Unusual CD4⁺CD28⁻ T Cells and Their Pathogenic Role in Chronic Inflammatory Disorders

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CD28 is a primary co-stimulatory receptor that is essential for successful T cell activation, proliferation, and survival. While ubiquitously expressed on naive T cells, the level of CD28 expression on memory T cells is largely dependent on the T-cell differentiation stage in humans. Expansion of circulating T cells lacking CD28 was originally considered a hallmark of age-associated immunological changes in humans, with a progressive loss of CD28 following replicative senescence with advancing age. However, an increasing body of evidence has revealed that there is a significant age-inappropriate expansion of CD4⁺CD28⁻ T cells in patients with a variety of chronic inflammatory diseases, suggesting that these cells play a role in their pathogenesis. In fact, expanded CD4⁺CD28⁻ T cells can produce large amounts of proinflammatory cytokines such as IFN-γ and TNF-α and also have cytotoxic potential, which may cause tissue damage and development of pathogenesis in many inflammatory disorders. Here we review the characteristics of CD4⁺CD28⁻ T cells as well as the recent advances highlighting the contribution of these cells to several disease conditions.

[Immune Network 2016;16(6):322-329]

Keywords: CD28, Co-stimulatory receptor, CD4⁺CD28⁻ T cells, Chronic inflammatory diseases, Cytotoxic potential

INTRODUCTION

CD4 T cells play a critical role in orchestrating immune responses by helping humoral and cellular immune cells (1). Successful CD4 T-cell activation is guaranteed by two signals; TCR stimulation as the primary signal (signal 1) and antigen-independent costimulation as the secondary signal (signal 2) (2,3). CD28 is a primary co-signaling receptor that transduces a costimulatory signal 2 and is essential for successful T cell activation, proliferation, and survival (3,4). Furthermore, the CD28 molecule is constitutively expressed on naive and memory T cells. However, in humans there is a marked loss of CD28 expression on terminally-differentiated effector memory T cells (5). Repeated antigenic stimulation over a lifetime results in the generation of this terminally-differentiated T-cell subset following extensive division (6). Thus, CD28 is progressively lost after replicative senescence with advancing age (7). The molecular mechanisms controlling CD28 expression and loss appear to be...
similar in both T-cell subsets, but the age-associated accumulation of CD28 T cells is more prominent in CD8 T cells than CD4 T cells (8,9). Expansion of CD8 CD28 T cells in part reflects the infectious burden from latent viral infections such as CMV and EBV and leads to generating memory inflation in the elderly (10). Accumulating evidence reveals that there is a significant age-inappropriate expansion of cytotoxic CD4 CD28 T cells in patients with a variety of chronic inflammatory diseases, suggesting that these cells might play a role in the pathogenesis of these immune disorders (11-15). Despite the lack of CD28 expression on these cells, they are not anergic, but rather respond to stimulation in a costimulation independent nature. Moreover, loss of CD28 is closely linked to changes in the transcriptional program resulting in production of potent effector cells exhibiting atypical cytotoxic capacity and inflammatory cytokine-producing potential (7,11,16).

The biological role of CD8 CD28 T cells has been extensively reviewed elsewhere. Therefore, this review will focus on physiological and pathological characteristics of CD4 CD28 T cells and recent advances in the understanding of the role of CD4 CD28 T cells in several disease conditions.

**CD4 CD28 T-CELL BIOLOGY**

The mechanism underlying loss of CD28 molecules

CD28 is constitutively expressed on all T cells at birth in humans, but its expression level is changed during activation and differentiation of T cells in a lifetime (4). Considering that the majority of CD28 T cells are of the terminally-differentiated memory subset and that these cells accumulate gradually with age, replicative senescence caused by repeated antigenic stimulation, such as latent viral infection, is considered to be a primary cause of CD28 loss in T cells. Indeed, in vitro culture experiments clearly demonstrated that highly purified CD28 T cells progressively lost CD28 expression after repeated TCR stimulation (9,17). In addition, the loss of CD28 also occurs when T cells are exposed to proinflammatory cytokines (18-21). Of interest, chronic exposure to TNF-α leads to downregulation of the CD28-specific initiator complex and consequently results in decreased CD28 expression in CD4 T cells at the transcriptional level (20,22,23). Furthermore, repeated antigenic stimulation leads to chronic inflammation, making proinflammatory cytokines such as TNF-α abundant and therefore, loss of CD28 due to both replicative senescence and cytokine exposure may not be mutually exclusive in inflammatory disorders (10). Moreover, increased TNF-α is one of the features of inflamming, which is a low-grade, chronic, systemic pro-inflammatory state frequently observed in the elderly that is associated with the unexpected upregulation of proinflammatory responses later in life (10,24-26). Although CD4 CD28 T cells are more resistant to age-associated phenotypic and functional changes than CD8 T cells, CD4 CD28 T cells is also increased with advancing age in healthy individuals (9). Therefore, it will be an intriguing question whether increased TNF-α in the elderly affects the accumulation of CD4 CD28 T cells with advancing age.

Of interest, loss of CD28 by T cells is exclusively observed in humans and non-human primates, and not in mice (7,27,28). Murine CD28 is expressed throughout the lifetime and in some strains its expression is even higher in geriatric mice than in younger mice (29). It should be noted that telomeres are ~10 fold longer in mice than in humans and mice have much shorter lifespan (30), suggesting that telomere erosion may not play a significant role in regulating murine T cell memory, especially loss of CD28. Telomerase is the enzyme that extends telomeres and is induced in T cells by repeated antigenic stimulation. It has been demonstrated that antigen-specific telomerase inducibility is dependent on CD28 expression and the decline in telomerase activity parallels the loss of CD28 expression in human T cells (17). In humans, induction of telomerase, the enzyme that extends telomeres, accompanies Therefore, a limited number of CD28 T cell studies have utilized human and non-human primate cells in vitro (31).

**Characteristics of CD4 CD28 T cells**

Functionally, CD4 CD28 T cells can be defined as typical Th1 cells, which produce a large amount of IFN-γ and TNF-α and express increased levels of the T-bet transcription factor (32-34)(manuscripts in preparation). Moreover, unlike conventional Th1 cells these cells also express the cytotoxic molecules perforin and granzyme B (33), and it has been suggested that their cytotoxic features are responsible for tissue damage and development of pathogenesis in many inflammatory diseases. There are still debates on antigen specificity and possible activation mechanism of CD4 CD28 T cell. Given their oligoclonality, ubiquitous antigens including auto-antigens have been suggested as specific antigens for CD4 CD28 T cell. Alternatively, other molecules such as ligands for receptor, cytokine, adhesion molecules rather than antigen
also have been suggested as their activation cues (35). CD4^+CD28^- T cells exhibit distinct surface expression profiles different from their CD28^+ counterparts (36). Furthermore, like CD8^+ T cells, CD4^+CD28^- T cells also exhibit a phenotype typical of senescent T cells. In addition, the expression of many natural killer cell receptors (NKRs), including CD11b, CD57, CD85j, NKG2D and KIR2DS2, is an important characteristic of CD4^+CD28^- T cells (9,12,37-39) and might be involved in modulating their functional activity. Loss of CD28 on CD4^+ T cells also coincides with decreased expression of CD40L and ICAM-1, implying a dysregulation of B-cell differentiation and immunoglobulin secretion and enhanced migratory potential, respectively (32). Virtually all CD4^+CD28^- T cells are lacking of the costimulatory CD27 receptor but overexpress CD70, the ligand for CD27, suggesting a possible role in modulating T cell activation (40). Moreover, recent studies demonstrated that CD4^+CD28^- T cells exhibit constitutive activation of p38 through the unconventional AMPK-TAB-1 signaling pathway. This finding suggests that DNA damage and dysregulation of glucose metabolism might be associated with characteristics of CD4^+CD28^- T cells (41,42).

**CLINICAL RELEVANCE OF CD4^+CD28^- T CELLS**

**CD4^+CD28^- T cells in rheumatoid arthritis (RA)**

CD4^+CD28^- T cells were initially identified and primarily characterized in patients with RA, a prototype systemic autoimmune disease that manifests through massive and chronic inflammatory infiltrates and bone destruction in joints (12,43-45). The frequency of circulating CD4^+CD28^- T cells in RA is positively correlated with disease severity and the presence of extra-articular manifestation (46). In early studies, the pathogenic role of these cells in RA was suggested by the strong cytotoxic potential, resistance to apoptosis, and tissue-infiltrating capacity of CD4^+CD28^- T cells (38,47-50). Furthermore, CD4^+CD28^- T cells are oligoclonal with limited TCR diversity and have shortened telomeres, implying that an expansion of these cells is a consequence of repeated exposure to the same antigen, possibly an autoantigen (35). However, it is difficult to prove whether the CD4^+CD28^- T-cell subset is a product of the autoreactive response because RA is a typical systemic autoimmune disease and its autoantigen is not yet defined (7). Rather, several studies have suggested that CD4^+CD28^- T cells in RA are probably not just autoreactive T cells. First, CD4^+CD28^- T cells are observed more infrequently in synovial fluid and extra-articular tissue than in peripheral blood of RA patients (43). Secondly, the dominant TCR-Vβ subsets of circulating CD4^+CD28^- T cells are often missing in synovial fluid (43). Finally, these cells tend to be more robustly stimulated by ubiquitous antigens, such as heat shock proteins or CMV antigens, than collagen, a putative autoantigen in RA. CD4^+CD28^- T cells also show no association with antibodies to anti-citrullinated protein, another putative autoantigen in RA, in the serum or synovial fluid (43,51,52). Moreover, accumulating evidence demonstrates that chronic stimulation with proinflammatory cytokines such as TNF-α and IL-15 is responsible for the expansion of CD4^+CD28^- T cells in humans (20,53,54).

The loss of CD28 is closely associated with the acquisition of multiple regulatory molecules on the T cell surface (9,16,55). Utilizing these regulatory molecules, the CD4^+CD28^- T cell subset on its own or through its interactions with other immune cells might contribute to the perpetuation and amplification of inflammatory responses. It has recently been shown that a variety of NKRs, such as NKG2D, KIR2DS2, 2B4, and DNAM-1, are upregulated by CD4^+CD28^- T cells, and activation of these NKRs can provide co-stimulatory signals during TCR activation (56). In addition, CD4^+CD28^- T cells in RA patients aberrantly sustain CD70 express for a longer time after T cell activation than do counterpart CD28^+ T cells. When expressed on CD4^+CD28^- T cells, CD70 can act as a bystander costimulatory signal to naive CD27^+CD4^+ T cells and facilitate their activation and proliferation though the CD70-CD27 interaction. This causes lowering of the activation threshold of CD4^+CD28^- T cells themselves as well as other immune cells, rendering cell activation completely independent of recognition of the appropriate antigenic peptide (40). Moreover, a majority of cytotoxic CD4^+CD28^- T cells in RA patients express CX,CRI, a specific chemokine receptor for CX,CL1, which is abundantly produced by fibroblast-like synoviocytes (FLS) in the RA synovium. Thus, these cells can selectively migrate into inflamed tissue through the CX,CL1-CX,CRI interaction, further supporting their pathogenic roles in patients with RA (57,58). In summary, several studies have provided substantial evidence for a contributing role of CD4^+CD28^- T cells in the pathogenesis of RA.

**CD4^+CD28^- T cells in vascular-related diseases**

The increased frequency of CD4^+CD28^- T cells has been associated with various types of cardiovascular diseases such as atherosclerosis and acute coronary syndromes.
These findings imply that CD4+ T cells are preferentially accumulated in coronary atherosclerotic plaques, but not in stable lesions (61). The expansion of CD4+CD28– T cells has also been associated with the recurrence of acute coronary events, showing that the increased frequency of these cells remains relatively stable after the acute episode in patients with UA (62). These findings imply that CD4+CD28– T-cell expansion is not just a consequence of the inflammatory response triggered by acute events, but rather reflects the chronic inflammatory response to certain viral infections or autoantigens (e.g. human heat shock protein 60), which are present in atherosclerotic plaques (15). In addition, a multivariate logistic regression analysis of ACS patients showed that the frequency of CD4+CD28– T cells is an independent predictor of future acute coronary events (62). Moreover, recent data demonstrated that statins as well as anti-TNF-α treatment can decrease the circulating CD4+CD28– T-cell level, suggesting that these cells are a promising therapeutic target for the prevention of acute coronary events (15,63).

In addition to the direct role of these cells in vascular disease, Betjes and colleagues recently demonstrated that the relative and absolute numbers of circulating CD4+CD28– T cells is massively expanded in patients with end-stage renal disease (ESRD) (14). In ESRD, the substantially elevated risk of cardiovascular diseases is closely linked to uremia-related immune activation such as hypercytokinemia and inflammation (64). Thus, this implies that the expanded CD4+CD28– T cell population also plays a pathogenic role in ESRD. Furthermore, patients with ESRD suffer a remarkably high risk for acute atherosclerotic vascular events shortly after kidney transplantation (65).

**CD4+CD28– T cells in solid organ transplantation**

Of interest, the clinical relevance of CD4+CD28– T cells was first explored in the late 1980s in renal transplant recipients by monitoring of their frequency (66). It was noted that there was an increase of CD4+CD28– T cells in patients after renal or liver transplantation and their expansion is associated with chronic graft rejection (66-68). Considering the primary role of CD28 as a co-stimulatory molecule for T cell activation, it was reasonable to target the CD28-B7 interaction for costimulation blockade therapies as an alternative to calcineurin inhibitor (CNI)-based therapy. Consequently, functional blockade of CD28 by belatacept, a human CTLA-4-Ig fusion protein, has recently become a clinically important immunosuppressive strategy. However, co-stimulation blockade resistant rejection (CoBRR) has increasingly been observed in kidney transplant patients and therefore, efforts to identify potential explanations for CoBRR are ongoing. It should be noted that the loss of CD28 on CD4 T cells has been implicated in promoting immunosuppression resistance and allograft rejection (69,70) due to the cytotoxic capacity of these cells. Very recently it was reported that increases in circulating CD4+CD28–CD57+ T cells prior to transplantation is associated with belatacept resistant rejection (BRR) post-transplantation (71,72). Furthermore, cytomegalovirus-related, cytotoxic CD4+CD28– T cells potentiate kidney allograft dysfunction by glomerular endothelial injury in an NKG2D-dependent manner. The two above-mentioned populations (CD4+CD57+ T cells in BRR and CD4+CD28– T cells in chronic CMV infection) exhibit remarkable overlap both phenotypically and functionally. Therefore, given their expansion in patients with ESRD under the influence of a previous CMV infection and the chronic inflammatory cytokine milieu, CD4+CD28– T cells are likely a nonclassical risk factor for atherosclerotic disease pre- and post-renal transplantation (73,74). However, further investigation regarding their contribution to graft rejection or tolerance is still necessary.

**CONCLUDING REMARKS**

Accumulation of CD28– T cells was initially considered a hallmark of age-associated changes in the human immune system. However, loss of CD28 on CD4 T cells also occurs in patients with chronic autoimmune diseases in an age-inappropriate manner (Fig. 1). Despite their restricted TCR diversity, shorter telomeres and
abundance at the inflamed site, it remains questionable whether the autoreactive T cell response is a major contributing factor for expansion of CD4⁺CD28⁻ T cells. Rather, recent studies suggest that repeated antigenic stimulation of T cells by chronic inflammation or latent CMV infections causes them to proliferate more rapidly and extensively resulting in the loss of CD28. Although most studies of CD28⁻ T cells have been conducted in humans, which are much more sensitive to loss of CD28 than mice are, development of an appropriate animal model will be required in order to investigate the underlying mechanisms of this phenomenon and its biological relevance in vivo. In this context, recent CD28 co-stimulation blockade therapy might provide invaluable information regarding the biological role of CD4⁺CD28⁻ T cells in physiological settings. Moreover, it is necessary to understand how CD28 loss is linked to the transcriptomic shift into pathogenic T cells. Better understanding of the molecular and functional features of CD4⁺CD28⁻ T cells will open new avenues to explore potential targets for intervention in a variety of chronic inflammatory diseases. Future research will be needed to investigate whether the accumulation of CD4⁺CD28⁻ T cells is generalized for various chronic inflammatory disorders and to evaluate their usefulness as a biomarker or a prognostic factor. Recovery of CD28 expression by TNF-α inhibition or selective depletion of CD4⁺CD28⁻ T cells by targeting specific surrogate molecules on their surface will be promising approaches for therapeutic intervention in various inflammatory disorders.

ACKNOWLEDGEMENTS

This research was supported by grants from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI13C0715 and HI13C0954 to W.W. L.).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

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