SUMMARY/MAIN FINDINGS IN PERSPECTIVE
Vascular injury is considered integral to the onset of atherosclerosis and acute atherothrombosis. There is an enormous body of experimental and clinical evidence demonstrating that the initial response to injury involves the activation of both inflammation and blood coagulation, which both serve as primary host-defense mechanisms to promote wound healing and maintain vascular integrity. Atherosclerosis is a chronic inflammatory disease. Blood coagulation and inflammation reciprocally interact in various clinical conditions. Acute inflammation can induce hypercoagulable states and plays a crucial role in the initiation of arterial thrombosis. Histopathological studies document an imbalance in the expression between pro-coagulant and anti-coagulant proteins within human atherosclerotic lesions – a significant increase in tissue factor (TF) and fibrinogen synthesis, accompanied by diminished tissue factor pathway inhibitor (TFPI) and thrombomodulin (TM) expression levels. Subclinical thrombosis is known to occur long before an atherosclerotic plaque ruptures, and is even found associated with mild luminal stenosis. In fact, current concepts of plaque vulnerability assert an important role for thrombosis in triggering atherosclerotic lesion destabilization. Nevertheless, the role of clotting in atherosclerosis remains unclear to date. In this thesis, using various ex vivo and in vivo methodological approaches, we aimed to elucidate the contribution of blood coagulation to atherosclerosis progression and plaque phenotype determination.

**Major Findings**

**Active Coagulation Network in the Atherosclerotic Vessel Wall – Relevance to Atherogenesis**

In Chapter 3, we investigated the presence of all coagulation proteins within the arterial vessel wall in relation to atherosclerosis progression. Besides focusing on the expression and overall distribution alone, we also provided novel evidence indicating that both early and advanced atherosclerotic lesions exhibit functional activity of key coagulation proteins such as TF, prothrombin, factor (F)X, and FXII. Furthermore, many of those enzymes were locally synthesized within the atherosclerotic plaques and co-localized with macrophages and vascular smooth muscle cells, suggesting an active, cell-based coagulation network during disease progression. Intriguingly, the primary finding of this study is that early atherosclerotic lesions exhibit a pronounced prothrombotic profile in comparison to stable advanced atheromas. Whereas it seems counterintuitive at first glance, these data suggest that local coagulation proteins may play an important role not only in the onset of atherothrombosis, but also in ath-
erogenesis and plaque phenotype determination. This phenomenon might be in part explained as a “response to injury” mechanism, which prevents atherosclerosis lesions from rupture during the early phases of atherosclerotic development. However, the persisting inflammation, which is an established feature of atherosclerosis progression, may trigger an overshooting host defense response, thus leading to a continuous coagulation activation, whereas the latter can reciprocally contribute to more local inflammation. The long-term net effects of such perpetual activation of the coagulation-inflammation axis may be detrimental for atherosclerotic plaque stability.

Accelerated In Vivo Thrombin Formation Independently Predicts the Presence and Severity of Coronary Atherosclerosis

In atherothrombosis, thrombin plays one of the most critical roles in initiating thrombus formation by promoting both the formation of fibrin, which is the ultimate product of the coagulation cascade, but also by acting as a powerful platelet-activating enzyme. Beyond its hemostatic roles, thrombin is also recognized as a potent cell-signaling effector molecule and pro-inflammatory mediator (Chapter 2). By selectively cleaving its G-protein-coupled protease-activated receptors (PARs), thrombin can initiate a plethora of pro-atherogenic and plaque-destabilizing effects such as inflammation, vascular smooth muscle cell migration and proliferation, leukocyte chemotaxis, proteolysis, apoptosis, angiogenesis, etc. PARs are abundantly expressed throughout various compartments in the arterial vessel wall, and overexpressed in atherosclerotic lesions. To help better understand the relationship between blood coagulation potential and atherosclerosis progression in vivo, in Chapter 4 we used a non-invasive vascular imaging modality (cardiac computed tomographic angiography (CCTA)) and studied the association between thrombin formation and the presence and severity of coronary atherosclerosis in patients with suspected coronary artery disease (CAD). The primary finding of this study is that increasing thrombin-antithrombin complex (TATc) levels, known as a sensitive marker for thrombin generation in vivo, are independently associated with the presence and severity of CAD, but also coronary calcification. Of note, TATc measurement was efficient in detecting even mild-grade coronary stenosis, whereas the incorporation of TATc as an additional test improved the predictive capacity of the Framingham cardiovascular risk stratification model. Nevertheless, because of the large inter-individual differences in humans, and the multifactorial nature of atherosclerosis, we do not anticipate that a single biomarker can substitute powerful
Thrombin Controls the Level of Inflammation Related to Atherosclerosis

In Chapter 6 we provide a mechanistic insight into the role of thrombin in atherogenesis. Here we demonstrate the in vivo significance of genetic alterations and pharmacologic inhibition of thrombin formation for the onset and progression of atherosclerosis, but also plaque phenotype determination. Genetically imposed hypocoagulability in transgenic mice inhibited atherosclerosis development and promoted atherosclerotic plaque stability, characterized by abundant collagen and vascular smooth muscle cell (VSMC) content, and diminished leukocyte infiltration. Conversely, hypercoagulability induced severe atherosclerosis progression, a marked inflammatory phenotype, plaque vulnerability and spontaneous atherothrombosis. Hypercoagulability-triggered plaque destabilization was mediated in a thrombin-dependent manner through an enhanced activation and oxidative burst response of neutrophils in the bone marrow, followed by an increased mobilization and infiltration of leukocytes into the atherosclerotic plaques, the latter attributed to pro-inflammatory chemokine and cytokines overexpression. Notably, this severe atherosclerosis phenotype was rescued by the administration of either synthetic specific thrombin inhibitor Dabigatran etexilate or a recombinant form of the natural anticoagulant activated protein C (APC), which both resulted in substantially reduced leukocyte intraplaque recruitment, attenuation of atherosclerotic lesion formation (by ~80%), diminished systemic inflammation and enhanced plaque stability.

Concluding Remarks and Future Perspectives

The main goal of the studies described in this thesis was to unravel part of the mechanisms through which blood coagulation affects atherosclerosis progression and contributes to the determination of atherosclerotic plaque phenotype. This thesis provides important new insights on the role of clotting proteins and coagulation-driven inflammation in atherogenesis. In particular, we here unmask a potentially significant role for thrombin in controlling the level of inflammation related to atherosclerosis onset and progression. Whereas our data clearly implicates the potential of thrombin inhibition in the prevention of atherosclerosis development in mice, fu-
tire research will be needed to establish its effects on the regression and reversibility of atherosclerotic lesions. Of note, numerous studies have shown that very low concentrations of thrombin are associated with beneficial physiological effects such as endothelial barrier protection, apoptosis reduction, and attenuation in leukocyte transmigration. Hence, learning the safety and efficacy of long-term thrombin inhibition in atherosclerosis becomes of crucial importance. In future, we also aim to explore the involvement of other key hemostatic proteins with strong cellular signaling capacity (e.g. FXa and TF-FVIIa complex). Overall, extensive studies of the blood coagulation-innate immunity-inflammation axis, and the various molecular pathways involved, may open up new therapeutic avenues in prevention and treatment of atherosclerosis. Antithrombotic therapy is the cornerstone in primary and secondary prevention of atherothrombosis, associated with a reduction in mortality rates by 30%. Despite that numerous clinical trials studying the effects of long-term administration of anticoagulants have failed to demonstrate beneficial side effects on atherosclerosis plaque growth and degree of stenosis, there are no clinical studies to date, which have investigated the effects of anticoagulant therapy on modulating plaque phenotype and stability. The development of new high-resolution vascular imaging modalities, including molecular magnetic resonance imaging (MRI), and the introduction of new specific coagulation inhibitors will overall enable us to more precisely investigate how alterations in blood coagulation activity can affect atherosclerosis development in humans. The potential clinical impact of our studies involves the unique opportunity to modify atherosclerosis phenotype and progression through the administration of novel classes of anticoagulants.

References

9. Esmon, C.T. The interactions between
SUMMARY
MAIN FINDINGS IN PERSPECTIVE