Atherosclerosis is the major cause of death in the Western world. The mechanism leading to atherosclerotic lesion formation has been tremendously updated in the last decades. From a merely degenerative concept, the pathogenesis of the disease is now well ascertained to be inflammatory. It is also recognized that the thrombotic complications of the disease are a consequence of the disruption of the atherosclerotic plaque due to the erosion of the endothelium or the rupture of the fibrous cap covering the lipid core within the plaque (127). The current morbidity and mortality and increasing age of the population require the development of new diagnostic and therapeutic strategies to treat early subclinical disease stages.

In addition to the endothelium, blood vessel walls are composed of many constituents such as matrix base-ment, smooth muscle cells, and pericytes. All these components form a cohesive tissue that requires, for proper function, a constant exchange of information via electrical and chemical signals. A key pathway that allows such communication is gap junction (GJ) 

The dysfunction of the endothelium, often induced by hypercholesterolemia and other cardiovascular risk factors, is the initial step of atherosclerosis. Atherosclerotic lesions are typically observed in arteri-

lesion formation (83). Beside selectins, activated ECs express integrins such as vascular cell-adhesion mole-
cule 1 (VCAM-1) in response to cholesterol accumula-
tion in the intima (23). T-cells and monocytes, expressing both the very late antigen 4 (VLA-4), bind thereafter to endothelium and transmigrate under the influence of chemokines such as macrophage colony-stimulating factor that is produced by low-density lipoprotein (LDL)-stimulated ECs and smooth muscle cells (SMCs) (99). SMCs may also express VCAM-1, promoting further recruitment and retention of mononuclear cells in the intima (72). Once migrated within the plaque, two phenotypes of macrophages appear: inflammatory macrophages and foam cells. The latter cells are characterized by the accumulation of cholesterol in their cytoplasm, resulting from the uptake of oxidized lipoprotein by these cells (113). Besides macrophages, T-cells are the second most important cell population in the atherosclerotic plaque (~10%) (61). They are important determinants in the disease by governing the transition from a latent plaque to a vulnerable plaque (46). CD4+ clones of T-cells isolated from atherosclerotic plaques recognize oxidized LDL (114), suggesting a local stimulation of T-cells by monocytes/macrophages in the plaque through major histo-compatibility antigen II. These T-cells express a pro-inflammatory (TH1) phenotype, suggesting that atherosclerosis is a T41-driven pathol-
ogy (114, 148). T51-type cytokines are predominant in plaques, but IL-4, a T24-type cytokine, is also pro-
duced by a few cells (37). Thus the initiation of athero-
sclerotic lesions is induced by many cell types within the vascular wall, circulating or resident.

Rupture of the Atherosclerotic Plaque is the Primary Cause of Sudden Cardiac Death

Acute coronary syndrome (ACS) results mostly of sud-
den luminal thrombosis (15), which may result from three different pathological processes: plaque rupture, erosion, and calcified nodules (128). Rupture occurs when the fibrous cap overlying a necrotic core is thin and broken, inducing luminal thrombosis. Lesions with erosion may also show a luminal thrombus but are often devoid of necrotic core or the core is isolated from the lumen by a thick fibrous cap (127). Calculated

Connexins in Vascular Wall

GJs mediate bidirectional passage of small molecules, a process essential for synchronizing contraction for the contractile units that make up the organ, and more than that. Connexins, as described (115), form transmembrane channels. Many cell types assemble into channels, each with its own connexin repertoire, thus dictating cell-cell communication and modulating cellular processes and responses, and thereby may play a role in the evolution of neointima (115).

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Thus Cx37 and Cx43 have been implicated in many aspects of vascular biology, however, the role of connexins in the plaque formation and rupture remains controversial.

Connexin 43

Connexin 43 (Cx43) is the most frequent gap junctional protein in mammalian cells and is the prototype for the gap junction channel (GJ). GJs mediate bidirectional passage of small molecules, a process essential for synchronizing contraction of the contractile units that make up the organs and tissues. Most cells assemble into channels, each with its own connexin repertoire, which dictates cell-cell communication and modulating cellular processes and responses, and thereby may play a role in the evolution of neointima.

However, the role of connexins in the plaque formation and rupture remains controversial. Cx37 and Cx43 have been implicated in many aspects of vascular biology, but the exact role of connexins in these processes is still under investigation.
Connexins have many functions in vascular physiology, such as conduction of vasomotor responses and tone among SMCs (20, 26, 33), capillary sprouting, and endothelial repair (65). Specifically, Cx40 is important for blood pressure regulation. Cx40−/− mice are hypertensive (27, 28, 104), possibly due to the requirement of the protein for the transmission of the endothelium-dependent vasodilator response (27). Furthermore, a link between Cx40 expression and renin secretion has been reported. Hence, the plasma titer of renin is increased in Cx40−/− mice due to a modified number and distribution of renin-secreting cells (62). Moreover, a polymorphism of Cx40 is linked to an increased risk of hypertension (35). In contrast, it seems that mice with an endothelial-specific deletion of Cx43 suffer from hypotension and bradycardia (73). Although contradictory results have been described as well (119). Interestingly, Cx43 is thought to be involved in the sensitivity to mechanic stimuli due to overexpression during shear stress, in particular in cardiac valves ECs (29, 58).

Deletion of connexins affects also the vasculature development. Indeed, Cx43−/− embryos die between days 9.5 and 10.5 (63) and Cx37−/− females are infertile (109). Cx37−/− mice do not display a noticeable vascular phenotype (34), but when two connexins are lacking, Cx37−/−/Cx40−/− pups die perinatally due to severe hypotension and bradycardia (73). Although contradictory results have been described (119). Cx43 is thought to be involved in the sensitivity to mechanic stimuli due to its overexpression during shear stress, in particular in cardiac valves ECs (29, 58).

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human or murine monocytic cells (2, 98), between human mononuclear cells and ECs, and between human mononuclear cells and SMCs (31, 98). Various connexins have been found in monocytic cells: Cx43 is present in mouse macrophage cell lines (2, 7, 16), in activated peritoneal hamster and mouse macrophages (2, 60), and in monocytic cells stimulated by tumor necrosis factor (TNF)-α or INF-γ, and Cx47 has been detected in human and mouse monocytes (135).

Lymphocytes form functional GJs. Electrical coupling has been observed after phytohemagglutinin-stimulation (56). Dye-coupling between phagocytes and ECs has also been reported (91). Cx43 is found in human blood-derived T- and B-cells and Cx37 (19, 103, 146) [natural killer (NK)] cells (92). Toluuid-dye-coupled T- and B-cells express both Cx40 and Cx43 (92). Neutrophils also play a role in atherosclerosis by contributing to the pathogenesis of the disease (93). Neutrophils have been described to express Cx37, Cx46, and Cx43, but not Cx32 (11, 146). After activation by Lipopolysaccharide (LPS) or TNF-α, human neutrophils are able to form morphological, but not dye-coupled, GJs (11). Functional GJs have been shown between ECs and neutrophils, and this was decreased by TNF-α (146). Thus connexins are expressed and GJ channels are functional in blood cells and reveal a communication network between circulating cells and vascular cells.

Expression of Connexins During Atherosclerosis is Altered and Participates in the Disease Progression

Atherosclerosis is a progressive disease, and Cx37, Cx40, and Cx43 expression patterns change during plaque formation (summarized in FIGURE 1) in murine and human atherosclerotic plaques (84). Here, we will report the role of GJs in atherosclerosis throughout the disease progression.

Endothelial dysfunction initiates the disease

The major risk factors for atherosclerosis are aging, hypertension, hyperlipidemia, smoking, and diabetes. These conditions influence endothelium biology. ECs display GJs, and connexin expression is tightly regulated. Deregulation can occur in different pathological conditions that will be discussed here.

Aging seems to induce a general decrease in connexin expression, with Cx40 being relatively undisturbed for a long time (138). Nicotine induces a decrease in Cx43 expression due to enhanced protein degradation (121). Hypertension is a cause of ECs dysfunction and a major risk factor of atherosclerosis. Hypertensive rats have a reduced endothelial expression of Cx37 and Cx43, but Cx40 expression is not modified (141). Moreover, cavedolin, a commonly used β-blocker for hypertension and cardioprotection, directly upregulates endothelial Cx43 independently of its antioxidant activity (141).

Oxidation products of lipoprotein-derived phospholipids upregulate Cx43 and downregulate Cx37 but do not affect Cx40 expression in murine endothelium of carotid arteries both in vivo and in culture (59). ECs are also sensitive to cholesterol titers. Increased GJ assembly between hepaticoma cells is observed after increasing the cholesterol level or treatment with LDL, or apolipoprotein B (85, 86). Besides the effect of cholesterol on Cx43 expression, membrane cholesterol content affects the GJ channel (57). Neutrophils also play a role in atherosclerosis by contributing to the pathogenesis of the disease (93). Neutrophils have been described to express Cx37, Cx46, and Cx43, but not Cx32 (11, 146). After activation by Lipopolysaccharide (LPS) or TNF-α, human neutrophils are able to form morphological, but not dye-coupled, GJs (11). Functional GJs have been shown between ECs and neutrophils, and this was decreased by TNF-α (146). Thus connexins are expressed and GJ channels are functional in blood cells and reveal a communication network between circulating cells and vascular cells.

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influences the gene expression profile of human vascular endothelial cells and enhances monocyte adhesion to ECs (131). Moreover, CRP upregulates Cx43 mRNA expression, as shown by microarray analysis (131). Activated ECs are at the crossroad of inflammation and hemostasis. Sepsis is a life-threatening state defined by an infection associated with a systemic inflammatory response syndrome (SIRS). As described above, TNF-α, which activates ECs by promoting expression of adhesion molecules. Thus treatment of ECs with TNF-α decreases the expression of Cx37 and Cx40 but does not change Cx43 expression (123). In keratinocytes, TNF-α decreases Cx43 expression, and inhibition of c-Jun NH2-terminal kinase (JNK) abolishes this effect (117). Activation of ECs (55, 123) or SMCs (84) with LPS, TNF-α, IL-1β, or IL-1α reduces the GJ communication. Finally, TNF-α induces closure of myoendothelial GJs in co-culture of human ECs and SMCs (54). This modifies both gating and permeability of GJs.

Thus a large range of stimuli modify GJ expression and enhance endothelial dysfunction, leading to atherosclerosis initiation.

**Leukocytes infiltrate the arterial intima**

As described above, TNF-α induces changes in expression of connexins in ECs that affect the migration of leukocytes in different inflammatory pathologies. Thus monocytes and lymphocytes are recruited early in atherosclerosis. A role for connexins in leukocytes migration studied in different inflammatory models and results are conflicting (19, 25). Thus diapedesis of neutrophils is increased or not by using connexin-mimetic peptides or pharmacological channel blockers (19, 103, 146). Using same modulators, monocytes transmigration is decreased (31), but lymphocytes transmigration is only modestly affected (91). Connexin expression is modulated during atherosclerosis. Endothelial Cx37 and Cx40 expression is considerably reduced in low-density lipoprotein receptor (LDLR)−/− mice on a cholesterol-rich diet for several months (64). Moreover, Cx37 is present in monocytes and macrophages in early and late atheroma (64) but also in medial SMCs beneath advanced atherosclerotic lesions (64). Thus atherosclerosis-susceptible ApoE−/− mice were fed with a high-cholesterol diet (5). By 10.220.33.6 on June 27, 2017
high-cholesterol diet (18). Altogether, all these results underline the importance of GJ communication for and during leukocytes infiltration in atherosclerosis.

**Smooth muscle cell migration in the growing phase**

During the growing phase, SMCs transmigrate from the media to the intima. There, they continue to grow and produce components of the extracellular matrix, leading to a strong fibrous cap covering the original plaque. In general, this cover safeguards the plaque from rupture. Macrophage and SMCs filled with lipids die within the plaque and release lipids. This process generates the typical lipid core of atherosclerotic lesions.

At this stage, two qualities of plaque exist. Plaques with a thin cap, prone to rupture, are composed of a large lipid core and numerous macrophages. Their phenotype is dependent on macrophage activity. Macrophage foam cells produce pro-inflammatory cytokines, amplifying inflammatory response that, in turn, induces macrophage proliferation and lipid uptake. Moreover, activated macrophages produce matrix metalloproteinases (MMPs) that degrade extracellular matrix and weaken the fibrous cap.

On the other hand, stable plaques, with larger fibrous cap and reduced lipid core, are less prone to rupture, and thus the stabilization of the plaque has become a potentially new therapeutic goal.

The expression of Cx43 differs in intimal SMCs and depends on the stage of the disease. Its expression is increased at early stage but decreased in advanced human plaques (8). Cx43 expression is also decreased in intimal SMCs in advanced lesions in the hypercholesterolemic rabbit (97) as well as in LDLR−/− mice (64). In addition, Cx37 appears in medial SMCs beneath advanced atherosclerotic lesions (64). Furthermore, Cx43 mRNA is present in foam cells of human and rabbit atherosclerotic carotid arteries (97, 98). The reduction of Cx43 expression inhibits the formation of atherosclerotic lesions in mice models. Cx43+/− LDLR−/− mice (under cholesterol-rich diet) display reduced atherosclerosis lesions, with smaller lipid cores and fewer macrophages and T-cells despite normal leukocyte blood counts (67). In addition, lesions have thicker fibrous caps containing more SMCs and interstitial collagen. Interestingly, activation of monocyctic cells, which are associated with the release of MMP-2 and -3, increases GJ communication through Cx43 channel (31). Enhanced MMP release may favor plaque rupture. Interestingly, TGFβ1 cells secrete more MMPs than TGFβ2 (6). Moreover, both Cx43 expression and dye-coupling between macrophages and T-cells increase in TGFβ1 CD4+ T-cells compared with Th0 and Th2 (98), pointing out a potent role of connexins in atherosclerotic plaque quality. Thus Cx43 targeting potentially favors plaque stabilization besides its action on plaque development (133).

Finally, Cx43 expression is enhanced in intimal thickening after acute vascular injury (16, 74, 96, 144). Interestingly, the time course of restenosis changes when the Cx43 is reduced (16, 74, 130). Somehow results are opposed, depending on whether the Cx43 expression is systematically reduced, leading to a reduced neointima formation (16), or deleted in SMCs only, leading to an increased neointimal formation (74). It should, however, be kept in mind that the specific deletion of Cx43 in SMCs reduces the expression of Cx43 in aortic endothelium (74), and specific deletion of Cx43 in ECs reduces Cx43 coding mRNA in the adjacent vascular smooth muscle layer (73). This reflects the complex GJ interactions between different cell types. Similar interactions are observed in Cx40−/− mice, supporting a tight relationship between different connexins in ECs.

Recently, it has been described that SMCs of pig coronary arteries show a heterogeneous phenotype, with both spindle-shaped SMCs (S-SMCs) and thomboïd SMCs (R-SMCs) (47). S-SMCs are predominant in the normal media, whereas R-SMCs are present in stent-induced intimal thickening. R-SMCs have an increased proliferative, migratory, and protelytic phenotype, and are involved in vascular tissue repair and restenosis. S-SMCs express Cx40, but R-SMCs do not. R-SMCs display higher Cx43 expression levels and more cell-to-cell coupling than S-SMCs. Moreover, S-SMCs treated with platelet-derived growth factor-BB acquire an R phenotype, with an increased expression of Cx43, and more cell-to-cell coupling than S-SMCs. Interestingly, this platelet-derived growth factor-BB-induced phenotypic change is prevented by a reduction of Cx43 expression by an antisense oligonucleotide (17). These findings suggest that Cx43 may also be an attractive target for local delivery strategies aimed at reducing restenosis.

**Connexins as Cardiovascular Risk Markers and Their Therapeutic Targets**

There is increasing attention to genetic variation in connexin genes and their relation to cardiovascular disease. A Cx37-1019C polymorphism has been reported to be a potential prognostic marker for carotid atherosclerotic plaque development in a Swedish population (9) and coronary artery disease in a Taiwanese and Swiss population (134, 139). In addition, a Chinese study showed that out of three studied polymorphisms in the Cx37 gene, the C allele contributes to an increased risk of coronary artery disease (43). In contrast, the Cx37-1019T polymorphism has been associated with acute myocardial infarction in a Japanese and Sicilian population (50, 77, 78, 137). Finally, the Cx-1019T polymorphism appears in a prospective study of death after acute coronary syndrome (68). The association of the polymorphism with CAD or MI has not been found in an Irish study group (52). Moreover, the Cx37-1019T polymorphism is not associated with carotid artery disease (43).

Connexin expression in human plaques (97) and in animal models (142) has been shown to play a pleiotropic effect in atherosclerosis. Indeed, the Cx43 and the Cx37 connexins have been shown to have an effect on plaque growth by reducing restenosis. Additionally, both Cx43 and Cx37 have been shown to be potential targets for local delivery strategies aimed at reducing restenosis.

Moreover, S-SMCs treated with platelet-derived growth factor-BB acquire an R phenotype, with an increased expression of Cx43, and more cell-to-cell coupling than S-SMCs. Interestingly, this platelet-derived growth factor-BB-induced phenotypic change is prevented by a reduction of Cx43 expression by an antisense oligonucleotide (17). These findings suggest that Cx43 may also be an attractive target for local delivery strategies aimed at reducing restenosis.
intimal thickening, 74, 96, 144). Arterial injury results in a reduced expression of Cx43 in aortic SMCs only, leading to a reduced expression of Cx43 in ECs (144). Importantly, SMCs of pig aorta express a predominant Cx43 phenotype, and thrombogenic injury results in the shedding of Cx43 from vascular cells. Consequently, the expression of Cx43 in ECs is increased after denudation injury and 28 days of neointima formation (144). In a rat model of balloon acute injury, an increased expression of Cx43 within the neointima is observed (144). In this review, we have summarized an emerging role of connexins in atherosclerosis and vascular injury in Cx43−/− mice and 7 and 14 days after balloon angioplasty (16). Together, these events led to a reduction of neointimal thickening after balloon-injured vascular injury in Cx43−/−LDLR−/− mice. Stents are often placed after balloon dilation in angioplasty with the intention to reduce restenosis. Endothelial cells, growing on metallic stent material, showed a decrease expression of von Willebrand factor, endothelial nitric synthase (eNOS), and Cx43, suggesting endothelial dysfunction (143). DES are potent in preventing restenosis. However, by reducing neointimal proliferation, DES appear to have a life-threatening side effect by enhancing the risk of thrombosis (107). It has been recently communicated that, besides its effect on inflammation, the deletion of Cx43 in mice increases platelet aggregation and decreases bleeding time and time survival to thromboembolism. Moreover, Cx43 communication is functional between platelets (4). Thus Cx43 and Cx43 modulation could be potent therapeutic targets to reduce the restenosis and thrombotic mortality induced by stents.

**Conclusion**

In this review, we have summarized an emerging role of connexins in all sorts of cardiovascular disease. Studies on knockout mice have unequivocally demonstrated the role of connexins in atherosclerosis and balloon-induced restenosis. Further genetic changes now include the development of specific connexin-targeting drugs for therapeutic applications.

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