implications for viral immune escape. *J Virol* 2005;79:7990–8003. [PubMed: 15956545]
94. Morrow G, Slobedman B, Cunningham AL, Abendroth A. Varicellazoster virus productively infects mature dendritic cells and alters their immune function. *J Virol* 2003;77:4950–9. [PubMed: 12663800]
95. Servet-Delprat C, Vidalain PO, Bausinger H, Manie S, Le Deist F, Azocar O, et al. Measles virus induces abnormal differentiation of CD40 ligand-activated human dendritic cells. *J Immunol* 2000;164:1753–60. [PubMed: 10657621]
96. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007;25:267–96. [PubMed: 17134371]
97. Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* 1998;92:4150–66. [PubMed: 9834220]
98. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* 1996;2:1096–103. [PubMed: 8837607]
99. Hodge JW, Abrams S, Schlom J, Kantor JA. Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules. *Cancer Res* 1994;54:5552–5. [PubMed: 7522961]
100. Liu X, Bai XF, Wen J, Gao JX, Liu J, Lu P, et al. B7H costimulates clonal expansion of, and cognate destruction of tumor cells by, CD8(+) T lymphocytes in vivo. *J Exp Med* 2001;194:1339–48. [PubMed: 11696598]
101. Wallin JJ, Liang L, Bakardjiev A, Sha WC. Enhancement of CD8+ T cell responses by ICOS/B7h costimulation. *J Immunol* 2001;167:132–9. [PubMed: 11418641]
102. Chen L, McGowan P, Ashe S, Johnston J, Li Y, Hellstrom I, et al. Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J Exp Med* 1994;179:523–32. [PubMed: 7507508]

103. Antonia SJ, Seigne J, Diaz J, Muro-Cacho C, Extermann M, Farmelo MJ, et al. Phase I trial of a B7-1 (CD80) gene modified autologous tumor cell vaccine in combination with systemic interleukin-2 in patients with metastatic renal cell carcinoma. *J Urol* 2002;167:1995–2000. [PubMed: 11956426]
104. Raez LE, Cassileth PA, Schlesselman JJ, Sridhar K, Padmanabhan S, Fisher EZ, et al. Allogeneic vaccination with a B7.1 HLA-A gene-modified adenocarcinoma cell line in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:2800–7. [PubMed: 15254047]
105. Chan L, Hardwick NR, Guinn BA, Darling D, Gaken J, Galea-Lauri J, et al. An immune edited tumour versus a tumour edited immune system: Prospects for immune therapy of acute myeloid leukaemia. *Cancer Immunol Immunother* 2006;55:1017–24. [PubMed: 16450142]
106. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734–6. [PubMed: 8596936]
107. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nat Immunol* 2002;3:611–8. [PubMed: 12087419]
108. Effros RB, Cai Z, Linton PJ. CD8 T cells and aging. *Crit Rev Immunol* 2003;23:45–64. [PubMed: 12906259]
109. Grubeck-Loebenstein B, Wick G. The aging of the immune system. *Adv Immunol* 2002;80:243–84. [PubMed: 12078483]
110. Murasko DM, Jiang J. Response of aged mice to primary virus infections. *Immunol Rev* 2005;205:285–96. [PubMed: 15882361]
111. Ely KH, Roberts AD, Kohlmeier JE, Blackman MA, Woodland DL. Aging and CD8+ T cell immunity to respiratory virus infections. *Exp Gerontol* 2007;42:427–31. [PubMed: 17197143]
112. Glezen WP, Greenberg SB, Atmar RL, Piedra PA, Couch RB. Impact of respiratory virus infections on persons with chronic underlying conditions. *Jama* 2000;283:499–505. [PubMed: 10659876]
113. Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, et al. Influenza-associated hospitalizations in the United States. *JAMA* 2004;292:1333–40. [PubMed: 15367555]
114. Effros RB, Dagarag M, Spaulding C, Man J. The role of CD8+ T-cell replicative senescence in human aging. *Immunol Rev* 2005;205:147–57. [PubMed: 15882351]
115. Xie D, McElhaney JE. Lower GrB+ CD62Lhigh CD8 TCM effector lymphocyte response to influenza virus in older adults is associated with increased CD28null CD8 T lymphocytes. *Mech Ageing Dev* 2007;128:392–400. [PubMed: 17570460]
116. Serra P, Amrani A, Yamanouchi J, Han B, Thiessen S, Utsugi T, et al. CD40 ligation releases immature dendritic cells from the control of regulatory CD4+ CD25+ T cells. *Immunity* 2003;19:877–89. [PubMed: 14670304]
117. Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT. CD4+ CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. *J Exp Med* 2003;198:889–901. [PubMed: 12975455]
118. Chiu BC, Stolberg VR, Zhang H, Chensue SW. Increased Foxp3(+) Treg cell activity reduces dendritic cell co-stimulatory molecule expression in aged mice. *Mech Ageing Dev* 2007;128:618–27. [PubMed: 17961632]
119. Grolleau-Julius A, Harning EK, Abernathy LM, Yung RL. Impaired dendritic cell function in aging leads to defective antitumor immunity. *Cancer Res* 2008;68:6341–9. [PubMed: 18676859]
120. Abrams JR, Lebwohl MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, et al. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J Clin Invest* 1999;103:1243–52. [PubMed: 10225967]
121. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med* 2003;349:1907–15. [PubMed: 14614165]
122. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A* 1997;94:8789–94. [PubMed: 9238056]

123. Levisetti MG, Padrid PA, Szot GL, Mittal N, Meehan SM, Wardrip CL, et al. Immunosuppressive effects of human CTLA4Ig in a non-human primate model of allogeneic pancreatic islet transplantation. *J Immunol* 1997;159:5187–91. [PubMed: 9548454]
124. Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobertm E, et al. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005;5:443–53. [PubMed: 15707398]
125. Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blanche G, et al. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005;353:770–81. [PubMed: 16120857]
126. Gramaglia I, Jember A, Pippig SD, Weinberg AD, Killeen N, Croft M. The OX40 costimulatory receptor determines the development of CD4 memory by regulating primary clonal expansion. *J Immunol* 2000;165:3043–50. [PubMed: 10975814]
127. Hendriks J, Gravestein LA, Tesselaar K, van Lier RA, Schumacher TN, Borst J. CD27 is required for generation and long-term maintenance of T cell immunity. *Nat Immunol* 2000;1:433–40. [PubMed: 11062504]
128. Bertram EM, Dawicki W, Sedgmen B, Bramson JL, Lynch DH, Watts TH. A switch in costimulation from CD28 to 4-1BB during primary versus secondary CD8 T cell response to influenza in vivo. *J Immunol* 2004;172:981–8. [PubMed: 14707071]
129. Hendriks J, Xiao Y, Rossen JW, van der Sluijs KF, Sugamura K, Ishii N, et al. During viral infection of the respiratory tract, CD27, 4-1BB, and OX40 collectively determine formation of CD8+ memory T cells and their capacity for secondary expansion. *J Immunol* 2005;175:1665–76. [PubMed: 16034107]
130. Soroosh P, Ine S, Sugamura K, Ishii N. Differential requirements for OX40 signals on generation of effector and central memory CD4+ T cells. *J Immunol* 2007;179:5014–23. [PubMed: 17911586]

Fig. 1.

The effect of CD28 costimulation on the quality and expansion of memory T cells. Activation of naïve T cells by APCs such as DC expressing MHC/peptide complexes and CD28 ligands, leads to intense proliferation and clonal expansion. Memory T cells generated after a primary response expand rapidly upon re-encountering antigen and CD28 costimulation. When memory cells re-encounter antigen without sufficient CD28 costimulation, they fail to fully expand and clear pathogens. If naïve T cells are activated by APCs in the absence of CD28 costimulation, there is limited clonal expansion or even anergy. The resulting memory population is normal in terms of quantity but quality is affected and these memory cells do not re-expand optimally upon antigen re-encounter.

Fig. 2.

Pathogens, tumors and immunotherapeutics can impair memory T cell activation. In contrast to a healthy immune response in which memory T cells receive both signal 1 and signal 2 (costimulation), the immune response against some viruses or tumors or during ageing may be impaired due to downregulation of costimulatory ligands. Blocking costimulation, although beneficial in autoimmunity and transplantation for diminishing the activation of auto- and alloantigen specific responses, may impair the memory T cell responses induced by previous vaccinations infections.