Transplant International

REVIEW

Cardiovascular disease after transplantation: an emerging role of the immune system

Steven Van Laecke, Thomas Malfait, Eva Schepers & Wim Van Biesen

Renal Division, Ghent University Hospital, Ghent, Belgium

Correspondence
Steven Van Laecke, Renal Division, Ghent University Hospital, C. Heymanslaan 10, 9000 Gent, Belgium.
Tel.:+3293324509;
fax:+3293323847;
e-mail: steven.vanlaecke@ugent.be

SUMMARY

Cardiovascular disease (CVD) after transplantation remains a major concern. Little is known about what drives the increased cardiovascular risk in transplant recipients apart from traditional risk factors. The immune system is involved in the pathogenesis of hypertension, atherosclerosis, and coronary artery disease in the general population. Recently, inhibition of interleukin 1 − β by canakinumab versus placebo decreased the incidence of cardiovascular events. Emerging evidence points to a role of adaptive cellular immunity in the development of CVD. Especially, expansion of pro-inflammatory and antiapoptotic cytotoxic $CD4^{+}CD28^{null}$ T cells is closely associated with incident CVD in various study populations including transplant recipients. The association of cytomegalovirus exposure with increased cardiovascular mortality might be explained by its capacity to upregulate these cytotoxic cells. Also, humoral immunity seems to be relevant for cardiovascular outcome in transplant recipients. Panel-reactive antibodies at baseline and donor-specific antibodies are independently associated with poor cardiovascular outcome after kidney transplantation. Cardiovascular effects of immunosuppressive drugs and statins do not only imply indirect positive or negative effects on traditional cardiovascular risk factors but also intrinsic immunological effects. How immunosuppressive drugs modify atherosclerosis largely remains elusive.

Transplant International 2018; 31: 689–699

Key words
atherosclerosis, cardiovascular, immunosuppressive, mortality, transplantation, Treg

Received: 23 December 2017; Revision requested: 12 February 2018; Accepted: 22 March 2018; Published online: 30 April 2018

Introduction

Both innate and adaptive immunity are important in the pathophysiology of cardiovascular disease (CVD) [1]. Compelling evidence, mainly derived from animal models or autoimmune diseases, supports the notion that immunological alterations directly promote atherosclerosis [1]. Immunological disturbances may also indirectly contribute to CVD by causing or amplifying traditional risk factors such as hypertension and diabetes [2]. Considering the high cardiovascular burden after transplantation, the role of immunodeficiency and the variable effects of immunosuppressive drugs on particularly the T-cell repertoire deserve further attention [3].

The simplified concept of atherosclerosis as a process of lipid deposition with macrophage infiltration and formation of foam cells has been reformulated. We should acknowledge that these models most likely but not certainly are valid for transplant recipients. Briefly, dyslipidemia still plays a central role as trapped oxidized low-density lipoprotein (ox-LDL) cholesterol particles

doi:10.1111/tri.13160

in the subendothelium trigger an immune response by recruiting monocyte-derived macrophages and lymphocytes (Fig. 1) [1]. Macrophages in the plaque fuel the production of pro-inflammatory cytokines such as interleukin (IL)-6 via the formation of foam cells and the activation of vascular smooth muscle cells (VSMC) [1]. Dendritic cells (DC) guide the differentiation of T cells toward maturation, anergy, death, or a regulatory (Treg) phenotype [4]. The response and distribution of T cells are not only anchored to the innate immune response but also to the local cytokine environment [1]. Inflammation will favor differentiation of T cells into pro-inflammatory TH1 cells with increased expression of tumor necrosis factor (TNF)-α and interferon (IFN)-γ rather than into antiatherogenic TH2 or Tregs [1]. In the peripheral blood of patients with CVD, the proportion of reactive effector memory T cells is consistently increased [4]. T cells in atherosclerotic lesions of patients with coronary artery disease (CAD) are characterized by ongoing antigen-driven immune response with ox-LDL, apolipoprotein B100-derived, and other unraveled antigens acting as neoepitopes promoting the development of high-affinity antibodies, resulting in local inflammation [1,4]. Adaptive immunity is pivotal not only in plaque formation, with a role of especially $CD4^+$ rather than less abundant activated $CD8^+$ T cells but also in plaque instability and rupture [5]. $CD4^+$ T lymphocytes, especially $CD4^+CD28^{null}$ T cells, accumulate in unstable plaque and endothelial erosions, potentially triggering coronary events [1,4]. In patients with acute coronary syndrome (ACS), effector T cells have molecular fingerprinting of recent IFN-γ signaling [6]. Atherosclerosis shares features with inflammatory and autoimmune diseases, while both infection and autoimmunity promote vascular inflammation and endothelial dysfunction [4].

It seems likely that the same mechanisms are at play in solid organ transplant (SOT) recipients. Genotypes associated with increased or decreased local or systemic production of, respectively, TNF-α and IL-10, the most potent downregulator of pro-inflammatory cytokines, were associated with more CVD in kidney transplant recipients (KTR) after adjustment for confounders including C-reactive protein (CRP) [3]. Genetic susceptibility to CAD has been associated with variable immunological responses of vascular cells to cytokines such as IFN-γ [7,8]. CRP sensitizes endothelial cells to T-cell-mediated cytotoxicity *in vitro* and pretransplant CRP values are associated with cardiac events in KTR [9]. In this review, we will discuss emerging immunological concepts in the pathophysiology of atherosclerotic disease. We aim at integrating recent clinical findings in SOT into experimental models and will compare studies among various SOT recipients.

The role of the costimulatory pathway in atherosclerosis

Crucial in the immune response is the costimulatory signal by the contact between antigen-presenting cell (APC) CD80/86 ligands with T-cell adhesion molecules (particularly CD28). Of interest here is angiotensin II-mediated overexpression of CD80/CD86 on DC, promoting inflammation and atherosclerosis [2]. Inhibition of these triggered DC decreases the accumulation of macrophages and activated T lymphocytes into the plaques decreasing secretion of IFN-γ, an important mediator of plaque destabilization. Cytotoxic lymphocyte antigen-4 (CTLA-4), a CD28 homolog albeit with functional dichotomy, and the CTLA-4/IgG1 fusion protein abatacept both attenuate the inflammatory response and the accelerated formation of atherosclerosis in mice by high-affinity binding to and downregulation of the CD80/86 ligands [10,11]. This seems relevant for SOT as belatacept blocks the costimulatory pathway due to the interaction between CD28 and CD80/86 ligands. Tregs suppress the immune response partially by CTLA-4-dependent downregulation of these ligands [12]. Apolipoprotein E knockout (ApoE-/) mice typically overexpress CTLA-4 and develop less atherosclerosis than control breeds [13]. Also, infusion of CTLA-4/IgG improves angiotensin II-induced hypertension in rats [2]. CTLA-4 bears potential as a relevant target in the therapeutic approach of atherosclerosis while CTLA-4 blockade by checkpoint inhibitors such as ipilimumab seem cardiotoxic [14].

The role of adaptive T-cell immunity

$CD4^+CD28^{null}$ cells

Emerging data point to a causal role of these terminally differentiated effector T cells in the pathophysiology of atherosclerosis and ACS, of which half of the patients have an abnormal T-cell distribution [15]. In healthy humans, the concentration of $CD4^+CD28^{null}$ cells, a subset of TH1 cells and the main biological indicator of immunological aging, is very low [16]. Permanent loss of CD28 expression on $CD4^+$ cells is antigen-driven or occurs through aging [17]. Inflammatory conditions such as diabetes, rheumatological disorders, and chronic kidney disease (CKD) share, often in conjunction with a

Transplant International 2018; 31: 689–699

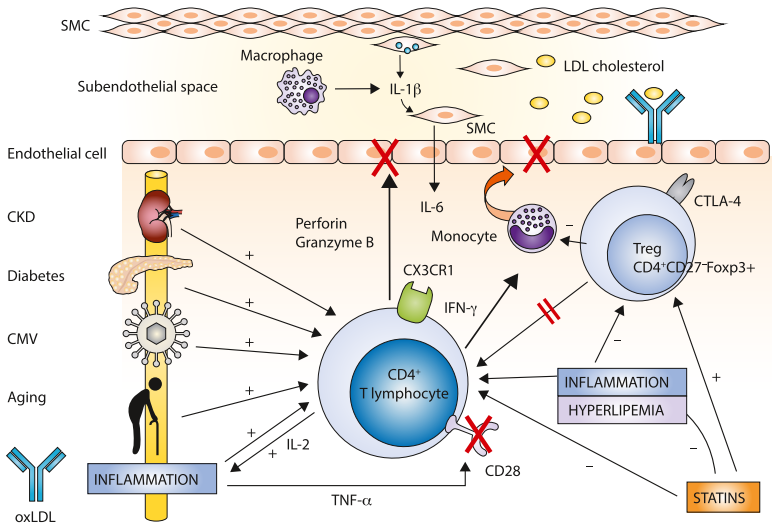

Figure 1 The role of adaptive immunity in cardiovascular disease after transplantation. Depicted is the imbalance between cardiotoxic $CD4^+CD28^{null}$ T cells which are dominant compared to cardioprotective Tregs both contributing to atherosclerosis and cardiovascular disease after transplantation. Subendothelial accumulation of LDL leads to recruitment of monocytes which transmigrate into the subendothelial space and differentiate into macrophages and foam cells which produce pro-inflammatory cytokines. SMC undergoes phenotypic changes during atherogenesis, proliferate, and migrate to atherosclerotic plaques. Macrophages and apoptotic VMSC produce IL-1β which activates endothelial cells. A central role for terminally differentiated $CD4^+CD28^{null}$ cells is likely as these cells are expanded in conditions as aging, diabetes, kidney dysfunction, and hyperlipemia which are prevalent in transplant recipients and are involved in atherosclerosis development and plaque destabilization. CMV infection and chronic rejection also contribute to the increase in these cells expressing endothelial homing receptors (CX3CR1) with endothelial tropism and pro-inflammatory characteristics perpetuating a positive loop. Hyperlipemia and inflammation have a bidirectional relationship, and antibodies against oxidized LDL (ox-LDL) are pathogenic. Ox-LDL and other antigens stimulate TH1 cells to secrete IFN-γ. Inflammation and hyperlipemia downregulate the anti-inflammatory regulatory T cells (Tregs) which counteract the effects of effector T cells. Statins induce expansion of Tregs and apoptosis of $CD4^+CD28^{null}$ cells but also indirectly affect both types of cells by their anti-inflammatory properties; CKD, chronic kidney disease; CMV, cytomegalovirus; CTLA-4, cytotoxic lymphocyte antigen-4; IFN-γ, interferon gamma; IL-1β, interleukin-1 beta; ox-LDL, oxidized low-density lipoprotein; SMC, smooth muscle cell; TNF-α, tumor necrosis factor alpha.

lower fraction of Tregs, an expansion of these cardiotoxic cells which are independently associated with all-cause, cardiovascular mortality, and/or cardiovascular events in various populations including patients with heart failure, unstable angina, and recurrent coronary events [18–20]. These plaque-destabilizing cells which catalyze inflammation are numerous in rupture-prone atherosclerotic plaques [18]. They share cytotoxic characteristics with $CD8^+$ T cells and natural killer (NK) cells as they produce cytolytic enzymes such as perforin and granzyme B and express the activating cytotoxicity receptor NKG2D, enabling them to kill endothelial cells and VSMC [21]. Also contributing to plaque destabilization they, unlike $CD4^+CD28^+$ T helper cells, express markers of endothelial homing such as the fractalkine receptor CX3C chemokine receptor 1 (CX3CR1) [21]. They are less resistant to pro-apoptotic effects of Tregs [15,16]. TNF-α, whose plasma concentration is associated with mortality in heart failure, downregulates CD28 expression on $CD4^+$ T lymphocytes and expands these cells, perpetuating a positive loop [22]. Traditional cardiovascular risk factors amplify $CD4^+CD28^{null}$ cells by inducing low-grade inflammation. In end-stage kidney disease (ESKD), their expansion was associated with preclinical atherosclerosis and increased arterial stiffness and cardiovascular mortality, while in patients with CKD stage 4 and 5, plasma $CD4^+CD28^{null}$ cells independently correlated with intima-media thickness (IMT) [23–25]. These cardiotoxic cells are of potential interest after transplantation. In KTR and especially those with pre-existing atherosclerotic disease, high levels of $CD4^+CD28^{null}$ cells at the time of transplantation predispose to atherosclerotic events the first year after transplantation, suggesting a role in plaque destabilization [26]. $CD4^+CD28^{null}$ cells tend to rise after kidney transplantation especially in conjunction with episodes of inflammation and in patients with delayed graft function and poorer kidney function or in patients with chronic rejection [21]. These cells are however not alloreactive in the absence of IL-15 explaining the absence of an association with acute rejection in KTR [27,28]. Also, $CD4^+CD28^{null}$ cells are higher in patients

with previous cytomegalovirus (CMV) exposure across different study populations [21,28,29], potentially explaining the exaggerated risk of CVD in CMV-seropositive transplant recipients [30]. Not unexpectedly $CD28^{null}$ effector T cells are not affected by the CD28 costimulation blocker belatacept. Also, tacrolimus and everolimus fail to suppress these cells *in vitro*, whereas mycophenolate mofetil (MMF) or corticosteroids decrease their proliferation [31]. Costimulatory blockade with abatacept decreased their concentration in patients with rheumatoid arthritis, most likely through its anti-inflammatory properties [32]. Other potentially cardioprotective drugs associated with lower counts of $CD4^{+}CD28^{null}$ cells are metformin and TNF-α blockers [16]. It remains uncertain whether the latter might modify CVD in patients with rheumatic disorders or inflammatory bowel disease [32]. In a nonrandomized controlled trial, patients with psoriasis treated with TNF-α blockers had a slower progression of coronary artery calcification [33]. At present, however, randomized controlled trials (RCTs) evaluating the effect of these drugs on $CD4^{+}CD28^{null}$ cells or Tregs and the ensuing association with cardiovascular events are lacking. By suppressing inflammation and the generation of cardiotoxic $CD4^{+}CD28^{null}$, anti-inflammatory drugs could decrease cardiovascular morbidity and mortality. In patients with previous myocardial infarction, inhibition of IL-1β by canakinumab versus placebo decreased recurrent cardiovascular events in line with a decrement of CRP albeit at the expense of more fatal infections [34].

Statins, which have pleiotropic immunomodulatory and anti-inflammatory effects, may reduce cardiovascular events in KTR [35]. Next to significant lipid lowering, they induce expansion of Tregs and apoptosis of $CD4^{+}CD28^{null}$ cells [16,36–38]. The latter might explain a significantly lower percentage of these cardiotoxic cells in patients with ACS with versus without statins [39]. In apparently healthy people without hyperlipemia but with elevated CRP, rosuvastatin decreased major cardiovascular events [40].

Regulatory T cells (CD4 $CD25^{+}Foxp3^{+}$) cells (Tregs)

These atheroprotective cells suppress several immune cells involved in atherosclerosis such as B cells, monocytes, and DC [4,12]. In patients with CAD and especially ACS, they are less common and dysfunctional [12]. Tregs are a major source of the antiatherogenic cytokines IL-10 and TGF-β contributing to powerful suppression of atherosclerosis in murine models [4,12]. Tregs influence cholesterol metabolism while oxidized LDL depletes Tregs *in vitro* [12]. Hyperlipidemic atherosclerosis-prone mice systematically harbor less Tregs than wild-type mice. Depletion of forkhead box P3 $(FOXP3)^{+}$ Tregs in murine models promotes atherosclerosis by modulating lipoprotein metabolism illustrating the bidirectionality between lipid metabolism and inflammation [41]. Also, engagement of CTL4-A on Tregs contributes to its anti-inflammatory profile [13]. In humans, their role in the development of CVD is less outlined. Their concentration and suppressive capacities are consistently lower in patients with ACS [15]. Considering the inhibitory role of Tregs not only in initiation of atherosclerosis but also in stabilization of established lesions, Tregs are currently being considered as treatment for atherosclerosis in the general population [12]. So far, data coupling kinetics of Tregs and cardiovascular outcome in SOT recipients are lacking. Individual effects of immunosuppressive drugs on Treg proliferation might be clinically relevant and especially considering future trials will engage the infusion of Tregs alongside concurrent immunosuppressive drugs. Tacrolimus, mycophenolate mofetil (MMF), and corticosteroids but not sirolimus reduce viability and proliferative capacity of *in vitro* expanded human Tregs [42]. Although the *in vivo* effects of these drugs on Tregs are not always consistent, mammalian target of rapamycin (mTOR) inhibitors mostly has beneficial effects, while CNI impairs their proliferation and function [43,44]. Effects of MMF and corticosteroids on Tregs are inconsistent and generally show less or absence of inhibition of Tregs, while corticosteroids even increase Tregs in autoimmune disease [45]. Conversion from CNI to MMF monotherapy in liver transplant recipients overturned the inhibitory effects of CNI on circulating Tregs [46]. Belatacept was associated with lower amounts of functionally impaired Tregs in KTR [47,48].

Studies on circulating T-cell subpopulations have methodological caveats, and so variable concentrations should be interpreted cautiously. It is uncertain whether findings in rodents are generalizable to humans [4]. Another limitation particularly in transplant recipients is confounding by kidney dysfunction. In patients with kidney allograft dysfunction, serum concentrations of pro-inflammatory cytokines such as IL-2, IL-17, and IFN-ɣ were higher than in subjects with stable kidney function in whom there was a higher expression of IL-4, IL-10, and Tregs [49]. Therefore, negative outcome in hyperproducers of pro-inflammatory cytokines can be confounded by graft outcome. Instead, chronic

inflammation due to rejection can contribute to atherosclerosis.

The role of humoral immunity

B2 cells aggravate atherogenesis through antibody-independent mechanisms that augment the action of pro-inflammatory cytokines [4]. Emerging data demonstrate that antibodies play an active role. A pathophysiological role for IgE in the development of atherosclerosis was suggested by both clinical and experimental data [50]. Antibodies against oxidized LDL enhance uptake of cholesterol and promote atherosclerosis [1]. Transcripts of endothelial stress disclose the endothelium as target for cellular immunity after transplantation, and non-HLA antibodies also disrupt endothelial homeostasis [51]. The role of humoral auto- and alloimmunity in the pathophysiology of CVD until recently was not well outlined [1]. In epidemiological studies, multiparity, and especially with different fathers, is associated with increased cardiovascular mortality, while blood transfusion from women with a history of pregnancy was associated with an increased mortality in male acceptors, providing indirect evidence for a role of alloimmunity [52,53]. Allogenic hematopoietic stem cell transplant recipients with versus without pretransplant HLA antibodies had more vascular complications [54]. Following KTR registry data, panel-reactive antibodies (PRA) at the time of transplantation were dose-dependently associated with cardiovascular mortality [55]. The analysis could support the causal paradigm that anti-HLA antibodies induce both a local and general pro-inflammatory state leading to endothelial injury. HLA class II antibodies alter the endothelial expression of the anti-inflammatory and antithrombotic glycoprotein thrombomodulin and lower its serum concentration in KTR [56]. Studies indirectly suggest a pro-atherogenic potential of HLA antibodies. Loupy and coworkers demonstrated that DSA in KTR were dose-dependently associated with increased cardiovascular mortality and cardiac events [57]. Endothelial damage by HLA antibodies or ischemia may lead to a pro-inflammatory phenotype and *de novo* expression of endothelial neoantigens contributing to immune activation and vascular remodeling [58]. In systemic lupus erythematosus, autoantibodies promote atherosclerosis and cardiac events. However, the presence of HLA and non-HLA antibodies rather than being causative may also reflect activation of adaptive immunity.

Nonrenal solid organ transplant recipients

Heart

Cardiac allograft vasculopathy (CAV) is mediated by both immune-mediated and nonimmune mechanisms. It is distinct of CAD and primarily affects the epicardial and intramural arteries presenting with concentric intimal layer thickening. Apart from cellular mechanisms [59], HLA antibodies recently were demonstrated to correlate with the later development of CAV but also with increased endothelial activation and IFN-ɣ transcripts on microarray analysis [60,61]. These alterations mimic the changes in developing CAD. Additionally, antibodies against cardiac myosin and vimentin are relevant for the development of rejection and vasculopathy [62]. Next to traditional cardiovascular risk factors, CAV is also associated with elevated CRP as proxy of increased inflammation and previous CMV infection [62,63]. In HTR, statins are the standard of care treatment to prevent the progression of CAV and decrease mortality [63–66]. Statins have immunomodulatory effects next to lipid lowering and decrease cytotoxicity of NK cells, and IFN-ɣ induction of MHC class molecules, possibly contributing to a lower rejection rate [63,64]. They inhibit the costimulatory pathway and chemotaxis of lymphocytes and macrophages, while inducing apoptosis of $CD4^{+}CD28^{null}$ cells [16,36–38]. Data on cardiotoxic $CD4^{+}CD28^{null}$ cells as far as we know are however lacking in HTR. In murine models, expansion of Tregs was demonstrated to improve cardiac graft survival [67].

Liver

The potential role of $CD4^{+}CD28^{null}$ cells or Tregs in the pathophysiology of CVD in liver transplant recipients largely remains elusive. Non-HLA autoantibody, preformed and especially high MFI, IgG3, or C1q-positive HLA DSA are associated with an increased mortality risk after liver transplantation [68–71]. It remains unclear whether the increased mortality is largely attributable to a higher rejection risk. Liver transplant recipients have a pro-atherogenic lipoprotein profile with higher IFN-ɣ and vascular cell adhesion molecules and lower protective serum IL-10 concentrations than controls the first year after transplantation [72,73]. A higher pretransplant and 12-month CRP concentration are associated with more cardiovascular events after transplantation [74].

Lung

Class I and class II post-transplant DSA are both associated with mortality after lung transplantation although cardiovascular death was unspecified or neglectable [75–77]. Pretransplant DSA are variably associated with overall mortality [78,79]. Of potential interest, bronchiolitis obliterans syndrome (BOS) is associated with increased peripheral blood NK, pro-inflammatory TH1 cells, and $CD4^+CD28^{null}$ cells with increased expression of TNF-α and IFN-γ [80,81], mediators of atherosclerosis. Statins inhibited T-cell alloimmune activation and decreased the production of pro-inflammatory cytokines inhibiting the development of BOS in rats [82]. The role of especially cellular immunity in the pathophysiology of CVD after lung transplantation remains yet unexplored.

Cytomegalovirus

Prior CMV infection is associated with an increased risk of cardiovascular death in transplant recipients, patients with ESKD, rheumatic disorders, and in healthy elderly people [23,30,83,84]. CMV infection in KTR is associated with a higher risk of arrhythmia, heart failure, and coronary stenosis [85], while it increases the risk of CAV in HTR [86,87]. KTR with post-transplant CMV replication developed more atherosclerotic events [88]. In patients with HIV, CMV IgG titers correlated with subclinical atherosclerosis [89].

Cytomegalovirus infection and its pro-inflammatory effects potentially modify atherosclerosis [84]. CMV replication upregulates production of MHC II antigens and pro-inflammatory cytokines causing endothelial damage [90]. CMV-specific $CD4^+$ T cells have enhanced dual expression of TNF-α and IFN-γ together with CX3CR1 directing them to activated endothelium [91].

Cytomegalovirus leaves a footprint of an aging T-cell repertoire as more $CD4^+$ and $CD8^+$ memory T cells in people with CMV infection have senescent-like characteristics [83]. CMV infection increases the fraction of cytotoxic $CD4^+CD28^{null}$ cells among different study populations [32]. Episodes of inflammation accrue the expansion of these cells. Endothelium is the key site of CMV latency and reactivation. Transplant recipients, especially those treated with ATG, seem more prone to these negative indirect effects of CMV with negative effects on atherosclerosis progression and cardiovascular outcome [92].

Cytomegalovirus prophylaxis was associated with a lower risk of cardiovascular death in KTR [93]. HTR receiving ganciclovir versus placebo had a lower incidence of CAV [94], while HTR receiving pre-emptive treatment versus prophylaxis had a faster progression [95]. In KTR, it remains yet unestablished whether CMV prophylaxis compared to the pre-emptive approach translates into a lower incidence of CVD [93].

Immunosuppressive drugs

The majority of the immunosuppressive drugs worsen traditional cardiovascular risk conditions including weight gain (corticosteroids), hyperlipemia (CNI, corticosteroids and mTOR inhibitors), glycemic control (CNI, corticosteroids and mTOR inhibitors), and hypertension (corticosteroids and CNI) [96]. The combination of different drugs of variable exposure hampers the interpretation of cardiovascular effects. Withdrawal of pro-atherogenic corticosteroids is not associated with lower mortality in KTR [97]. The interpretation can be flawed by a higher incidence of rejection necessitating boluses of corticosteroids and/or higher exposure to CNI. In the general population, corticosteroid use is associated with CVD [98,99] although the association with incident CAD seems modest and with cerebrovascular disease paradoxically negative [99].

Pro-atherogenic drug effects possibly are attenuated by beneficial effects on vascular inflammation.

Abatacept and belatacept

In animal models, modulation of costimulatory and coinhibitory signals has beneficial cardiovascular effects. Abatacept decreases $CD4^+CD28^{null}$ cells in patients with rheumatoid arthritis [32]. In a murine model of heart failure, this beneficial effect on cardiac dysfunction depended upon induction of the anti-inflammatory IL-10 [100]. In humans, it was associated with a decreased risk of myocardial infarction [33]. In KTR, belatacept was associated with a lower incidence of DSA versus cyclosporine, which coincided with a lower incidence of cardiac events and overall mortality [101].

Rituximab

Adding up to theoretical benefits of corticosteroid avoidance, this drug improved endothelial dysfunction and IMT in patients with rheumatoid arthritis [102]. Paradoxically, it was associated with acute coronary events early after administration attributed to an increase in cytokines including TNF-α in a subset of these patients and increased cardiovascular mortality the

Transplant International 2018; 31: 689–699

first 3 years after kidney transplantation [103,104]. This could mirror previous findings demonstrating an excessive increase in cellular rejection early after kidney transplantation in accordance with a rise of cytokines [105]. Moreover, rituximab accelerated CAV without a higher incidence of treated rejection in a recent placebo-controlled RCT in unsensitized HTR [106]. Rituximab can possibly increase autoimmunity by decreasing immunoregulatory B cells. Rituximab-specific IgE antibodies and TH2 suggestive of type 1 hypersensitivity have been demonstrated [107]. Other monoclonal antibodies such as bevacizumab and trastuzumab were also associated with cardiac events possibly due to autoimmunity [107]. This conundrum highlights that the role of B cells in allo- and autoimmunity is complex, not always unambiguous and sometimes modified by unrecognized conditions.

Polyclonal induction

B-cell-depleting antibodies and neutralization of B-cell-activating factor (BAFF) were atheroprotective in mice [4,108]. The situation seems more complex for antithymocyte globulin (ATG), which was associated with pro-apoptotic effects and a longitudinal decrease in $CD4^{+}CD28^{null}$ cells in KTR [109], opposing studies demonstrating an increase in parallel with a rise of cardiovascular mortality [92,110]. ATG accelerates immunosenescence, especially in patients with CMV infection [92,110]. ATG also at least temporarily expands peripheral Tregs [111]. Induction with ATG versus basiliximab was associated with a lower incidence of DSA in SOT [112]. Prolonged CD4 lymphopenia, possibly following ATG treatment, has been associated with atherosclerosis progression and cardiovascular death in other populations [109]. In HTR, ATG attenuated plaque progression the first year after transplantation [113].

Calcineurin inhibitors

Calcineurin inhibitors (CNI) have pro-atherogenic properties primarily because of effects on traditional cardiovascular risk factors [96]. They promote vascular inflammation in murine models by increasing cytokine production through upregulation of TLR4 signaling [114]. CNI impairs Treg proliferation and function after liver and kidney transplantation [43,44]. Treatment with tacrolimus versus cyclosporine is associated with less DSA formation [112]. In a RCT, withdrawal of CNI in KTR did not improve carotid IMT despite better blood pressure control [115]. CNI withdrawal in KTR was not associated with decreased mortality [116].

Mycophenolate

In analogy with belatacept, MMF has no effect on traditional cardiovascular risk factors. In a RCT in patients with symptomatic carotid stenosis, a short course of MMF attenuated plaque inflammation, decreased pro-inflammatory gene expression, and increased circulating Tregs [117]. MMF was associated with lower CRP concentrations in KTR [118]. In liver transplant recipients, use of MMF was associated with a lower cardiovascular mortality [119]. HTR randomized to MMF versus azathioprine has slower 1-year progression of intimal thickening [120].

mTOR inhibitors

Despite unfavorable metabolic effects, mTOR inhibitors have antiproliferative and cardioprotective properties. HTR allocated to everolimus versus MMF had beneficial effects on intima proliferation at months twelve [121]. CNI avoidance strategies demonstrated beneficial effects on CAV progression, likely due to inhibitory effects on endothelial cells and fibroblasts or by reduction in CMV infection [122]. Also, in parallel with less CAV progression, there was a greater decline of soluble TNFR-1, in HTR allocated to everolimus versus CNI [123].

In KTR, data are less convincing despite beneficial effects of mTOR inhibitors on cardiac hypertrophy [124]. Cardiovascular outcome or mortality is not lower compared to CNI [116]. Mostly short-term trials focusing on renal endpoints are not powered to detect differences in cardiovascular outcome. Registry data even demonstrated an increased overall mortality in KTR with an mTOR inhibitor-based regimen [125]. In a meta-analysis of individual patient data, KTR on mTOR inhibitors had a higher incidence of CVD although death-censored graft survival was not different [126]. Interestingly, conversion of KTR from tacrolimus to sirolimus not only increased Tregs but also paradoxically increased indirect donor T-cell reactivity activating pro-inflammatory genes, in line with *in vivo* and *in vitro* studies in mice [127,128]. It is unclear to what extent drug-induced hyperlipidemia can counteract cardioprotective properties. Early (<12 months) conversion from CNI to mTOR inhibitors was associated with a higher incidence of DSA [129].

Conclusion and future prospects

Mounting evidence points to a crucial role of cellular and humoral immunity in the pathogenesis of CVD and mortality in the general population. This is supported by interventional studies demonstrating beneficial effects of anti-inflammatory drugs on both surrogate outcomes and cardiovascular events. In SOT, the situation is more complex as potentially protective effects of immunosuppressive drugs are being offset by pro-atherogenic drug effects. Potentially, relevant biomarkers are longitudinal measurement of circulating cardiotoxic $CD4^{+}CD28^{null}$ T cells and atheroprotective Tregs which participate in plaque formation and destabilization and whose respectively increased and decreased concentration is associated with active inflammation in atherosclerosis. Also, the causal role of alloantibodies (including DSA), autoantibodies, and of genetic traits in donor and acceptor should be further explored. Possibly, integration of these and other markers can further contribute to the individualization of immunosuppression after transplantation in the era of personalized medicine.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no conflicts of interest.

REFERENCES

1. Gistera A, Hansson GK. The immunology of atherosclerosis. *Nat Rev Nephrol* 2017; **13**: 368.
2. Rodriguez-Iturbe B, Pons H, Johnson RJ. Role of the immune system in hypertension. *Physiol Rev* 2017; **97**: 1127.
3. La Manna G, Cappuccilli ML, Cianciolo G, *et al.* Cardiovascular disease in kidney transplant recipients: the prognostic value of inflammatory cytokine genotypes. *Transplantation* 2010; **89**: 1001.
4. Ammirati E, Moroni F, Magnoni M, Camici PG. The role of T and B cells in human atherosclerosis and atherothrombosis. *Clin Exp Immunol* 2015; **179**: 173.
5. Ducloux D, Bamoulid J, Crepin T, Rebibou JM, Courivaud C, Saas P. Post-transplant immune activation: innocent bystander or insidious culprit of post-transplant accelerated atherosclerosis. *Cell Transplant* 2017; **26**: 1601.
6. Liuzzo G, Vallejo AN, Kopecky SL, *et al.* Molecular fingerprint of interferon-gamma signaling in unstable angina. *Circulation* 2001; **103**: 1509.
7. Melk A, Schildhorn C, Hömme M, *et al.* Association of single nucleotide polymorphisms on chromosome 9p21.3 with cardiovascular death in kidney transplant recipients. *Transplantation* 2013; **95**: 928.
8. Harismendy O, Notani D, Song X, *et al.* 9p21 DNA variants associated with coronary artery disease impairs interferon-γ signaling response. *Nature* 2011; **470**: 264.
9. Krüger B, Walberer A, Debler J, *et al.* Is inflammation prior to transplantation predictive for cardiovascular and renal outcomes? *Atherosclerosis* 2010; **210**: 637.
10. Gartner D, Jeffery LE, Sansom DM. Understanding the CD28/CTLA-4 (CD152) pathway and its implications for costimulatory blockade. *Am J Transplant* 2014; **14**: 1985.
11. Ewing MM, Karper JC, Abdul S, *et al.* T-cell co-stimulation by CD28-CD80/86 and its negative regulator CTLA-4 strongly influence accelerated atherosclerosis development. *Int J Cardiol* 2013; **168**: 1965.
12. Foks AC, Lichtman AH, Kuiper J. Treating atherosclerosis with regulatory T cells. *Arterioscler Thromb Vasc Biol* 2015; **35**: 280.
13. Matsumoto T, Sasaki N, Yamashita T, *et al.* Overexpression of cytotoxic T-lymphocyte-associated antigen-4 prevents atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 2016; **36**: 1141.
14. Varricchi G, Marone G, Mercurio V, Galdiero MR, Bonaduce D, Tocchetti CG. Immune checkpoint inhibitors and cardiac toxicity: an emerging issue. *Curr Med Chem* 2017; https://doi.org/10.2174/0929867324666170407125017. [Epub ahead of print]
15. Flego D, Liuzzo G, Weyand CM, Crea F. Adaptive immunity dysregulation in acute coronary syndromes. *J Am Coll Cardiol* 2016; **68**: 2107.
16. Dumitriu E. The life (and death) of CD4+ CD28(null) T cells in inflammatory diseases. *Immunology* 2015; **146**: 185.
17. Suarez-Alvarez B, Rodríguez RM, Schlangen K, *et al.* Phenotypic characteristics of aged CD4+ CD28null T lymphocytes are determined by changes in the whole-genome DNA methylation pattern. *Ageing Cell* 2017; **16**: 293.
18. Liuzzo G, Biasucci LM, Trotta G, *et al.* Unusual $CD4^{+}$ $CD28^{null}$ T lymphocytes and recurrence of acute coronary events. *J Am Coll Cardiol* 2007; **50**: 1450.
19. Koller L, Richter B, Goliasch G, *et al.* CD4+CD28null cells are an independent predictor of mortality in patients with heart failure. *Atherosclerosis* 2013; **230**: 414.
20. Sulzgruber P, Koller L, Winter MP, *et al.* The impact of CD4+CD28null T-lymphocytes on atrial fibrillation and mortality in patients with chronic heart failure. *Thromb Haemostasis* 2017; **117**: 349.
21. Shabir S, Smith H, Kaul B, *et al.* Cytomegalovirus-associated CD4+ CD28 null cells in NKG2D-dependent glomerular endothelial injury and kidney allograft dysfunction. *Am J Transplant* 2016; **16**: 1113.
22. Bagley J, Yuan J, Chandrakar A, Iacomini J. Hyperlipidemia alters regulatory T cell function and promotes resistance to tolerance induction through costimulatory molecule blockade. *Am J Transplant* 2015; **15**: 2324.
23. Betjes MG, de Wit EE, Weimar W, Litjens NH. Circulating pro-inflammatory CD4posCD28null T cells are independently associated with cardiovascular disease in ESRD patients. *Nephrol Dial Transplant* 2010; **25**: 3640.

24. Sun Z, Ye H, Tang B, *et al.* Prevalence of circulating CD4+CD28null T cells is associated with early atherosclerotic damage in patients with end-stage renal disease undergoing hemodialysis. *Hum Immunol* 2013; **74**: 6.
25. Yadav A, Lal A, Jha V. Cytotoxic CD4+CD28null T lymphocytes, systemic inflammation and atherosclerotic risk in patients with chronic kidney disease. *Nephron Clin Pract* 2012; **120**: 185.
26. Betjes MG, Weimar W, Litjens NH. Circulation CD4+CD28null T cells may increase the risk of an atherosclerotic vascular event shortly after kidney transplantation. *J Transplant* 2013; **2013**: 841430.
27. Dedeoglu B, Meijers RW, Klepper M, *et al.* Loss of CD28 on peripheral T cells decreases the risk for early acute rejection after kidney transplantation. *PLoS One* 2016; **11**: e0150826.
28. Dedeoglu B, Litjens NHR, Klepper M, *et al.* CD4+ CD2null T cells are not alloreactive unless stimulated by interleukin-15. *Am J Transplant* 2018; **18**: 341.
29. Slot MC, Kroon AA, Damoiseaux JGMC, *et al.* CD4+CD28null T Cells are related to previous cytomegalovirus infection but not to accelerated atherosclerosis in ANCA-associated vasculitis. *Rheumatol Int* 2017; **37**: 791.
30. Desai R, Collett D, Watson CJ, Johnson PJ, Moss P, Neuberger J. Impact of cytomegalovirus on long-term mortality and cancer risk after organ transplantation. *Transplantation* 2015; **99**: 1989.
31. Demmers M, 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.
32. Broadley I, Pera A, Morrow G, Davies KA, Kern F. Expansions of cytotoxic CD4+ CD28- T cells drive excess cardiovascular mortality in rheumatoid arthritis and other chronic inflammatory conditions and are triggered by CMV infection. *Front Immunol* 2017; **8**: 195.
33. Hjuler KF, Bøttcher M, Vestergaard C, Bøtker HE, Iversen L, Kragballe K. Association between changes in coronary artery disease progression and treatment with biologic agents for severe psoriasis. *JAMA Dermatol* 2016; **152**: 1114.
34. Ridker PM, Everett BM, Thuren T, *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017; **377**: 1119.
35. Palmer SC, Navaneethan SD, Craig JC, *et al.* HMG CoA reductase inhibitors (statins) for kidney transplant recipients. *Cochrane Database Syst Rev* 2014; **28**: CD005019.
36. Forero-Pena DA, Gutierrez FR. Statins as modulators of regulatory T-cell biology. *Mediators Inflamm* 2013; **2013**: 167086.
37. Link A, Selejan S, Hewera L, Walter F, Nickenig G, Böhm M. Rosuvastatin induces apoptosis in CD4(+)CD28 (null) T cells in patients with acute coronary syndromes. *Clin Res Cardiol* 2011; **100**: 147.
38. Raemer PC, Kohl K, Watzl C. Statins inhibit NK-cell cytotoxicity by interfering with LFA-1-mediated conjugate formation. *Eur J Immunol* 2009; **39**: 1456.
39. Giubilato S, Liuzzo G, Brugaletta S, *et al.* Expansion of CD4+CD28null T-lymphocytes in diabetic patients: exploring new pathogenetic mechanisms of increased cardiovascular risk in diabetes mellitus. *Eur Heart J* 2011; **32**: 1214.
40. Ridker PM, Danielson E, Fonseca FA, *et al.* Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008; **359**: 2195.
41. Klingenberg R, Gerdes N, Badeau RM, *et al.* Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. *J Clin Invest* 2013; **123**: 1323.
42. Scotta C, Fanelli G, Hoong SJ, *et al.* Impact of immunosuppressive drugs on the therapeutic efficacy of ex vivo expanded human regulatory T cells. *Haematologica* 2016; **101**: 91.
43. Miroux X, Morales O, Ghazal K, *et al.* In vitro effects of cyclosporine A and tacrolimus on regulatory T-cell proliferation and function. *Transplantation* 2012; **94**: 123.
44. Akimova T, Kamath BM, Goebel JW, *et al.* Differing effects of rapamycin or calcineurin inhibitor on T-regulatory cells in pediatric liver and kidney transplant recipients. *Am J Transplant* 2012; **12**: 3449.
45. Karagiannidis C, Akdis M, Holopainen P, *et al.* Glucocorticosteroids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004; **114**: 1425.
46. Demirkiran A, Sewgobind VD, van der Weijde J, *et al.* Conversion from calcineurin inhibitor to mycophenolate mofetil-based immunosuppression changes the frequency and phenotype of CD4+FOXP3+ regulatory T cells. *Transplantation* 2009; **87**: 1062.
47. Alvarez Salazar EK, Cortés-Hernández A, Alemán-Muench GR, *et al.* Methylation of FOXP3 TSDR underlies the impaired suppressive function of Tregs from long-term belatacept-treated kidney transplant patients. *Front Immunol* 2017; **8**: 219.
48. Levitsky J, Miller J, Huang X, Chandrasekaran D, Chen L, Mathew JM. Inhibitory effects of belatacept on allospecific regulatory T-cell generation in humans. *Transplantation* 2013; **96**: 689.
49. Tsaur I, Gasser M, Aviles B, *et al.* Donor antigen-specific regulatory T-cell function affects outcome in kidney transplant recipients. *Kidney Int* 2011; **79**: 1005.
50. Wang J, Cheng X, Xiang MX, *et al.* IgE stimulates human and mouse arterial cell apoptosis and cytokine expression and promotes atherogenesis in Apoe-/- mice. *J Clin Invest* 2011; **121**: 3564.
51. Dragun D, Catar R, Philippe A. Non-HLA antibodies against endothelial targets bridging allo- and autoimmunity. *Kidney Int* 2016; **90**: 280.
52. Lv H, Wu H, Yin J, Qian J, Ge J. Parity and cardiovascular disease mortality: a dose-response meta-analysis of cohort studies. *Sci Rep* 2015; **5**: 13411.
53. Caram-Deelder C, Kreuger AL, Evers D, *et al.* Association of blood transfusion from female donors with and without a history of pregnancy with mortality among male and female transfusion recipients. *JAMA* 2017; **318**: 1471.
54. Detrait M, Dubois V, Sobh M, *et al.* Impact of anti-HLA antibodies on allogeneic hematopoietic stem cell transplantation outcomes after reduced-intensity conditioning regimens. *Exp Hematol* 2012; **40**: 792.
55. Sapir-Pichhadze R, Tinckam KJ, Laupacis A, *et al.* Immune sensitization and mortality in wait-listed kidney transplant candidates. *J Am Soc Nephrol* 2016; **27**: 570.
56. Beland S, Vallin P, Désy O, Lévesque E, De Serres SA. Effects of alloantibodies to human leukocyte antigen on endothelial expression and serum levels of thrombomodulin. *J Thromb Haemostasis* 2017; **15**: 1020.
57. Loupy A, Vernerey D, Viglietti D, *et al.* Determinants and outcomes of accelerated arteriosclerosis: major impact of circulating antibodies. *Circ Res* 2015; **117**: 470.
58. Lion J, Taflin C, Cross AR, *et al.* HLA class II antibody activation of endothelial cells promotes Th17 and disrupts regulatory T lymphocyte expansion. *Am J Transplant* 2016; **16**: 1408.
59. Kobashigawa JA, Miller L, Yeung A, *et al.* Does acute rejection correlate

with the development of transplant coronary artery disease? A multicenter study using intravascular ultrasound. Sandoz/CVIS investigators. *J Heart Lung Transplant* 1995; **14**: S221.
60. Loupy A, Duong Van Huyen JP, Hidalgo L, *et al.* Gene expression profiling for the identification and classification of antibody-mediated heart rejection. *Circulation* 2017; **135**: 917.
61. Tran A, Fixler D, Huang R, Meza T, Lacelle C, Das BB. Donor-specific HLA alloantibodies: impact on cardiac allograft vasculopathy, rejection, and survival after pediatric heart transplantation. *J Heart Lung Transplant* 2016; **35**: 87.
62. Raichlin ER, McConnell JP, Lerman A, *et al.* Systemic inflammation and metabolic syndrome in cardiac allograft vasculopathy. *J Heart Lung Transplant* 2007; **26**: 826.
63. Sieg A, Weeks P, Krustchinsky L, Rajapreyar I. Statin therapy in cardiac allograft vasculopathy progression in heart transplant patients: does potency matter? *Transplant Rev* 2016; **30**: 178.
64. Kobashigawa JA, Katznelson S, Laks H, *et al.* Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med* 1995; **333**: 621.
65. Vallakati A, Reddy S, Dunlap ME, Taylor DO. Impact of statin use after heart transplantation: a meta-analysis. *Circ Heart Fail* 2016; **9**: e003265.
66. Wu AH, Ballantyne CM, Short BC, *et al.* Statin use and risks of death or fatal rejection in the Heart Transplant Lipid Registry. *Am J Cardiol* 2005; **95**: 367.
67. Wolf D, Schreiber TH, Tryphonopoulos P, *et al.* Tregs expanded in vivo by TNFRSF25 agonists promote cardiac allograft survival. *Transplantation* 2012; **94**: 569.
68. O'Leary JG, Demetris AJ, Philippe A, *et al.* Non-HLA antibodies impact on C4d staining, stellate cell activation and fibrosis in liver allografts. *Transplantation* 2017; **101**: 2399.
69. McCaughan JA, Robertson V, Falconer SJ, Cryer C, Turner DM, Oniscu GC. Preformed donor-specific HLA antibodies are associated with increased risk of early mortality after liver transplantation. *Clin Transplant* 2016; **30**: 1538.
70. O'Leary JG, Gebel HM, Ruiz R, *et al.* Class II alloantibody and mortality in simultaneous liver-kidney transplantation. *Am J Transplant* 2013; **13**: 954.
71. O'Leary JG, Kaneku H, Banuelos N, Jennings LW, Klintmalm GB, Terasaki PI. Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. *Am J Transplant* 2015; **15**: 1003.
72. Chhatrala R, Siddiqui MB, Stravitz RT, *et al.* Evolution of serum atherogenic risk in liver transplant recipients: role of lipoproteins and metabolic and inflammatory markers. *Liver Transpl* 2015; **21**: 623.
73. Alvares-da-Silva MR, de Oliveira CP, Stefano JT, *et al.* Pro-atherosclerotic markers and cardiovascular risk factors one year after liver transplantation. *World J Gastroenterol* 2014; **20**: 8667.
74. Watt KD, Fan C, Therneau T, Heimbach JK, Seaberg EC, Charlton MR. Serum adipokine and inflammatory markers before and after liver transplantation in recipients with major cardiovascular events. *Liver Transpl* 2014; **20**: 791.
75. Snyder LD, Wang Z, Chen DF, *et al.* Implications for human leukocyte antigen antibodies after lung transplantation: a 10-year experience in 441 patients. *Chest* 2013; **144**: 226.
76. Morrell MR, Pilewski JM, Gries CJ, *et al.* De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. *J Heart Lung Transplant* 2014; **33**: 1288.
77. Safavi S, Robinson DR, Soresi S, Carby M, Smith JD. De novo donor HLA-specific antibodies predict development of bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* 2014; **33**: 1273.
78. Brugière O, Suberbielle C, Thabut G, *et al.* Lung transplantation in patients with pretransplantation donor-specific antibodies detected by Luminex assay. *Transplantation* 2013; **95**: 761.
79. Verleden SE, Vanaudenaerde BM, Emonds MP, *et al.* Donor-specific and -nonspecific HLA antibodies and outcome post lung transplantation. *Eur Respir J* 2017; **50**: 1701248.
80. Hodge G, Hodge S, Holmes-Liew CL, Reynolds PN, Holmes M. Bronchiolitis obliterans syndrome is associated with increased peripheral blood natural killer and natural killer T-like granzymes, perforin, and T-helper-type 1 pro-inflammatory cytokines. *J Heart Lung Transplant* 2012; **31**: 888.
81. 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. *Clin Exp Immunol* 2013; **173**: 150.
82. Ropponen JO, Syrjälä SO, Hollmén M, *et al.* Effect of simvastatin on development of obliterative airway disease: an experimental study. *J Heart Lung Transplant* 2012; **31**: 194.
83. Spyridopoulos I, Martin-Ruiz C, Hilkens C, *et al.* CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: results from the Newcastle 85+ study. *Aging Cell* 2016; **15**: 389.
84. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. *Am J Epidemiol* 2010; **172**: 363.
85. Humar A, Gillingham K, Payne WD, Sutherland DE, Matas AJ. Increased incidence of cardiac complications in kidney transplant recipients with cytomegalovirus disease. *Transplantation* 2000; **70**: 310.
86. Johansson I, Andersson R, Friman V, *et al.* Cytomegalovirus infection and disease reduce 10-year cardiac allograft vasculopathy-free survival in heart transplant recipients. *BMC Infect Dis* 2015; **15**: 582.
87. Delgado JF, Reyne AG, de Dios S, *et al.* Influence of cytomegalovirus infection in the development of cardiac allograft vasculopathy after heart transplantation. *J Heart Lung Transplant* 2015; **34**: 1112.
88. Courivaud C, Bamoulid J, Chalopin JM, *et al.* Cytomegalovirus exposure and cardiovascular disease in kidney transplant recipients. *J Infect Dis* 2013; **207**: 1569.
89. Parrinello CM, Sinclair E, Landay AL, *et al.* Cytomegalovirus immunoglobulin G antibody is associated with subclinical carotid artery disease among HIV-infected women. *J Infect Dis* 2012; **205**: 1788.
90. Roman A, Manito N, Campistol JM, *et al.* The impact of the prevention strategies on the indirect effects of CMV infection in solid organ transplant recipients. *Transplant Rev (Orlando)* 2014; **28**: 84.
91. Pachnio A, Ciaurriz M, Begum J, *et al.* Cytomegalovirus infection leads to development of high frequencies of cytotoxic virus-specific CD4+ T cells targeted to vascular endothelium. *PLoS Pathog* 2016; **12**: e1005832.
92. Ducloux D, Courivaud C, Bamoulid J, *et al.* Polyclonal antithymocyte globulin and cardiovascular disease in kidney transplant recipients. *J Am Soc Nephrol* 2014; **25**: 1349.
93. Opelz G, Döhler B. Reduced rate of cardiovascular death after cytomegalovirus prophylaxis in renal transplant recipients. *Transplantation* 2015; **99**: 1197.

94. Valantine HA, Gao SZ, Menon SG, *et al.* Impact of prophylactic immediate posttransplant ganciclovir on development of transplant atherosclerosis: a post hoc analysis of a randomized, placebo-controlled study. *Circulation* 1999; **100**: 61.
95. Potena L, Grigioni F, Magnani G, *et al.* Prophylaxis versus preemptive anti-cytomegalovirus approach for prevention of allograft vasculopathy in heart transplant recipients. *J Heart Lung Transplant* 2009; **28**: 461.
96. Chakkera HA, Sharif A, Kaplan B. Negative cardiovascular consequences of small molecule immunosuppressants. *Clin Pharmcol Ther* 2017; **102**: 269.
97. Haller MC, Royuela A, Nagler EV, Pascual J, Webster AC. Steroid avoidance or withdrawal for kidney transplant recipients. *Cochrane Database Syst Rev* 2016; **22**: CD005632.
98. Wei L, MacDonald TM, Walker BR. Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease. *Ann Intern Med* 2004; **141**: 764.
99. Souverein PC, Berard A, Van Staa TP, *et al.* Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. *Heart* 2004; **90**: 859.
100. Kallikourdis M, Martini E, Carullo P, *et al.* T cell costimulation blockade blunts pressure overload-induced heart failure. *Nat Commun* 2017; **8**: 14680.
101. Vicenti F, Rostaing L, Grinyo J, *et al.* Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med* 2016; **374**: 333.
102. Kerekes G, Soltész P, Dér H, *et al.* Effects of rituximab treatment on endothelial dysfunction, carotid atherosclerosis, and lipid profile in rheumatoid arthritis. *Clin Rheumatol* 2009; **28**: 705.
103. Sharif K, Watad A, Bragazzi NL, *et al.* Anterior ST-elevation myocardial infarction induced by rituximab infusion: a case report and review of the literature. *J Clin Pharm Ther* 2017; **42**: 356.
104. Macklin PS, Morris PJ, Knight SR. A systematic review of the use of rituximab as induction therapy in renal transplantation. *Transplant Rev* 2015; **29**: 103.
105. Clatworthy MR, Watson CJ, Plotnek G, *et al.* B-cell depleting induction therapy and acute cellular rejection. *New Engl J Med* 2009; **360**: 2683.
106. Chandraker A, Kobashigawa JA, Stehlik J, *et al.* Rituximab induction in cardiac transplantation is associated with accelerated coronary artery vasculopathy: CTOT11. [abstract]. *Am J Transplant* 2016; **16**: 403.
107. Kounis NG, Soufras GD, Hahalis G. Adverse cardiac events to monoclonal antibodies used for cancer therapy: the risk of Kounis syndrome. *Oncoimmunology* 2015; **3**: e27987.
108. Tsiantoulas D, Diehl CJ, Witztum JL, Binder CJ. B cells and humoral immunity in atherosclerosis. *Circ Res* 2014; **114**: 1743.
109. Duftner C, Dejaco C, Hengster P, *et al.* Apoptotic effects of antilymphocyte globulins on human pro-inflammatory CD4+CD28-T cells. *PLoS One* 2012; **7**: e33939.
110. Bamoulid J, Staeck O, Crépin T, *et al.* Anti-thymocyte globulins in kidney transplantation: focus on current indications and long-term immunological side effects. *Nephrol Dial Transplant* 2017; **32**: 1601.
111. De Serres S, Sayegh MH, Najafian N. Immunosuppressive drugs and Tregs: a critical evaluation!. *Clin J Am Soc Nephrol* 2009; **4**: 1661.
112. O'Leary JG, Samaniego M, Barrio MC, *et al.* The influence of immunosuppressive agents on the risk of de novo donor-specific HLA antibody production in solid organ transplant recipients. *Transplantation* 2016; **100**: 39.
113. Azarbal B, Cheng R, Vanichsarn C, *et al.* Induction therapy with antithymocyte globulin in patients undergoing cardiac transplantation is associated with decreased coronary plaque progression as assessed by intravascular ultrasound. *Circ Heart Fail* 2016; **9**: e002252.
114. Rodrigues-Diez R, González-Guerrero C, Ocaña-Salceda C, *et al.* Calcineurin inhibitors cyclosporine A and tacrolimus induce vascular inflammation and endothelial activation through TLR4 signaling. *Sci Rep* 2016; **6**: 27915.
115. Mourer JS, de Koning EJ, van Zwet EW, Mallat MJ, Rabelink TJ, de Fijter JW. Impact of late calcineurin inhibitor withdrawal on ambulatory blood pressure and carotid intima media thickness in renal transplant recipients. *Transplantation* 2013; **96**: 49.
116. Karpe KM, Talaukikar GS, Walters GD. Calcineurin inhibitor withdrawal or tapering for kidney transplant recipients. *Cochrane Database Syst Rev* 2017; **7**: CD006750.
117. Van Leuven SI, van Wijk DF, Volger OL, *et al.* Mycophenolate mofetil attenuates plaque inflammation in patients with symptomatic carotid artery stenosis. *Atherosclerosis* 2010; **211**: 231.
118. Wong BM, Huang M, Zaltzman JS, Prasad GV. Mycophenolate mofetil and C-reactive protein in renal transplant recipients. *Transplantation* 2005; **83**: 48.
119. D'Avola D, Cuervas-Mons V, Martí J, *et al.* Cardiovascular morbidity and mortality after liver transplantation: the protective role of mycophenolate mofetil. *Liver Transpl* 2017; **23**: 498.
120. Kobashigawa JA, Tobis JM, Mentzer RM, *et al.* Mycophenolate mofetil reduces intimal thickness by intravascular ultrasound after heart transplant: reanalysis of the multicenter trial. *Am J Transplant* 2006; **6**: 993.
121. Eisen HJ, Kobashigawa J, Starling RC, *et al.* Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. *Am J Transplant* 2013; **13**: 1203.
122. Fine NM, Kushwaha SS. Recent advances in mammalian target of rapamycin inhibitor use in heart and lung transplantation. *Transplantation* 2016; **100**: 2558.
123. Arora S, Andreassen AK, Andersson B, *et al.* The effect of everolimus initiation and calcineurin inhibitor elimination on cardiac allograft vasculopathy in de novo recipients: one-year results of a Scandinavian randomized trial. *Am J Transplant* 2015; **15**: 1967.
124. Stoumpos S, Jardine AG, Mark PB. Cardiovascular morbidity and mortality after kidney transplantation. *Transpl Int* 2015; **28**: 10.
125. Isakova T, Xie H, Messinger S, *et al.* Inhibitors of mTOR and risks of allograft failure and mortality in kidney transplantation. *Am J Transplant* 2013; **13**: 100.
126. Knoll GA, Kokolo MB, Mallick R, *et al.* Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. *BMJ* 2014; **349**: g6679.
127. Gallon L, Traitanon O, Sustento-Reodica N, *et al.* Cellular and molecular immune profiles in renal transplant recipients after conversion from tacrolimus to sirolimus. *Kidney Int* 2015; **87**: 828.
128. Schmitz F, Heit A, Dreher S, *et al.* Mammalian target of rapamycin (mTOR) orchestrates the defense program of innate immune cells. *Eur J Immunol* 2008; **38**: 2981.
129. Grimbert P, Thaunat O. mTOR inhibitors and risk of chronic antibody-mediated rejection after kidney transplantation: where are we now? *Transpl Int* 2017; **30**: 647.