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Cholesterol efflux in the transplant patient

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Abstract

Purpose of review—Cholesterol metabolism is increasingly recognized in inflammatory diseases including transplantation. This review discusses the mechanistic underpinnings that tie macrophage cholesterol efflux capacity of high-density lipoprotein to chronic rejection in transplanted patients.

Recent findings—Animal studies suggest that administration of apolipoprotein A-I, the main protein constituent of high-density lipoprotein, can prevent transplant atherosclerosis. apoA-I administration increases cholesterol efflux capacity of high-density lipoprotein. In patients with cardiac allograft vasculopathy, decreased cholesterol efflux capacity has been associated with poorer survival. In addition, reduced cholesterol efflux capacity in recipients, pre-transplant, has been associated with development of cardiac allograft vasculopathy and renal allograft survival.

Summary—These recent findings raise the hypothesis that increasing cholesterol efflux may prevent chronic rejection and improve allograft survival after transplant. Reconstituted high-density lipoprotein significantly increases cholesterol efflux capacity and is currently in clinical development for traditional atherosclerosis. Clinical trials of reconstituted high-density lipoprotein administration in transplantation should be performed.

Keywords

transplantation; cholesterol efflux; high-density lipoprotein; macrophage

Introduction

Solid organ transplantation is a lifesaving therapy for many patients with various end stage diseases. In 2017 alone, nearly 30,000 organ transplants have been performed in the United States, including kidney, liver, heart and lung transplants[1]. After brain death of an organ donor, surgical removal of the organ, organ preservation and reperfusion, significant ischemia/reperfusion (IR) injury occurs leading to innate immune activation. IR injury leads to a rapid, macrophage-dependent influx of neutrophils to the allograft, followed by pro-inflammatory monocyte-derived macrophages[2]. As inflammation resolves, macrophages play a critical role in phagocytosis of necrotic and apoptotic cells, a process that is critical to

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Conflicts of interest

The authors have no conflicts of interest.

the resolution of IR injury. Therefore, pathways affecting macrophage function may play a significant role in allograft function.

Metabolic stress such as diabetes or dyslipidemia may significantly affect immune activation and wound healing. In particular, the role of cholesterol metabolism in organ transplantation and innate immune activation is increasingly recognized. Exogenous and intracellular lipids can inhibit macrophage function and stimulate pro-inflammatory responses[3]. As evidenced by the beneficial effects of HMG-CoA reductase inhibitors (statins) in cardiac transplant recipients[4], lipids may be a critical mediator of inflammation in organ transplant recipients. Recent studies have also highlighted the role of cholesterol efflux capacity (CEC), which has been linked to cardiac allograft vasculopathy[5] and renal allograft failure[6]. The ability of high-density lipoprotein (HDL) particles to promote cholesterol efflux from macrophages is the rate-limiting step in reverse cholesterol transport, and CEC is associated with coronary artery disease[7–9] which itself is increasingly recognized as an inflammatory disease with the recent findings that anti-inflammatory therapy with canakinumab reduced recurrent cardiovascular events[**10].

Therapies that increase CEC may suppress innate immune activation in the setting of inflammatory diseases, organ transplantation, and coronary artery disease (CAD). Administration of reconstituted HDL therapies, such as CSL112[11, 12], can robustly increase CEC and is currently under study as a therapeutic target for secondary prevention in CAD. CEC raising therapy could benefit organ transplant recipients by mitigating early IR injury, attenuating innate immune activation or chronic rejection, and finally by decreasing risk of cardiovascular death, a common cause of death in multiple organ transplant recipients. Given the available pre-clinical and animal studies, as well as the safety and potency of CEC raising therapies, clinical trials are needed to evaluate whether increasing CEC would clinically benefit organ transplant recipients.

CEC in cardiac allograft vasculopathy

While overall survival after cardiac transplantation continues to improve, conditional survival after 1 year has not improved in the past decade[13]. Cardiac allograft vasculopathy (CAV) is a major cause of graft failure among heart transplant recipients and an important cause of mortality after 1 year. When one considers that CAV itself is a major risk factor for long-term graft failure[14], and hence deaths due to graft failure are at least partially attributable to CAV, CAV can be considered one of the leading causes of transplant mortality. CAV arises due to both immunologic and non-immunologic factors[15, 16]. However, the role of lipid deposition and resulting inflammation in CAV is increasingly recognized. Like native coronary artery disease (CAD), CAV lesions exhibit significant macrophage accumulation and lipid deposition[17]. Clinically, CAV is diagnosed via angiography where it is seen as a diffuse concentric fibrous intimal hyperplasia along the length of coronary vessels. Although angiography is the standard screening test for CAV, intra-vascular ultrasound (IVUS) and optical coherence tomography (OCT) are sensitive tools to diagnose early CAV in the clinical and research arenas[18].

Mechanisms to reduce the risk and progression of CAV are critical given its prevalence among heart transplant recipients and its significant morbidity and mortality. Statins have been shown to slow progression of CAV and have become standard of care in the post-transplant population[4, 19, 20]. In addition to the clear benefits of statins in this population, a number of pre-clinical interest spawned interest in whether cholesterol metabolism pathways may play an important role in CAV pathogenesis.

Multiple animal studies support that pharmacologic or genetic increases in apoA-I, the main protein constituent of HDL particles, can prevent transplant arteriopathy. The seminal studies were from the Fisher group, who in 2001 showed that HDL could remodel transplanted aortic segments from hypercholesterolemic mice[21]. Lesions transplanted into mice overexpressing human apoA-I versus control mice demonstrated reduced macrophage content and increased smooth muscle cells, primarily in the superficial subendothelial layer. Further studies determined that apoA-I mimetic peptides that augment cholesterol efflux[22] could prevent murine cardiac allograft vasculopathy[23, 24]. The De Geest group went on to describe the mechanism by which apoA-I increases circulating endothelial progenitor cells that incorporate into the allograft, resulting in reduced neointima formation. Furthermore, a requirement for the HDL scavenger receptor SR-BI attenuation of murine CAV was demonstrated via bone marrow transplantation studies[25]. These pre-clinical data support a role for apoA-I and peptides that stimulate cholesterol efflux in the development of transplant arteriopathy. However, a specific requirement for cholesterol efflux as opposed to other possible anti-inflammatory functions of apoA-I has not been established.

However, human studies also support a role for HDL-mediated CEC in CAV progression. First, consensus exists across multiple studies suggesting reduced cholesterol efflux relative to HDL-cholesterol mass in transplant patients[26, 27]. In 2014, the De Geest group published an important cross-sectional study[27] to determine associations between CAV and CEC in heart transplant recipients between 5 and 15 years post-transplant⁹. At baseline, serum total cholesterol and LDL were lower in transplant patients compared to healthy controls. HDL cholesterol levels were not statistically significant. Diagnosis of CAV was made using the International Society for Heart and Lung Transplantation (ISHLT) nomenclature based on angiography[28]. Interestingly, patients with CAV had higher CRP levels compared to those without CAV; however, there were no observed differences in CEC between transplant patients with or without CAV. In addition, the vasculoprotective function of HDL was lower among all heart transplant patients compared to healthy controls, but no differences based on CAV status were observed. The authors concluded that CEC was not associated with CAV but that HDL functionality was lower in the transplant population compared to healthy controls. Limitations of this study included its cross-sectional design and utilization of standard angiography for CAV diagnosis, which is less sensitive than intravascular ultrasound (IVUS). Therefore, patients with subclinical CAV may have been missed, confounding the comparisons between those with and without CAV.

We recently published the effects of reduced CEC on clinical outcomes and progression of CAV[5]. In a study of 35 patients with angiographic CAV, we found that reduced CEC, measured at the time of CAV diagnosis, was inversely associated with survival. Furthermore, cyclosporine levels checked at the time of cardiac catheterization showed an inverse

correlation with CEC, supporting previous findings that cyclosporine inhibits the ATP-binding cassette transporter[29, 30], which is critical for the majority of cholesterol efflux. HDL cholesterol levels were not significantly different between survivors and non-survivors with CAV, and, importantly, the associations between CEC and survival in CAV patients persisted after adjustment for HDL-C mass, consistent with the concept that HDL particle functionality is more clinically relevant than absolute cholesterol mass.

To understand whether CEC was associated with CAV progression, we measured CEC pre-transplant in the Clinical Trials in Organ Transplantation 05 (CTOT05) study. Reduced CEC was associated with CAV based on change in maximal intimal thickness (MIT) as determined by IVUS one year post-transplant (versus the early post-transplant IVUS study). Interestingly, pre-transplant CEC was inversely associated with baseline values of MIT and total atheroma volume. Importantly, these associations were also independent of HDL-C mass. We hypothesize that reduced CEC pre-transplant may predispose the allograft to enhanced ischemia-reperfusion injury and accelerated CAV. Limitations of our study included that our two cohorts were selectively used to answer two separate questions. First, we studied a group of patients with CAV nearly one decade after transplant at a single center. We then performed a second study in 81 patients from CTOT05. While this approach certainly was not without controversy[31], we contend that our studies are hypothesis generating and will require further validation in subsequent studies.

Cholesterol efflux and other organ transplants

Cardiac allograft vasculopathy exhibits similar pathophysiology to chronic rejection after other solid organ transplants. Furthermore, cardiovascular disease is a significant cause of morbidity and mortality in transplant recipients who may have multiple cardiovascular risk factors. In particular, renal transplant recipients exhibit higher rates of cardiovascular disease¹¹. In addition, graft failure, frequently due to transplant arteriopathy, remains a significant cause of morbidity in this population. The mechanism is similar to coronary vasculopathy, with increased macrophage accumulation and endothelial inflammation, resulting in accelerated atherosclerosis. Recently, a prospective study of 495 renal transplant recipients showed that CEC was inversely associated with renal allograft failure, independent of HDL-C or apoA-I levels. Interestingly, overall cardiovascular mortality and all-cause mortality were not significantly associated with CEC.

Conclusions and future directions

Early allograft injury, metabolic risk factors, and immune activation are key mediators of acute and chronic allograft injury. Studies in cardiac and renal transplant patients suggest the hypothesis that enhancing cholesterol efflux capacity may prevent allograft loss. In the case of cardiac transplant recipients, augmenting cholesterol efflux by administering reconstituted HDL may reduce chronic rejection, which manifests as cardiac allograft vasculopathy. Given that CAV remains a leading cause of graft failure and mortality in heart transplant patients, reducing CAV burden could lead to a mortality benefit in cardiac transplant recipients.

Given the similar pathophysiology of chronic rejection manifesting as arteriopathy in multiple solid organ transplants, it is tempting to speculate the cholesterol efflux may play a critical role in preventing early allograft injury that then primes the allograft for chronic rejection. One outstanding question, therefore, is whether CEC plays a role in chronic rejection in other organ transplants, including lung and liver. Furthermore, future studies will clarify the role of CEC in cardiac transplant recipients given somewhat conflicting data thus far.

Nonetheless, therapies that enhance cholesterol efflux are relatively safe and currently in clinical development for native coronary artery disease. One such drug, CSL112, is an intravenous formulation of reconstituted HDL that significantly increases CEC, well beyond the distribution observed in transplant recipients who develop CAV. While a clinical trial of CEC increasing therapy in transplant recipients seems warranted, multiple important questions will need to be answered including whether to administer CEC raising therapy prior and/or subsequent to transplant surgery, and how often CEC raising therapy might need to be administered. While increasing evidence points to a critical role for the metabolic milieu of the transplant recipient, little is known about how the donor profile may also affect long term outcomes. Although logistically difficult, further studies will need to clarify the potential role for donor metabolic disease in transplant outcomes.

Finally, whether other steps in reverse cholesterol transport are critical in transplant patients is unknown. One possibility is that the allograft ischemia reperfusion injury results in acceleration of arteriosclerosis early after transplant, in which case CEC and the functional characteristics of recipient macrophages may both be key, while other steps in reverse cholesterol transport may be less relevant. Alternatively, it is possible that reverse cholesterol transport reduces early lipid accumulation in plaque. Finally, it is possible that other HDL modifications, for example symmetric dimethyl arginine[32], may play a role in reducing cholesterol efflux or other HDL anti-inflammatory functions. Further understanding of the mechanisms by which cholesterol efflux may prevent post-transplant allograft loss and chronic rejection will be necessary in order to develop efflux raising therapies in the transplant setting.

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Key points

- Pre-transplant cholesterol efflux capacity of high-density lipoprotein is associated with cardiac allograft vasculopathy
- Pre-transplant cholesterol efflux capacity is associated with renal allograft survival
- Strategies that increase cholesterol efflux should be studied further in transplantation