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Transforming growth factor- β and atherosclerosis: interwoven atherogenic and atheroprotective aspects

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Abstract

Age-related progression of cardiovascular disease is by far the largest health problem in the US and involves vascular damage, progressive vascular fibrosis and the accumulation of lipid-rich atherosclerotic lesions. Advanced lesions can restrict flow to key organs and can trigger occlusive thrombosis resulting in a stroke or myocardial infarction. Transforming growth factor-beta (TGF- β) is a major orchestrator of the fibroproliferative response to tissue damage. In the early stages of repair, TGF- β is released from platelets and activated from matrix reservoirs; it then stimulates the chemotaxis of repair cells, modulates immunity and inflammation and induces matrix production. At later stages, it negatively regulates fibrosis through its strong antiproliferative and apoptotic effects on fibrotic cells. In advanced lesions, TGF- β might be important in arterial calcification, commonly referred to as “hardening of the arteries”. Because TGF- β can signal through multiple pathways, namely the SMADs, a MAPK pathway and the Rho/ROCK pathways, selective defects in TGF- β signaling can disrupt otherwise coordinated pathways of tissue regeneration. TGF- β is known to control cell proliferation, cell migration, matrix synthesis, wound contraction, calcification and the immune response, all being major components of the atherosclerotic process. However, many of the effects of TGF- β are essential to normal tissue repair and thus, TGF- β is often thought to be “atheroprotective”. The present review attempts to parse systematically the known effects of TGF- β on both the major risk factors for atherosclerosis and to isolate the role of TGF- β in the many component pathways involved in atherogenesis.

Keywords

Transforming growth factor; Atherosclerosis; Cardiovascular disease; Restenosis; Gene expression

Introduction

Cardiovascular disease is by far the largest chronic health problem in the US, causing significant morbidity, mortality and a public health burden. The pathophysiology of cardiovascular disease probably involves chronic vascular inflammation, which triggers inflammatory cell infiltration, lipid accumulation and progressive vascular fibrosis, ultimately culminating in atherosclerotic lesions. Advanced lesions can restrict flow to key organs and if they rupture, can precipitate occlusive thrombosis, resulting in a stroke and myocardial infarction (MI). The present review will examine the role of transforming growth factor- β (TGF- β) as a major orchestrator of the fibroproliferative response to tissue damage.

Atherosclerosis

Atherosclerosis, derived from the Greek “athero” for gruel and “skleros” for hard, is a vessel wall disease, principally in the arterial bed, which is characterized by gruel-like cholesterol-rich lesions protruding from and to a lesser degree penetrating into, the medial layers of the artery. Particularly at advanced stages, the cholesterol-rich regions are encapsulated in a dense fibrous matrix and this fibrous matrix can become significantly calcified in both diffuse and focal deposits. Thus, the principal embodiment of cardiovascular disease consists in a raised “fibro-fatty” lesions, which occlude blood flow and trigger thrombosis. Atherosclerotic vascular changes are a major contributing factor to life-threatening events such as MI, stroke, aneurysm and pulmonary embolism.

Epidemiology

Whereas major advances have been made in preventing, detecting and treating atherosclerotic diseases, cardiovascular diseases remain the single largest cause of death worldwide. In 2007, roughly one-third of all deaths in the US were attributable to heart disease, cerebrovascular disease and hypertension (Xu et al. 2010). Americans have an approximately 50% greater risk of dying of cardiovascular disease than cancer (Xu et al. 2010). There are roughly 3000 heart attacks per day in US, one every 28 s. It is poorly appreciated that 50% of first attacks are fatal, resulting in about one death every minute from heart attacks (Roger et al. 2011). The incidence of coronary and aortic atherosclerosis is more severe in males than in females and increases strongly with age, such that, by age 65, up to 50% of the coronary artery surface can be covered by raised atherosclerotic lesions (Vihert 1976; Fig. 1).

In a “lucky” subset of patients, recurrent chest pain, termed angina, will necessitate medical attention prior to a fatal infarction. If detected and treated quickly, almost 90% of patients survive even major coronary artery blockage. Although public health and pharmaceutical advertising has driven awareness of preventable risk factors such as hypertension, smoking and hypercholesterolemia, more than 50% of victims have no overt risk factors for atherosclerosis. Recent analysis of the Get With The Guidelines (GWTG) database demonstrates that about 70% of patients admitted for acute coronary syndrome have normal levels of triglycerides, low density lipoprotein (LDL) and cholesterol and in about half of those patients, the high density lipoprotein (HDL) level corresponds to the normal values

established by the American Heart Association (Sachdeva et al. 2009) suggesting that we have yet to identify major risks for heart disease. The lipid profile might be a risk predictor predominantly in people over the age of 65 and to a lesser degree in younger populations (Villines et al. 2010). However, if diagnosed, coronary disease is readily treatable by diet, exercise, risk factor management and medical and interventional strategies.

Atherosclerosis is statistically associated with several preventable risk factors, such as hypertension, hypercholesterolemia, smoking and diabetes. Of equal or greater overall impact are several unpreventable risks: advancing age, gender and a family history of early heart attacks. The impact of age on atherosclerosis is shown in Fig. 1, which illustrates the amount of atherosclerosis in the aorta of people as a function of age and gender.

Pathophysiology

The prevailing theories have suggested that atherosclerosis arises from chronic or repetitive injury to the vessel wall; this damage triggers a hyperplastic response with prominent inflammatory components (Ross 1993). Agents that cause chronic vascular injury include elevated lipids, especially in their oxidized/modified forms, hydrocarbons from smoking, elevated glucose from diabetes, elevated blood pressure and shear forces attributable to hypertension and potentially autoimmune or infectious damage. Whereas chronic vascular injury is tolerated and efficiently repaired in young individuals, repair is less efficient in older individuals, leading to the accumulation of repair cells, excess extracellular matrix, inefficient removal of cholesterol-rich debris, negative remodeling (contraction) of the artery (see below) and persistent inflammation. As shown in Fig. 2, plaques located near the lumen and contained only by thin fibrous caps can easily rupture and release cholesterol and procoagulant-rich debris, leading to life-threatening complications including thrombosis, MI and stroke.

Stable versus unstable lesions: the critical issue of plaque stability

Although raised atherosclerotic lesions are indisputably an undesirable feature, they are surprisingly both ubiquitous and generally asymptomatic. Autopsy studies have demonstrated that almost 75% of young healthy Korean War soldiers had raised lesions in their coronary arteries (Enos et al. 1955), a key finding supported by more recent autopsy studies observing at least 20% of males younger than 35 with histologically diagnosed coronary artery disease (Joseph et al. 1993). Likewise, the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study detected raised fibro-fatty lesions in the arteries of children as young as 10–12 years old (Strong 1993). However, in adults, lesions can begin to occlude significant amounts of the lumen diameter, creating regions of turbulent flow, which activate the endothelium and platelets, predisposing to platelet adhesion and thrombosis. Atherosclerotic lesions can be roughly divided histologically into two types: stable, in which there is a thick fibrous “cap” that isolates any fibro-fatty debris, or unstable, in which the cholesterol-rich debris is separated by only a thin fibrous cap from the circulating blood (Fig. 2). These unstable lesions in coronary and carotid arteries are so-called “widow makers” because any type of physical stress, such as elevated blood pressure or transient viral or bacterial infection can cause physical dissection or “rupture”. Plaque rupture exposes the blood to the lesion’s lipid-laden matrix, which is rich in tissue factors and other

strongly procoagulant molecules, resulting in the active propagation of thrombus. The typical lesion from elderly adults often demonstrates histological evidence of multiple rounds of rupture, thrombosis and re-endothelialization. However, in too many cases, the plaque rupture leads to a significant occlusive thrombus at the rupture site or the thrombus breaks free and becomes occlusive in a smaller distal artery in the heart or brain, causing MI or stroke, respectively.

Plaque stability or instability might reflect fundamentally different pathological processes that differ between lesions and among people. For instance, some recent evidence has been presented that people who form keloids, a fibroproliferative disease, are also more likely to have an increased intimal/medial thickness, which is probably a reasonable clinical measure of intimal fibroproliferative progression. Mechanistically, it is common to categorize stable lesions as “fibro-proliferative”, whereas unstable lesions are “fibro-fatty” or principally inflammatory in nature. There is ample reason to believe that TGF- β signaling can modulate the fibrotic versus inflammatory components of the lesion. A recent examination of circulating levels of TGF- β and platelet-derived growth factor (PDGF) in patients with stable versus unstable carotid artery lesions suggested that low TGF- β levels were associated with plaque instability, probably by favoring inflammatory matrix degradation and reducing matrix synthesis (Borrelli et al. 2006). Likewise, immunohistochemical staining of stable and unstable human coronary and carotid lesions suggested that TGF- β was higher in stable lesions (Cipollone et al. 2004; Jiang et al. 2004). Furthermore, even within an “unstable” lesion, the critical events surrounding plaque rupture are thought to be determined by apoptosis or necrosis of the thin fibrous cap, thereby further weakening the cap and allowing rupture (Kolodgie et al. 2001). Numerous studies have demonstrated that TGF- β can modulate susceptibility to apoptosis in vascular cells (Hyman et al. 2002).

Component factors in the etiology of atherosclerosis

As discussed, a complex and multifactorial disease such as atherosclerosis must be parsed into its component parts, with a keen awareness of the fact that, in a given patient, one or more of those components might predominate as a cause. Based on epidemiological data, 50%–70% of the cases of atherosclerosis might be explained based on known risk factors. In the following sections, the effects of TGF- β on the major etiologic components of atherosclerosis will be discussed.

Atherosclerosis/restenosis as a defective wound repair

Rats and rabbits develop vascular lesions after balloon injury, although in young adult animals, these lesions spontaneously regress over a period of weeks. In older rats, however, balloon injury causes a more exuberant fibroproliferative response and lesions that persist after re-endothelialization (Stemerman et al. 1982), possibly because of intrinsic age-related changes in the artery wall (Hariri et al. 1986, 1988; Stemerman et al. 1982). Evidence from the PDAY and other studies also strongly suggest that atherosclerotic lesions are reversible in young human adults but become more persistent in older individuals (Strong 1995). Serial angiographic analysis in humans indicates that restenosis occurs in most patients after balloon angioplasty but in the majority of patients, the lesions spontaneously regress (Mehta

et al. 1995). The reason that some patients heal without persistent hyperplasia, whereas others develop occlusive vascular hyperplasia in response to the same injury, is not currently known. A plausible theory is that atherosclerosis and restenosis partially reflect an underlying failure in the regression of wound repair attributable to defects in the antiproliferative and apoptotic processes (Rembold 1996).

Origin of fibroproliferative cells of human vascular lesions

Atherosclerotic lesions are composed of several cell types, some of which are associated transiently with the lesion. Monocyte-derived macrophages are a predominant population of cells in early lesions. These cells scavenge oxidized lipoprotein complexes and can become “foam cells” with visible cytoplasmic lipid vacuoles (Nakashima et al. 2007). The predominant cell in late lesions is often called a “smooth muscle cell (SMC)” because of its cytoskeletal expression of contractile, smooth muscle α -actin. However, several cell types express smooth muscle α -actin (Buoro et al. 1993), most notably the blood-derived myofibroblasts that are prominent in many non-vascular wounds and scars (Schmitt-Graff et al. 1994). Whereas SMC can migrate from the damaged arterial media through disrupted elastic lamina toward the lumen to close perceived breaches, it is equally likely that both adventitial and blood-derived cells migrate and differentiate into SMC-like myofibroblasts designed for vascular repair. This possibility is supported by the finding that lesion-like coatings form on stainless-steel surfaces such as left-ventricular assist devices exposed to blood (Vranken et al. 2008). These lesion-like coatings are composed of SMC-like cells that are phenotypically similar to the cells composing advanced fibrous lesions. Indeed, SMC can be isolated in vitro by allowing whole blood of healthy volunteers to adhere to collagen-coated plastic in the presence of PDGF (Simper et al. 2002). The collagen-coated plastic attracts and binds “smooth-muscle-like outgrowth cells”.

Vascular damage response: angioplasty and restenosis

The primary treatment options for patients with clinical and angiographic evidence of coronary atherosclerosis are coronary bypass surgery or angioplasty. Balloon angioplasty, typically with endovascular stent placement, is performed on more than 650,000 patients per year in the US and achieves initial reperfusion in more than 95% of cases. Given its high success rate, short hospitalization and minimally invasive nature, angioplasty is likely to remain a treatment of choice for coronary and peripheral atherosclerosis. However, the initially high success rate of angioplasty is often followed by progressive fibroproliferative reclosure of the artery, termed “restenosis”, within 3–6 months post-angioplasty. Recently, drug-eluting stents have been developed to reduce the frequency of this complication.

Restenosis mimics some aspects of the atherosclerotic process, because it is a highly-defined, acute, vascular insult triggering a hyperplastic response. Balloon angioplasty causes severe mechanical arterial injury, often creating intimal “flaps” and fissures, with endothelial denudation and accompanying mural thrombus. These flaps and fissures are consumed within a fibroproliferative lesion over a period of 3–6 months. The course of injury-induced intimal hyperplasia probably involves: (1) endothelial injury and dissection of the elastic lamina, (2) mural thrombosis, (3) recruitment of SMC-like myofibroblasts, probably derived from circulating monocytes or fibrocytes, (4) increased cell proliferation within the

neointima, (5) accumulation of extracellular matrix, (6) re-endothelialization, (7) partial or complete resolution via apoptosis (Bochaton-Piallat et al. 1995) and (8) tissue remodeling involving wound contraction. Histologically, restenotic lesions are principally fibrotic, often being termed “arteriosclerotic” and can largely reclose an artery in the absence of drug-eluting stents. The potential role of TGF- β and SMAD signaling in restenosis has recently been reviewed (Suwanabol et al. 2011).

Hyperlipidemia: TGF- β as cargo and target

Recent data indicate that active TGF- β is found at high levels in apoE3-containing HDL particles, suggesting that a constitutive level of TGF- β signaling is a part of normal vascular biology and not uniquely associated with disease states (Tesseur et al. 2009). However, cholesterol can interfere with TGF- β /receptor interaction in several cultured cell types such as bovine aortic endothelial cells (BAEC) and thus hypercholesterolemia could blunt the normal signaling of TGF- β (Chen et al. 2008). Conversely, statins, which lower cholesterol, increase the effect of TGF- β on vascular cells, such as BAEC (Chen et al. 2007), probably because of the ability of statins to regulate the rho family of G protein signaling factors. Statins are also known to afford some renoprotection by decreasing oxidative stress and TGF- β production (Zhou et al. 2008). Furthermore, the induction of severe hyperlipidemia in apoE knockout mice causes an elevation in circulating TGF- β 1 levels (Zhou et al. 2009).

Hypertension: TGF- β effects on vessel wall compliance

The role of TGF- β in hypertension is documented by an extensive literature that is well beyond the scope of this review. However, several basic effects of TGF- β can be briefly described. Genetic variants in the TGF- β /bone morphogenic protein (BMP) pathways are known to cause various hypertensive states, such as pulmonary hypertension and lead to vascular restructuring, such as hereditary hemorrhagic telangiectasia (Upton and Morrell 2009). Mechanistically, TGF- β , derived either from platelets or from autocrine/paracrine production, increases the collagen content of arteries relative to elastin and, thus, has the effect of stiffening arteries, thereby reducing their compliance to pulsatile flow and increasing blood pressure (Fleenor et al. 2010). Furthermore, TGF- β affects endothelial cells in numerous ways, notably altering the nitric oxide pathway to impair flow-induced relaxation (Buday et al. 2010). Polymorphisms in TGF- β are associated with essential hypertension in Chinese ethnic subgroups (He et al. 2010). Elevated TGF- β levels associated with TGF- β single nucleotide polymorphisms might also be a contributing factor to renal disease, a major cause of hypertension (August et al. 2009).

Inflammation: TGF- β as an immunomodulator

Early theories of atherosclerosis focused on vascular damage and lipid storage, whereas more recent theories propose that subacute chronic inflammation of the vessel wall also plays a significant role in disease pathology. As recently reviewed by Rocha and Libby (2009), inflammation might play similar roles in promoting atherosclerosis and obesity. In both cases, oxidized LDL and free fatty acids mobilize pro-inflammatory adipokines and cytokines. In the vessel wall, interferons promote monocyte/macrophage infiltration, endothelial activation and thrombosis. Microarray analysis confirms that interferon-related genes are activated during coronary artery restenosis (Zohnhofer et al. 2001).

Vascular repair programs probably recapitulate the embryonic development program for vascular tissue. The regression phase of repair is critical to limit and prevent excessive growth and is heavily dependent on the appropriate differentiation of repair cells, growth inhibition and “editing” by the apoptosis of unnecessary cells. Some of the key pathways involved in repair-associated regression include Fas/Fas ligand, endogenous glucocorticoids, relaxin, small lipid intermediates such as lipoxins and ceramides and the TGF- β system. Among the most potent known anti-inflammatory factors is TGF- β , which suppresses inflammation in a variety of cell types. Further, other anti-inflammatory and antifibrotic agents, such as glucocorticoids (Wen et al. 2002) and relaxins (Heeg et al. 2005), partially exert their effect by interacting with the TGF- β /SMAD pathway. In vascular SMC, TGF- β suppresses inflammatory markers such as inducible nitric-oxide synthase and interleukin 6 via the SMAD3 pathway (Feinberg et al. 2004). The role of TGF- β and SMAD signaling in atherogenesis and vascular inflammation has previously been reviewed by Feinberg and Jain (2005).

Vascular remodeling: the stealth effect of TGF- β

To a large degree, atherosclerosis and arteriosclerosis are misunderstood diseases. Although a lesion within an arterial lumen would seem to obviously occlude blood flow, this is indeed only one aspect of the defect. Surprisingly, a direct negative correlation has not been found between the size of the lesion and the size of the remaining lumen. For example, after coronary angioplasty, the progressive reclosure of the artery is not related to the growth of the lesion but to a decrease in the diameter of the artery as a whole, termed “negative remodeling” (Meine et al. 1999). Negative remodeling of arteries is circumferential narrowing, probably attributable to contractile functions of the smooth muscle and the SMC-like myofibroblasts, which are specifically designed for contracting wounds and laying an additional matrix to solidify the closure (Wang et al. 2007). An extensive literature, encompassing almost a 1000 publications, documents that TGF- β is one of the prime movers in the differentiation of fibroblasts or blood-borne fibrocytes (Abe et al. 2001) into myofibroblasts (Evans et al. 2003; Gabbiani 2003) and for stimulating the contractile function of the wound myofibroblasts (Malmstrom et al. 2004). Thus, potentially the most untoward effect of elevated or dysregulated TGF- β signaling would be to stimulate contractile “negative remodeling” of the artery (Fig. 3). In essence, the myofibroblasts are closing a wound, which unfortunately happens to be a coronary, carotid, or other vital artery.

Thrombosis: both cause and effect of TGF- β action

As discussed below, TGF- β is released from the α -granules of platelets along with other key factors, such as PDGFs, which cooperate to stimulate the vascular repair program at sites of perceived breach. The physical and chemical insults to an artery at sites of high turbulence can activate the endothelium and increase the adhesion and activation of platelets, thereby releasing active TGF- β (Fig. 3). Further, activated endothelium and activated lesion cells produce TGF- β , which is not a “classic” member of the coagulation cascade, like tissue factor but has indirect effects on coagulation. Most notably, TGF- β can regulate the activation of the endothelium (Goumans et al. 2002) and is extensively studied for its ability to induce plasminogen activator inhibitors (PAI), especially PAI-1, which has the effect of decreasing the thrombolytic effect of plasminogen activators that help to dissolve fibrin clots

(Fujii et al. 1991). Thus, tissues with high TGF- β and thus PAI-1, would be less efficient at clearing thrombus, thereby tipping the balance toward thrombosis, which is the major proximal cause of MI. Elevated PAI-1 also has the untoward effect of decreasing matrix breakdown, which is a major element of plaque progression (Otsuka et al. 2006).

Diabetes: the AGE/RAGE connection to TGF- β

The obesity epidemic in the US is associated with an alarming increase in the incidence of Type II diabetes and the related metabolic syndrome of lipid abnormalities. Diabetes is among the most powerful and preventable risk factor for atherosclerosis, MI and stroke (Lundberg et al. 1997; Weitzman et al. 2004). Whereas many possible connections can be envisioned, a relatively straightforward hypothesis is that some of the untoward effects of diabetes are mediated by the induction of TGF- β . Elevated plasma glucose causes non-enzymatic glycosylation of plasma proteins, called advanced glycation end-products (AGEs). These AGEs interact with specific receptors (RAGEs) on many cells, including the vascular endothelium, which activates the endothelium and perivascular cells to produce TGF- β and other cytokines (Heidland et al. 2001). AGE/RAGE effects are accelerated by interactions with TGF- β signaling to converge on a signal transducer and activator of transcription 5 (STAT5) signaling (Brizzi et al. 2004). Thus, TGF- β is an important component of an “activation program” stimulated by diabetic elevations in blood glucose.

Arterial calcification: ectopic effects of the TGF- β family

A histological and clinical hallmark of atherosclerosis is arterial calcification, which has led to the pathognomonic phrase “hardening of the arteries”. Intravascular ultrasound has shown that 73% of coronary lesions contain calcifications (Mintz et al. 1998). While being characteristic of advanced lesions in elderly persons, vascular calcifications exhibit potentially important variations between races, ethnicities and vascular beds and associations with environmental risk factors such as diabetes, smoking and hypercholesterolemia. Considerable evidence suggests that TGF- β family members, particularly the BMPs, are important modulators of vascular calcification (Jeziorska 2001; Simionescu et al. 2005), probably mostly by creating an extracellular matrix that is amenable to mineralization and by favoring differentiation toward osteoblast-like lineages (Wang et al. 2010; Fig. 3).

Production and localization of TGF- β 1 during the repair of vascular damage

Sources of TGF- β activity in the vascular lesion

The TGF- β family includes TGF- β 1, β 2 and β 3 and several TGF- β -like proteins, such as BMPs, inhibins, activins and growth/differentiation factors (GDFs), such as myostatin (GDF8). Essentially all cells in the arterial wall can produce one or more TGF- β family members during vascular damage. Active TGF- β 1 is released from activated platelets, which are present at any type of thrombotic event, or occurs even at low levels upon an activated endothelium. TGF- β 1 circulates in plasma at biologically active levels in the range of 2–12 ng/ml, whereas TGF- β 2 and β 3 are essentially undetectable (Wakefield et al. 1995). TGF- β 1 and β 2 isoforms are detected within the subendothelial space of lesions (Scott et al. 1997)

and the $\beta 1$ and $\beta 3$ isoforms are detected at high levels in the smooth muscle, macrophage and foam cells of fibro-fatty streaks (Bobik et al. 1999). Infiltrating cells, such as macrophages, neutrophils and lymphocytes, can all produce TGF- β s and facilitate their release from the extracellular matrix by the action of proteases such as plasmin. Likewise, TGF- β s are produced by the SMC-like cells, which compose the fibroproliferative regions of human vascular lesions.

Localization of TGF- β s in the vascular lesion

TGF- $\beta 1$ is produced as a 26-kDa dimer, which reassociates with a precursor sequence to form a latent complex (Lyons et al. 1990). Latent TGF- β can be activated by acidic conditions (Lawrence et al. 1984), plasmin (Lyons et al. 1988), transglutaminase (Kojima et al. 1993) and thrombospondin (Schultz-Cherry et al. 1994). TGF- β and its latent complex bind to several key extracellular matrix proteins including fibronectin (Mooradian et al. 1989), thrombospondin (Murphy-Ullrich et al. 1992) and type IV collagen (Paralkar et al. 1991). The latent form of TGF- β localizes to the extracellular matrix (Flanders et al. 1989). Thus, latent TGF- β is a “cryptocrine” factor, which can be activated by local proteolytic activity (Falcone et al. 1993b).

TGF- $\beta 1$ binds heparin (McCaffrey et al. 1989, 1992), which prevents binding of TGF- $\beta 1$ to the activated form of α_2 -macroglobulin, the principal soluble inactivating pathway for TGF- β (McCaffrey et al. 1989). This might be related to the mechanism by which heparin inhibits the proliferation of SMC (Grainger et al. 1993; McCaffrey et al. 1989). At sites of vascular injury, TGF- $\beta 1$ is released from degranulating platelets in the mural thrombus. In addition, repair cells produce higher levels of TGF- $\beta 1$ in restenotic lesions (Nikol et al. 1992) and in balloon-injured rat carotid arteries (Majesky et al. 1991). Interestingly, TGF- β signaling can be generated from non-TGF ligands because recent data show that thrombin, generated during acute or chronic procoagulant states, can transactivate the TGF- β Type I receptor via proteinase-activated receptor-1 (PAR-1), a thrombin receptor (Burch et al. 2010).

TGF- β receptors in atherosclerosis

TGF- $\beta 1$ binds and activates a pair of transmembrane receptors (Type I and II), which possess intracellular serine/threonine kinase activity and which mediate downstream effects of TGF- β and can be sequestered on the cell surface by Type III proteoglycan receptors.

Type III membrane receptors

Several membrane proteoglycans show high affinity for TGF- $\beta 1$: betaglycan (Andres et al. 1989), endoglin, biglycan and decorin (Yamaguchi et al. 1990). The binding of TGF- $\beta 1$ to these Type III membrane proteins has no known signaling function but might represent a reservoir of TGF- $\beta 1$, which can later be laterally transferred to Type I and II receptors (Yamaguchi et al. 1990).

TGF- $\beta 1$ signaling receptors

TGF- $\beta 1$ binds to two high-affinity cell-surface receptors. The presence or absence of these receptors generally correlates with the gain or loss of responsiveness to TGF- $\beta 1$, suggesting

that the receptor level is an important mode of regulation (Boyd et al. 1990). The Type II receptor (T β R-2) is a 60-kDa protein, which is glycosylated to approximately 80 kDa (Lin et al. 1992). The amino acid sequence predicts a cysteine-rich extracellular domain, a single transmembrane domain and a cytoplasmic domain with serine/threonine kinase activity. T β R-2 has constitutive serine/threonine kinase activity in the absence of ligand (Wrana et al. 1994). The Type I receptor (T β R-1) is a member of a family of transmembrane serine/threonine kinases. The principal human T β R-1 is the activin-like kinase 5 (ALK5) receptor encoding a 53-kDa membrane protein (Franzen et al. 1993). Unlike T β R-2, the kinase function of T β R-1 is inactive until it is phosphorylated by T β R-2 (Wrana et al. 1994).

Heteromeric signaling

Extensive evidence indicates that many effects of TGF- β 1 are mediated by T β R-1/T β R-2 heteromeric complexes (Wrana et al. 1992). For instance, when T β R-1 is overexpressed in cells lacking any TGF- β 1 receptors, no increase occurs in radiolabeled-iodinated TGF- β 1 (125 I-TGF- β 1) binding unless T β R-2 is also overexpressed (Ebner et al. 1993; Fassing et al. 1994). Transfection of T β R-2 into a class of mutant cells apparently lacking both T β R-2 and T β R-1 revealed that T β R-1 was present but was unable to bind 125 I-TGF- β 1 in the absence of T β R-2 (Wrana et al. 1992). Thus, T β R-1 requires T β R-2 in order to bind TGF- β 1 and T β R-2 requires T β R-1 to transduce the signal via the phosphorylation of receptor SMAD intermediates.

Intracellular TGF- β signaling: many paths to many outcomes

One of the puzzling and initially controversial, aspects of the biology of TGF- β was the startling number of cellular responses and the degree to which many of the responses seemed to act with relative autonomy from other TGF-induced effects. We now understand that the complexity of the TGF- β signal derives from: (1) multiple receptors, (2) multiple signaling pathways that diverge just below the receptor level, (3) divergence within the pathways, (4) extensive secondary effects of TGF- β and (5) feedback control of signaling, which creates temporal “disconnects” in the effects, disconnects that are however essential as subroutines within the overall response program. An overview of the major TGF- β signaling pathways is shown in Fig. 4 and more in-depth descriptions can be found in two excellent reviews on the subject (Yokote et al. 2006; Zhang 2009).

SMAD family of signaling intermediates

The ligand-activated receptor complex, probably a heterotetramer of two T β R-1s (ALK5) and two T β R-2s, phosphorylates SMAD2 or 3 (pSMAD2, pSMAD3), which are then competent to bind SMAD4. Rat SMC express SMAD1–SMAD5 and coexpression of SMAD2 and SMAD4 produces the most pronounced induction of PAI-1 after TGF- β treatment (Ikeda et al. 2003). The pSMAD3/SMAD4 complex can interact directly with certain promoter sites, whereas the pSMAD2/SMAD4 complex requires adaptors to bind promoter regions and to activate important TGF- β intermediates such as connective tissue growth factor (CTGF; Gressner et al. 2009), a PDGF-like factor that further affects growth and matrix production by the cells (Grotendorst 1997). pSMAD3/SMAD4 complexes induce transcriptional activation of a number of promoters, including the cyclin-dependent kinase

inhibitors (CDKIs), such as p15 (Rich et al. 1999), p21 (Pardali et al. 2005) and p27 (Pierelli et al. 2000), which block the normal interaction of cyclins with cyclin-dependent kinases (CDKs) and growth arrest in G1 occurs. Extracellular matrix production is mediated by nuclear factor-1 (NF-1)-responsive transcriptional promoters in genes such as $\alpha 2(I)$ collagen (Rossi et al. 1988) or by adaptor-related protein-1 (AP-1) regions of other genes such as PAI-1 (Keeton et al. 1991). Consistent with its role in TGF- β signaling, pSMAD3 signaling is markedly upregulated in restenotic femoral arteries compared with primary lesions (Edlin et al. 2009).

Non-SMAD signaling: the kinase pathway

TGF- β modulates vessel wall functions via SMAD-dependent and SMAD-independent pathways (Khan et al. 2007; Zhang 2009). The T β R-1 (ALK5) has kinase activity toward SMAD2 and SMAD3 but also contains a kinase-independent recognition site for tumor necrosis factor (TNF) receptor-associated factor-6 (TRAF6), which activates several members of the mitogen-activated protein (MAP) kinase (MAPK) family, including TGF-activated kinase 1 (TAK1, MAP3K7), which can then further activate both the p38 kinase pathway and, importantly, the jun N-terminal kinase-1 (JNK1, MAPK8) pathway. In primary vascular smooth muscle cells, the growth inhibitory effect of TGF- β is at least partially dependent on this p38 kinase pathway (Seay et al. 2005).

TGF- β signaling via the Rho/ROCK pathway

A second SMAD-independent pathway involves engagement of the small guanosine triphosphate hydrolases (GTPases), particularly Rho kinase and its downstream effectors, such as Rho-associated coiled-coil containing protein kinase 1 (ROCK1). Rho kinase activation induces TGF- β 2 and endothelin production in hepatic stellate cells (Shimada et al. 2011) and SMAD and Rho pathways combine to regulate PAI-1 induction (Samarakoon and Higgins 2008). A consensus effect of Rho signaling is the modulation of cytoskeletal restructuring necessary for such key functions as macrophage chemotaxis (Kim et al. 2006) or contractile activity, which is a major component of vascular remodeling (Lee et al. 2008; Miura et al. 2006; Rivera et al. 2007) and may be vital to the transdifferentiation of myofibroblasts (Smith et al. 2006). TGF- β -induced stress fiber formation, characteristic of contractile myofibroblasts, is mediated by Rho signaling via the neuroepithelial transforming 1 (NET1) guanine nucleotide exchanger (Shen et al. 2001). In certain cell types, the growth inhibitory actions of TGF- β require Rho/ROCK signaling, which inhibits cdc25a and CDK2, thereby causing G1/S arrest (Bhowmick et al. 2003). Dominant-negative inhibition of rhoA (Chen et al. 2009) or Rho inhibitors (Matsumoto et al. 2004) blocks neointimal formation via suppression of the TGF- β pathway.

Modulation of TGF- β signals in the vascular environment

Tuning the TGF- β signal in time and space

The TGF- β signal is carefully modulated at several different levels: (1) receptor kinase modulators, (2) SMAD modulators and (3) transcriptional modulators. TGF- β signaling is significantly regulated by negative feedback loops, which dampen signal propagation and define temporal boundaries. For example, TGF- β , via SMADs, induces SMAD7, which in

turn has negative effects on signaling at the receptor level. SMAD-specific E3 ubiquitin protein ligases (SMURFs) 1 and 2, which are also induced by TGF- β , can downregulate receptor SMADs (rSMADs 2,3). The c-Ski and SnoN proteins have both positive and negative effects on TGF- β signaling. Generally, they appear to exert a negative effect (Takahata et al. 2009; Xu et al. 2000) by stabilizing inactive SMAD3/SMAD4 complexes at SMAD-sensitive promoters (Suzuki et al. 2004). C-Ski can block both transactivation by SMADs and the repressive function of SMAD3/SMAD4 at the c-Myc promoter and thus, is capable of blocking the cytostatic effect of TGF (Liu et al. 2008; Suzuki et al. 2004). C-Ski is targeted for proteosomal degradation by Arkadia (RNF111; Le Scolan et al. 2008; Yuzawa et al. 2009) and SnoN is marked for degradation by the anaphase promoting complex (APC; Wan et al. 2001) and thus, both Arkadia and APC are potentially important modulators.

Key balance between profibrotic versus antiproliferative and apoptotic effects of TGF- β

Tissue biogenesis and homeostasis after injury reflect the balance between cell proliferation and matrix production versus growth inhibition, apoptosis and matrix degradation. The profibrotic effects of TGF- β are well established as being mediated by SMAD response elements in the promoters of matrix-related genes such as collagens, fibronectin, plasminogen activator inhibitor and proteoglycans. Thus, inhibition of T β R-1 ALK5 kinase activity abrogates transcriptional activation of essentially all SMAD-dependent gene induction.

The antiproliferative effect of TGF- β is more complicated. TGF- β inhibits cell proliferation at the G1/S boundary in a variety of cell types (Kletsas et al. 1995). Currently, five related hypotheses are being considered for the way that TGF induces growth arrest: (1) repression of c-Myc transcription, (2) induction of CDKIs, especially p21, (3) repression of E2F1, (4) SMAD-independent signaling via the MAP or JNK pathway and (5) as discussed above, SMAD-independent signaling via Rho/ROCK modulation of cdc25a/CDK2. These are not truly independent theories because the relationship between c-Myc and p21, for instance, is complicated and both are related to E2F1 activation. Antiproliferative signaling by TGF- β is the major “checkpoint” for limiting the fibroproliferative expansion of vascular lesions and wounds in general; therefore, its mechanism must be clearly elucidated.

SMAD-dependent repression of c-Myc transcription—Early investigations suggested that the cytostatic effect of TGF- β could be inhibited by overexpression of the c-Myc proto-oncogene in 10 T1/2 and AKR-2B fibroblasts (Alexandrow et al. 1995). The c-Myc promoter contains a TGF- β inhibitory element (TIE), which downregulates c-Myc transcription when bound by pSMAD3, SMAD4 and E2F4 (Frederick et al. 2004; Yagi et al. 2002). Repression of c-Myc is cytostatic and has the additional effect of modulating other key factors. The short-lived oncoprotein c-Myc induces the AP4 transcription factor, which represses the CDKI p21 (Jung et al. 2008). Thus, TGF- β downregulates c-Myc, causing derepression (upregulation) of p21 (Claassen and Hann 2000). Microarray, serial analysis of gene expression, and reporter gene analyses have identified approximately 500 putative c-Myc target genes, approximately 220 of which are down-regulated, including the CDKIs p15 (CDKN2B), p21 (CDKN1A) and p27 (CDKN1B) and the T β R-2 (TGFR2; Zeller et al. 2003). Consistent with a possible role for c-Myc in the fibroproliferative expansion of

lesions, microarray analysis has revealed higher expression of c-Myc in stenotic saphenous bypass grafts compared with normal unaffected veins of the same patient (Hilker et al. 2003).

SMAD-dependent induction of p21—Whereas repression of c-Myc might play an important role in the cytostatic effect of TGF- β , p21 itself is an important target of TGF- β signaling. First, as discussed above, c-Myc repression allows unimpeded activation of p21. In turn, p21 (a CDKI) blocks CDK4 activity and thus retinoblastoma (Rb) phosphorylation, which is required for E2F1-dependent cell cycle progression. However, SMADs cooperate with specificity protein-1 (SP1) at a cluster of SP1 sites in the proximal region of the p21 promoter to stimulate p21 transcription directly (Datto et al. 1995; Pardali et al. 2000). In cell lines with deletion of p21, TGF- β is mitogenic instead of cytostatic (Bachman et al. 2004). Thus, the combined effect of the SMAD-induced repression of c-Myc and the SMAD/SP1-induced activation of p21 might produce a level of p21 that is cytostatic.

E2F1 as a target of TGF- β —A recent study in human Burkitt lymphoma cells suggested a third possible mechanism for the cytostatic effect of TGF- β (Spender and Inman 2009). CA45BL Burkitt lymphoma cells have a c-Myc translocation (t(8:14)) placing it under the control of the immunoglobulin heavy chain promoter, so that it is unresponsive to TGF- β . CA45BL cells do not induce p15 or p21 in response to TGF- β but exposure to TGF- β (1 ng/ml) inhibits their growth by 50%. The expression of E2F1 decreases and the E2F1 promoter is repressed in response to TGF- β in CA45BL cells. Whereas the role of E2F1 in cell cycle progression is well established, this is the only report, to our knowledge that TGF- β directly downregulates the expression of E2F1. Additional studies are required to determine whether this observation is unique to CA45BL cells, or whether it is a more general phenomenon.

TGF- β activation of protein kinases—A large body of evidence suggests that SMAD-independent activation of downstream protein kinase effectors plays a role in the antiproliferative effect of TGF- β . The T β R-1 (ALK5) has an adaptor sequence that recognizes the E3 ubiquitin ligase TRAF6 in a kinase-independent manner, causing TRAF6 activation by autoubiquitinylation (Sorrentino et al. 2008). TRAF6-mediated ubiquitinylation of TAK1 at Lys 34 correlates with TAK1 activation. TAK1 then phosphorylates and activates both the JNK and p38 MAPK (Yamashita et al. 2008). In primary mouse aortic SMC, inhibition of T β R-1 (ALK5) kinase by SB431542 and inhibition of p38 MAPK by S203580 attenuated the antiproliferative effect of TGF- β (Seay et al. 2005).

Cross-talk between the pathways—These seemingly independent components of the TGF- β response might be inter-related in the following manner. SMAD3 is phosphorylated at its C-terminus by the T β R-1 but is also phosphorylated in the linker region that joins the MAD homology domains MH1 and MH2. Whereas C-terminal phosphorylation activates SMAD3 binding to SMAD4 and subsequent transactivation of target genes, phosphorylation in the linker region occurs at consensus proline/serine sites, which are recognized by the prolyl cis/trans isomerase, NIMA-interacting-1 (PIN1).

SMAD3 linker phosphorylation by casein kinase, MAPK and JNK can have a negative effect on SMAD3 activity by affecting SMAD3 stability and reducing the DNA-binding ability of the SMAD3/SMAD4 complex, inhibiting JNK suppressed SMAD3 linker phosphorylation and increasing C-terminally phosphorylated SMAD3 and its activity at the p21 promoter (Nagata et al. 2009). A second point of crosstalk between these pathways is that both p38 and JNK1 directly phosphorylate p21 and phospho-p21 is relatively protected from ubiquitin-mediated proteasomal degradation, which allows p21 to accumulate after TGF- β /SMAD transcriptional activation (Kim et al. 2002). Thus, the MAPK pathway interacts in important ways with the SMAD pathway. However, SMAD activation is an important component of the cytostatic response to TGF- β , as indicated by the effects of small molecule inhibition of the T β R-1 kinase (Seay et al. 2005), SMAD3 knockout cells (Kohn et al. 2010) and dominant-negative SMAD3 mutants (Liu et al. 1997).

Effects of TGF- β s on vascular cells

TGF- β exerts potent and diverse actions on each of the cell types involved in vascular disease (Saltis et al. 1996). TGF- β frequently exerts bifunctional effects that are dependent upon the context in which the particular cell type encounters the TGF- β signal. This allows TGF- β to orchestrate an entire repair process that involves opposite effects at different times (Grainger 2007).

Endothelial cells

The proliferation of endothelial cells tends to be strongly inhibited by TGF- β . This inhibition is associated with decreased proliferation and migration (Bell and Bell 1989; Heimark et al. 1986) and increased synthesis of extracellular matrix and proteoglycans (Chen et al. 1987). The in vitro antiproliferative and antimigratory effect of TGF- β may explain the in vivo effects of TGF- β , including delayed re-endothelialization of a denuded artery (Madri et al. 1989). Importantly, TGF- β can have a dose-dependent bifunctional effect on angiogenesis induced by the vascular endothelial growth factor and fibroblast growth factor (Pepper et al. 1993). This is consistent with the observation that TGF- β stimulates endothelial migration and proliferation at low concentrations but inhibits both at higher concentrations (Bobik 2006). Conceptually, this in vitro dose-dependent effect probably mimics an in vivo concentration gradient of TGF- β , which possibly acts to attract cells from a distance (low ligand concentration) but to retain them at the site of injury (high ligand concentration) during vascular repair. A potentially important recent observation is that TGF- β can influence the transdifferentiation of bone-marrow-derived circulating endothelial progenitor cells into SMC-like cells (Fadini and Tjwa 2010).

Smooth muscle cells

Vascular SMC compose the bulk of the normal arterial tunica media and myofibroblasts, which can express the smooth muscle α -actin isoforms, are a major constituent of the atherosclerotic lesion. TGF- β signaling is a critical aspect of the differentiation of pluripotent cells into the smooth muscle lineage (Fadini and Tjwa 2010; Sinha et al. 2004, 2009). In adult human SMC, TGF- β potently inhibits migration and proliferation of SMC (Assoian and Sporn 1986; Bjorkerud 1991). However, in rat aortic SMC, TGF- β stimulates

the production and release of mitogens, such as PDGF, which can have mitogenic effects (Battagay et al. 1990), particularly on quiescent or highly confluent SMC. SMC from the chick embryonic cardiac neural crest are of ectodermal origin and are thought to compose the proximal portions of the coronary arteries. TGF- β stimulates the growth of these ectodermal cells but inhibits the growth of mesodermal cells (Topouzis and Majesky 1996). TGF- β also strongly modulates the phenotype of human SMC via effects on cytoskeletal actin reorganization and cell spreading (Bjorkerud 1991).

The effects of TGF- β on apoptosis are cell-type-dependent (Pollman et al. 1999) and context-dependent. Under some conditions, TGF- β can induce apoptosis of vascular SMC (Redondo et al. 2007) and endothelial cells (Hogg et al. 1999; Hyman et al. 2002). However, when apoptosis is initiated by other factors, TGF- β can promote cell survival. Cells from human vascular lesions appear prone to spontaneous apoptosis (Bennett et al. 1997) when first isolated; however, lesion-derived cells are typically completely resistant to TGF- β -induced apoptosis once established in cell culture (McCaffrey et al. 1999). TGF- β -induced apoptosis might be critical for wound or lesion regression after vascular repair is complete.

Macrophages

Macrophages play a key “scavenger” role in atherosclerosis and respond to TGF- β in a complex manner that is dependent upon their activation state. TGF- β suppresses the conversion of monocytes to macrophages (Tsunawaki et al. 1988), a key step in atherogenesis. Brief treatment with TGF- β stimulates migration, whereas longer exposure inhibits chemotaxis of cells in the monocyte/macrophage lineage via the Rho pathway (Kim et al. 2006). In activated macrophages, TGF- β stimulates the production of urokinase and, subsequently, of plasmin (Falcone et al. 1993a). Macrophages and active TGF- β colocalize in vascular lesions, suggesting that macrophages activate latent TGF- β via proteolysis (McCaffrey et al. 1999; Ross et al. 1990). Recently, deficiency in GDF15, a TGF- β family member, has been shown to attenuate lesion development in LDL receptor knockout (LDLR^{-/-}) mice in a T β R2-dependent pathway that culminates in reduced macrophage chemotaxis (de Jager et al. 2011), suggesting that this divergent member of the TGF- β family is somewhat pro-atherogenic. Interestingly, glucocorticoids induce mature macrophages to express T β R-2 and thereby activate a multi-step activation program reminiscent of lesion macrophages, suggesting that stress-induced, or even therapeutic, glucocorticoids might paradoxically accelerate atherosclerosis (Gratchev et al. 2008).

Lymphocytes

Lymphocyte proliferation is potently suppressed by TGF- β under most conditions tested (Ahuja et al. 1993) and TGF- β is regarded as one of the most potent immunosuppressive factors in the body (Bonecini-Almeida et al. 2004). TGF- β induces apoptosis in B and T lymphocytes in culture at very low concentrations (Lomo et al. 1995). Within the vascular lesion, lymphocytes tend to express T β R-1 but not T β R-2, suggesting that limited resistance to TGF- β might be required for their survival and function in the plaque (McCaffrey et al. 1999). Deliberately disabling T β R2 selectively in lymphocytes leads to more aggressive atherosclerosis attributable to accelerated vessel wall infiltration in ApoE (Robertson et al. 2003) and LDLR (Gojova et al. 2003) knockout mice.

Expression of TGF- β s and receptors during vascular repair

Animal models

Circulating TGF- β might modulate the ability of the vessel wall to respond to injury. Apo(a), which interferes with the normal activation of TGF- β 1 in the artery wall, contributes to excessive cell proliferation in animal models of lipid-induced injury (Grainger et al. 1994). After injury to the rat carotid, the level of TGF- β 1 mRNA in the vessel wall increases within 6 h, peaks at 24 h and can remain elevated for up to 2 weeks, coinciding with the development of the fibroproliferative neointima (Majesky et al. 1991). TGF- β 1 production increases in arteries in response to mechanical injury (Majesky et al. 1991), hypercholesterolemia (Ross et al. 1990) and deoxycorticosterone/salt hypertension (Sarzani et al. 1989). Both T β R-1 and T β R-2 for TGF- β 1 increase after balloon injury to the rabbit (Kanzaki et al. 1995). Mice lacking SMAD3 show enhanced neointimal hyperplasia, attributable to increased cellularity but with reduced extracellular matrix deposition (Yokote et al. 2006).

Human atherosclerosis

Immunohistochemical analysis indicates that the expression of TGF- β 1 and TGF- β 3 is associated with SMC, macrophages and foam cells in early human vascular lesions. Expression of the T β R-1 (Alk5) and T β R-2 is elevated in the early fibro-fatty streak (Bobik et al. 1999). However, active TGF- β 1 and its receptors are expressed to a limited extent in isolated areas of advanced atherosclerotic lesions (Bobik et al. 1999; McCaffrey et al. 1999). Most advanced fibrous lesions express low and variable levels of the T β R-1 and, generally, much lower levels of T β R-2. Elevated SMAD3 expression has been documented in the smooth-muscle-like cells of human femoral restenotic lesions compared with primary aortic atherosclerotic populations and experimental transfection of SMAD3 into human aortic SMC increases proliferation in vitro (Edlin et al. 2009; Tsai et al. 2009).

Aneurysms, pulmonary hypertension and other vascular pathologies

Although not the main subject of this review, it is worth noting that TGF- β has a well-established role in other vascular pathologies. Overall, mutations in the TGF- β pathway increase significantly the risk of affected individuals and carriers for cardiovascular disease and fibrotic disorders (Akhurst 2004). Whereas, in most cases, atherosclerosis leads to the progressive occlusion of arteries, in some cases, it can weaken the artery and cause partial dissection and dilation, called aneurysms. The role of TGF- β in the initiation and progression of aortic aneurysms has recently been reviewed, with defective TGF- β signaling being implicated at several levels of disease progression (Jones et al. 2009). Extensive evidence also indicates that abnormalities in the BMP, BMP receptor and TGF/BMP signaling pathway lead to congenital and acquired pulmonary hypertension, a devastating disease in which progressive fibroproliferative thickening of the pulmonary vessels decreases vessel elasticity and increases pulmonary artery pressure to dangerous levels (Machado et al. 2006; Perros et al. 2005). Mutations in the Type III family of TGF- β receptors, such as endoglin, have also been implicated in microvascular anomalies such as hereditary hemorrhagic telangiectasias (Chaouat et al. 2004).

Circulating/soluble levels of TGF- β in atherosclerosis

TGF- β has important autocrine, paracrine and endocrine effects and thus, we should consider the impact of variation in the soluble and circulating levels of TGF- β . Accumulating evidence suggests that the plasma level of TGF- β is reduced in patients with atherosclerosis (Grainger et al. 1995; Stefoni et al. 2002; Tashiro et al. 2002). In the vessel wall, TGF- β is largely interstitial and matrix-associated and its level is also reduced in atherosclerotic regions. These results suggest that TGF- β signaling is generally lower in patients with atherosclerosis (Grainger 2007). The combination of reduced levels and reduced responsiveness would greatly reduce the overall activity in the TGF- β pathway in the atherosclerotic environment. These ideas have led to a “protective cytokine” theory of atherosclerosis, a proposal that emphasizes the role of reduced TGF- β bioactivity in generating a pro-atherosclerotic environment (Grainger 2004).

Manipulating TGF- β and TGF- β receptors in vascular disease

Overexpression of TGF- β in a rat injury-induced hyperplasia model, via direct transfection of TGF- β cDNA into the artery wall at the time of vascular injury, markedly increases intimal and medial thickness, principally by increasing the extracellular matrix component (Nabel et al. 1993). In rats, infusion of exogenous TGF- β at the time of balloon catheter injury also causes a two-fold increase in the thickness of the neointima and increases the content of matrix in the lesion (Majesky et al. 1991). Rabbit arteries infused with exogenous TGF- β show a similar fibrotic response to vascular injury (Kanzaki et al. 1995). Consistent with a profibrotic activity of TGF- β , infusions of a neutralizing antibody to TGF- β at the time of balloon catheter injury to the rat aorta markedly reduces intimal hyperplasia and fibrosis (Wolf et al. 1994). Small molecule inhibitors of the T β R-1 (ALK5) kinase prevent myofibroblast induction and vascular fibrosis in the rat carotid injury model (Fu et al. 2008).

However, inhibition of the TGF response has a different outcome in more complex animal models of atherosclerosis, which have a much greater inflammatory component than simple balloon injury models. In ApoE^{-/-} mice with selective TGF- β overexpression in the heart, TGF- β is generally protective against lesion development by inhibiting lesion growth, suppressing inflammatory cell infiltration and increasing the matrix composition to form more stable lesions (Frutkin et al. 2009). However, when the effect of TGF- β on the immune system is preferentially targeted, the opposite effect on lesion development is observed. The expression of dominant negative T β R-2 in T-cells in ApoE (Robertson et al. 2003) and LDLR (Gojova et al. 2003) knockout mice or injection with a soluble T β R-2 (Mallat et al. 2001) strongly promotes atherosclerosis, demonstrating that TGF- β maintains a tonic inhibitory effect on the immune system that minimizes the vessel wall response to hypercholesterolemia. Likewise, injection of a soluble T β R-2 into ApoE^{-/-} mice also reduces lesion size and switches lesion-type from fibrotic to inflammatory (Lutgens et al. 2002). Thus, to oversimplify greatly, TGF- β has a strong profibrotic but a powerful anti-inflammatory effect on the vessel wall. Therefore, the effect of TGF- β is context-dependent and is heavily determined by the nature of the vascular injury.

Asymmetries in the production and response to TGF- β 1: the “TGF- β paradox”

In several other diseases, investigators have noted anomalies between elevated levels of TGF- β growth factor versus a marked decrease in one or more of the TGF- β responses that should be observed. In asthma, for instance, the pulmonary levels of TGF- β appear elevated and yet the immunosuppressive effect appears decreased (Rook 2001). Likewise, in cancer, TGF- β levels are frequently increased and yet the antiproliferative effects are almost invariably lost (Tian and Schiemann 2009). In several viral conditions, TGF- β production is elevated but the antiproliferative effect is lost, thus creating asymmetries whereby the production of TGF- β and the response to TGF- β are moving independently and often in opposite directions; hence, our attention is drawn to “the TGF- β paradox”.

The sharp increase in atherosclerotic disease with age is consistent with direct evidence that underlying age-related changes in the vascular wall exacerbate vascular injury (Hariri et al. 1986; Stemerman et al. 1982), partially because of age-related resistance to inhibitors such as TGF- β (Bochaton-Piallat et al. 1993; Hariri et al. 1988; McCaffrey et al. 1988). SMC derived from old animals produce normal amounts of both active and latent TGF- β 1 (McCaffrey and Falcone 1993). However, the old SMC show no inhibition of DNA synthesis in response to TGF- β 1 over the range 0–5 ng/ml, whereas young SMC are inhibited by 50% at 50 pg/ml (McCaffrey and Falcone 1993). However, the old SMC retain the ability to induce extracellular matrix protein in response to TGF- β . A similar age-dependent response to TGF- β has been observed in SMC derived from the spontaneously hypertensive rat (Saltis et al. 1994). In human lesion-derived cells, the antiproliferative response to TGF- β is lost within a few passages in culture and, indeed, is often converted to a mitogenic response to TGF- β (McCaffrey et al. 1995). However, the profibrotic response to TGF- β is not lost and is actually exaggerated in the lesion cells compared with cells from a normal artery (McCaffrey et al. 1995).

In both rat and human cells, this acquired resistance is associated with a preferential down-regulation of T β R-2 (McCaffrey et al. 1999; McCaffrey and Falcone 1993), although additional changes in negative regulators of signaling, such as SMURF-2, have been observed (Gagarin et al. 2005). Transfection of T β R-2 partially corrects the response of human lesion-derived cells and old rat SMC (McCaffrey et al. 1995) suggesting that the intracellular signaling system remains at least partially functional. A small subset of patients possess lesion cells with acquired mutations in a microsatellite region of T β R-2 (McCaffrey et al. 1997; Clark et al. 2001). However, despite changes in T β R-2 levels, a TGF- β /SMAD signal is clearly transmitted in the lesion-derived cells, because they show a strong profibrotic response and a mitogenic response to TGF- β . The mitogenic response is possibly attributable to a SMAD2-dependent induction of CTGF and PDGF by TGF- β (Battagay et al. 1990). The selective loss of the antiproliferative response has direct implications for lesion progression and possibly for other fibrotic disease and for cancer and viral infections.

A systems model for the future

Although the focus of this review is the narrow, albeit substantial, subject of TGF- β in atherosclerosis, we would be unwise to consider TGF- β or atherosclerosis in the absence of their respective contexts. TGF- β is one of several important families of factors that coordinate the vascular repair process and any schemes for the future must be able to integrate the other major signaling systems that undoubtedly play a role in atherosclerosis: coagulation factors, chemokines, PDGFs, CTGF, interferons, AGE/RAGE and toll-like receptors, to name just a few.

Likewise, atherosclerosis is commonly discussed as if it is a single disease with one common causality. Empirically, we know that several, largely unrelated defects can cause atherosclerosis. From the human mutations in LDLR and ATP-binding cassette transporters and from animal models of ApoE and LDLR knockouts, we know that lipid dysfunctions can create atherosclerotic disease. However, defects in the lamin A gene, causing Hutchinson-Guilford progeria, cause rapid and profound atherosclerosis in children who typically die at the age of 12–14 years from MI but without obviously dysregulated lipid profiles (Eriksson et al. 2003; Merideth et al. 2008). Diseases such as systemic lupus erythematosus (Harats et al. 1999), antiphospholipid syndrome (Belizna et al. 2008) and Sjogrens disease (Vaudo et al. 2005) and studies on heat shock protein 60 autoantibodies (Knoflach et al. 2005) suggest that autoimmunity could be an independent contributor to atherosclerosis. Thus, to understand such diseases, or indeed any disease, we must carefully parse each contributing component of the disease and integrate the diverse systems that impact upon that component. Ultimately, in treating the disease, we will need accurate diagnostic tools not only to identify the presence of disease but also to diagnose which disease components are the most significant in a particular patient.

Summary

A substantial body of evidence indicates that TGF- β is a key modulator of normal and abnormal vascular repair and that dysfunctions in this pathway promote a pro-inflammatory, pro-fibrotic and pro-atherosclerotic environment. As diagrammed schematically in Fig. 4, TGF- β signaling is tightly regulated at multiple levels. Reduced TGF- β activity/signaling is a feature of atherosclerosis, as evidenced by low TGF- β activity in vessel walls and low levels of circulating TGF- β in the plasma of affected patients. However, smooth-muscle-like cells in the lesion are capable of producing active TGF- β and no shortage of matrix-derived or platelet-derived TGF- β is apparent. Concurrent with altered TGF- β activity, lesion cells display defects in the cytostatic response to TGF- β . These defects might be symptomatic of additional changes in the phenotype of lesion cells, such as resistance to the effects of glucocorticoids (Bray et al. 1999) or fas ligation (Yang et al. 2007). TGF- β resistance, on the one hand, may allow repair cells to tolerate hostile environments and repair vascular damage but if unchecked, the failure to limit the repair process could, on the other hand, have adverse effects on the artery wall. Thus, an understanding of the molecular mechanisms that regulate vascular repair, including TGF- β signaling, is crucial for the development of treatments for occlusive vascular disease. Although we might be tempted to label TGF- β as

either an “atheroprotective” or “atherogenic” factor, TGF- β is more likely to play a central role in both normal and pathological vascular repair.

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Fig. 1.
Effect of age and gender on the degree of atherosclerosis in the human aorta. Autopsy studies measured the area of the human aorta exhibiting raised atherosclerotic lesions as a function of the age and gender of the subject. Adapted from Supplementary data in Vihert (1976)

Fig. 2. Histological appearance of stable and unstable human atherosclerotic lesions. **a, b** Human carotid artery atherosclerotic lesions excised by surgical endarterectomy; formalin-fixed paraffin-embedded hematoxylin/eosin-stained sections. **a** Stable lesion exhibiting a thick fibrous cap covering a largely acellular, fibrous lesion with significant lesion calcification. **b** Unstable lesion in which a thin fibrous cap covers a large cholesterol-rich lesion with significant inflammatory infiltrates

Fig. 3.

Schematic model of TGF- β involvement in atherosclerosis. A hypothetical atherosclerotic lesion creates progressive stenosis of a coronary artery. TGF- β activity can be generated by the release of active TGF- β from degranulating platelets activated by adhesion to inflamed endothelium. TGF- β can also be produced by most of the cells that compose the lesion. TGF- β induces the transcriptional activation of mitogens, such as platelet-derived growth factor and connective tissue growth factor and extracellular matrix genes, such as collagens, fibronectin and thrombospondins. In parallel, TGF- β suppresses the breakdown of the extracellular matrix by inducing the transcription of plasminogen activator inhibitors and metalloprotease inhibitors, leading to accumulation of the fibrotic matrix. The matrix becomes amenable to calcification by cells pushed along the osteoblastic lineage, partially by the action of TGF- β and related bone morphogenic proteins. TGF- β also modulates the activation state of immune cells entering the lesion and regulates their differentiation into specialized phagocytic and fibroblastic phenotypes. The action of TGF- β on the differentiation of progenitors into myofibroblasts creates contractile cells that progressively narrows the lumen of the artery in a process termed “negative remodeling”

Fig. 4.

Schematic diagram of the major TGF- β signaling pathways in vascular cells. TGF- β interacts with three types of cell-surface receptors: Type III receptors (T β R3) are proteoglycans, such as endoglin (ENG), which bind TGF- β and act as a peri-membraneous reservoir but have no known signaling functions; Type II receptors (T β R2) are constitutive serine-threonine kinases, which activate Type I receptors (T β R1), particularly ALK5, in tetrameric complexes induced by the TGF- β ligand. T β R1 phosphorylates receptor SMADs (SMAD2/3), which then bind to the common SMAD4 (SMAD4), are translocated to the nucleus and interact with a variety of cofactors to activate or repress transcription. In particular, c-Myc (C-Myc) can be repressed by SMAD3-containing complexes, whereas transcription of cyclin-dependent kinase (CDK) inhibitors such as p15, p21 and p27 can be directly activated or indirectly activated by c-Myc repression. In a SMAD-independent pathway (MAPK mitogen-activated protein kinase), T β R1 has a kinase-independent binding site for TRAF6,

which ubiquitinates and activates TGF-activated kinase 1 (*TAK1*) to influence signaling in the jun N-terminal kinase-1 (*JNK*) and MAPK pathways. In a third pathway (*Rho*), receptor activation can activate Rho GTPases, which couple to guanine nucleotide exchange factors (*GEF*), such as neuroepithelial transforming 1 (*NET1*), to activate Rho-associated coiled-coil containing protein kinase (*ROCK*) and Lim kinase, causing inactivation of cdc25a and CDK2, favoring cell cycle arrest