This Review is part of a thematic series on Transplant Vasculopathy, which includes the following articles:

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William Baldwin and Jordan Pober, Guest Editors

Interferon-\(\gamma\) Axis in Graft Arteriosclerosis

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Abstract—Cardiac transplantation is the most effective treatment for advanced heart failure. Despite improvements in immunosuppression therapy that prevent acute rejection, cardiac allografts fail at rates of 3% to 5% per posttransplant year. The hallmark morphological lesion of chronically failing cardiac allografts, also seen in chronic renal and liver graft failure, is luminal stenosis of blood vessels, especially of conduit arteries. Late graft failure results from widespread secondary ischemic injury to the graft parenchyma rather than direct immune-mediated damage. Although this process affects the entire graft vasculature, graft arteriosclerosis is a suitable term to describe the problem because it applies to different types of failing organs and because it emphasizes the central feature, namely an accelerated form of arterial injury and remodeling. The precise pathogenesis of graft arteriosclerosis is unknown. In this review, we make the case that the signature T-helper type 1 cytokine, interferon (IFN)-\(\gamma\), is a key effector in graft arteriosclerosis, which, together with the IFN-\(\gamma\)-inducing cytokine interleukin-12 and IFN-\(\gamma\)-inducible chemokines such as CXCR3 ligands, constitute a positive feedback loop for T-cell activation, differentiation, and recruitment that we refer to as the IFN-\(\gamma\)-axis. We evaluate the evidence to support this hypothesis in clinical observational and experimental animal studies. Additionally, we examine the regulation of IFN-\(\gamma\) production within the artery wall, the effects of IFN-\(\gamma\) on vessel wall cells, and the outcome of therapeutic agents on IFN-\(\gamma\) production and signaling. These observations lead us to suggest that new therapies for graft arteriosclerosis should be optimized which focus on reducing IFN-\(\gamma\) synthesis or actions. (Circ Res. 2007;100:622-632.)

Key Words: interferon-\(\gamma\) ■ coronary arteries ■ cardiac transplantation ■ T cells ■ vascular cells

Graft arteriosclerosis (GA), a relatively rapid and progressive loss of lumen in allograft conduit arteries, is the major cause of late cardiac allograft failure. Early loss of cardiac allografts in humans is generally caused by a destructive host immune response against graft parenchymal or vascular cells termed acute rejection. Modern immunosuppressive agents are reasonably effective at controlling acute rejection, and 1 year cardiac allograft survival now routinely approaches 90%.

However, there is a steady loss of 3% to 5% of grafts per year thereafter that has not been significantly impacted by modern immunosuppressive regimens. These clinical observations strongly suggest that chronic graft failure is likely to arise from different mechanisms from acute graft rejection.

The pathological hallmark of chronic cardiac graft loss is luminal stenosis and occlusion of conduit arteries. At the time of heart failure, these lesions exhibit significant intimal expansion in a concentric pattern and diffusely involve the...
entire arterial tree from epicardial coronaries through intramyocardial branches. Intimal thickening may even be observed in veins, leading many investigators to call this process “cardiac allograft vasculopathy.” Because the arterial lesions are the key cause of graft ischemic injury and loss, our preference is to refer to this disease process as “graft arteriosclerosis,” emphasizing the central importance of arterial disease. The intimas of the stenotic arteries with advanced GA usually lack the necrotic cores of typical atheroma and also differ in their distribution from atheromatous plaques, which are typically eccentric, focal, and largely confined to proximal epicardial coronaries. It is also uncommon for transplant arteriosclerotic vessels to fissure or thrombose and death results from progressive ischemic cardiomyopathy rather than acute myocardial infarction.

It is important to realize that this original picture of GA was drawn from anatomic analysis of end-stage lesions examined at necropsy. Serial investigation of early stages of disease by intravascular ultrasound and other modalities has told a somewhat different story. Early lesions in epicardial arteries may, in fact, be eccentric and focal, i.e., they may more closely resemble classical atheroma in their pattern of distribution, although not in morphology. Also, like atherosclerotic vessels, graft arteriosclerotic vessels may initially show compensatory outward remodeling, preserving the lumen until later stages, when intimal expansion can no longer be accommodated and/or outward remodeling is halted or even reversed. Importantly, even at the early stages of GA, when lesions are structurally compensated, affected graft arteries show dysfunction in endothelial-dependent relaxation and sometimes in smooth muscle cell contractility and relaxation to exogenous mediators. The mechanistic link of endothelial cell (EC) or of vascular smooth muscle cell (VSMC) dysfunction to intimal expansion and to compensatory or pathological remodeling of vessel diameter is unknown, but because these same links are also exhibited in stenotic atherosclerotic vessels, this sequence is likely to represent a common progression pattern for the arterial response to injury.

Pathogenesis of GA

Insights into the pathogenesis of GA have come both from studies of patients and from animal models. Because similar-appearing lesions are found in human heart, kidney, and liver allograft arteries, there is a consensus that GA in different organs share a common pathogenesis. There is also a broad consensus that GA is, in large part, an alloimmune process. This conclusion is based on the clinical observations that vascular disease typically ends at the suture lines and, in experimental studies, that GA does not develop in grafts involving immunodeficient recipients or syngeneic donor-recipient pairs. The dominant role of alloimmunity is why we classify GA as a manifestation of chronic vascular rejection. However, there is also a consensus view that nonimmune factors significantly contribute to GA. The best evidence for this conclusion comes from human kidney allograft studies showing that delayed graft function, a result of perioperative ischemic injury, strongly predisposes toward chronic allograft nephropathy, a pattern of late graft failure characterized by GA and graft parenchymal fibrosis. Similarly, kidneys from cadaver donors, which are adversely affected by brain death, show impaired long-term survival compared with organs from unrelated living donors independent of any other factors. This interplay between peritransplant events with late graft failure and GA has suggested 2 explanations. One is a “burden of injury” model, which posits that every form of damage to a graft artery contributes to stenosis, alloimmunity being just 1 of several factors. The alternative view, to which we ascribe, is an “immune modulation” model in which injured tissues release mediators that modify the adaptive immune rejection response. A similar idea has been proposed in the cancer field to explain how tumor cell necrosis leads to antitumor immunity.

In this review, we have chosen to focus on the disease process that develops within conduit arteries because this is the most significant lesion leading to graft failure. However, we do not intend to imply that vascular lesions do not occur elsewhere within the heart. Indeed, this is why many authors refer to the process as a cardiac allograft vasculopathy. Lesions in the microvessels or veins may have a different appearance from those that develop in coronary arteries for 2 reasons. First, it is possible that microvascular or venous ECs may have properties that differ from those of arterial ECs as relates to interactions with T cells and other leukocytes. For example, venular and venous EC readily express vascular cell adhesion molecule-1, whereas arterial and capillary ECs do not, and cardiac microvascular ECs more readily express major histocompatibility complex (MHC) molecules than large vessel ECs. The second point is that the development of arteriosclerosis depends on the response of VSMCs to immune mediators, and these cells are much more abundant in the arteries than in the rest of the vasculature. The immunologic responses of particular vascular cells may lead to the selective accumulation of leukocytes that are relatively resistant to conventional immunosuppression. Little is known regarding the unique identity of leukocytes in GA. In a related pair of studies, sequencing analysis of T-cell receptor transcripts from the coronary arteries of a limited number of patients with GA demonstrated oligoclonal populations of T cells in the majority and relatively heterogeneous populations of T cells in the minority of explanted hearts with severe GA. In some patients, from whom the relevant specimens were available, the same clones of T cells were found in multiple conduit arteries, as well as in endomyocardial biopsies of acutely rejecting parenchyma from years earlier. These findings indicate that a few clones of alloreactive T cells may cause both acute rejection and GA in certain cases but that not all GA is associated with T-cell oligoclonality and may also result from nonspecific recruitment and/or activation of bystander T cells by the inflamed vasculature.

It is generally accepted that allograft rejection is an example of adaptive immunity. Adaptive immune responses may activate a variety of different effector mechanisms to deal with various types of microbes. The specific contributions of the various effector mechanisms of adaptive immunity to clinical GA are uncertain. Experimental models in rodents are useful for identifying factors that can cause arteriosclerotic changes in graft arteries, but arteriosclerosis is...
a common end point of arterial injury, and animal models generally cannot establish that a specific mechanism is actually operational in the clinical setting. With this limitation in mind, it is useful to know that various mouse models have provided evidence consistent with a role for a number of adaptive immunity effector molecules, such as alloantibodies, death receptors, and their ligands (eg, tumor necrosis factor [TNF] or Fas ligand), other T cell–derived cytokines molecules (perforin, granzymes), various T cell–derived cytokines (eg, interferon [IFN]–γ, interleukin [IL]–4 and transforming growth factor [TGF]–β) and signaling molecules (eg, CD40 ligand), and loss of regulatory T cells. Although it is not the only factor that can cause arteriosclerosis, we view the evidence for a pathogenetic role of IFN-γ in GA to be particularly compelling. The senior author (J.S.P.) originally proposed the hypothesis that sustained allogeneic reactions localized in the walls of coronary arteries, which histologically resemble chronic delayed-type hypersensitivity responses rather than acute cytolytic rejection reactions, is the cause of GA. This view emphasized the key role of IFN-γ derived from the localized chronic immune reaction and its effects on infiltrating leukocytes. Our subsequent work, discussed below, has led to the conclusion that IFN-γ can also act directly on vessel wall cells to promote arteriosclerotic changes. In the remainder of the review, we summarize the evidence supporting a pathogenetic role of IFN-γ, discuss the regulation of IFN-γ production, describe the mechanisms of IFN-γ actions on vessel wall cells, examine the effects of therapeutic agents on IFN-γ synthesis and responses, and suggest how this hypothesis may be tested and exploited in clinical transplantation.

Evidence for a Pathogenetic Role of IFN-γ in GA

There is substantial observational evidence that immune responses resulting in IFN-γ production are associated with the development of GA in clinical cardiac transplantation. The primary cellular source of IFN-γ is a subset of CD4+ effector T cells designated as T-helper type 1 (or Th1); other cells capable of producing significant quantities of IFN-γ are CD8+ effector T cells, natural killer (NK) cells, NKT cells, and possibly B cells, dendritic cells (DCs), or macrophages (MOs). Expansion of circulating Th1 cells is associated with endothelial dysfunction after cardiac transplantation, a predictor of GA. The propagation of Th1 cells from endomyocardial biopsies also correlates with the subsequent development of GA. More directly, induction of IFN-γ transcripts in endomyocardial biopsies precedes the development of GA. In support of this finding, increased expression of transcripts for the IFN-γ–inducible chemokines IP-10 (also known as CXCL10), I-TAC (CXCL11), and Mig (CXCL9) in endomyocardial biopsies also predict GA. A common limitation of these studies is that immune mechanisms were not directly assessed within the conduit coronary arteries, and immune responses may differ in peripheral, parenchymal, and arterial compartments. The most relevant findings are those derived from specimens of arteriosclerotic coronary arteries from postmortem studies or explanted hearts at the time of retransplantation. Notably, an increased expression of the IFN-γ–inducible chemokines RANTES (CCL5) and I-TAC and an increased expression of the Th1–associated chemokine receptor CXCR3 have been described in arteriosclerotic coronary arteries. The most convincing evidence for an association of IFN-γ with GA was a recent detailed inventory of infiltrating leukocytes and cytokines in arteriosclerotic coronary arteries. van Loosdrecht et al found that the expression of IFN-γ, IFN-γ–inducible chemokines (Mig, IP-10, I-TAC, RANTES, and fractalkine/CX3CL1), and markers of IFN-γ–secreting Th1 cells (CXCR3, CCR5, and CX3CR1) were increased in epicardial coronary arteries from transplanted hearts with documented GA compared with referent specimens. The activation of Th1 responses was specific for GA as the expression of the Th2 cytokine IL-4 and the Th3 cytokine IL-10 were similar to that of arteries from transplanted hearts without GA or nontransplanted hearts. Further analysis of the specimens revealed that IFN-γ and Th1–associated chemokine receptors and their ligands were expressed in the intima and adventitia but not in the media, which had a 5-fold lesser leukocytic infiltrate. Finally, concomitant studies of the myocardium revealed a lesser expression of CXCR3, CCR5, and CX3CR1, and of chemokines binding to these receptors.

The critical role of IFN-γ in the pathogenesis of GA is further supported by experimental studies. In mouse models of allogeneic cardiac transplantation, serologic neutralization or genetic absence of IFN-γ markedly reduces the extent of intimal expansion. IFN-γ has also been implicated as a contributor to arteriosclerosis in other mouse models, eg, genetic hyperlipidemias caused by deficiency of apolipoprotein E or of low-density lipoprotein receptors. We have reached similar conclusions with our investigations in a chimeric human–animal model in which human coronary arteries are interposed into the aortae of severe combined immunodeficient (SCID) mouse hosts that are subsequently reconstituted with allogeneic human peripheral blood mononuclear cells. In this model, intimal expansion and outward vascular remodeling in response to allogeneic human T cells is dependent on IFN-γ, and neutralization of IFN-γ can prevent T cell–mediated EC and VSMC dysfunction. IFN-γ–inducible chemokine production by vascular cells is associated with the recruitment of Th1 cells, and immunosuppressive agents that prevent intimal expansion also reduce IFN-γ synthesis and responses. Finally, administration of human IFN-γ alone, in the absence of leukocytes, is sufficient to induce arteriosclerosis in the transplanted human arteries.

Control of IFN-γ Synthesis in GA

Because we propose that IFN-γ is a central mediator of GA, we next review how its production is regulated within the artery wall. IFN-γ is made principally by T cells of the adaptive immune system and by NK cells of the innate immune system. The responses of adaptive immunity are much larger and sustained than those of innate immunity, presumably because antigen-activated T cells undergo clonal expansion and because T cells are more long-lived than NK cells. Chronic processes, like GA, are therefore much more likely to be adaptive than innate immune responses. However, under certain experimental conditions in which adaptive
immune responses are absent, eg, organ transplantation between parental donors and F1 hybrid recipients, NK cells of the innate immune system are necessary, but not sufficient, to result in GA by nonspecifically activating bystander T cells via an IFN-γ-dependent mechanism.42

Adaptive immune responses are principally driven by the response to foreign antigen, and our discussion of the control of IFN-γ synthesis will therefore begin with a consideration of the sources of antigen in GA. Anatomically, the arterial wall appears isolated from the surrounding tissue, and its involvement by a rejection process may be uncoupled from the response to the parenchyma. Within the artery, graft ECs are a significant source of antigenic stimulation for the host immune response because these cells display MHC molecules and costimulators and have the capacity to activate resting T-cell populations in vitro.43 However, there are important species differences that must be considered in the interpretation of experimental findings.44 Most notably, human ECs express both class I and class II MHC molecules in vivo and typically lack B7-1 (CD80) and B7-2 (CD86) costimulators, depending primarily on lymphocyte function–associated antigen (LFA)-3 (CD58) to activate T cells. In contrast, murine ECs express only class I MHC, and may express B7-1 or B7-2 but not LFA-3 because mice and rats lack the gene encoding this protein. In culture, human ECs can directly present their MHC molecules to allogeneic CD4+ and CD8+ T cells, effectively activating cytokine elaboration and proliferation from resting memory T cells.45 Murine ECs activate CD8+ but not CD4+ effector T cells, even when they are induced to express class II MHC molecules in response to IFN-γ.46,47 Instead, IFN-γ–treated murine ECs may predominantly activate CD4+ CD25+ regulatory T cells,48 a finding we have not been able to replicate with human ECs (J.S. Pober, unpublished observations, 2006). VSMCs are a second major cell population within the graft artery wall, and these cells also appear to show species differences in their interactions with T cells. Human VSMCs fail to activate resting allogeneic T cells and may actually inhibit their activation;49 in contrast, murine VSMC appear to be immunogenic and can activate T cells that mediate vasculitis.50,51 In summary, although murine VSMCs favor IFN-γ production by T cells and murine ECs do not,50,51 we have not been able to confirm such observations using human systems.

In addition to direct interactions of host T cells with graft vascular cells, both graft and host “professional” antigen-presenting cells (APCs), such as DCs and MØs, may be present within the vessel wall and participate in alloimmunity by presenting antigens to T cells. This could involve a direct pathway of alloantigen presentation (involving display of graft MHC molecules on graft-derived professional APCs), or a “semi-direct” pathway (involving display of intact MHC molecules acquired from graft-cell membrane fragments by host APCs), or an indirect pathway (involving processing of graft proteins and display of peptides derived from these protein antigens on host MHC molecules by host APCs).52 Alloreactive T cells responsive by the direct (or semi-direct) pathway are present at high frequencies within the human naïve and memory T-cell pools in peripheral blood. The numbers of circulating T cells that respond to alloantigen via the indirect pathway is usually quite low before transplantation and a rise in the frequency of these cells has been correlated with chronic rejection.53 This change correlates with the loss of graft-derived APCs, often referred to as passenger leukocytes. Circulating T cells may not be the relevant population and the frequency of alloreactive T cells within the vessel wall itself have not been analyzed. In addition to presenting antigen, these professional APCs may provide cytokines or other immunomodulatory molecules that can influence the host T-cell responses to graft vascular cells.54 Little is known about the role of MØs and DCs in GA.

In adaptive immune responses to allografts, IFN-γ is made by both antigen-activated CD4+ and CD8+ T cells.55 In general, CD4+ T cells produce larger amounts of this cytokine, especially after differentiation into effector cells that specialize in IFN-γ secretion. As we noted earlier, such cells are typically described as constituting the T H1 subset. IL-12 (and possibly related cytokines) favors the development of naïve CD4+ T cells into T H1 effector cells during an initial encounter with cognate antigen.56 Memory T cells can also contribute to the IFN-γ–secreting pool of effector T cells. The differentiation of naïve T cells into T H1 effector cells that secrete cytokines such as IL-4, IL-5, and IL-13 are favored by the actions of IL-4, whereas the differentiation of regulatory T cells that produce inhibitory cytokines (such as IL-10 or TGF-β1) may be favored by the actions of IL-10. The cytokines that favor these alternative pathways of naïve T-cell differentiation inhibit IFN-γ production by and T H1 differentiation of naïve CD4+ T cells. The same cytokines may also affect further differentiation of as-yet-uncommitted effector cells, known as T H0 cells. In addition, IFN-γ–producing effector CD4+ T cells may arise either from activation of central memory cells (identified as CD45RO+CCR7−, and L-selectin+ and already committed to the T H1 pathway) within the secondary lymphoid organs or from activation of effector memory cells (identified as CD45RO+CCR7− and L-selectin−) within a peripheral tissue such as an allograft.57 Although it is uncertain to what extent the further differentiation of committed memory cells of either type may be influenced by cytokines, conditions may exist for their selective activation. CD8+ T cells typically elaborate less IFN-γ than CD4+ T H1 cells, but it is synthesized by the majority of cytolytic T lymphocytes (CTLs), sometimes called T H1 cells. Indeed, CTLs that make other cytokines are viewed as unusual, and IFN-γ production has often been used to enumerate antigen-specific CTLs.58,59 IL-12 is a key cytokine for CTL differentiation from resting CD8+ T cells.56 As discussed above, the ability of IL-12 and IL-18 to promote the antigen-driven development of T H1 immunity is well established. More recently, it has become clear that IL-12 and IL-18 can also activate T cells in an antigen-independent fashion.60 Studies of IFN-γ production by murine CD4+ T cells have characterized separate IL-12/IL-18 and T-cell receptor signaling pathways.61 Interactions between innate and adaptive stimuli for cytokine responses by murine CD8+ T cells have been investigated in a viral infection model in which both IL-12/IL-18 and antigen result in IFN-γ production, but only T-cell receptor signaling induces IL-2 secretion, which is pivotal for T-cell clonal
expansion.\textsuperscript{62} We have recently described that IL-12 and IL-18 may nonspecifically activate IFN-γ production by coronary artery–infiltrating T cells and/or exacerbate antigen-driven T-cell responses within the arterial wall.\textsuperscript{63} IL-12 and IL-18, but not other monokines, elicited secretion of IFN-γ and IFN-γ-inducible chemokines in human coronary arteries maintained in organ culture. T cells, not innate immunocytes, were the principal source of IFN-γ in response to IL-12/IL-18 within the arterial wall. CD8\textsuperscript{+} T cells produced more IFN-γ than CD4\textsuperscript{+} T cells following IL-12/IL-18 stimulation, and naïve CD8\textsuperscript{+} T cells were as responsive as memory CD8\textsuperscript{+} T cells. This inflammatory response did not require, but was synergistic with and primed for, T-cell receptor signals. Finally, IL-12/IL-18–stimulated T cells displayed a cytokine-producing, nonproliferating, and noncytolytic phenotype. Thus, circulating monokines may provide a mechanistic link between peripheral inflammation and T\textsubscript{H1}-type cytokine production in coronary arteriosclerosis. An important implication of these nonspecific mechanisms of T-cell activation is that they may evade normal tolerance control, such as natural regulatory T cells.

Although cytokines are proposed to be the dominant signals that drive particular pathways of T-cell differentiation, the characteristics of the APC, which presents the antigen to the T cell, is also known to be important. DCs are the major cell populations that present antigens to naïve T cells, and some investigators have proposed that DCs may be divided into those that favor T\textsubscript{H1} responses (DC1), those that favor T\textsubscript{H2} responses (DC2), or those that favor regulatory T cells (tolerogenic DCs).\textsuperscript{64,65} Tolerogenic DCs are sometimes referred to as “immature” DCs, but definitive evidence for the idea that this is merely a state of inadequate DC maturation is not compelling. It is even less clear whether other types of APCs, including MØs and ECs, fall into such categories and, if so, what mediators would regulate these phenotypes. Recently, it has been reported that IL-10 in combination with IFN-α can induce tolerogenic ECs, which share with tolerogenic DCs the expression of immunoglobulin-like transcript (ILT)-3.\textsuperscript{66} The ligand for ILT-3 is unknown, but its engagement by ILT-3 appears to deliver inhibitory signals to T cells. It is also possible that ECs, which not only can present antigens but can recruit circulating T cells,\textsuperscript{43} may acquire characteristics that favor selective recruitment of effector or effector memory cells already committed to the T\textsubscript{H1}, T\textsubscript{H2}, or regulatory cell lineages. IFN-γ itself, as well as by regulatory cytokines, such as TGF-β1 or IL-10.\textsuperscript{71} As noted above, these cytokines exert some effects directly on T cells, shutting off IFN-γ secretion, but IL-4 and regulatory cytokines also exert effects on APCs, eg, turning off IL-12 production. The signals which initiate these alternative responses are less clear than those which turn on the IFN-γ axis; the T\textsubscript{H2} response has sometimes been described as a default pathway. Conditions that induce tolerance rather than aggressive alloimmunity are a subject of much current investigation.

**Tissue Injury and the IFN-γ Axis**

IL-12 has emerged as a key initiator of IFN-γ responses by both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells, as well as by NK cells of the innate immune response.\textsuperscript{56} It stimulates production of IFN-γ from activated T cells (an action that may be enhanced by cotreatment with another cytokine, IL-18), and it drives T cells into effector cells that selectively produce IFN-γ. IL-12 may share some of these actions with 2 related cytokines, IL-23 and IL-27, but more recent studies have emphasized a more specialized role for these more recently described molecules.\textsuperscript{72} IL-12 is principally made by professional APCs, such as DCs and MØs, but these cells must be stimulated to do so. The best described inducers of IL-12 secretion by APCs are microbial products that bind to Toll-like receptors (TLRs) on APCs, such as lipopolysaccharide, which signals via TLR4. This response is augmented by IFN-γ itself, providing positive feedback. Lipopolysaccharide induction of IL-12 is an example of how the innate immune response to a microbial product can influence the outcome of an adaptive immune response. In our hands, lipopolysaccharide does not induce IL-12 production by ECs,\textsuperscript{73} although others have asserted that ECs can make IL-12 in response to CD40 ligand.\textsuperscript{74} The recognition and activation of innate immunity by microbial mediators (often referred to as pathogen-associated molecule patterns or PAMPs), which, in turn, activates adaptive immunity is sometimes referred to as the “stranger hypothesis.”\textsuperscript{75} Molecules released by tissue injury...
may have similar effects, an idea sometimes known as the “danger hypothesis.” 76 We hypothesize that tissue injury in the absence of microbial products can affect the way the adaptive immune system responds to an allograft. 77 It has been shown that lengthening the interval between transplantation and the introduction of effector cells into an immune-deficient mouse attenuates the alloimmune rejection response, supporting the suggestion that the status of the graft is a determinant of rejection. 78 Putative mediators produced by tissue injury are less well characterized than are pathogen-associated molecule patterns. One possible candidate is high-mobility group box 1 (HMGB1) protein, which is released from the nuclei of necrotic (but not apoptotic) cells. 79 HMGB1 may also be secreted by professional APCs independently of cell injury. DCs do so as a consequence of TLR-triggered maturation, 80 whereas MØs secrete HMGB1 in response to cytokines, especially IFN-γ and TNF, as well as in response to TLR ligands. 81 There is one report, not yet confirmed, that cultured EC will secrete HMGB1 in response to TNF, but injury was not assessed in this study. 82 In MØs, secretion of HMGB1 involves hyperacetylation of the protein whereas release by injury does not. 83 HMGB1 has been shown to favor murine T h 1 differentiation. 80 The principal receptor for HMGB1 is RAGE (an acronym for Receptor for Advanced Glycation End-products), although it has also been proposed to interact with cells via TLR2 and TLR4. The receptor on T cells is thought to be RAGE. 84 It is not known whether HMGB1 can act on human T cells to influence cytokine production or differentiation. HMGB1 may also act on APCs. In the vessel wall, it is of interest that in addition to being released by ECs, HMGB1 has been reported to activate human ECs. 85, 86 Other mediators generated by tissue injury, such as IL-1, complement fragments (eg, C5a) and uric acid, may also affect IFN-γ production or Th 1 differentiation. The general point is that injury may, acting via specific mediators, bias the adaptive alloimmune response to favor production of IFN-γ by vessel wall infiltrating T cells, leading to arteriosclerosis.

**Effects of IFN-γ on Vessel Wall Cells**

IFN-γ has multiple effects on vascular cells through orchestration of a remarkable spectrum of distinct cellular programs. Many hundreds of genes are regulated by IFN-γ, and the complexity of the signaling responses is accentuated by cell-specific transcriptional programs and by several IFN-γ-regulated genes that are themselves components of transcription factors. 41 Despite the extraordinary complexity of the IFN-γ response, the major function that can be attributed to this cytokine is regulation of immunity for pathogen resistance. The properties of IFN-γ include stimulation of antiviral and bactericidal activity, enhancement of antigen presentation, recruitment of leukocytes, and more general effects on cell proliferation and apoptosis. Microarray analysis of human cell lines and primary vascular cells reveal a wide range of transcripts that are regulated by IFN-γ, including many with understandable immunomodulatory functions as well as a variety of genes whose functional significance remains obscure. 87, 88

In general, IFN-γ induces a proinflammatory phenotype of ECs and VSMCs through the induction and upregulation of membrane receptors and secretory products. As previously discussed, ECs effectively present antigens to T cells, and we have shown that IFN-γ enhances this response by increasing the expression of class I and II MHC molecules 89, 90 and accessory molecules that mediate assembly and peptide loading, eg, transporter associated with antigen processing 1 (TAP1). 91 The expression of MHC molecules is greater on human ECs than VSMCs and is dependent on IFN-γ. 92, 93 T-cell activation by ECs is also influenced by IFN-γ–mediated regulation of classical costimulator molecules, such as CD40 and CD40 ligand, 92, 93 and more recently identified costimulators, eg, programmed death ligand (PDL)-1. 94 Adhesion molecules that play a role in the immune synapse between ECs and T cells and the recruitment of T cells, eg, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, are also upregulated by IFN-γ. 90 Additionally, IFN-γ induces the expression of chemokines in human ECs, such as CXCR3, CCR5, and CX3CR1 ligands, that have a role in Th 1 cell activation and recruitment. 27, 38 As mentioned above, despite a similar expression of IFN-γ–inducible proinflammatory molecules, human VSMCs do not activate T cells and can inhibit T-cell responses to ECs. 49 One explanation is that IFN-γ induces a far greater expression of the enzyme indoleamine 2,3-dioxygenase in VSMCs than in ECs and the depletion of tryptophan in the microenvironment by indoleamine 2,3-dioxygenase leads to inhibition of T-cell activation and proliferation (M.C.C. Cuffy, G.T. Tellides, unpublished observations, 2006). This observation may largely explain why the VSMC-rich media is the least infiltrated compartment of the vessel wall in GA.

Unlike the consistent regulation of inflammatory factors with a relatively predictable cellular phenotype, the effects of IFN-γ on vascular cell survival are variable and condition dependent. IFN-γ was generally viewed as an anti-proliferative cytokine for viral-infected cells, cancer cells, and primary cultured cells, including ECs and VSMCs. 95–100 However, there have been occasional reports that IFN-γ can promote VSMC growth by potentiating growth factor signaling under certain in vitro conditions, such as serum deprivation. 50, 101–104 Similarly, IFN-γ occasionally results in the proliferation of other mesenchymal cell types 105–108 and even ECs. 109 We believe that the in vivo setting represents the gold standard to assess whether IFN-γ has pro- or antiproliferative effects on vascular cells. As previously mentioned, human arteries exposed to human IFN-γ in SCID/beige mice demonstrate unequivocal evidence of VSMC division and intimal expansion. 40 Because IFN-γ effects are species restricted, it is likely that the proarteriosclerotic effects in our model are direct actions of IFN-γ on vascular cells in the absence of leukocytes. Our more recent work using human coronary artery grafts and adenoviral-mediated human IFN-γ administration confirm a robust proliferation of VSMCs that is dependent on mammalian target of rapamycin (mTOR) signaling (Y.W. Wang, G.T. Tellides, unpublished observations, 2006).

In the same in vivo model, we also detect apoptosis of VSMCs. One possible scenario is that IFN-γ–induced cell death
precedes or even triggers subsequent cell proliferation. Administration of agonistic antibodies against human Fas to immunodeficient mouse recipients of human artery grafts reveals that IFN-γ primes VSMCs to Fas-induced apoptosis, in part by relocation of Fas to the cell surface. Others have reported that IFN-γ induces apoptosis of VSMC via diverse intermediate molecules, including nitric oxide, peroxynitrite, gelsolin, TNF, and loss of insulin-like growth factor-1 receptor. In contrast, another group found differing results: that IFN-γ–treated human VSMCs show a lower basal rate of apoptosis and that treatment of VSMCs with IFN-γ significantly attenuate reactive oxygen species–induced apoptosis.

In summary, IFN-γ regulates a wide range of genes with seemingly opposing effects that is echoed by contrasting in vitro cellular phenotypes that are condition- and cell type specific. Even under in vivo conditions and in the absence of other cell types, IFN-γ induces disparate pro- and antiinflammatory and pro- and antiapoptosis effects on vascular cells. This paradox has been noted by other authors who have recently reviewed cytokine-mediated GA and native atherosclerosis who emphasize temporal and spatial divergent effects of IFN-γ and use the terms “contradictory,” “complex,” “bimodal,” “yin and yang,” and “Janus faces” to describe the role of IFN-γ in the arteriosclerotic process. Further investigations using therapeutic agents that inhibit IFN-γ are required to dissect the significance of this cytokine in clinical disease.

Effects of Therapeutic Agents on IFN-γ Production

Immunosuppressive drugs, such as calcineurin inhibitors (eg, cyclosporine and tacrolimus), mTOR inhibitors (eg, sirolimus, also known as rapamycin, and everolimus), and purine synthesis inhibitors (eg, mycophenolate mofetil and azathio- prine) prevent T-cell activation, clonal proliferation, and, consequently, the production of cytokines, including IFN-γ, in allografts. As previously discussed, despite the prevention of acute rejection by optimal immunosuppression, IFN-γ and IFN-γ–inducible chemokines may still be detected in graft biopsy specimens. IFN-γ responses are also detected in other arteriosclerotic conditions in the absence of immunosuppression, such as atherosclerosis and in-stent restenosis of native coronary arteries. In addition to suppressing T-cell proliferation and cytokine production, the mTOR inhibitors have potent antiproliferative effects on VSMCs. In clinical trials, everolimus or sirolimus, in combination with cyclosporine, reduce the incidence of GA (defined by intimal thickening using intravascular ultrasound) compared with azathioprine and cyclosporine. Sirolimus-coated stents also decrease intimal proliferation and restenosis in atherosclerotic coronary arteries. The beneficial effect of mTOR inhibitors on the arteriosclerotic process is assumed to result from inhibition of VSMC proliferation, but a primary immunosuppressive effect may provide an alternative or complementary explanation for these observations. Few studies have directly compared the effects of mTOR inhibitors on T cells versus VSMCs as responsible for their antiarteriosclerotic properties. We have investigated this issue in our chimeric humanized immunodeficient mouse models. Sirolimus at a low dose of 0.1 mg/kg per day is partially effective at preventing alloimmune-mediated remodeling of human arteries in SCID/beige mouse recipients and displays synergistic beneficial effects at this dose in combination with cyclosporine. A higher dose of sirolimus of 0.5 mg/kg per day completely prevents intimal expansion of human arteries in response to allogeneic human T cells and reduces circulating and intra-graft levels of IFN-γ. In contrast, a 3-fold higher dose of sirolimus of 1.5 mg/kg per day is required to inhibit mTOR signaling, VSMC proliferation, and intimal expansion in response to adenoviral-mediated administration of human IFN-γ to SCID/beige mice bearing human coronary artery grafts in the absence of human leukocytes (Y.W. Wang, G.T. Tellides, unpublished observations, 2006). These results suggest that inhibition of T-cell proliferation and cytokine production are more readily inhibited by immunosuppressive and immunomodulatory agents than cytokine-induced VSMC proliferation.

It is an important point that GA develops in patients who are treated with calcineurin inhibitors like cyclosporine and tacrolimus. This raises the question of whether T-cell responses, especially IFN-γ production, in response to allogeneic ECs are resistant to the effect of these drugs. This issue has been somewhat contentious. Our group first reported that ECs do provide signals that allow polyclonally activated T cells to resist the inhibitory effects of cyclosporine. Subsequently, Batten et al reported that human EC did not confer resistance to allogeneic T-cell responses because they lacked CD80 and CD86 and could not costimulate T cells through CD28, a previously described mechanism of resistance to cyclosporine. Calcineurin is a calcium-activated protein phosphatase, and calcineurin inhibitors work by preventing NFAT from being dephosphorylated. Phospho-NFAT is unable to enter the cell nucleus, reducing cytokine gene transcription. Most recently, Murphy and Hughes found that EC use wnt-5a to interact with frizzled-5 on T cells, thereby inhibiting glyogen synthase kinase-3β in the T cells. This enzyme normally antagonizes calcineurin effects on NFAT, rephosphorylating this transcription factor and driving it from the nucleus. The result of glyogen synthase kinase-3β inhibition is to enhance the actions of calcineurin, prolonging the residence of active NFAT in the nucleus and effectively decreasing the efficacy of cyclosporine. Although this question has not been definitively settled, there is now both evidence and a mechanism to support the idea that ECs do confer resistance on T cells to calcineurin inhibitors.

In contrast to the controversy regarding the cyclosporine-sensitivity of cytokine production by T cells to allogeneic ECs, there is general agreement that an inflammatory pathway of IFN-γ production by human and murine T cells is cyclosporine resistant. IFN-γ production by murine CD4+ T cells in response to IL-12/IL-18 is dependent on p38 mitogen-activated protein kinase (MAPK) activation. IL-12/IL-18–induced synthesis of IFN-γ by human peripheral T cells is also inhibited by sirolimus. We have demonstrated that IL-12/IL-18–dependent IFN-γ secretion from human coronary artery–infiltrating T cells is prevented by a p38 MAPK inhibitor, partially diminished by sirolimus, and is not affected by cyclosporine or the 3-hydroxy-3-
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mutually exclusive, and IFN-γ secretion, such as we have recently reported for inducible nitric oxide synthase production, may also be resistant to conventional immunosuppression. The emerging data regarding more effective therapy against innate functions of T cells provide a rationale for combination immunosuppressive drug regimens to successfully prevent IFN-γ production and responses in allografts.

**Strategies for Clinical Investigations to Test the IFN-γ Axis Hypothesis of GA**

The clinical data that support a key effector role for the IFN-γ axis in GA are the positive correlations between IFN-γ production or responses and the development of GA. Ultimately, specific inhibition of IFN-γ synthesis and actions are required to determine whether these associations are causal in human disease. Immunosuppressive drugs lack the specificity to unambiguously assign a nonredundant causal role of IFN-γ in GA. Biologic agents that may be useful in testing the hypothesis have been recently tested in safety and efficacy trials for autoimmune conditions. Treatment with a humanized monoclonal antibody against IL-12 induces clinical responses and remissions in patients with active Crohn’s disease and is associated with decreased Th1 responses at the site of disease. Similarly, a humanized monoclonal antibody directed against IFN-γ is well tolerated by patients with Crohn’s disease and has a beneficial effect on disease activity. Besides neutralizing antibodies or similar strategies with soluble cytokine receptors, we have recently demonstrated that the IFN-γ axis may be inhibited in an experimental model of GA by preventing IP-10 binding to low-affinity glycosaminoglycan (GAG) sites on vascular cells and consequently decreasing the transendothelial migration and arterial recruitment of CXCR3+ Th1 cells. Currently, there is a great deal of interest by a number of groups in developing synthetic GAG derivatives capable of competitively binding cytokines and chemokines and in producing modified chemokines with an alteration in the GAG-binding site such that they do not oligomerize on EC surfaces. Thus, multiple opportunities exist to pharmacologically interrupt the amplification and pathologic sequelae of the IFN-γ axis in GA and possibly improve the long-term outcomes of cardiac transplantation.

**Conclusion**

We have summarized the broad and convincing evidence that supports a causal relationship between the IFN-γ axis and GA, and we have provided an understanding of the initiators and effectors of this cytokine cascade within the arterial wall. We acknowledge that this is a relatively narrow viewpoint, biased by our own work, and necessary to present a focused and in-depth review of a single pathogenetic mechanism. We emphasize that the many effector molecules of adaptive immunity that may potentially injure allograft arteries are not mutually exclusive, and IFN-γ–secreting Th1-type cells may also express other cytokines, signaling molecules, cytotoxic molecules, and death receptor ligands, as well as promote the differentiation of antibody-producing B cells. We refer the reader to the other excellent reviews in this thematic series on Transplant Vasculopathy for further insight into alternative or complimentary theories. Only specific biologic therapy that targets the IFN-γ axis in the clinical setting can reveal whether it plays a critical or redundant role in GA and, by inference, in other arteriosclerotic diseases.

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**Disclosures**

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