CD4\textsuperscript{+}CD28\textsuperscript{null} T cells in coronary artery disease: when helpers become killers

Ingrid E. Dumitriu, Ernesto Trallero Araguás, Christina Baboonian, and Juan Carlos Kaski* 

Cardiovascular Biology Research Centre, Division of Cardiac and Vascular Sciences, St George's University of London, Cranmer Terrace, London SW17 0RE, UK

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1. Introduction

Coronary artery disease (CAD), a manifestation of atherosclerosis continues to be the most common cause of death in the developed world.\textsuperscript{1,2} Atherosclerosis is predominantly an inflammatory process that involves both the innate and adaptive arms of the immune system.\textsuperscript{3-5} T lymphocytes, the main 'soldiers' of the adaptive immune system, are consistently found in atherosclerotic lesions and contribute to their growth and activity.\textsuperscript{6} In the early stages of atherosclerosis, T cells are involved in the initiation and progression of the disease, while in more advanced stages they contribute to the destabilization of atherosclerotic lesions.\textsuperscript{7,8} Most of the T cells present in atherosclerotic plaques are CD4\textsuperscript{+} helper T lymphocytes.\textsuperscript{9} Detailed analysis of CD4\textsuperscript{+} T cells in CAD revealed the expansion of an unusual subset of lymphocytes, characterized by the lack of the CD28 costimulatory receptor and therefore named CD4\textsuperscript{+}CD28\textsuperscript{null} T cells.\textsuperscript{10} Recent studies suggested that CD4\textsuperscript{+}CD28\textsuperscript{null} T cells mediate plaque instability and recurrence of acute coronary events.\textsuperscript{11} In this review, we summarize the current knowledge on the biology of CD4\textsuperscript{+}CD28\textsuperscript{null} T cells and their role in the pathogenesis of CAD. In addition, we postulate that a better understanding of CD4\textsuperscript{+}CD28\textsuperscript{null} T cells might provide clinically effective strategies for the diagnosis, prevention, and treatment of patients with CAD.

2. CD4\textsuperscript{+}CD28\textsuperscript{null} T cells

CD28 is a T lymphocyte membrane receptor that transduces signals delivered by co-stimulatory molecules of the B7 family, such as CD80 (B7.1) and CD86 (B7.2).\textsuperscript{12,13} Activation of naïve T cells requires not only the recognition of antigen presented by dendritic cells (DCs) but also the interaction of B7, present on DCs with the CD28 receptor. The only costimulatory receptor constitutively expressed on naïve T cells is CD28, and therefore CD28 is pivotal for the induction and maintenance of T cell-mediated immune responses.\textsuperscript{13} In the absence of co-stimulatory signals transduced by CD28, T cells enter a state of anergy (functional unresponsiveness).\textsuperscript{14} Co-stimulation through the CD28 receptor controls the expression of interleukin-2 (IL-2) receptors and the production of IL-2 by activated T cells, which enable their proliferation and survival.\textsuperscript{12,13}

CD4\textsuperscript{+}CD28\textsuperscript{null} T cells differ from conventional CD4\textsuperscript{+}CD28\textsuperscript{+} helper T lymphocytes in both phenotype and function (Table 1). CD4\textsuperscript{+}CD28\textsuperscript{null} T cells are terminally differentiated and have pro-inflammatory functions characterized by the production of high levels of interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), and IL-2.\textsuperscript{15} In addition, CD4\textsuperscript{+}CD28\textsuperscript{null} T cells are cytotoxic and effectively kill endothelial cells \textit{in vitro}.\textsuperscript{16} This is mediated by cytolytic enzymes, such as perforin, granzyme A, and granzyme B expressed by CD4\textsuperscript{+}CD28\textsuperscript{null} T cells.\textsuperscript{17} These cytolytic enzymes are usually present in cytotoxic CD8\textsuperscript{+} T lymphocytes and natural killer (NK) cells, whereas classical CD4\textsuperscript{+} T cells lack them. Another particular feature of CD4\textsuperscript{+}CD28\textsuperscript{null} T cells is the expression of the C-type lectin receptor NKG2D.
and of a variety of NK cell-related receptors that belong to the killer immunoglobulin-like receptor (KIR) family. Another receptor expressed by NK cells, which is also present on CD4⁺CD28null T lymphocytes, is the receptor for the chemokine fractalkine (CX3CR1). These receptors may endow CD4⁺CD28null T cells with the ability to respond to novel environmental cues that provide survival signals. Moreover, CD4⁺CD28null T cells have been shown to be resistant to apoptotic cell death in spite of normal expression of death-inducing receptors such as CD95 (Fas). This resistance to apoptosis, which is mediated by the up-regulation of the anti-apoptotic protein Bcl-2, may also explain the unusual longevity and persistence of CD28null T cells (Figure 1). In addition to losing the expression of the CD28 receptor, CD4⁺CD28null T cells also fail to express the CD40 ligand molecule and are therefore unable to provide helper signals required for the production of antibodies by B cells.

In healthy individuals, CD4⁺CD28null T cells represent a minor subset usually accounting for about 0.1–2.5% of the CD4⁺ T cells. Aging, infections, and chronic inflammatory diseases associate with the expansion of this peculiar T cell subset. In elderly individuals, CD4⁺CD28null T cells are used as a marker of immunosenescence. Their increased frequency in these subjects correlates with the development of autoimmune phenomena and defective B cell responses characterized by impaired production of antibodies.

### 3. The role of CD4⁺CD28null T cells in systemic autoimmune diseases

One of the best characterized autoimmune diseases associated with increased frequencies of CD4⁺CD28null T cells is rheumatoid arthritis (RA). Studies in patients with RA showed CD4⁺CD28null T cells to be increased in over one-third of patients. Furthermore, expansion of the CD4⁺CD28null T cell subset correlated directly with an increased frequency of extra-articular involvement like rheumatoid vasculitis, and the clinical severity of the disease. These results suggest that the expansion of

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**Table 1** Characteristic features of CD4⁺CD28null T cells

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<th>Surface markers</th>
<th>CD28⁺ T cells</th>
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<td>Sensitivity to suppression by Treg cells</td>
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**Figure 1** Generation of CD4⁺CD28null T cells in coronary artery disease (CAD). Antigens such as heat shock proteins (HSPs) that are expressed in the atherosclerotic plaques are presented by antigen-presenting cells like dendritic cells (DCs) to CD4⁺CD28⁺ T cells (A). Following repeated rounds of activation by DCs, some CD4⁺CD28⁺ T cells lose the expression of CD28 and undergo oligoclonal expansion (B). The precise mechanisms that underlie the loss of the CD28 receptor are not known. CD4⁺CD28null T cells are resistant to apoptosis induction (C) and relatively insensitive to the suppression mediated by regulatory T cells (Treg cells) (D). These factors also contribute to the expansion and accumulation of CD4⁺CD28null T cells in CAD (E). MHC, major histocompatibility complex; TCR, T cell receptor.
CD4⁺CD28null T cells does not represent just an epiphenomenon but could have a critical role in the pathogenesis of the disease. Interestingly, in contrast to classical CD4⁺ T lymphocytes, CD4⁺CD28null T cells express CX3CR1 which provides signals that support the activation of these cells in vitro.⁴⁰ Compared to CD28⁺ T lymphocytes, CD4⁺CD28null T cells isolated from patients with RA induced a significantly higher proliferation of synoviocyte lines established from rheumatoid synovium.⁴¹ This effect was mediated through CX3CR1 and amplified by the production of TNF-α from CD4⁺CD28null T cells. The excessive proliferation of synovial fibroblasts causes bone and cartilage destruction in RA. Therefore, these in vitro studies suggest that CD28null T cells promote bone and cartilage destruction in RA. However, recent studies failed to confirm the presence of CD4⁺CD28null T cells in the synovial tissue and fluid of patients with RA,⁴² contradicting previous results.¹⁷,¹⁸ Further studies are thus required to clarify the contribution of CD4⁺CD28null T cells to joint destruction in RA. The CD4⁺CD28null T cell subset is also expanded in other autoimmune diseases like Wegener’s granulomatosis, systemic lupus erythematosus (SLE), and active Crohn’s disease.³²,³³

4. CD4⁺CD28null T cells in coronary artery disease

Recent discoveries highlighting the role of inflammation and immune responses in atherosclerosis have led to a better understanding of the mechanisms underlying CAD development and novel clinical approaches to this disease.³,⁴,³⁴ The most common forms of presentation of CAD are chronic stable angina (CSA) and the acute coronary syndrome (ACS). The ACS, usually triggered by unstable atherosclerotic plaque rupture, manifests clinically as unstable angina (UA) or myocardial infarction and represents a major clinical emergency. Of interest, some ACS patients experience a single acute event in their life, while others have repetitive potentially life-threatening acute episodes, despite receiving optimal treatment. Therefore, the characterization of the cellular and molecular mechanisms that regulate the transition from stable to unstable atherosclerotic lesions and the recurrence of acute coronary events is critical for the development of therapeutic strategies aimed at plaque stabilization and the prevention of CAD progression.

Compared to CSA and control subjects, UA patients have an increased frequency of T cells that produce IFN-γ.⁷,¹⁰ As CD4⁺CD28null T cells are known to produce high levels of IFN-γ, their frequency was investigated in patients with CAD. Indeed, it was found that the CD4⁺CD28null T cell subset was significantly increased in the peripheral blood of patients with UA when compared with CSA.³⁵ Furthermore, the frequency of CD4⁺CD28null T cells correlated directly with that of IFN-γ-secreting lymphocytes, suggesting that CD28null T cells are likely to be the main source of IFN-γ in patients with UA.³⁵ As seen in RA, the expansion of the CD4⁺CD28null T cells in UA patients was oligoclonal, suggesting chronic antigen stimulation by a common antigen.³⁵ In support of this hypothesis is the finding that the amino acid sequence of T cell receptor (TCR) β-chains of CD4⁺CD28null T cell clones generated from different UA patients shows a high degree of similarity.³⁵ Furthermore, CD4⁺CD28null T cell clones generated from CAD patients recognize heat shock protein 60 (HSP60), an antigen which is present in atherosclerotic plaques.³⁶ Therefore, the expansion of CD4⁺CD28null T cells in UA may be the result of repeated antigen stimulation by HSP and other yet unidentified antigens.

The expansion of CD4⁺CD28null T cells remains relatively stable over time after the acute episode in patients with UA, suggesting that this expansion is not just the mere consequence of the inflammatory response triggered by the acute events.¹¹ Indeed, the frequency and clonal identity of CD4⁺CD28null T cells remained unchanged 3 months after myocardial infarction. Moreover, CD4⁺CD28null T cells have been shown to accumulate preferentially in unstable ruptured atherosclerotic plaques and not in stable lesions.³⁵ The selective invasion of unstable lesions indicates that this particular subset of CD4⁺ T lymphocytes may have a direct role in plaque destabilization following local activation by antigens.³⁵ Indeed, CD4⁺CD28null T cells isolated from UA patients have the ability to lyse endothelial cells in vitro, an event that is known to trigger plaque destabilization and rupture.¹⁶,³⁷

Recently, it was shown that an increased frequency of CD4⁺CD28null T cells is an independent predictor of future acute coronary events in UA patients.¹¹ Patients with UA, who experienced recurrent acute events, had a median frequency of CD4⁺CD28null T cells four-fold higher than those with a single acute coronary episode over a 4 year follow-up.¹¹ Moreover, compared with CSA patients, the frequency of CD4⁺CD28null T cells was ~nine-fold higher in UA patients.¹¹ Another recent study demonstrated that the expansion of the CD4⁺CD28null T cells correlates with the extent of CAD.⁸ In addition, the inflammatory marker neopterin, which is specifically expressed by activated monocytes and macrophages, was significantly increased and correlated with the extent of CAD.⁸

5. CD4⁺CD28null T cells in accelerated atherosclerosis driven by autoimmunity

Patients with autoimmune diseases like RA and SLE have an increased risk of developing atherosclerosis.³⁹ Traditional risk factors cannot completely account for the increased incidence of atherosclerosis in these patients and it has been proposed that inflammatory and immune mechanisms have a major role. CD4⁺CD28null T cells are a component of the immune response that mediates the vascular damage as observed both in autoimmune disorders and CAD.¹⁰,⁴⁰ Therefore, this T cell subset may be involved in the development of accelerated atherosclerosis in patients with autoimmune disorders. Indeed, RA patients with an expansion of CD4⁺CD28null T cells have an increased risk of developing vasculitic phenomena.⁴¹ Endothelial damage is the initiating factor in the development of atherosclerotic lesions, leading to vascular dysfunction and the progression of atherosclerosis that results in cardiovascular disease, acute coronary events, and stroke.⁴²,⁴³ Endothelial dysfunction in patients with RA and other autoimmune conditions manifests as impaired flow-mediated vasodilatation (FMD) and increased intima-media thickness (IMT), markers of early atherosclerosis.⁴¹ Interestingly, both abnormal FMD
and increased IMT are more marked in RA patients with an expansion of the CD4+CD28null T cells than in those without.41 Thus, CD4+CD28null T cells may induce endothelium dysfunction and contribute to the acceleration of atherosclerotic process and cardiovascular complications observed in subjects with autoimmune diseases. CD4+CD28null T cells can cause vascular damage either directly by killing endothelial cells or indirectly through macrophage activation.16

6. The origins of CD4+CD28null T cells

Little is known about the precise origin of CD4+CD28null T cells. Environmental and genetic factors have been implicated in the expansion of this subset.24 In addition to TCRs, CD4+CD28null T cells express a variety of receptors belonging to the KIR family, which are normally present on NK cells. Therefore, it has been hypothesized that CD28null T cells might derive from a NK cell precursor.20 Another population of cells characterized by co-expression of TCRs and NK cell receptors are the NKT cells,44 which have been implicated in cardiovascular disease.45,46 In murine models, NKT cells have been shown to accelerate atherosclerosis.45 However, patients with CAD are reported to have decreased frequency of circulating NKT cells compared with controls.47 These contrasting results may be due to the recruitment of NKT cells from the peripheral blood into the plaques in CAD patients. Further studies that assess the frequency of NKT cells in atherosclerotic plaques are therefore required. Furthermore, as cytokines produced by NK cells could affect CD4+CD28null T cells, it would be of interest to assess the relationship between these two unconventional T cell subsets in the plaque environment.

The absence of other molecules characteristic of NK cells such as CD16 indicates that CD28null cells may belong to the same lineage as classic CD4+CD28 T cells (Figure 1). Indeed, experimental data showed that CD28null T lymphocytes are terminally differentiated and resemble cells in replicative senescence, which have characteristic shortened telomeres.24,48 They may therefore derive from the same precursors as CD28+ as a result of repeated antigen stimulation. Data showing that several cytokines influence the expression of CD28 by human T cells provide further support to this hypothesis. The exposure of T cell lines and clones to TNF-α in vitro induces the down-regulation of CD28.49 Furthermore, primary CD4+ T cells exposed to TNF-α express reduced levels of CD28.50 Noteworthy, the production of TNF-α is increased in the elderly, and in patients with RA or ACS, conditions known to associate with an increased frequency of CD4+CD28null T cells.3,24,50 The cytokine IL-12 has been shown to induce the re-expression of functional CD28 molecules on the surface of CD4+CD28null T cells by restoring the transcription of the CD28 gene.51 This suggests that cytokines could be used to modulate the frequency of CD4+CD28null T lymphocytes in patients with autoimmunity or CAD.

7. Antigen specificity of CD4+CD28null T cells

The oligoclonal expansion of the CD4+CD28null T subset suggests that repetitive stimulation by a limited number of antigens triggers the generation and accumulation of these cells.25,35 Identification of the antigens that drive the expansion of CD4+CD28null T cells could be of clinical relevance. In the early phases of atherogenesis, the recruitment of T cells into the lesions is mediated by non-specific inflammatory signals and is antigen-independent.4 In advanced stages, local antigens present in the atherosclerotic plaques trigger the clonal expansion of T cells, including CD4+CD28null T lymphocytes.4 Indeed, T cells specific for antigens like oxidized LDL (oxLDL), HSPs, or Chlamydia, have been isolated from atherosclerotic plaques.52–54 Results obtained from animal models of atherosclerosis that allow detailed characterization of T cells isolated from atherosclerotic lesions in different stages of the disease support this hypothesis.55 In these studies, T cells isolated from mature vs. earlier plaques use different TCRs with specificity for distinct antigens. Furthermore, the T cells recruited in the early stages of atherogenesis are heterogeneous, while advanced lesions associate with the selective expansion of T cells specific for a limited number of local antigens.55

The activation and expansion of antigen-specific T cells is induced by antigen-presenting cells (APCs) (Figure 1). These specialized cells take up antigens and display them in combination with major histocompatibility complex (MHC) molecules for recognition by T lymphocytes.56 The most potent APCs are represented by DCs, which are the only APCs capable to activate naive T cells.56 Other cells like macrophages and B lymphocytes can function as APCs but this requires their previous activation.56 All these APCs have been identified in atherosclerotic lesions, where they might provide signals required for the activation and expansion of T cells.3,57–59 In addition, endothelial and smooth muscle cells present in the atherosclerotic plaques constitutively express MHC class II molecules, which could enable them to present antigens as well.60 Two main types of DCs have been characterized in humans (myeloid and plasmacytoid)61 and both have been identified in human atherosclerotic lesions.59 The precise location where DCs present antigens to T cells and trigger their activation is still not known. It can either happen locally, in the plaques, or in the regional lymph nodes following migration of DCs that have taken up local antigens. Results from in vivo models of atherosclerotic lesion regression demonstrated that unstable plaques associate with reduced emigration of cells resembling DCs into the regional lymph nodes.62

The antigens that trigger the activation and expansion of CD28null T lymphocytes in CAD are not fully characterized. Several infectious diseases have been implicated as cause for atherosclerosis.63 CD4+ T lymphocytes that recognize proteins derived from various micro-organisms such as Chlamydia pneumoniae, Herpes simplex, Helicobacter pylori and Cytomegalovirus (CMV) have been identified.52,64–66 In addition to antigens derived from microorganisms, endogenous antigens like oxLDL and HSPs have also been implicated in atherogenesis.53,54,67 These endogenous antigens are highly expressed in atherosclerotic plaques and their levels correlate with the severity of atherosclerosis.68,69 Interestingly, it has been demonstrated that >50% of the CD4+CD28null T cell clones derived from patients with ACS recognize HSP60, while this was not observed in stable coronary disease.56 When stimulated with autologous HSP60, CD28null T cell clones produce IFN-γ and perforin.38 Recent results suggest that following
recognition of HSP60 on APCs. CD4+CD28null T cell clones from CAD patients induce the lysis of target cells through signals delivered by both TCR and KIR2DS2 receptors (Behnam et al., submitted for publication). Further studies are required to better characterize the importance of antigen-specific vs. antigen-independent activation of CD4+ T cells in general and of CD4+CD28null T cells in particular. This would allow the identification of the immunodominant epitopes that drive the immune response in CAD and autoimmune conditions.

8. CD4+CD28null T cells and plaque destabilization

The acute and most severe manifestations of CAD, i.e. myocardial infarction and sudden death, result from the rupture of a plaque which causes the formation of a thrombus and sudden occlusion of the artery. The presence of activated immune cells, thinning of the fibrous cap, disruption of the collagen matrix, and apoptosis of smooth muscle cells have been implicated in the destabilization of atherosclerotic lesions leading to plaque rupture. Among immune cells, macrophages have a pivotal role in plaque rupture through the release of extracellular matrix degrading enzymes like metalloproteinases (MMPs). CD4+CD28null T cells have been shown to accumulate preferentially in unstable atherosclerotic plaques, therefore being well located to induce plaque destabilization (Figure 2).

The mechanisms that regulate the preferential accumulation of CD4+CD28null T cells in atherosclerotic plaques are not yet understood. It remains to be investigated whether this subset is expanded locally in the plaques or in the regional lymph nodes by APCs like DCs. Another possible mechanism is that CD4+CD28null T cells are continually recruited from the circulation. Chemokines like fractalkine, that are present in the plaques, may be actively involved in the recruitment of CD4+CD28null T cells. Studies in RA patients have shown that CD28null cells are the only CD4+ T lymphocytes that express CX3CR1. However, the expression of CX3CR1 in the CD4+CD28null T cell subset in plaques from CAD patients has not yet been characterized. A study evaluating the surface expression of CX3CR1 in circulating cells reported an expansion of a CD8+ T cell subset expressing CX3CR1 in CAD patients compared with controls. However, the frequency of CX3CR1+CD4+ T lymphocytes was similar in patients and controls. Detailed studies that specifically investigate the expression of CX3CR1 on CD4+CD28null vs. CD4+CD28+ T cell subsets are warranted.

Most T cells present in atherosclerotic plaques, including CD4+CD28null T lymphocytes, produce IFN-γ. IFN-γ increases the recruitment of T cells and macrophages into atherosclerotic lesions, promotes the differentiation of macrophages into foam cells and the activation of macrophages and other APCs. Activated macrophages release MMPs and other enzymes that cause plaque rupture. Furthermore, CD4+CD28null T cells can also contribute to plaque disruption as they can directly lyse endothelial cells, vascular smooth muscle cells and of the extracellular matrix results in the destabilization and rupture of atherosclerotic plaques that causes acute coronary events (E).

Figure 2  Pathogenic effects of CD4+CD28null T cells in coronary artery disease. CD4+CD28null T cells secrete high amounts of interferon-γ that induces the activation of macrophages (A), which in turn release metalloproteinases that degrade the extracellular matrix (B). Secretion of tumour necrosis factor-α by activated macrophages contributes to the downregulation of CD28 by CD4+ T cells and the generation of CD4+CD28null T cells (C). Furthermore, CD4+CD28null T cells mediate direct lysis of endothelial cells and possibly vascular smooth muscle cells by releasing cytolytic enzymes (perforin, granzymes) (D). Destruction of endothelial cells, vascular smooth muscle cells and of the extracellular matrix results in the destabilization and rupture of atherosclerotic plaques that causes acute coronary events (E).
cells and smooth muscle cells (SMCs) due to their cytotoxic function. The imbalance between the synthesis and degradation of extracellular matrix components like collagens is another mechanism that contributes to the destabilization of atherosclerotic plaques. IFN-γ, which is secreted in high levels by CD4⁺CD28null T cells, has been shown to increase the number of MHC class II molecules expressed by SMCs, enhancing the chronic inflammatory process in atherosclerosis. In addition, IFN-γ potently inhibits the collagen production by fibroblasts and the proliferation of SMCs. The combination of increased inflammation and decreased collagen synthesis compromises the plaque stability and could culminate in plaque rupture.

9. Modulation of CD4⁺CD28null T cells and new therapeutic approaches

As the CD4⁺CD28null T cell subset appears to have important contributions to the pathogenic mechanisms that underlie CAD, modulation of these cells could provide rational tools for patient management. Here, we summarize the effects of therapeutic agents in use in atherosclerosis and CAD on CD4⁺CD28null T cells.

Statins are potent inhibitors of cholesterol biosynthesis that have dramatically improved the management of ischaemic cardiovascular disease. Although most of the beneficial actions of these agents are due to their lipid lowering effects, some beneficial effects seem to be due to their anti-inflammatory and anti-thrombotic properties. Statins decrease the production of MMPs and pro-inflammatory cytokines from activated macrophages. Recent results suggest that statins reduce the expansion of CD4⁺CD28null T cells in UA patients. Patients with UA who were taking statins in addition to the standard anti-ischaemic treatment had significantly fewer circulating CD4⁺CD28null T cells in comparison to patients on anti-ischaemic medication alone. As the frequency of CD4⁺CD28null T cells is preferentially increased in UA in comparison with CSA, patients with unstable atherosclerotic lesions could benefit from tailored therapy with statins. Additional longitudinal studies are required to establish the best timing to initiate statin treatment in order to achieve long-lasting effects on the CD4⁺CD28null T cell subset and to prevent the recurrence of acute coronary episodes.

The pro-inflammatory cytokine TNF-α has pro-atherogenic effects in murine models of atherosclerosis. In healthy middle-aged men increased TNF-α levels correlate with the severity of early carotid atherosclerosis. Treatment with TNF-α antagonists has dramatically improved the clinical course and outcome of patients with RA. Patients with RA suffer from accelerated atherosclerosis and increased cardiovascular complications. TNF-α blockade was shown to improve the endothelial function, as assessed by FMD in these patients. Of note, TNF-α can drive the generation of CD4⁺CD28null T cells in vitro. Patients with CAD have increased levels of circulating TNF-α, and high levels of TNF-α associate with an increased frequency of CD4⁺CD28null T lymphocytes in UA. Therefore, the effect of selective blockade of TNF-α on CD4⁺CD28null T cells was investigated in patients with UA. The expression of CD28 by T lymphocytes increased significantly after ex vivo incubation of these cells with anti-TNF-α antibodies, suggesting that the expansion of CD4⁺CD28null T cells in patients with UA may be reduced by selective blockade of TNF-α. However, as these findings were generated using an ex vivo system, clinical studies are required to assess whether the in vitro findings are reproduced in patients.

Recent research has underscored the crucial role of regulatory T (Treg) cells in the maintenance of immune homeostasis and prevention of pathogenic responses. Naturally occurring CD4⁺CD25⁺ Treg cells are a subpopulation of lymphocytes that suppress the development and progression of disorders associated with chronic inflammation, like autoimmunity. Alterations in the number, phenotype, and function of Treg cells have been found in autoimmune diseases. Recently, the frequency and the function of Treg cells have been shown to be altered in CAD as well. Treg cells were significantly decreased in the peripheral blood of patients with ACS as compared with the patients with CSA and healthy controls. In addition, the suppressive function of Treg cells isolated from ACS patients was dramatically reduced. These results suggest that Treg cells have important roles in conferring protection against plaque destabilization. Little is known, however, about the relationship between Treg cells and CD4⁺CD28null T lymphocytes (Figure 1). A small proportion of normal healthy individuals have high frequencies of circulating CD4⁺CD28null T lymphocytes (between 5 and 13% instead of 0.1 and 2.5%) in the absence of any deleterious effects. This may indicate that if the function of Treg cells is normal, CD4⁺CD28null T cells are kept in check. Interestingly, CD4⁺CD28null T cells from patients with autoimmune disorders have been shown to be less capable of suppression in comparison to patients with ACS. As reported by Thewissen et al., CD4⁺CD25high Treg cells were not able to suppress the proliferation of CD4⁺CD28null T cells induced by stimulation with antibodies anti-CD3. However, CD4⁺CD25high Treg cells significantly suppressed the production of IFN-γ by CD4⁺CD28null T cells, although the suppressive effect was less prominent than that observed on CD4⁺CD28⁺ T cells. The decreased susceptibility of CD4⁺CD28null T cells to the suppressive effects of Treg cells may favour the uncontrolled expansion of CD28null T lymphocytes in autoimmune disorders and possibly in CAD. Data obtained from animal models of atherosclerosis indicate that Treg cells have strong inhibitory effects on both the initiation and progression phase of atherogenesis. Furthermore, cytokines like transforming growth factor-β (TGF-β) and IL-10 that have important atheroprotective roles are also involved in the maintenance and function of Treg cells. Therefore, disequilibrium between regulatory and pathogenic T cells is thought to be crucial for plaque development and destabilization. Work done in murine models of atherosclerosis indicated that induction of oral tolerance to antigens involved in atherogenesis such as oxLDL and HSP60 results in an increased number of Treg cells. The increased number of Treg cells correlated significantly with the reduction in size of arterial plaques. These results suggest that the activation and expansion of Treg cells in vivo may provide new therapeutic means for the treatment of atherosclerosis and CAD. However, as CD4⁺CD28null T cells appear to be unique to humans, these animal studies do not provide any information on therapies that augment Treg cells in atherosclerosis and their effects on the CD4⁺CD28null T cell subset.
10. Conclusions and future directions

CD4⁺CD28null T cells have been identified in various physiological and pathological situations such as aging, autoimmune, and cardiovascular diseases. Characterization of the phenotype and function of these cells revealed their complex contribution to the development of immune suppression in aged individuals and of tissue lesions in autoimmunity and CAD. However, the precise mechanisms that regulate the activation, expansion, and recruitment of CD4⁺CD28null T cells in CAD are far from being completely understood. Further prospective studies that assess the frequency of CD4⁺CD28null T cells in patients with stable and unstable coronary disease will help understand the temporal relationship between the expansion of the CD28null subset and the development of acute coronary events. Therapeutic trials employing statins or anti-TNF-α antibodies that have effects on CD4⁺CD28null T cells may provide useful data to assess the clinical implications of the modulation of this peculiar cell subset. Targeting CD4⁺CD28null T cells in addition to other inflammatory mediators in CAD may help prevent and improve the outcome of ACS.

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