Characteristics of Chronic Rejection in Heart Transplantation: Important Elements of Pathogenesis and Future Treatments

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Although 85,000 heart transplantations have been performed worldwide, coronary allograft vasculopathy (CAV), which is a phenomenon of chronic rejection, is still a serious problem. Because CAV involves all the allograft arteries, angioplasty, stenting or bypass grafting are not practical treatment options. Therefore, CAV is the biggest long-term limitation in cardiac allograft recipients. Although the cause of CAV is mostly immunologic, non-immune pathways also contribute to its development. Several cytokines, chemokines and adhesion molecules play a critical role in the process. Cell adhesion, migration and proliferation of bone marrow progenitor and other cells are involved in its development. Although there is not an established clinical strategy for preventing or treating CAV, recent investigations have provided some promising methodologies. Progress in DNA technology, such as antisense oligodeoxynucleotides (ODNs) to regulate the transcription of disease-related genes, has an important role in its therapeutic applications. Antisense ODN transfection preventing CAV in experimental cardiac allografts has been reported for the first time. The ODN strategy has not only been useful in the experimental studies, but is also a novel clinical strategy for gene therapy. The pathological and immunological characteristics of CAV and some promising methodologies for prevention of the disease are reviewed. (*Circ J* 2010; **74**: 233–239)

Key Words: Arteriosclerosis; Gene therapy; Transplantation

Hearth transplantation is a common surgical procedure in humans; almost 85,000 operations have been performed worldwide over the past 40 years.¹ However, coronary allograft vasculopathy (CAV), which is a phenomenon of chronic rejection, is still a serious problem.^{1,2} Because CAV involves all the allograft arteries, angioplasty, stenting or bypass grafting are not practical treatment options. Therefore, CAV is the biggest long-term limitation in cardiac allograft recipients. CAV in cardiac allografts is especially problematic because the heart exhibits more severe disease than other transplanted organs.^{3–5}

Several cytokines and adhesion molecules promote rejection. CAV is characterized by intimal thickening comprised of proliferative smooth muscle cells (SMCs) and extracellular matrix (ECM) with relatively intact internal and external elastic laminae. Although the cause of CAV is mostly immunologic, nonimmune pathways also contribute to its development. Inflammatory cells, such as lymphocytes, macrophages, neutrophils and eosinophils, are all variably present in evolving lesions. In the early stages, there is a subendothelial accumulation of mononuclear cells associated with markers of

endothelial cell (EC) activation. Because endothelial or perivascular cellular immune injury induces persistent allograft vascular damage, inflammatory cells and activated ECs secrete growth factors to recruit and activate SMCs.^{3,6–9} Recently, the intimal SMCs in CAV lesions are known to derive in part from circulating host cells. Although these cells share a number of markers with medial SMCs, they are modulated cells with a proliferative and synthetic phenotype.^{3,6,10,11}

This review article describes the characteristics of CAV, identifying the important elements of its pathogenesis in the process. Finally, a section on prevention and/or treatment of CAV is included, to represent the new approaches to future therapies.

Clinical Findings of CAV

Because transplant hearts are denervated, the clinical symptoms of ischemia are not useful for diagnosing early CAV. Coronary angiography (CAG) is still the standard methodology for the diagnosis of CAV in the clinical setting. However,

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CAG is relatively insensitive and underestimates the lesions because CAV involves vessels diffusely. Instead, intravascular ultrasound (IVUS) and coronary computed tomography (CT) are the preferred methods of quantitatively assessing intimal thickening. IVUS is one of the most sensitive tools for diagnosing CAV because it reveals both the actual lumen diameter and the thickness of the neointima. The examinations have proven the high frequency of CAV: 90% of patients develop CAV within 10 years. Of note, CAV development does not correlate with either the number of episodes or the severity of cellular rejection, and vascular lesions occur despite adequate immunosuppression to prevent acute rejection.^{4,12} However, the IVUS is invasive and thus associated with the risk of complications, whereas contrast CT can provide a comprehensive and noninvasive evaluation of CAV. It may be useful as a single noninvasive procedure, not only for CAV evaluation but also for assessment of allograft function and other complications.¹³

Pathology of CAV

Pathologically, CAV involves the entire length of the transplanted arterial vasculature. CAV is characterized by concentric intimal thickening comprised of SMCs and ECM, with relatively intact internal and external elastic laminae. The vascular media and adventitia are relatively unaffected. Inflammatory cells are variably present in evolving lesions, but calcification and atheroma formation are also uncommon.³

Based on the registry of the International Society for Heart and Lung Transplantation 2009, risk factors for CAV within 8 years are: donor history of hypertension; induction therapy with interleukin (IL)-2 antagonist or OKT3; pretransplant diagnosis of coronary artery disease; increasing number of HLA-DR mismatches etc.¹ Other risk factors include obesity, diabetes, hyperlipidemia, viral infection and hyperhomocysteinemia.³

Allografts are subject to multiple injuries, by immune and nonimmune factors. Although many factors contribute to

CAV development, any of the various injuries can independently induce CAV. Immune injury is known to play a critical role in CAV pathogenesis. Although cellular immune responses are central in CAV development, humoral injury is also recognized as a critical component. The predominant effector cells are macrophages; they secrete proinflammatory cytokines that influence the proliferation of smooth muscle-like cells (SMCs) and fibroblasts and the ECM synthesis that results in CAV formation (**Figure 1**). Recipient T cells require 2 signals to be activated: 1 signal involves host T-cell receptor interaction with the donor major histocompatibility complex (MHC) and the other signal involves ligation of costimulatory molecules. The nature and efficiency of both signals affect the development of CAV.^{14,15}

Nonimmune risk factors, including ischemia, diabetes and hypertension, correlate with the intimal thickening of CAV. Periods of ischemia are significantly important for promoting CAV; the effects are attributable to ischemic ECs, which increases local platelet accumulation. Ischemic injury to ECs can also increase MHC and adhesion-molecule expression.^{16,17} Cytomegalovirus is known to be associated with an increased incidence of CAV, which in turn is associated with enhancement of multiple genes associated with wound repair and angiogenesis.^{18,19}

Costimulatory Signals

Costimulatory signals are important for stimulation of T cells and inflammatory cell recruitment into the allograft.²⁰ It has been reported that CAV correlates with expression of adhesion molecules, including intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule-1, and the E- and P-selectins.^{21,22} Besides facilitating EC interactions, lymphocyte integrins can also act as costimulatory molecules during antigen presentation. Although blockade of ICAM-1 and leukocyte function associated antigen-1 interactions induces allograft tolerance,²³ the absence of this signal suppresses but does not completely prevent CAV development.²⁴ It has been reported that CTLA-4 blockade of B7-CD28 interactions attenuated CAV.²⁵ The inducible costimulator (ICOS) mole-

cule on T cells can also influence CAV development by directly interacting with the ICOS-ligand on medial SMCs activated by IL-1 β .^{26,27} We also found that blockade of selectin,²⁸ LIGHT,²⁹ and 4-1BB³⁰ attenuated CAV, whereas PD blockade accelerated its development.³¹ Because each costimulatory signal has a different effect on CAV development, investigation of anti-costimulatory therapy should evaluate different organs and conditions.

Cytokines

Cytokines mediate local and systemic immune responses. Interferon (IFN)- γ is the most important player because it activates macrophages, augments inflammatory cell recruitment and amplifies immune responses.³² Despite ongoing acute rejection, the absence of IFN- γ abrogates CAV.³³ In association with IFN- γ -induced cytokines, the IFN- γ and IL-12 relationship is a critical element in CAV pathogenesis because medial SMCs synthesize IFN- γ following stimulation by IL-12 and IL-18.³⁴ Although IFN- γ blocks medial SMC proliferation in vitro, IFN- γ directly induces intimal hyperplasia in the absence of inflammatory cells.³⁵ Other proinflammatory cytokines downstream of IFN- γ also play an important role. We reported that with both tumor necrosis factor (TNF) receptor-1 and -2 deficiencies CAV did not develop, despite normal host inflammatory cell IFN- γ production.³⁶ Because host TNF-receptor deficiency did not reduce CAV, the effect was not attributable to TNF signaling on host inflammatory cells.

Chemokines

Chemokines are known to recruit and activate inflammatory cells. They are classified into 4 groups: CC, CXC, XC, and CX3C. The roles of these molecules in acute rejection and CAV have been investigated.³⁷ RANTES is a well-charac-

terized CC chemokine implicated in the pathogenesis of both atherosclerosis and CAV.³⁸ Other chemokines, the IFN- γ -inducible IP10, Mig, and I-TAC, are critical for their strong association with CAV.^{39,40} Moreover, targeted deletion of CCR1 reduces both acute rejection and the development of CAV.⁴¹

Sources of Cells in CAV

Although SMLCs are similar in appearance to medial SMCs, they are phenotypically modulated cells with different genetic expression profiles. We have shown that medial SMC have a contractile function, whereas SMLC exhibit a more embryonic, synthetic phenotype.¹⁰ Previously, CAV intimal cells were assumed to derive from the migration and proliferation of donor medial SMCs. However, several groups have shown that intimal SMLCs can also be of host origin. In animal models, the proportion of host origin SMLCs in intimal lesions exceeds 90%.^{42–44} However, in gender-mismatched cardiac allografts, Y-chromosome in situ hybridization identified only 3–15% host-derived intimal SMLCs.^{45,46} These results demonstrate that both host bone marrow and non-bone marrow precursors contribute to CAV development. Therefore, therapies against CAV may need to account for different cell origins, routes of recruitment, and differentiation pathways.

Prevention and Treatments

Clinically Applicable Treatments

Studies have shown that HMG-CoA reductase inhibitors (statins) play an important role in the prevention of CAV. The effects of statins are mediated by multiple immunogenic effects. Simvastatin reduces proinflammatory cytokine activity, improves coronary endothelial function and increases the area of the coronary artery lumen.⁴⁷ Calcium-channel blockers

Figure 3. Representative findings in allograft coronary arteries. (A) Effects of E2F decoy compared with controls (B) on the intimal hyperplasia that defines coronary allograft vasculopathy. (Modified from *Circ Res* 2000; **87**: 1063–1068 with permission.⁷¹)

Figure 4. Summary of the pathological and immunological characteristics of CAV (black) and some promising methodologies for prevention (blue). CAV, coronary allograft vasculopathy; CMV, cytomegalovirus; ECM, extracellular matrix; NF- κ B, nuclear factor- κ B; SMLC, smooth muscle-like cell.

are also known to delay the progression of CAV and enhance coronary vasodilation.⁴⁸ Angiotensin-converting enzyme (ACE) inhibitors partially improve allograft endothelial dysfunction, oxidative stress and endothelin activation. They are also associated with plaque regression and improved graft

survival.^{49,50} Recently, a synergistic beneficial effect of a calcium-channel blocker and an ACE inhibitor on the development of CAV was described.⁵¹ Other drugs, such as vitamins and flavonoids, might have the potential to restore allograft endothelial function.^{52,53}

Cyclosporin A (CyA) significantly suppresses acute cardiac allograft rejection, but has not been effective in preventing CAV.⁵⁴ Although tacrolimus provides superior prevention of acute rejection compared with CyA, CAV development was similar between CyA- and tacrolimus-treated groups.⁵⁵ It has been reported that mycophenolate mofetil significantly decreased both first-year maximal intimal thickness⁵⁶ and the incidence of CAV.⁵⁷ Recently, proliferation signal inhibitors were shown to have significant effects on preventing CAV. Sirolimus has delayed the progression of CAV in some clinical trials.^{58,59} Another proliferation signal inhibitor, everolimus, also prevented CAV development.⁶⁰ However, as there is not yet a report of the long-term effect of proliferation signal inhibitors in suppressing CAV, further observation studies are needed.

Treatments Under Investigation

Possible targets to prevent CAV are chemokines, cytokines, costimulatory signals, cell proliferation and apoptosis. It has been reported that the induction of transient mixed hematopoietic chimerism leads to long-term heart allograft survival and CAV did not develop in MHC disparate monkeys without chronic immunosuppression.⁶¹ However, tolerance induction and systemic immune modulation may cause adverse effects; limited treatment of transplant organs is ideal for the long-term survival of recipients. Thus, we have investigated the effects of a limited gene transfer system to allografts for the prevention of CAV.⁶²

Recent progress in DNA technology, such as antisense oligodeoxynucleotides (ODNs) to regulate the transcription of disease-related genes, has shown that the technology will have an important role in therapeutic applications⁶³ (Figure 2). In our study, we first selected antisense cdk2 kinase ODN, because the enzyme plays an important role in cell transition through the G1/S phase.⁶⁴ We then showed that treatment with antisense cdk2 kinase ODN significantly reduced CAV.⁶⁵ Next, we transfected the allografts with antisense PCNA ODN, because PCNA plays an essential role in cell transition through both the G1/S and G2/M phases.⁶⁶ We observed limited neointimal formation in antisense PCNA ODN-transfected allografts.⁶⁷ We also targeted apoptosis because it is a prominent feature of atherosclerosis.⁶⁸ We clarified that antisense *bcl-x* ODN suppresses CAV with increased TUNEL-positive cells.⁶⁹

Transfection of cis-element double-stranded DNA, termed "decoy", has been reported as a useful method for gene therapy. The transcription factor E2F regulates multiple cell-cycle regulatory genes, which are critical to the process of cell growth and proliferation.⁷⁰ To clarify the effects of E2F decoy ODN on CAV, we used both murine and monkey cardiac transplant models, which showed that E2F decoy ODN transfer into the allografts specifically abolishes E2F activity and inhibits CAV⁷¹ (Figure 3). Another critical transcription factor, nuclear factor- κ B (NF- κ B), plays a pivotal role in the coordinated transcription of multiple inflammatory genes.⁷² We demonstrated that NF- κ B decoy ODN inhibits CAV effectively and of note, the NF- κ B decoy ODN attenuated both chronic and acute rejection.⁷³

Although the decoy ODN strategies have not yet been used in clinical transplantation, the effects have been investigated in coronary bypass grafting and coronary angioplasty in the clinical setting. It was reported that ex vivo E2F decoy ODN transfection in vascular grafts suppresses neointimal hyperplasia after cardiac bypass surgery.^{74,75} We also tried NF- κ B decoy ODN transfection of the site in coronary arteries after

stent implantation in humans and the results demonstrated that the strategy is clinically promising.^{76–78} Thus, further studies should be conducted in other transplant models to explore the clinical utility of this technology for prevention of CAV.

Summary

Although improved surgical techniques have resulted in excellent graft function,⁷⁹ CAV remains a problem to be resolved by treatment. Because heart transplantation in Japan has been very limited by a severe shortage of donors,⁸⁰ preventing CAV is critical for the prognosis of recipients. We have described the pathological and immunological characteristics of CAV. Several cytokines, chemokines and adhesion molecules play a critical role in the process. Cell adhesion, migration and proliferation of bone marrow and other cells are involved in its development. Although there is not yet an established clinical strategy for preventing or treating CAV, recent investigations have provided some promising methodologies (Figure 4). Therefore, further studies are needed to clarify the clinical prevention of CAV.

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