This Review is part of a thematic series on Transplant Vasculopathy, which includes the following articles:
Allograft Vasculopathy Versus Atherosclerosis
Antibody and Complement in Transplant Vasculopathy
Interferon-γ Axis in Graft Arteriosclerosis

Vascular Remodeling in Transplant Vasculopathy

Chemokines and Transplant Vasculopathy
Stem Cells and Transplant Vasculopathy

William M. Baldwin III and Jordan Pober, Guest Editors

Vascular Remodeling in Transplant Vasculopathy

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Abstract—As therapeutic strategies to prevent acute rejection progressively improve, transplant vasculopathy (TV) constitutes the single most important limitation for long-term functioning of solid organ allografts. In TV, allograft arteries characteristically develop severe, diffuse intimal hyperplastic lesions that eventually compromise luminal flow and cause ischemic graft failure. Traditional immunosuppressive strategies that check acute allograft rejection do not prevent TV; indeed 50% of transplant recipients will have significant disease within five years of organ transplantation, and 90% will have significant TV a decade after their surgery. TV can involve the entire length of the transplanted arterial bed, including penetrating intraorgan arterioles. Indeed, the luminal narrowing of such penetrating vessels may be the most functionally significant because arterioles represent the major contributors to tissue vascular resistance. Because of the diffuseness of TV involvement in the allograft vascular bed, the only currently definitive therapy requires re-transplantation. Nevertheless, as we better understand the pathogenesis and critical mediators of these lesions, pharmacological advances can be anticipated. Other articles in this thematic review series focus on the specifics of the inciting injury, the cytokines and chemokines that drive TV development, and the nature of the recruited cells in TV lesions, as well as the pathogenic similarities between TV and other vascular lesions such as atherosclerosis. This review focuses on the mechanisms of vascular wall remodeling in TV, including the intimal accumulation of smooth muscle–like cells and associated extracellular matrix, medial smooth muscle cell degeneration, and adventitial fibrosis. A brief overview highlights the aneurysmal changes that can accrue when vessel wall inflammation has a cytokine profile distinct from the typical proinflammatory interferon-γ–dominated milieu. (Circ Res. 2007;100:967-978.)

Key Words: transplant vasculopathy ■ intimal hyperplasia ■ smooth muscle cells ■ negative remodeling

We originally hypothesized, more than a decade ago, that transplant vasculopathy (TV) represented an ineffective delayed-type-hypersensitivity response directed against donor endothelial cells (ECs) and medial smooth muscle cells (SMCs). Although the alloresponse does not effectively eliminate the donor vascular wall cells, injured and dysfunctional ECs and SMCs will nevertheless participate in the recruitment and activation of medial SMCs to progressively occlude the vascular lumen.

Although a number of the details have since been refined, subsequent work has largely borne out the original hypothesis. Donor arterial ECs and SMCs are preserved in long-term allografts. Whether these cells persist because hosts develop an attenuated alloagraft response or because the grafts...
otherwise accommodate to humoral mediators, ECs and SMCs potentially can act as on-going targets of an alloresponse. Interestingly, even in experimental situations lacking persistent alloimmunity (eg, because of immune cell depletion or retransplant into a major histocompatibility complex–matched recipient), a single transient episode of rejection sufficiently drives subsequent TV development.\(^7,8\) This result suggests that secondarily recruited cells (eg, macrophages), once activated, may suffice to maintain an environment in which TV can progress. Indeed, the observations that TV can also occur following ischemic injury in isografts\(^9\) and that nonimmunologic factors influence TV development\(^10\) indicate that a variety of vascular wall insults lead to a final common pathway of intimal hyperplasia.

We and others showed that the development of TV requires the archetypal Th1 cytokine interferon-\(\gamma\) (IFN-\(\gamma\)); TV lesions do not develop in the setting of congenital absence or monoclonal antibody blockade of IFN-\(\gamma\).\(^8,11,12\) In an elegant experiment using donor human arterial grafts in immunodeficient mice, Tellides et al further demonstrated that IFN-\(\gamma\) alone, without associated inflammation, is sufficient to induce TV-like lesions.\(^13\) However, despite almost a decade of subsequent investigation, the relevant IFN-\(\gamma\) pathway(s) underlying TV development remain ill defined. As discussed elsewhere in this review series (G. Tellides and J. Pober), the potent pleiotropic cytokine IFN-\(\gamma\) affects the activation state of multiple cell types, and also regulates major histocompatibility complex and costimulator molecule expression and the production of cytokines, chemokines, adhesion molecules, and extracellular matrix (ECM). Classically a product of Th1 and Tc1 T-cell subsets as well as natural Killer (NK) cells, IFN-\(\gamma\) also can be produced by activated macrophages (forming an autocrine loop),\(^14\) and by SMCs stimulated by interleukin (IL)-12 and IL-18.\(^15\) Although we cannot pinpoint a unique IFN-\(\gamma\) effect (and indeed the effectiveness of IFN-\(\gamma\) blockade may result from multiple short-circuited pathways), therapies that modulate IFN-\(\gamma\) production or activity (eg, thiazolidinediones\(^16\)) might effectively ameliorate TV.

Although IFN-\(\gamma\) is both necessary and sufficient to induce the vasculopathy, other proinflammatory cytokines, potentially downstream of IFN-\(\gamma\), also may participate in TV development. Thus, we showed that grafts genetically deficient in both forms of the tumor necrosis factor (TNF) receptor also failed to develop TV, despite normal levels of host inflammatory cell IFN-\(\gamma\) production.\(^17\) Because the absence of TNF receptors on host cells had no effect on TV, the effect was not attributable to TNF signaling on host inflammatory cells or SMC precursors. Rather, absence of TNF signaling in donor vascular wall cells might reduce allograft vessel recruitment of SMC precursors to form the intimal lesion. In that setting, intimal SMCs could not contribute to the local IFN-\(\gamma\) milieu to generate an autonomous loop of SMC and macrophage activation culminating in TV.

Although researchers had long tacitly assumed that the intimal cells in TV were entirely derived from underlying donor medial SMCs, we and others demonstrated that intimal SMCs can originate from the host. In fact, in a number of animal models, the majority of the intimal SMCs are host-derived (summarized elsewhere\(^18,19\)). Moreover, up to 15% of these intimal SMCs stemmed from bone marrow (BM) precursor cells.\(^20-22\) Subsequently, others determined that circulating host precursors can also contribute to human heart and kidney transplant TV,\(^23,24\) and even to human atherosclerotic plaque\(^25\); a significant percentage of these were also BM-derived.\(^26\)

An important caveat in these studies is that the frequency of host SMCs found in the intimal lesions will likely vary as a function of the severity of vascular injury.\(^18,19\) In rodent heterotopic heart or aortic interposition models, the numbers of host origin SMCs in intimal lesions routinely exceed 90%. However, immunosuppression is usually minimal or absent in these models, and donor vessel ECs and SMCs frequently sustain moderate-to-severe injury; in that context, intimal cells must necessarily originate from host sources.\(^18,19\) On the other hand, in the setting of the traditional immunosuppression used for human solid organ transplantation, substantially less vascular injury is likely to occur. Indeed, using in situ hybridization techniques to identify Y chromosomes in human sex-mismatched cardiac allografts, a number of researchers have consistently identified only on the order of 3% to 15% host-derived intimal SMCs\(^23,26-27;\) for unclear reasons, renal allografts were approximately 2-fold more plentiful.\(^24\) Regardless of the host or donor origin, what emerges from these studies is that intimal smooth muscle cells can derive from multiple sources, and simple recruitment from donor medial cells is probably not a tenable paradigm. Both BM and non-BM precursors are important contributors, and therapeutic interventions will need to account for different cells of origin, routes of recruitment, and differentiation pathways. Additional details regarding the nature of the intimal smooth muscle cells are presented elsewhere in this review series.

### Vascular Wall Remodeling

Normal arterial walls contain 3 distinct layers or tunicae (Figure 1). The innermost tunica, the intima, consists of a layer of endothelium facing the lumen and residing on a basement membrane that overlies a thin ECM substrate. Deep to this layer, and demarcated by an internal elastic lamina, lies the media, composed of relatively quiescent SMCs admixed with ECM. Generally accepted as the vessel wall component responsible for maintaining vasomotor tone, the media accomplishes this function by contracting or relaxing in response to a variety of metabolic and hormonal stimuli (eg, from the adjacent ECs).\(^28\) The outer layer, frequently demarcated from the media by an external elastic lamina, is the adventitia, composed of myofibroblasts, scattered inflammatory cells, a few autonomic nerve fibers, and associated ECM. Tradition accords the adventitia only a structural supportive role (in larger arteries it will also contain the vasa vasorum, literally meaning “vessels of the vessels,” that perfuse the outer portion of the media). However, increasing evidence suggests that the adventitia participates directly in the regulation of arterial vasomotor tone.\(^29,30\)

In the course of TV development, distinct effects in the 3 vascular wall layers can influence luminal diameter (Figure 1). Such effects are grouped broadly into effects that result in negative remodeling (ie, reduce the luminal caliber) or positive remodeling (ie, that improve luminal diameter). For
example, in early atherosclerosis, luminal flow remains essentially unchanged, despite focal intimal thickening, because of associated positive remodeling (or “compensatory enlargement”) of the vessel wall.31 This remodeling occurs by SMC turnover and also through ECM degradation (via matrix metalloproteinases [MMPs] and other proteolytic enzymes) and rebuddressing, putatively driven by local changes in wall stress.32–34

Recruitment, proliferation, and ECM synthesis of SMCs within the intima eventually intrude on luminal flow. In early stages of TV, inflammatory cell recruitment also contributes to luminal restriction.35,36 Because vascular resistance varies inversely with the fourth power of the radius (ie, a 50% reduction in the radius will increase the resistance 16-fold), relatively small changes attributable to intimal thickening, particularly over long segments of the arterial wall, can profoundly impact myocardial perfusion pressures. Medial SMC apoptosis and medial remodeling can partially compensate this effect, along with remodeling of the adventitial ECM, thus theoretically maintaining luminal caliber. Indeed, inadequate vascular remodeling may predict TV compromise more accurately than intimal hyperplasia.37 Moreover, if adventitial fibrosis supervenes, the vessel cannot expand, resulting in a net negative remodeling. Changes in vascular tone, ie, increased medial SMC contraction or relative inability to stretch and dilate, will also significantly affect negative remodeling of the intima and adventitia. The following section examines each of these 4 components affecting luminal flow in turn.

Intimal Thickening

Circulating EC precursors that can participate in vascular wall remodeling have been described for almost a decade.38,39 Interestingly, the replacement of microvascular (eg, capillary) ECs with such circulating host precursors might underlie long-term host immunologic indifference to allografts.40–42 In comparison, the demonstration of a similar circulating source of intimal SMCs has emerged only more recently. As discussed above, in animal models of TV (and to a lesser extent in human TV), intimal SMCs are host derived.20,22,43,44 Moreover, a proportion of these intimal cells originate from host BM-derived cells,22 consistent with the demonstration of a BM-derived mesenchymal stem cell.45–47 That intimal SMCs can derive from circulating precursors also agrees with the long-standing observation that neointimal SMCs exhibit a phenotype distinct from medial SMCs.48–50 Intimal SMCs proliferate and elaborate ECM more akin to embryonic and fetal artery SMCs and share a number of morphological features with them.51 Besides the phenotypic distinctions, evidence suggests that intimal SMCs are clonal and potentially have a unique derivation,52 as they can express hematopoietic markers including CD34, Thy-1, c-kit, and flt-3.53 Because numerous important functional differences exist between medial SMCs and neointimal cells, we refer to the intimal population as smooth muscle–like cells (SMLCs).22 Although growth and differentiation of intimal SMLCs have considerable relevance to vascular disease, their development from precursor cells remains undetermined.52

Role of Chemokines and Adhesion Molecules in SMLC Recruitment

Because a proportion of SMLCs do not derive from the donor vessel media or ingrowth from adjacent host vessels,22 they must access graft vessels from the circulation. This can occur either via the luminal surface or an abluminal approach, and formal evidence exists for both, at least in an animal vein graft model.54 The principle route of SMLC entry into the vessel has more than just academic interest, because such a pathway becomes a potential therapeutic target. For example, if SMLCs enter arterial walls by mechanisms analogous to those used for inflammatory cell recruitment,55 a distinct cohort of chemokines and adhesion molecules may be involved (see Figure 2).

Early histologic evaluation in animal models find that the first smooth muscle α-actin-positive cells of host origin in TV lesions are arrayed on the luminal side of the intima.56 Although this does not prove direct entry from the lumen, it does at least suggest rapid transmigration to the newly forming intima. Such a luminal egress also bypasses the potential barriers imposed by the internal and external elastic laminae and by the ECM of the media. Although the elastic laminae are fenestrated and the medial ECM is largely porous,57 vascular transmigration would likely still be somewhat slowed. It is also noteworthy that neointimal formation, at least in coronary artery bypass vein grafts, begins only after EC loss and is temporally followed by apparent SMC precursor recruitment at the sites of injury.58 Such an observation suggests that frank endothelial injury and/or denudation could be an important proximal step that allows luminal attachment of SMLC precursors, perhaps via platelet intermediaries. Platelet adhesion can readily occur at high shear forces such as are present in injured arteries,59 and scattered reports associate platelet activation with TV development.60 If luminal adhesion of SMLCs occurs through such pathways,
thrombosis and platelet adhesion could be the relevant therapeutic targets to block TV onset.61

Conversely, for SMLC recruitment to occur in the setting of the high flow and shear stresses of an arteriolar circulation with an intact endothelium,3 it is not unreasonable that SMLC precursors access vessel walls via their abluminal face. Such an hypothesis is consistent with the observation that inflammatory cells appear to coalesce around arteries from the surrounding parenchyma during TV development12 and the likelihood that EC/mononuclear inflammatory cell adhesion will resemble EC–SMLC interactions. In postcapillary venules, where the majority of inflammatory cells exit the circulation, shear forces are only 1 to 3 dyne/cm². Although mononuclear cells can bind at shear forces up to 5 to 15 dyne/cm², and neutrophils can bind under shear forces up to 40 dyne/cm², firm adhesion requires several seconds to minutes.62 Moreover, the shear stress in many human arterial beds (20 to 80 dyne/cm²) exceeds the limits of mononuclear cells adhesion.63 Finally, with pulsatile flow, the forces on arterial walls constantly change, and shear stress gradients exceed significantly laminar shear forces. Consequently, based on flow considerations alone, it can be argued that SMLCs approach the intima by egress from microvasculature in the abluminal face of an artery; moreover, such an approach will very likely involve SMLC–EC adhesion molecule pairs and chemotactic stimuli.

Chemokines and Chemokine Receptors

Chemokines, small-molecular-weight (8 to 10 kDa) secreted proteins, classically associate with the recruitment of inflammatory cells at sites of injury. However, they likely also participate in the recruitment and activation of SMLCs in TV lesions. Despite a systematic classification based on the spacing of paired cysteine residues (eg, CCL1-28 and CXCL1-16),64,65 most of the chemokines discussed below are designated with names developed from their original assayed function (see the Table). Chemokine receptors, G-protein-coupled 7-transmembrane-spanning proteins with discrete cellular distributions and binding specificities, are systematically numbered as CCR1 to -11 and CXCR1 to -6. Recent reviews have discussed the function of these molecules in allograft rejection,64,66 and their role in TV is specifically detailed by Hancock in this series (see also Figure 2). In addition to recruitment, chemokines can also activate cells directly and/or potentiate the effects of other activating signals67; consequently, chemokines contribute significantly to cellular proliferation at sites of recruitment.

CXC chemokines are subdivided on the basis of an amino-terminal Glu–Leu–Arg (ELR) motif. ELR–CXC chemokines (eg, IL-8) attract neutrophils and act as potent angiogenic factors that promote the migration and proliferation of ECs. Conversely, CXC chemokines lacking ELR characteristically recruit lymphocytes and are angiostatic.68,69 Among the non-ELR CXC chemokines, IFN-γ strongly induces IP10 (IFN-inducible protein 10), Mig (monokine induced by IFN-γ), and I-TAC (IFN-inducible T-cell chemoattractant); this induction is significant because these chemokines associate with TV development,70 and the absence of IFN-γ abrogates TV.8,12 All vascular wall cells (ECs, SMCs, and macrophages) secrete IP10, whereas ECs and macrophages preferentially express I-TAC and Mig.71 These 3 CXC chemokines recruit activated memory T cells by binding to the CXCR3 receptor expressed on those cells, and T-cell recruitment is likely the most important role played by CXCR3 in TV development (see also Figure 1).68–70

CC chemokines were originally described as chemoattractants involved in recruiting and activating mononuclear inflammatory cells.69 Monocyte chemoattractant protein (MCP)-1, synthesized by ECs, SMCs, and macrophages, is prototypical of these chemokines and classically associates with monocyte, T-cell, and NK cell recruitment via CCR2 interactions.69,72,73 CCR2 activity likely participates in a
various chronic inflammatory diseases, including atherosclerosis,\textsuperscript{74,75} RANTES (regulated on activation, normal T cell expressed and secreted), another well-characterized CC chemokine expressed by mononuclear inflammatory cells, platelets, and neointimal SMLCs in vascular lesions, likely plays a role in both atherosclerotic and TV pathogenesis.\textsuperscript{76}

The plethora of chemokines and receptors, as well as the apparent promiscuity of chemokine–receptor interactions,\textsuperscript{68} raises the relevant concern that no single chemokine (eg, MCP-1) or receptor (eg, CCR2) might have a central and nonredundant role in any given inflammatory process. Fortunately, it appears that isolated MCP-1 or CCR2 ablations in genetically deficient mice materially impact monocyte recruitment and activation in delayed-type hypersensitivity lesions,\textsuperscript{77,78} as well as attenuate atherosclerosis.\textsuperscript{79,80} In experimental transplantation models, monoclonal antibody blockade of the IFN–γ–induced chemokine Mig diminished allograft rejection,\textsuperscript{81} and blockade of fractalkine/CX3CR1 interactions prolonged murine cardiac allograft survival.\textsuperscript{82}

**Chemokine Recruitment of Smooth Muscle Cells**

Although chemokine function classically associates with recruiting and activating inflammatory cells, we increasingly appreciate that many nonhematopoietic cells also express chemokine receptors (eg, ECs, epithelial cells, astrocytes, mesangial cells, and neurons) and that these receptors participate in a variety of processes, including angiogenesis, tumor metastasis, and wound healing (reviewed elsewhere\textsuperscript{68}). As early as 1998, functional CCR1 and CCR2 receptors were identified on cultured vascular SMCs,\textsuperscript{83} and functional CCR5 subsequently was demonstrated on normal human aortic and coronary vascular SMCs.\textsuperscript{84} Perhaps more significantly, SMCs from diseased vascular tissue showed increased CCR1 and CCR2 levels relative to SMCs derived from nonlesional tissue.\textsuperscript{85} Importantly, these seminal observations suggest that SMCs express chemokine receptors that potentially influence smooth muscle recruitment and activation in pathologic states. Interestingly, newer therapies that show promise in reducing TV severity may be beneficial because they can interfere with smooth muscle recruitment and activation.\textsuperscript{85,86}

The full spectrum of chemokine receptors reported on SMCs is listed in the Table. Immunohistochemistry has shown CXCR3 on SMCs in human CNS atherosclerotic plaques,\textsuperscript{87} and differential display demonstrated CXCR4 expression on hematopoietic stem cells that differentiated into SM-like cells in a TV model.\textsuperscript{88} SMCs also expressed CCR2, CCR3, and CCR5 in various pathologic lesions including human atherosclerotic plaques,\textsuperscript{89} and functional CCR5 expression by aortic media was shown in vivo in an experimental model of atherosclerosis.\textsuperscript{90} In experimental transplantation models, monoclonal antibody blockade of the IFN–γ–induced chemokine Mig diminished allograft rejection,\textsuperscript{81} and blockade of fractalkine/CX3CR1 interactions prolonged murine cardiac allograft survival.\textsuperscript{82}

### Summary of Chemokine Receptors Potentially Involved in SMC Recruitment and Activation

<table>
<thead>
<tr>
<th>Chemokine Receptors</th>
<th>Cellular Receptor Expression</th>
<th>Ligands</th>
<th>Cell Sources of Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXC receptors (CXCR1 to CXCR6)</td>
<td>Memory T cells (Th1), B cells, mesangial cells, SMCs\textsuperscript{77}</td>
<td>Mig, IP-10, I-TAC</td>
<td>ECs, SMCs, macrophages, lymphocytes</td>
</tr>
<tr>
<td>CXCR3</td>
<td>Memory T cells (Th1), B cells, mesangial cells, SMCs\textsuperscript{77}</td>
<td>Mig, IP-10, I-TAC</td>
<td>ECs, SMCs, macrophages, lymphocytes</td>
</tr>
<tr>
<td>CXCR4</td>
<td>T cells, DCs, monocytes, neutrophils, platelets, astrocytes, ECs, SMCs\textsuperscript{26,89}</td>
<td>SDF-1α, -1β, PBSF</td>
<td>ECs, SMCs, macrophages, lymphocytes, fibroblasts, stromal cells</td>
</tr>
<tr>
<td>CC receptors (CCR1 to CCR11)</td>
<td>Monocytes, T cells (Th2), SMCs\textsuperscript{33}, DCs (immature), neutrophils, eosinophils, mesangial cells, platelets</td>
<td>LD78-α, -β, MIP-1α, -1β, -1δ, -5, Lcn-1, RANTES; MCP-2, -3, -4; HCl4; NCC-1, -2, -3, CKβ1, -β8, -β10; HCC-1, -2, -3, MCF1; MARC, C10</td>
<td>ECs, SMCs, macrophages, lymphocytes, mast cells, platelets, various tumor cells</td>
</tr>
<tr>
<td>CCR1</td>
<td>Monocytes, T cells (Th2), SMCs\textsuperscript{33}, DCs (immature), neutrophils, eosinophils, mesangial cells, platelets</td>
<td>MCP-1, -2, -3, -4, -5, MCAF; HCl4; NCC-1; CKβ10</td>
<td>ECs, SMCs, macrophages, lymphocytes</td>
</tr>
<tr>
<td>CCR2</td>
<td>Monocytes, DCs (immature), T cells (Th2), basophils, NK, ECs, SMCs\textsuperscript{26,89}, fibroblasts</td>
<td>RANTES, eotaxin-1, -2, -3, MCP-2, -3, -4; HCl4; NCC-1; -2, -3; CKβ1, -β6, -β10; HCC-1, -2, -3, MCF; MIP-1β, -4α, -5, Lkn-1; MIPF-2; IMAC; TSC-1</td>
<td>ECs, SMCs, macrophages, lymphocytes, mast cells, platelets</td>
</tr>
<tr>
<td>CCR3</td>
<td>Eosinophils, basophils, T cells (Th2), DCs, platelets, SMCs\textsuperscript{83}</td>
<td>RANTES, eotaxin-1, -2, -3, MCP-2, -3, -4; HCl4; NCC-1; -2, -3; CKβ1, -β6, -β10; HCC-1, -2, -3, MCF; MIP-1β, -4α, -5, Lkn-1; MIPF-2; IMAC; TSC-1</td>
<td>ECs, SMCs, macrophages, lymphocytes, mast cells, platelets</td>
</tr>
<tr>
<td>CCR5</td>
<td>Monocytes, T cells (Th1), DCs, NK, thymocytes, SMCs\textsuperscript{83,89}</td>
<td>LD78-α, -β, MIP-1α, -1β, RANTES; Act-2; G-26; HCl4; HCl21; H400; LAG-1; SISY; MAD-5; MCP-2, -4; eotaxin; NCC-1, -2; CKβ1, -β10; HCC-1, -3, MCF</td>
<td>ECs, SMCs, macrophages, lymphocytes, mast cells, platelets</td>
</tr>
</tbody>
</table>

SMCs in pathologic lesions use only a subset of the 6 CXC receptors and 11 CC receptors. Act-2, activator protein-2; CK indicates chemokine; DC, dendritic cell; HCC, hemofiltrate cc chemokine; IMAC, inner-mitochondrial membrane anion channel; Lkn, leukotactin; MAD-5, MAX dimerization protein-5; MARC, mast cell activation-related chemokine; MCAF, macrophage chemotactic activating factor; MCF1, macrophage chemotactic inhibitory factor; MIPF, macrophage procoagulant inducing factor; LAG-1, lymphocyte activation gene-1; PBSF, pre-B-cell growth-stimulating factor; SDF, stromal cell–derived factor; SISY, skin immune system γ; TSC-1, tuberous sclerosis complex-1.
Activated lymphocytes and macrophages classically synthesize chemokines that participate in SMC recruitment and activation (Figure 2 and the Table). However, in addition to inflammatory cells, ECs and SMCs are important sources of RANTES, MCP-1, and macrophage inflammatory protein (MIP)-1 (Table). Thus, chemokine expression by injured or dysfunctional vascular wall cells could conceivably drive SMC recruitment (and/or proliferation) in TV, in the absence of ongoing rejection. In support of the potential importance of CCR1 in TV, targeted deletion of CCR1 reduced both acute parenchymal rejection as well as allograft vasculopathy. To date, no other chemokine receptor expressed on SMCs besides CCR1 has been shown to participate in TV pathogenesis by deletion or blocking experiments. RANTES, C10, mast cell activation–related chemokine, MCP-2, and MIP1-α and -γ constitute the major known murine CCR1 ligands; as is typical, these chemokines also bind to other chemokine receptors besides CCR1 (see the Table).

**Adhesion Molecules in Rejection and SMLC Recruitment**

Besides chemokine–chemokine receptor interactions, the recruitment of inflammatory cells into allografts requires adhesion to activated vascular beds. Unsurprisingly, therefore, EC expression of multiple adhesion molecules (eg, intercellular adhesion molecule [ICAM]-1, vascular cell adhesion molecule [VCAM]-1, E- and P-selectin) correlates with both acute rejection and TV. E- and P-selectins promote rolling of host leukocytes by associating with sialyl Lewis X moieties on certain surface glycoproteins. Fibrin adhesion occurs via ICAM-1 and VCAM-1 interactions. ICAM-1 binds to inflammatory cell lymphocyte function–associated antigen (LFA)-1 (CD11a/CD18) and macrophage antigen (MAC)-1 (CD11b/CD18) β2 integrin heterodimers expressed on all leukocytes, whereas VCAM-1 binds predominantly to the very-late antigen (VLA)-4 α5β1 integrin expressed mainly on mononuclear cells. Although isolated reports suggest that adhesion molecule blockade reduces acute rejection, and ICAM-1–deficient grafts have less TV, others have found no effect, or even exacerbation of alloimmune injury, from interference with adhesion molecule interactions. Indeed, the inability of most researchers to inhibit rejection by blocking leukocyte–EC interactions likely results from the multiplicity of adhesion molecules and/or the persistent generation of chemokines that overwhelm the adhesion blockade.

Notably, whereas the role of selectin and integrin adhesion molecules in hematopoietic cell recruitment is well documented, the expression and role of adhesion molecules on mesenchymal or smooth muscle precursor cells remains less well characterized. Thus, β1 integrins that bind to EC adhesion molecules are indeed expressed on mesenchymal stem cells and on SMCs as well (reviewed elsewhere). However, these β1 integrins primarily participate in SMC migration through the ECM, not current evidence suggests a role for these adhesion molecules in the attachment of smooth muscle precursors to ECs in vascular intimal lesions.

CD44, a highly charged membrane glycoprotein expressed ubiquitously on leukocytes, BM precursor cells, and nonhematopoietic cells, including SMCs, represents another adhesion molecule increasingly recognized as important in inflammation. CD44 specifically binds hyaluronan, an ECM glycosaminoglycan whose synthesis is increased because of ischemia and other tissue damage; CD44 and hyaluronan interactions thereby allow the localization of mononuclear inflammatory cells to sites of injury. Additionally, CD44 likely participates in SMC recruitment to sites of vascular injury. It is therefore interesting that hyaluronan administration significantly blocked TV development, and apoE- mice congenitally deficient in CD44 developed significantly less atherosclerosis. In both cases, CD44–hyaluronan blockade resulted in diminished inflammatory cell recruitment, so that an effect on SMCs alone is difficult to assess. Nevertheless, Cuff et al speculate that the beneficial effects also involved modulation of the ability of SMCs to achieve the “synthetic” state characteristic of intimal SMLCs, but a role in recruiting smooth muscle precursors was not suggested.

**Medial Remodeling**

Under the influence of arterial pressures, any loss of medial SMCs or medial ECM degradation in excess of synthesis can yield positive remodeling (dilation) of the vessel wall (Figure 1). Such vessel wall compensatory changes can preserve luminal diameters in atherosclerotic plaque. Conversely, collagenous scarring of the media results in a stiffer vessel with a net negative effect on remodeling.

Host alloreactive T cells and antibodies, as well as infiltrating macrophages, can all contribute to medial SMC apoptosis. Additionally, host effector cells can direct matrix remodeling through their production of lysosomal cysteine proteases and MMP. Conversely, a relative overproduction of protease inhibitors such as cystatin C or tissue inhibitors of metalloproteinase (TIMP) potentially inhibits ECM degradation and thus contributes to negative remodeling. Furthermore, medial SMCs and ECs directly synthesize a variety of MMPs and TIMPs.

**Constrictive Adventitial Fibrosis**

Intravascular ultrasound has been instrumental in demonstrating the role of adventitial fibrosis in negative remodeling in a variety of forms of vascular injury, including atherosclerosis and balloon injury restenosis. Typically, the technique measures luminal diameters at discrete sites along the vessel; intravascular ultrasound also assesses accurately the relative thickness of intima and media at each location. The various contributions of each layer are then compared with proximal and distal reference points. Even in the setting of intimal hyperplastic lesions, adventitial ECM remodeling that decreases the diameter of the external elastic lamina can maintain vessel luminal caliber. Conversely, when the external elastic lamina does not remodel to accommodate a larger diameter, the same thickness of intimal lesions associate with functional luminal stenoses. Regardless, negative remodeling attributable to inadequate expansion of the external elastic lamina (and/or adventitial fibrosis) explains only a minor subset of luminal stenoses, roughly 15% of cases.

Similar results apply to TV. In that setting, the perivascular cuffing of alloreactive inflammatory cells seen...
in transplanted organs might induce adventitial scarring and contraction in a manner comparable to wound healing. With time, the perivascular inflammation recedes, and likely would contribute less to ongoing vessel remodeling. This hypothesis is supported by longitudinal studies that demonstrated the dominant effect of early (within the first year) “vessel shrinkage,” i.e., negative remodeling driven largely by adventitial scarring; later luminal stenosis results more often from intimal hyperplasia. As discussed above, Th1 cytokines, specifically IFN-γ, induce vascular intimal hyperplastic lesions in TV as well as more typical atherosclerosis and vein graft stenosis. Besides mediating the recruitment and activation of inflammatory cells, IFN-γ and other proinflammatory mediators (e.g., TNF-α) theoretically drive the chemokine and adhesion molecule expression that may underlie SMLC accumulation in the intima. Interestingly, however, negative remodeling attributable to adventitial fibrosis is more likely mediated by transforming growth factor-β and, in fact, should be attenuated by a cytokine milieu rich in IFN-γ and TNF-α.

The intravascular ultrasound demonstration of functional stenosis attributable to negative adventitial remodeling in TV significantly preceded the biochemical and cellular evidence of this pathogenic pathway. Some of that story has since been fleshed out. Proliferation and increased matrix synthesis occurs in adventitial myofibroblasts in response to vascular injury including hypoxia and overdistention. Moreover, adventitial myofibroblasts express functional endothelin receptors. Besides potently inducing cell contraction, endothelin enhances mitogenic activity and ECM formation, both of which contribute to adventitial fibrosis.

**Vasoconstriction**

Impaired vasodilation and/or augmented vasoconstriction of the vessel media also contributes functionally to negative vascular remodeling. The interplay of vasorelaxant and vasoconstrictive mediators such as nitric oxide (NO) and endothelin, respectively, modulate luminal diameter. ECs, inflammatory cells, and myofibroblasts all participate in the production of these factors and therefore to vascular tone. Moreover, adventitia-derived cells contract in response to endothelin, thus contributing to vasoconstriction. Notably, ECs in transplanted organs show attenuated responses to vasodilators. Moreover, an early constrictor response to acetylcholine (a marker of EC dysfunction) correlates with subsequent TV development. Certainly, chronic increases in vascular tone contribute materially to functional stenoses that impede perfusion. Moreover, persistent (albeit potentially reversible) increases in vessel tone, resulting from dysfunctional endothelium in the setting of an allograft, conceivably could participate in the subsequent development of irreversible TV simply by increasing local wall shear stress.

**Vascular Wall Aneurysm Formation in a Th2-Predominant Environment**

Although IFN-γ (and other Th1 cytokines) is causally implicated in stenosing vascular lesions, recent human and animal studies suggest that vascular aneurysmal disease, particularly abdominal aortic aneurysms (AAAs), may result from vascular inflammatory processes dominated by Th2 cytokine expression. This final section briefly examines vascular wall changes in the context of a Th2 cytokine milieu. Aneurysm formation represents the polar opposite of stenosis in the pathologic spectrum of vascular remodeling (Figure 3). Intriguingly, it could also underlie the positive remodeling seen in the early stages of atherosclerosis and TV, although such a relationship between aneurysm and vascular remodeling has not been formally established.

**ECM Degradation in Aneurysms**

Human AAA lesions are typically infrarenal and fusiform and are characterized histologically by chronic inflammatory cell infiltrates, medial attenuation, and elastic tissue fragmentation. The inflammation variably includes neutrophils, T and B lymphocytes, macrophages, mast cells, and NK cells distributed throughout the entire wall thickness and involving any adherent luminal thrombus. Inflammatory cells infiltrating the vascular wall secrete a number of inflammatory mediators including cytokines, eicosanoids, reactive oxygen species, antibodies, and complement. Moreover, these cells, as well as the ECs and SMCs, produce a host of proteases, including MMP, plasminogen activators, and cysteine and serine proteases (reviewed elsewhere). Although medial and adventitial collagen synthesis increases in the early stages of aneurysm formation, a marked increase in degradative enzymes characterizes later-stage disease, with ECM catabolism (most notably elastin) exceeding synthesis. Protease expression occurs at much higher concentrations in aneurysms compared with stenotic atherosclerotic arteries and presumably participates in the loss of ECM integrity that culminates in vascular dilation. Even inflammatory cells in the luminal thrombus produce MMP-9 and urokinase-type plasminogen activator; in turn, plasmin produced in the process of modulating local thrombosis activates MMP. Concurrently, protease inhibitors are diminished in aneurysms; for example, cystatin C (an inhibitor of the elastolytic cathepsins S and K) is decreased in human AAA.
Immune Aspects of Aneurysms

The characteristics of the cellular immune response in vascular lesions likely influence the resulting pathologic alterations. T-cell recruitment with expression of proinflammatory Th1 cytokines typically characterizes early atherogenesis and stenotic atherosclerotic plaque, as well as TV (see above). In native human vascular disease (not TV), atherosclerotic lesions typically exhibit greater inflammatory cell infiltration than do stenotic lesions. In AAAs, CD4 T cells predominate over CD8 T cells, and although B cells occur less frequently, AAAs often exhibit focal immunoglobulin deposition, suggesting a possible humoral immune contribution. In both stenotic atherosclerotic and aneurysmal lesions, macrophages theoretically function as antigen-presenting cells and thereby influence local adaptive immunity. However, it remains uncertain whether the accumulated lymphocytes and antibody incite lesion formation, or simply accrue in response to some other injury. Nevertheless, innate immune elements such as macrophages also provide potent sources of collagenases and elastases. Moreover, the cytokine environment produced by the recruited inflammatory cells likely influences the activity of tissue macrophages and directs the nature of their subsequent response. Interestingly, evolving AAA lesions showed increased Th2 responses relative to stenotic atheromas.

Patterns of Cytokine Expression in Stenotic Versus Aneurysmal Disease

Most AAA lesions arise in the context of atherosclerosis, a chronic inflammatory lesion driven by T-lymphocyte effector mechanisms, including cytokines that potentially influence downstream events such as ECM synthesis or degradation. T lymphocytes are broadly grouped as either CD4 (helper) Th1 cells and CD8 (cytotoxic) Tc1 cells, which characteristically produce IFN-γ, or Th2 and Tc2 cells, which characteristically secrete IL-4, IL-5, and IL-13. Besides being mutually antagonistic for each other, Th1 cytokines tend to induce cellular inflammatory responses including macrophage activation, whereas Th2 cytokines play important roles in distinct inflammatory processes, particularly in the pathogenesis of allergies. The extrinsic cytokine stimuli and intracellular pathways that promote Th1 versus Th2 differentiation are reasonably well understood (these are beyond the limits of this discussion, but are reviewed nicely elsewhere). Regrettably, the specific triggers that determine whether an inflammatory response to a particular antigen(s) in a given individual will be predominantly Th1 or Th2 remain an elusive mystery. Nevertheless, Th1 versus Th2 patient-specific responses to particular stimuli are invoked to explain, eg, allergies (Th2-dominated inflammation), tuberculous (Th1-like) versus lepromatous (Th2-like) forms of leprosy, and reflux esophagitis (Th1-like) versus Barrett’s esophagitis (Th2-like).

Besides the cytokine mediators already described, eicosanoids and reactive oxygen species also contribute to AAA pathogenesis. In this regard, it is noteworthy that prostaglandin D, and reactive oxygen species both initiate and/or augment Th2-related inflammation.

Cytokines Regulate Protease Activities Associated With AAA Development

Cytokines regulate MMP, serine protease, and cathepsin expression. Indeed, both Th1 and Th2 cytokines (eg, IFN-γ or IL-4) induce or inhibit the expression of specific MMP, depending on the particular experimental conditions. Thus, IFN-γ induces MMP-9 in human melanoma cells but inhibits MMP-9 and -12 production by macrophages. Conversely, IL-4 inhibits MMP-1 production by human macrophages and induces MMP-12 expression by murine macrophages. Similar, and variable, Th1 and Th2 effects apply to other cell types and their associated protease products (reviewed elsewhere). In all cases, the specific cytokine effects have been assessed in vitro, and it is likely that the effects in a mixed cytokine milieu in vivo differ distinctly.

Aortic Aneurysm Formation In Vivo Correlates With a Th2-Dominant Cytokine Environment

The lack of a satisfactory animal model for aneurysmal disease hinders our understanding of the pathogenic mechanisms for AAA formation. Although elastase or angiotensin II infusion or local CaCl2 treatment all result in some vascular dilation, each uses a nonphysiologic agent to artificially expand the aortas, often without the same inflammatory components or ECM degradation that occur in human disease. Moreover, the roles of specific chemokine or cytokine mediators in driving aneurysm formation remain unexamined.

Because of the observations in human AAAs, we hypothesized that localized inflammation specifically elaborating a Th2 cytokine milieu might induce vascular aneurysm formation. Although the specific trigger(s) that tips atherosclerotic inflammation toward a Th2 predominance is unclear, some of the known risk factors for AAA (eg, smoking, genetic predilection) are likely involved. To test the hypothesis that Th2-dominated inflammation would promote aneurysm formation, we used an immunologically driven model, murine aortic transplantation, to focus local inflammation in allograft aortic segments.

Aortic interposition grafts in wild-type hosts developed IFN-γ–predominant responses in the allograft segment and culminated in typical TV with intimal hyperplasia. Notably, even complete medial SMC apoptosis attributable to rejection did not result in aneurysm formation, presumably because the ECM remained relatively intact. On the other hand, aortic transplantation into hosts lacking the IFN-γ receptor led to IL-4–dominated responses. In that setting, the allografts developed severe aneurysmal dilation associated with ECM degradation and, in particular, exhibited elastin loss similar to that seen in human AAA. Elastin destruction resulted from macrophage MMP-12 production driven by the local Th2 cytokine milieu and, in particular, by IL-4. Blockade using anti-IL4 antibodies or transplant recipients concurrently deficient in IL-4 reduced AAA formation in aortic allografts, suggesting important regulatory roles for Th1 and Th2 cytokines in modulating matrix remodeling.

Although this AAA model raises a number of interesting implications regarding aneurysm formation, and vascular remodeling in general, the results are controversial (see the excellent commentary by Curci and Thompson). First, the
model involves an aggressive alloresponse in an immunomodulated animal that may not bear much resemblance to human AAA disease. Second, the results contradict a substantial body of work pointing to a critical role for IFN-γ in driving atherosclerotic aneurysm formation. Although the involvement of IFN-γ can be explained as relevant only in initiating vascular wall inflammation, not in its subsequent evolution, additional work will be required to resolve the debate.

Regardless, it is intriguing to speculate that the early positive remodeling seen in atherosclerosis or TV is driven by a Th-specific inflammatory milieu. Certainly, the fact that the Th1 cytokine IFN-γ is associated with TV would suggest that Th2-induced positive remodeling may be beneficial. Interestingly, there are numerous case reports of ascending aortic aneurysms in patients following heart transplantation (including those in whom no other apparent risk factors were present[164]), and AAA incidence increases following transplantation and its attendant immunomodulation.[165] However, there are no reports of coronary artery aneurysms in transplanted hearts, as might be expected if a subset of patients were to diverge to an intragraft Th2 response. Overall, the possibility of exploiting Th2-dominated pathways to affect positive remodeling is an interesting area for future experimental exploration and could represent a therapeutic approach for moderating the occlusive effects of end-stage TV.

Summary

The luminal changes in TV result from an integration of negative and positive remodeling involving all 3 layers of the vessel wall; the net outcome in the majority of long-term allografts involves a progressive encroachment on the arterial diameter, resulting in perfusion compromise. Such narrowing results from a combination of intimal hyperplasia (involving the recruitment and proliferation of host SMLCs), adventitial scarring, and increased medial tone. Medial SMC dropout and/or ECM remodeling, as well as expansion of adventitial ECM elements potentially offset the stenosing effects, at least in the short term. However, current therapeutic approaches in solid-organ allografts cannot maintain these compensatory changes, and intimal hyperplasia eventually supervenes. Although unlikely to become a viable approach in TV prevention, the possibility of augmenting positive remodeling by modulating cytokine environments (eg, to a Th2-dominant milieu) is intriguing.

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Disclosures

None.

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