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CD28 Negative T cells: is their loss our gain?

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Abstract

CD28 is a primary costimulation molecule for T cell activation. However, during the course of activation some T cells lose this molecule and assume a CD28-independent existence. These CD28⁻ T cells are generally antigen-experienced and highly differentiated. CD28⁻ T cells are functionally heterogeneous. Their characteristics vary largely on the context in which they are found and range from having enhanced cytotoxic abilities to promoting immune regulation. Thus, CD28 loss appears to be more of a marker for advanced differentiation regardless of the cytotoxic or regulatory function being conducted by the T cell. CD28⁻ T cells are now being recognized as playing significant roles in several human diseases. Various functional CD28⁻ populations have been characterized in inflammatory conditions, infections, and cancers. Of note, the recent introduction of costimulation blockade-based therapies, particularly those that inhibit CD28-B7 interactions, has made CD28 loss particularly relevant for solid organ transplantation. Certain CD28⁻ T cell populations seem to promote allograft tolerance whereas others contribute to alloreactivity and costimulation blockade resistant rejection. Elucidating the interplay between these populations and characterizing the determinants of their ultimate function may have relevance for clinical risk stratification and personal determination of optimal post-transplant immune management.

Introduction

Lymphocyte activation has long been known to require antigen-dependent and antigen-independent cell surface signals. The foundations of this knowledge derive from the two signal models of Bretscher and Cohn, and Lafferty and Cunningham that gave rise to experiments in the 1980s defining antigen stimulation as signal 1 and antigen-independent costimulation as signal 2. In that context, CD28 was the first, and remains most extensively studied, costimulatory molecule. CD28 is constitutively expressed on naïve T cells. It has also been observed on the surface of plasma cells, neutrophils and eosinophils, though its function on these cells remains incompletely defined. For T cells, CD28 costimulation greatly enhances the response to antigen. It stabilizes the immune synapse, reduces the number of TCR-antigen engagements required to reach the cell's activation threshold, and in keeping with the fundamental postulates of the two-signal model, enables T cell activation

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and survival. CD28 engagement on CD4 T cells increases the T cell sensitivity to antigen receptors, greatly increases the cytokine production (mostly IL-2), and promotes cell survival through inducing expression of anti-apoptotic proteins including Bcl-XL.

Despite the importance of CD28 for lymphocyte activation and survival, some antigen-experienced T cells lose CD28, and subsequently can be re-activated without CD28 engagement. These CD28⁻ T cells have generally been characterized as antigen specific and terminally differentiated, and are often described as being memory T cells (TMs). Interestingly, loss of CD28 on lymphocytes appears to be reasonably conserved in higher mammalian species particularly in the context of apoptosis.¹ As humans age and consequently augment their antigen experience, they accumulate CD28⁻ T cells, mostly within the CD8 subset. These cells have decreased antigen receptor diversity, compromised antigen-induced proliferation, and are limited by a shorter replicative lifespan, though they exhibit enhanced cytotoxic and regulatory functions. These characteristics may contribute to the immune incompetence in the elderly, as manifested by susceptibility to latent viral reactivation, and compromised responses to novel pathogens, cancer cells, and vaccines.²

Importantly, CD28 loss is becoming an increasingly scrutinized topic in the context of solid organ transplantation, particularly in the advent of costimulation blockade therapies. Belatacept, a fusion protein that inhibits CD28-B7 interaction, has proven to be a reasonable alternative to calcineurin-inhibitor-based therapy, but has increasingly been recognized to be ineffective in a sizeable minority of kidney transplant patients.³ An emerging theory around this so-called costimulation blockade-resistant rejection (CoBRR) is that patients accumulate CD28⁻ T cells through repeated antigen stimulation, and that these cells become indifferent to the effects of belatacept and define a lymphocyte population most capable of conferring CoBRR. Indeed, it has been recently shown that belatacept's immunosuppressive effect, in contrast to that of tacrolimus, weakens with increasingly matured effector cells.⁴ Although costimulation blockade-based immunotherapies boast superior side effect profiles compared to calcineurin inhibitor-based therapies,³ overcoming the CoBRR hurdle is critical to their generalized use. This review will focus on CD28 loss in humans unless otherwise noted. CD28 loss is poorly characterized in mice, but it is a major feature defining TEMs in non-human primates (NHPs).⁵

CD28⁻ Cellular Immunology

Loss of CD28 expression in T cells

CD8⁺ T cells play a central role in the recognition and clearance of intracellular pathogens. Memory CD8⁺ T cells are generated in smaller quantities, and are maintained chronically for defense against subsequent exposures to the same antigens, enabling a faster and more vigorous response. Repeated antigen stimulations induce progressive reduction in CD28 expression on the surface of CD8⁺ T cells, eventually generating a population of highly antigen-experienced CD8⁺CD28⁻ T cells with shortened telomeres.² When considering an initial antigen exposure, CD28 clearly helps insure that CD8 T cell responses are initiated solely in the proper context, that being when antigen is being presented by a B7-expressing antigen presenting cell (APC) that has been activated through innate signals of cell injury or pathogen presence. Properly presented antigen is deemed deleterious, and elicits an

appropriate immune response culminating in the retention of a small population of CD8 TMs. Teleologically, survival after an antigen exposure suggests that the immune response was indeed appropriate, and subsequent responses to that antigen should, evolutionarily, also be appropriate, even before harm has been evoked. Thus, the requirement for costimulation might be considered an unnecessary redundancy delaying a protective anamnestic response. Hence, CD28 loss may be a means of TM learning that increases the efficiency of responses to common environmental antigens.

CD28 loss in CD4 T cells is less well characterized compared to that in CD8 T cells. CD4⁺CD28⁻ T cells are much less common than CD8⁺CD28⁻ T cells. They express less CD154, and thus are less capable of providing B cell help.⁶ CD4⁺CD28⁻ T cells have been shown to exhibit autoreactivity. These cells possess significant IFN γ cytoplasmic stores and express CD161, a tissue invasion molecule, suggesting that they may generally play a pro-inflammatory role.² Interestingly, although CD4 TEMs are mostly CD28⁺ in humans, there exists a significant CD28⁻ CD4 TEM population in NHPs. This difference likely contributed to the TeGenero clinical trial failure in 2006, where human volunteers experienced a massive CD4 TEM-mediated CD28 superagonist effect whereas the NHPs did not.⁷

The precise molecular mechanism of CD28 downregulation is yet to be adequately described.⁸ CD28 is regulated by a transcriptional initiator element (Inr) that, when disrupted, can lead to CD28 downregulation. Specifically, tumor-necrosis factor- α has been shown to be an Inr inhibitor.⁶ Additionally, FasL has been shown to induce CD28 downregulation in Jurkat cells through transcription blockade, thus linking CD28 downregulation to apoptotic pathways. Further elucidation of the mechanisms mediating CD28 downregulation will be of particular value in understanding the impetus of CD28 loss.

Surface phenotype in CD28⁻ T cells

CD28⁻ T cells exhibit distinct surface expression profiles. In CD8 T cells, surface expression of CD57 (also referred to as HNK-1, Leu7, or L-2) increases with decreasing CD28 expression.⁹ CD28 loss and increased CD57 expression have also been observed on CD4 T cells in the context of chronic immune activation. Interestingly, patients with costimulation blockade resistant rejection (CoBRR) may be more likely to have a CD28⁻CD57⁺ CD4 surface phenotype.¹⁰ Compared to naïve T cells, TMs express higher levels of CD2, CD11a, and CD44. Specifically, CD8⁺ TEMs with low CD28 express particularly high levels of the adhesion molecule CD2. This CD8⁺CD2^{hi}CD28⁻ T cell population has been shown in alloreactivity assays to contain high numbers of cells with polyfunctional cytokine (IFN γ , TNF and IL-2) production and cytotoxic effector molecule (CD107a and granzyme B) expression.¹¹

Interestingly, CD8⁺CD28⁻ T cells also express a number of NK-related receptors that include Killer Inhibitory Receptors (KIR) and NKG2D.² Both KIR and NKG2D are associated with TCR-independent cytotoxicity in CD8⁺CD28⁻ T cells, suggesting that CD8⁺CD28⁻ T cell subsets may contribute to innate immunity. Thus, the surface phenotype of CD28⁻ T cells suggests that they are cytotoxic, alloreactive, and may contribute to innate immunity.

Clinical relevance of CD28⁻ T cells

CD28⁻ T cells in human physiology

CD28 loss has been associated with many physiologic clinical findings in humans. Normal aging is directly correlated with the oligoclonal accumulation of CD8⁺CD28⁻ T cells.² This is presumably driven by the cumulative exposure to persistent antigens.^{6,12} At birth, virtually all human T cells express CD28. In young adults, up to 20-30% of their CD8 T lymphocytes lose CD28 expression. In individuals over 80 years old, over 50-60% of their CD8 T cells lose CD28 expression.¹³ Similar trends have been observed in CD4 T cells, though the effect is less drastic. It is thought that common chronic viral infections including human cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) contribute to the CD8⁺CD28⁻ T cell population expansion.²

Since CD8⁺CD28⁻ T cells are oligoclonal, their accumulation implies a concomitant restriction in the range of antigenic specificities within the CD8 T cell compartment.¹⁴ This consequently leads to an immune system compromised by limited antigenic diversity. Furthermore, age-related thymic involution and its related reduced output in naïve CD28⁺ T cells may also contribute to an aged and weakened immune phenotype. Young adults who have been thymectomized during early childhood for life-saving heart surgery exhibit a T cell profile that is strongly skewed towards oligoclonal memory cells.¹⁵ Interestingly, these patients exhibited high levels of TMs with CD57 expression, a phenotype that is strongly associated with immune aging and CD28 loss. Finally, CD8⁺CD28⁻ T cell expansion in the elderly has been correlated with a skewing towards the Th1 cytokines, resulting in an insufficient antibody response to influenza vaccination.¹⁶ Taken together, the accumulation of antigen experience over time, along with age-related thymic involution contributes to the accumulation of CD28⁻ T cells, which has been associated with the immunocompromised profile of the elderly, as illustrated by insufficient immune response to novel pathogens, tumor cells and vaccines.

CD28⁻ CD8 T cells in human pathology

Much evidence suggests that CD8⁺CD28⁻ T cells play a significant role in human pathology. Increased CD8⁺CD28⁻ T cells have been associated with chronic viral infections including HIV, hepatitis C virus, and human parvovirus B19.² CD28 loss may also serve as a prognostic indicator for viral infections. Higher CD8⁺CD28⁻ T cell populations in early-stage HIV infection is correlated with faster progression to the AIDS. Additionally, higher frequencies of CD8⁺CD28⁻ T cells in HIV-infected women are associated with subclinical carotid artery disease.²

Increased CD8⁺CD28⁻ populations have also been observed in malignancies. Elevated levels of these cells were observed in both the tumor and the blood of patients with patients with solid and hematogenous tumors.² The role of CD28⁻ T cells in these settings appear to be heterogeneous. In melanoma patients, CD8⁺CD28⁻ T cells express perforin, suggesting that they could contribute to the anti-tumor immune response.¹⁷ In contrast, CD8⁺CD28⁻ T cells from lung cancer patients express elevated FOXP3 and have been shown to play an immunoregulatory role in the antitumor response.¹⁸ Tumor infiltrating CD8⁺CD28⁻ T

regulatory lymphocytes have also been implicated in inhibiting T cell proliferation and cytotoxic function in a number of other human cancers.¹⁹ Thus, CD8⁺CD28⁻ T cells exhibit both cytotoxic and immunoregulatory phenotypes that vary across different malignancies. While it is tempting to speculate that there is a causal association between these cells and cancer pathogenesis, it is also possible that the accumulation of CD28⁻ cells is an epiphenomenon, driven by repeated antigen exposure and its associated chronic inflammation that both gives rise to this T cell phenotype and independently predisposes patients to malignant cell transformation.

A similarly heterogeneous CD8⁺CD28⁻ T cell phenotype is observed in autoimmune diseases. CD28 loss is associated with multiple sclerosis, type 1 diabetes, Graves' disease, ankylosing spondylitis, and rheumatoid arthritis.² There is evidence that there is increased CD8⁺CD28⁻ T cell frequency in patients with Graves' disease and ankylosing spondylitis, and that these cells confer cytotoxicity and contribute to the autoimmune response.^{20,21} In rheumatoid arthritis patients, clinical response to abatacept is associated with a concomitant decrease in CD8⁺CD28⁻ T cells, further implicating these cells as contributors to the autoimmunity. Despite this, systemic lupus erythematosus patients were found to have reduced CD8⁺CD28⁻ T cells, which suggests a potentially immunoregulatory function for these cells.²² Importantly, regulatory CD8⁺CD28⁻ T cells have been shown with adoptive transfer models to play an indispensable role in preventing autoimmune encephalomyelitis in mice.²³ Therefore, CD8⁺CD28⁻ T cells may play either cytotoxic or immunoregulatory roles in autoimmune diseases.

CD4⁺CD28⁻ T cells in human pathology

Like CD8 T cells, CD4 T cells also appear to associate with a number of human diseases, especially those affecting the vascular system. Increased CD4⁺CD28⁻ T cells have been associated with unstable atherosclerotic, acute coronary events, and ischemic stroke.² These cells also serve as an independent predictor of mortality in chronic heart failure (CHF) patients.²⁴ CD4⁺CD28⁻ T cells produce significant amounts of IFN γ and perforin, which is thought to promote atheromatous plaque destabilization. Mechanistically, the KIRs expressed on CD4⁺CD28⁻ T cells have been implicated in contributing to this proinflammatory phenotype.^{25,26} Interestingly, patients with end-stage renal disease suffer remarkably high risk for acute atherosclerotic vascular events shortly after kidney transplantation.²⁷ Traditional risk factors such as smoking, hypertension, and hypercholesterolemia insufficiently account for the magnitude of this risk.^{28,30} These patients have a significantly expanded CD4⁺CD28⁻ T cell population that reach up to 50% of the total CD4 T cell population.²⁶ Perhaps certain immunosuppression regimens combined with the accumulation of CD4⁺CD28⁻ T cells are responsible for the adverse cardiovascular outcomes observed in transplant patients.

Transplantation and CD28⁻ T cells

Role of CD8⁺CD28⁻ T cells transplantation

Allogeneic solid organ transplants have been associated with oligoclonal expansion of CD8⁺CD28⁻ T cells.^{12,31,33} These cells have been demonstrated to play a strong

immunosuppressive role in the context of allograft rejection. A distinct population of antigen specific CD8⁺CD28⁻ T cells can be generated *in vitro* by repeatedly stimulating human peripheral blood mononuclear cells with allogeneic or xenogeneic APCs. The resulting T suppressors (Ts) confer immunosuppression by inducing down-regulation of costimulatory molecules (CD80 and CD86) and up-regulation of inhibitor receptors (ILT3 and ILT4) in APCs.^{34,36} These Ts are also evident clinically. Elevated Ts levels in adult liver transplant patients have been associated with better graft function and reduced rejection rates.³¹ Additionally, non-rejecting transplant patients were observed to have Ts that induced ILT3 expression and reduced CD80/86 expression on APCs whereas rejecting transplant patients had lower amounts of Ts in circulation that did not induce this tolerogenic APC profile.^{36,39} These findings suggest that there exists a CD8⁺CD28⁻ T suppressor subset that confers immunosuppression and thus promotes allograft tolerance.

The relevance of these CD8⁺CD28⁻ Ts cells extends further to the efficacy of alemtuzumab as an induction agent. CD8 T cells recover significantly faster than CD4 T cells after alemtuzumab induction, and initially comprise disproportionately of CD8⁺CD28⁻ cells.⁴⁰ These CD8⁺CD28⁻ T cells have been shown *in vitro* to suppress the proliferation of CD4 T cells, which are associated with allograft rejection.^{41,42} Indeed in one study, alemtuzumab-treated patients with the lowest levels of CD8⁺CD28⁻ T cells were associated with kidney allograft rejection.⁴⁰

An obstacle of induction therapy involves the presence of viral antigens. Lymphocyte repopulation in the presence of a persistent antigen may result in activation and exhaustion of the engaged T-cell clones, and ultimately in enhanced nonspecific alloimmunity.⁴³ Indeed, CMV contributes to T cell aging in patients with renal failure and has been associated with increased CD28⁻ TMs.⁴⁴ Despite these barriers, it has been recently demonstrated that combination therapy with alemtuzumab and belatacept can effectively maintain kidney allografts without steroids or CNIs.⁴⁵ Perhaps lymphocyte depletion results in a naïve skewed T cell repertoire that is most responsive to belatacept. These data together show that CD28 loss may confer differential susceptibility to induction agents. There is no evidence that these cells are absolutely resistant to any agent, but a modest differential susceptibility appears to change repertoire population in a clinically significant way.

Role of CD4⁺CD28⁻ T cells transplantation

Despite the tolerance-inducing qualities of CD8⁺CD28⁻ T cells, CD28 loss in CD4 T cells have been implicated in promoting immunosuppression resistance and allograft rejection.^{41,42} The emergence of T cell costimulation blockade (CoB)-based immunosuppression therapies in solid organ transplantation has drawn much interest to CD28 expression on T cells. Belatacept, a B7-specific fusion protein, blocks CD28-B7 costimulation and prevents kidney allograft rejection with a superior side effect profile compared with CNI-based therapies (Benefit trial). However, it has been ineffective in a sizable minority of patients. It is thought that the antigen-experienced TMs that exhibit CD28 downregulation are the primary drivers of Belatacept resistant rejection (BRR). Patients with BRR are more likely to have a CD4⁺CD28⁻CD57⁺ surface phenotype, a phenomenon that is not observed in patients treated with traditional calcineurin inhibitor

therapies (Espinosa 2014, unpublished).⁴⁶ Additionally, it has been shown that proliferation of CD4⁺CD28⁻ T cells reactive with renal tubular epithelial cells (RTECs) is resistant to tacrolimus and everolimus, which suggests a potential CD28⁻ T cell mediated mechanism for organ rejection under standard immunosuppression therapy.⁴⁷

In summary, CD8⁺CD28⁻ T cells appear to promote immunoregulation and CD4⁺CD28⁻ T cells appear to confer cytotoxicity in the context of transplantation. However, given the observation of independent immunoregulatory and immunogenic subsets within CD4s and CD8s in malignancies and autoimmune conditions, it is more likely that a similar paradigm exists for CD28 loss in transplantation. In other words, both CD4 and CD8 T cells consist of heterogeneous CD28⁻ subsets that contribute both to immunosuppression and immunoreactivity in transplantation (Figure 1). Indeed it has been shown *in vitro* that a specific CD8⁺CD28⁻CD2^{hi} T cell population is both highly alloreactive and resistant to belatacept.¹¹ The ultimate phenotype is driven by which subsets predominate. Perhaps once an antigen specific cell is deemed useful in a regulatory or cytotoxic context, it can lose CD28 and subsequently only require TCR engagement for activation. In any event, a more comprehensive understanding of this phenomenon will be critical to developing more fine-tuned immunosuppression therapies. Appropriately, many novel methods of targeting CD28 in the context of transplantation are currently being investigated.⁴⁸

Concluding Remarks

Though it is tempting to generalize the functional consequences of CD28 loss in T cells, it is becoming apparent that different CD28⁻ T cell populations exhibit variable phenotypes in different human conditions, including transplantation. A heterogeneous mix of immunoregulatory and cytotoxic CD28⁻ T cells has been delineated in solid organ transplant patients. The drivers that ultimately push the phenotype one way or another remain to be fully elucidated. Interestingly, CD28⁻ T cells also appear to exhibit different susceptibilities to induction therapies, which may change the T cell repertoire in clinically meaningful ways.

One would be remiss to assume that loss of CD28 on T cells does not result in the activation of compensatory costimulation pathways. In fact, multiple such pathways have been already suggested in CD28⁻ T cells.^{49,51} Finally, there exists the possibility that CD28⁻ T cells may just be residual cells from prior antigen exposures that do not offer any unique clonotypes, and thus are largely dispensable.¹⁴ In any event, achieving a higher resolution of understanding in this interplay of costimulation and coinhibitory pathways in the context of CD28 loss will play an immense role in therapeutic development for many human diseases.

Works cited

1. Diaz D, Chara L, Chevarria J, et al. Loss of surface antigens is a conserved feature of apoptotic lymphocytes from several mammalian species. *Cellular immunology*. 2011; 271:163–72. [PubMed: 21745657]
2. Strioga M, Pasukoniene V, Characiejus D. CD8⁺ CD28⁻ and CD8⁺ CD57⁺ T cells and their role in health and disease. *Immunology*. 2011; 134:17–32. [PubMed: 21711350]
3. Vincenti F, Charpentier B, Vanrenterghem Y, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study).

American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2010; 10:535–46.

4. Xu H, Perez SD, Cheeseman J, Mehta AK, Kirk AD. The allo- and viral-specific immunosuppressive effect of belatacept, but not tacrolimus, attenuates with progressive T cell maturation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2014; 14:319–32.
5. Pitcher CJ, Hagen SI, Walker JM, et al. Development and homeostasis of T cell memory in rhesus macaque. Journal of immunology. 2002; 168:29–43.
6. Vallejo AN. CD28 extinction in human T cells: altered functions and the program of T-cell senescence. Immunological reviews. 2005; 205:158–69. [PubMed: 15882352]
7. Eastwood D, Findlay L, Poole S, et al. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells. British journal of pharmacology. 2010; 161:512–26. [PubMed: 20880392]
8. Ma S, Ochi H, Cui L, He W. FasL-induced downregulation of CD28 expression on jurkat cells in vitro is associated with activation of caspases. Cell biology international. 2003; 27:959–64. [PubMed: 14642527]
9. Merino J, Martinez-Gonzalez MA, Rubio M, Inoges S, Sanchez-Ibarrola A, Subira ML. Progressive decrease of CD8high+ CD28+ CD57– cells with ageing. Clinical and experimental immunology. 1998; 112:48–51. [PubMed: 9566789]
10. Espinosa J. CD4+ T Cell Differentiation and Belatacept Resistant Rejection. American Transplant Congress. 2013
11. Lo DJ, Weaver TA, Stempora L, et al. Selective targeting of human alloresponsive CD8+ effector memory T cells based on CD2 expression. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2011; 11:22–33.
12. Focosi D, Bestagno M, Burrone O, Petrini M. CD57+ T lymphocytes and functional immune deficiency. Journal of leukocyte biology. 2010; 87:107–16. [PubMed: 19880576]
13. Fagnoni FF, Vescovini R, Mazzola M, et al. Expansion of cytotoxic CD8+ CD28– T cells in healthy ageing people, including centenarians. Immunology. 1996; 88:501–7. [PubMed: 8881749]
14. Weinberger B, Welzl K, Herndler-Brandstetter D, Parson W, Grubeck-Loebenstein B. CD28(–)CD8(+) T cells do not contain unique clonotypes and are therefore dispensable. Immunology letters. 2009; 127:27–32. [PubMed: 19715728]
15. Sauce D, Larsen M, Fastenackels S, et al. Evidence of premature immune aging in patients thymectomized during early childhood. The Journal of clinical investigation. 2009; 119:3070–8. [PubMed: 19770514]
16. Saurwein-Teissl M, Lung TL, Marx F, et al. Lack of antibody production following immunization in old age: association with CD8(+)CD28(–) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. Journal of immunology. 2002; 168:5893–9.
17. Casado JG, Soto R, DelaRosa O, et al. CD8 T cells expressing NK associated receptors are increased in melanoma patients and display an effector phenotype. Cancer immunology, immunotherapy : CII. 2005; 54:1162–71. [PubMed: 15889254]
18. Meloni F, Morosini M, Solari N, et al. Foxp3 expressing CD4+ CD25+ and CD8+CD28– T regulatory cells in the peripheral blood of patients with lung cancer and pleural mesothelioma. Human immunology. 2006; 67:1–12. [PubMed: 16698419]
19. Filaci G, Fenoglio D, Fravega M, et al. CD8+ CD28– T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. Journal of immunology. 2007; 179:4323–34.
20. Sun Z, Zhong W, Lu X, et al. Association of Graves' disease and prevalence of circulating IFN-gamma-producing CD28(–) T cells. Journal of clinical immunology. 2008; 28:464–72. [PubMed: 18704663]
21. Schirmer M, Goldberger C, Wurzner R, et al. Circulating cytotoxic CD8(+) CD28(–) T cells in ankylosing spondylitis. Arthritis research. 2002; 4:71–6. [PubMed: 11879540]
22. Tulunay A, Yavuz S, Direskeneli H, Eksioglu-Demiralp E. CD8+CD28–, suppressive T cells in systemic lupus erythematosus. Lupus. 2008; 17:630–7. [PubMed: 18625635]

23. Najafian N, Chitnis T, Salama AD, et al. Regulatory functions of CD8+CD28– T cells in an autoimmune disease model. *The Journal of clinical investigation*. 2003; 112:1037–48. [PubMed: 14523041]
24. Koller L, Richter B, Goliasch G, et al. CD4+ CD28(null) cells are an independent predictor of mortality in patients with heart failure. *Atherosclerosis*. 2013; 230:414–6. [PubMed: 24075776]
25. Zal B, Kaski JC, Akiyu JP, et al. Differential pathways govern CD4+ CD28– T cell proinflammatory and effector responses in patients with coronary artery disease. *Journal of immunology*. 2008; 181:5233–41.
26. Betjes MG, Meijers RW, de Wit LE, Litjens NH. A killer on the road: circulating CD4(+)/CD28null T cells as cardiovascular risk factor in ESRD patients. *Journal of nephrology*. 2012; 25:183–91. [PubMed: 22135034]
27. Betjes MG, Weimar W, Litjens NH. Circulating CD4(+)/CD28null T Cells May Increase the Risk of an Atherosclerotic Vascular Event Shortly after Kidney Transplantation. *Journal of transplantation*. 2013; 2013:841430. [PubMed: 24288592]
28. Aull-Watschinger S, Konstantin H, Demetriou D, et al. Pre-transplant predictors of cerebrovascular events after kidney transplantation. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2008; 23:1429–35.
29. Muntner P, He J, Astor BC, Folsom AR, Coresh J. Traditional and nontraditional risk factors predict coronary heart disease in chronic kidney disease: results from the atherosclerosis risk in communities study. *Journal of the American Society of Nephrology : JASN*. 2005; 16:529–38. [PubMed: 15625072]
30. AM, de Mattos; Prather, J.; Olyaei, AJ., et al. Cardiovascular events following renal transplantation: role of traditional and transplant-specific risk factors. *Kidney international*. 2006; 70:757–64. [PubMed: 16788687]
31. Lin YX, Wang LL, Yan LN, et al. Analysis of CD8+CD28– T-suppressor cells in living donor liver transplant recipients. *Hepatobiliary & pancreatic diseases international : HBPDI*. 2009; 8:241–6. [PubMed: 19502162]
32. Gorochoff G, Debre P, Leblond V, Sadat-Sowti B, Sigaux F, Autran B. Oligoclonal expansion of CD8+ CD57+ T cells with restricted T-cell receptor beta chain variability after bone marrow transplantation. *Blood*. 1994; 83:587–95. [PubMed: 7506956]
33. Klaus G, Mostert K, Reckzeh B, Mueller TF. Phenotypic changes in lymphocyte subpopulations in pediatric renal-transplant patients after T-cell depletion. *Transplantation*. 2003; 76:1719–24. [PubMed: 14688522]
34. Liu Z, Tugulea S, Cortesini R, Suciu-Foca N. Specific suppression of T helper alloreactivity by allo-MHC class I-restricted CD8+CD28– T cells. *International immunology*. 1998; 10:775–83. [PubMed: 9678758]
35. Ciubotariu R, Colovai AI, Pennesi G, et al. Specific suppression of human CD4+ Th cell responses to pig MHC antigens by CD8+CD28– regulatory T cells. *Journal of immunology*. 1998; 161:5193–202.
36. Chang CC, Ciubotariu R, Manavalan JS, et al. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nature immunology*. 2002; 3:237–43. [PubMed: 11875462]
37. Colovai AI, Mirza M, Vlad G, et al. Regulatory CD8+CD28– T cells in heart transplant recipients. *Human immunology*. 2003; 64:31–7. [PubMed: 12507812]
38. Cortesini R, Suciu-Foca N. The concept of "partial" clinical tolerance. *Transplant immunology*. 2004; 13:101–4. [PubMed: 15380540]
39. Manavalan JS, Kim-Schulze S, Scotto L, et al. Alloantigen specific CD8+CD28– FOXP3+ T suppressor cells induce ILT3+ ILT4+ tolerogenic endothelial cells, inhibiting alloreactivity. *International immunology*. 2004; 16:1055–68. [PubMed: 15226269]
40. Trzonkowski P, Zilvetti M, Chapman S, et al. Homeostatic repopulation by CD28–CD8+ T cells in alemtuzumab-depleted kidney transplant recipients treated with reduced immunosuppression. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2008; 8:338–47.

41. Pearl JP, Parris J, Hale DA, et al. Immunocompetent T-cells with a memory-like phenotype are the dominant cell type following antibody-mediated T-cell depletion. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2005; 5:465–74.
42. Trzonkowski P, Zilvetti M, Friend P, Wood KJ. Recipient memory-like lymphocytes remain unresponsive to graft antigens after CAMPATH-1H induction with reduced maintenance immunosuppression. *Transplantation*. 2006; 82:1342–51. [PubMed: 17130784]
43. Stock P, Kirk AD. The risk and opportunity of homeostatic repopulation. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2011; 11:1349–50.
44. Meijers RW, Litjens NH, EA de Wit, et al. Cytomegalovirus contributes partly to uraemia-associated premature immunological ageing of the T cell compartment. *Clinical and experimental immunology*. 2013; 174:424–32. [PubMed: 23962178]
45. Kirk AD, Guasch A, Xu H, et al. Renal transplantation using belatacept without maintenance steroids or calcineurin inhibitors. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2014; 14:1142–51.
46. Legendre CM, Forbes RD, Loertscher R, Guttman RD. CD4+/Leu-7+ large granular lymphocytes in long-term renal allograft recipients. A subset of atypical T cells. *Transplantation*. 1989; 47:964–71. [PubMed: 2472027]
47. Demmers MW, Baan CC, Janssen M, et al. Substantial proliferation of human renal tubular epithelial cell-reactive CD4+CD28null memory T cells, which is resistant to tacrolimus and everolimus. *Transplantation*. 2014; 97:47–55. [PubMed: 24157471]
48. Poirier N, Blanche G, Vanhove B. CD28-specific immunomodulating antibodies: what can be learned from experimental models? *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2012; 12:1682–90.
49. Hodge G, Hodge S, Ahern J, Holmes-Liew CL, Reynolds PN, Holmes M. Up-regulation of alternate co-stimulatory molecules on proinflammatory CD28null T cells in bronchiolitis obliterans syndrome. *Clinical and experimental immunology*. 2013; 173:150–60. [PubMed: 23607447]
50. Zhu Y, Yao S, Iliopoulou BP, et al. B7-H5 costimulates human T cells via CD28H. *Nature communications*. 2013; 4:2043.
51. Moro-Garcia MA, Alonso-Arias R, Lopez-Larrea C. Molecular mechanisms involved in the aging of the T-cell immune response. *Current genomics*. 2012; 13:589–602. [PubMed: 23730199]

Figure 0001 **Figure 0002**

Figure 1. Distinct CD28⁻ T cell populations may promote cytotoxicity or immunoregulation Both CD4 and CD8 T cells can downregulate surface CD28 expression through both physiologic and pathologic pathways. These CD28⁻ T cells may ultimately acquire cytotoxic or immunosuppressive phenotypes. (A) Specifically in CD8 T cells, CD28⁻ FOXP3 T cells confer immunosuppression via APC modification. CD57⁺ CD28⁻ T cells have been associated with resistance to belatacept. CD2hiCD57⁺CD28⁻ T cells produce multiple cytotoxic cytokines, and KIR and NK2D receptors suggest potential for TCR-independent innate immune activity. (B) In CD4 T cells, loss of CD154 precludes B cell help, resulting in incompetent antibody response.⁶ CD57⁺ CD28⁻ T cells have been associated with resistance to belatacept, and CD161 and KIR are associated with inflammation and TCR-independent immune activity, respectively. The ultimate fate of transplanted allografts may depend on the interplay between these cytotoxic and immunoregulatory CD28⁻ T cell subsets.