Cyclophilin A and EMMPRIN (CD147) in cardiovascular diseases

Peter Seizer*, Meinrad Gawaz, and Andreas E. May

1. Introduction

In 1984, cyclophilin A (CyPA) was characterized as a specific intracellular binding protein for cyclosporin A (CsA). The immunosuppressive activity of CsA is mediated by an intracellular CsA–CyPA complex, which blocks calcineurin-dependent activation of nuclear factor activating T-cell (NFAT). Notably, this immunosuppressive effect strictly depends on the binding of the CsA–CyPA complex to calcineurin. The physiological functions of CyPA (in the absence of CsA) are completely different. CyPA has an enzymatic activity as a peptidyl–prolyl cis–trans isomerase (PPIase) catalysing cis–trans isomerization of peptidyl–prolyl bonds at proline residues. CyPA can induce chemotaxis and signalling via two distinct pathways. CyPA is secreted by various cell types upon stimulation, e.g. LPS-activated macrophages and -stimulated smooth muscle cells, or released upon cell death. Extracellularly, CyPA represents a potent chemotactic factor for leucocytes (T-cells, monocytes, and neutrophilic and eosinophilic granulocytes). Yurchenko et al. identified the extracellular MMP inducer EMMPRIN (CD147) as a surface receptor for extracellular CyPA. Studies with mutant CyPA proteins show that CyPA can induce chemotaxis and signalling via two distinct pathways: (i) extracellular binding to CD147 and (ii) PPIase activity. There are hints that extracellular CyPA activity requires a pre-activated state of leucocytes, which fits to the observation that constitutive EMMPRIN expression on resting vascular cells is low or even absent.
EMMPRIN has been intensively characterized as an MMP regulator on tumour cells being essentially involved in metastasis and tumour invasion. The first description of EMMPRIN was reported by Biswas and colleagues as a tumour cell-derived collagenase stimulatory factor. Initially, EMMPRIN was characterized as the only known ligand for itself (homotypic binding). In the meantime, a variety of soluble ligands including pathogens or well-known proinflammatory agents (e.g., S100A9) have been identified. EMMPRIN is widely expressed on cardiovascular cell types. Signalling via EMMPRIN can involve activation of nuclear factor kappa B, PI3 kinase, and extracellular signal-regulated kinases (ERK) 1/2 in a cell-dependent manner. Currently, the exact molecular mechanism of CyPA/CD147 interaction has not been characterized in detail. Based on initial experiments with mutants of CyPA lacking PPlase activity, it has been supposed that the enzymatic activity is required for EMMPRIN-mediated signalling. Additionally, Song et al. reported that the EMMPRIN-binding site of CyPA overlaps with the PPlase active site. Interestingly, mutants of CyPA with a conserved EMMPRIN-binding site, but with a missing enzymatic activity, still revealed a strong chemotactic effect, indicating that the chemotactic effect of CyPA can be directly mediated via binding to EMMPRIN.
1.3 Extracellular CyPA and EMMPRIN as mediators of inflammation in non-cardiovascular mouse models

The pathophysiological relevance of CyPA/EMMPRIN interaction for inflammatory processes has been studied in various non-cardiovascular animal models: in a mouse model of allergic lung inflammation, CyPA levels were found enhanced in bronchoalveolar lavages compared with control animals and CyPA was identified as an important driver of T-cell recruitment. 30,44 Systemic treatment with an EMMPRIN-blocking antibody dramatically reduced lung inflammation, which supports the role of EMMPRIN as the corresponding receptor for CyPA in this model. 81 In addition, in a mouse model of rheumatoid arthritis, antibody treatment directed against the EMMPRIN–CyPA interaction (clone RL 73.2) resulted in severely reduced TNF-α and myeloperoxidase levels in joints and reduced disease severity score as well. 45 Based on these and other studies, the interaction of extracellular CyPA with EMMPRIN represents an established pair of inflammatory factors in autoimmune diseases. 46,47 In addition, EMMPRIN is recognized as an essential mediator of neuroinflammation regulating leukocyte transmigration and MMP release into the CNS. 51

2. Vascular remodelling

Inflammation plays a critical role in the pathogenesis of cardiovascular diseases. 52,53 First, in vivo evidence for a relevance of CyPA in cardiovascular disease was reported by Satoh et al., 13 after carotid ligation, intimal and medial hyperplasia were significantly reduced in Ppia−/− mice lacking CyPA. This reduction could be ascribed to a reduced smooth muscle cell proliferation and a reduced recruitment of inflammatory cells (CD45+) to the vascular wall. 12 The same group has generated Apoe−/−/Ppia−/− double knockout mice and has treated the mice with Angiotensin II (Ang II) over 4 weeks. Interestingly, while the Apoe−/−/Ppia−/+ developed abdominal aortic aneurysms, CyPA-deficient mice (Apoe−/−/Ppia−/−) were nearly completely protected against aneurysm formation. 13 In this model, the main mechanism was attributed to inflammatory activities of vascular smooth muscle cell-derived extracellular CyPA, which was required for ROS generation and MMP-2 activity. 13 Both are key players for aneurysm formation. Additionally, expression of inflammatory cytokines such as interleukin-6 and recruitment of inflammatory cells into the vascular walls were dramatically reduced in the absence of CyPA. 12 Nevertheless, EMMPRIN is expressed in human samples of aortic aneurysms and remains to be investigated. 54

3. Atherosclerosis

Atherosclerosis is an inflammatory disease. 55 MMP activity is thought to promote both atheroprogression and plaque rupture. 56–59 Both CyPA and EMMPRIN have been histopathologically identified in atherosclerotic lesions and appear to co-localize with infiltrated monocytes and macrophages. 11,15,60 We and others could show that MT1-MMP and MMP-9 become up-regulated on monocytes upon EMMPRIN–EMMPRIN interaction. 62,63 CyPA–EMMPRIN interaction regulates MMP-9, MT-1–MMP, and M-CSF in a model of foam cell formation. 11 Apoe−/−/Ppia−/− reveal a severely reduced atherosclerosis due to a reduced CyPA-driven cardiovascular inflammation. 14 In CyPA-deficient ApoE−/− mice, reduced atherosclerotic lesions were associated with a reduced presence of inflammatory cells, supporting the relevance of CyPA as a chemotactic agent. Transplantation of CyPA-deficient bone marrow cells into ApoE−/− mice did not significantly inhibit the development of atherosclerosis when compared with ApoE−/− mice, reconstituted with wild-type cells. These data suggest that non-bone marrow cell-derived CyPA seems to play a predominant role in the development of atherosclerosis. In this study, further experiments using GFP-expressing bone marrow cells revealed that non-bone marrow cell-derived CyPA is essential for an effective recruitment of inflammatory cells to the vascular wall. However, it has been mentioned by the authors that, in ApoE−/− mice with any reconstituted bone marrow cells, the development of atherosclerosis was reduced by ~50% when compared with untreated ApoE−/− mice. 14 Thus, a role of bone marrow cell-derived CyPA in atherosclerosis, especially in later stages of atherosclerosis, cannot be completely excluded by these data.

T-cells play an important role in atherosclerosis. 64 EMMPRIN has also been described on FoxP3+CD45RO+CTLA4+-activated human regulatory T-cells and was described as a reliable marker to identify this specific subtype of regulatory T-cells. 65 Based on the fact that the transfer of regulatory T-cells attenuates atherosclerosis in ApoE−/− mice, 66 the role of EMMPRIN for T-cell-regulated inflammatory processes in atherosclerosis awaits future investigation.

However, both extra- and intracellular CyPA seem to play a role in atherosclerosis: in CyPA-deficient mice, a decreased low-density lipoprotein uptake into the vascular wall could be detected. This could be ascribed to a reduced expression of scavenger receptors in the vascular wall such as CD36 and SR-A. The exact mechanism how intracellular CyPA regulates the expression of scavenger receptors is unclear. 14 In addition, in vivo endothelial cells lacking CyPA revealed a reduced expression of vascular cell adhesion molecule-1. Further experiments with human umbilical vein endothelial cells revealed that the endothelial nitric oxide (NO) synthase (eNOS) production was affected by intracellular CyPA via reduction of Kruppel-like factor 2 expression and repression of eNOS transcription. 14 Finally, endothelial cells lacking CyPA were more resistant to TNF-induced apoptosis (Figure 2). In addition, it has been reported that intracellular CyPA can interact with apoptosis-inducing factor upon cerebral hypoxia ischaemia to form a proapoptotic DNA degradation complex leading to neuronal cell death in vitro and in vivo. However, the authors did not differentiate between intra- and extracellular CyPA in their in vivo model by using CyPA-deficient mice. 67 Extracellular CyPA promotes endothelial cell apoptosis in association with increased c-Jun N-terminal kinase and p38 activities. 68 Notably, in neurological disorders like Alzheimer’s disease, extracellular CyPA reveals neuroprotective effects. 69

Consistent with the above-discussed animal data, CyPA is enhanced in patients with Type 2 diabetes as well as in the plasma of patients with symptomatic coronary artery disease and correlates well with the severity of coronary artery disease as well as with the amount of classical cardiovascular risk factors (Figure 3). 3,21

4. Platelet function and (athero)thrombosis

EMMPRIN has recently been identified a characterized on platelets. 41 EMMPRIN is located in the alpha granules in the open canalicular system (according to electron microscopy and sucrose gradient ultracentrifugation). Binding of recombinant soluble EMMPRIN induces platelet degranulation with enhanced surface expression of CD40L and P-selectin, suggesting that EMMPRIN–EMMPRIN interaction
activates platelets. Incubation of platelets with monocytes leads to an NF-κB-dependent induction of inflammatory cytokines (e.g. IL-6 and TNF-α) and cell activation. NFκB: nuclear factor ‘kappa-light-chain-enhancer’ of activated B-cells; PI: phosphoinositid; ERK: extracellular signal-regulated kinases; Th: T-helper cell type; VCAM: vascular cell adhesion molecule; JAK: Januskinase; STAT: signal transducers and activators of transcription; ROS: reactive oxygen species; MMP: matrix metalloproteinase; eNOS: endothelial nitric oxide synthase.

5. Myocardial infarction

Monocytes in patients with acute myocardial infarction reveal an enhanced EMMPRIN surface expression, suggesting a role of EMMPRIN in plaque instability via the above-described mechanisms. In a mouse model of myocardial ischemia and reperfusion, Pπia−/− mice showed reduced infarct size which was accompanied by a preserved left ventricular ejection fraction. In addition, neutrophil and monocyte recruitment was dramatically reduced. Comparable effects were noted in Pπia−/− mice treated with mAb anti-CD147. In vitro data revealed the involvement of EMMPRIN as a main chemotactic receptor. The role of EMMPRIN was further supported by the fact that the combination of Pπia−/− mice with anti-CD147 treatment did not yield further protection after ischaemia and reperfusion. In this study, the inhibition of EMMPRIN via an antibody applied immediately before the reopening of the infarct-related artery (left anterior descending) still led to myocardial protection upon ischaemia and reperfusion. Thus, it is tempting to speculate that CyPA and EMMPRIN are potential targets in preventing ischaemia and reperfusion injury. Indeed, it has been reported that treatment with CsA, an inhibitor of CyPA, reduces infarct size in patients with acute myocardial infarction. However, cyclosporine A is a potent inhibitor of cyclophilin D as well, which is a powerful mediator of ischaemia and reperfusion injury.

6. Inflammatory and non-inflammatory cardiomyopathies

ApoE−/− mice develop a hypertrophic cardiomyopathy upon treatment with Angiotensin II. Notably, in ApoE−/− Pπia−/− mice, cardiac hypertrophy was significantly reduced compared with ApoE−/− Pπia+/+. Cardiac fibroblasts lacking CyPA revealed a reduced ROS production, proliferation, and migration upon stimulation with Angiotensin II. Furthermore, addition of CyPA directly induced hypertrophy (measured as [3H]leucine incorporation) of cardiomyocytes in vitro. These data suggest CyPA as an important mediator of angiotensin II-induced cardiac hypertrophy.

In a mouse model of coxsackie virus B3-induced myocarditis, CyPA and EMMPRIN were found up-regulated and CyPA was found to be required for an effective recruitment of macrophages and T-cells to the infected myocardium. The deficiency of CyPA or the

Figure 2  Cell-specific effects of CyPA in cardiovascular cell types. (A) Extracellular CyPA acts via EMMPRIN and other unknown identified CyPA receptors depending on the respective cell type. (B) Intracellular CyPA is involved in cell signalling, Ca2+ homeostasis, and cell activation. NFκB: nuclear factor ‘kappa-light-chain-enhancer’ of activated B-cells; PI: phosphoinositid; ERK: extracellular signal-regulated kinases; Th: T-helper cell type; VCAM: vascular cell adhesion molecule; JAK: Januskinase; STAT: signal transducers and activators of transcription; ROS: reactive oxygen species; MMP: matrix metalloproteinase; eNOS: endothelial nitric oxide synthase.
pharmacological inhibition of CyPA in wild-type mice reduced myocardial infiltration with inflammatory cells and reduced the remodelling process leading to fibrosis.77 Consistent with these animal data, an up-regulation of myocardial EMMPRIN and CyPA expression was noted in patients with inflammatory cardiomyopathies, suggesting these two proteins as novel biomarkers for inflammatory cardiomyopathy.78 Finally, recent data suggest that the enhanced presence of CyPA in endomyocardial biopsies predicts a poor prognosis in patients with non-ischaemic cardiomyopathies.79

7. Conclusion and future directions

In conclusion, CyPA is an important mediator of cardiovascular inflammation and seems to be a suitable target for prohibiting or treating inflammatory cardiovascular processes. Despite many in vitro studies using CsA as a blocking agent against CyPA, CsA is not an ideal candidate to block CyPA-mediated effects in vivo because of the immunosuppressive properties of the CyPA–CsA complex. CsA has been successfully applied in patients with acute myocardial infarction.74 58 patients were treated either with saline or with CsA (2.5 mg per kg) on top of the current guideline-conform treatment. Serum parameters for infarct size such as troponin or creatine kinase showed a significant reduction in the CsA-treated group. Notably, no adverse side effects were observed. Moreover, in patients with systemic lupus erythematosus, patients treated with CsA revealed an reduced carotid intima-media thickness compared with risk-adjusted healthy controls, which may support the concept that blocking cyclophilins can attenuate human atherosclerosis.80 However, there are discrepant findings about CsA-mediated effects on atherosclerosis.81 The complex effect of CsA on cardiovascular cells including lipoprotein metabolism and the inhibition of other cyclophilins apart from CyPA may account for this discrepancy.81 Thus, modified CsA derivatives are needed to achieve a more compartment-specific inhibition of cyclophilins.

The non-immunosuppressive cyclosporin A analogue NIM811 is such a compound blocking intra- and extracellular cyclophilins without affecting calcineurin activity.77,82 It has been used in several in vivo studies.47 However, it can pass the cell membrane and block intracellular cyclophilins such as cyclophilin D.75 Thus, NIM811 may cause side effects by affecting intracellular cyclophilins.

Based on these considerations, a modified CsA analogue has been designed, which cannot pass the cell membrane and, therefore, specifically blocks extracellular cyclophilins such as cyclophilin D.75 Thus, NIM811 may cause side effects by affecting intracellular cyclophilins.

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