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Review

Extracellular cyclophilins in health and disease☆

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ABSTRACT

Background: Extracellular cyclophilins (eCyPs) are pro-inflammatory factors implicated in pathogenesis of a number of inflammatory diseases. Most pathogenic activities of eCyPs are related to their chemotactic action towards leukocytes, which is mediated by eCyP receptor on target cells, CD147, and involves peptidyl–prolyl *cis–trans* isomerase activity of cyclophilins. This activity is inhibited by cyclosporine A (CsA) and non-immunosuppressive derivatives of this drug. Accumulating evidence for the role of eCyPs in disease pathogenesis stimulated research on the mechanisms of eCyP-initiated events, resulting in identification of multiple signaling pathways, characterization of a variety of effector molecules released from eCyP-treated cells, and synthesis of CsA derivatives specifically blocking eCyPs. However, a number of important questions related to the mode of action of eCyPs remain unanswered.

Scope of review: In this article, we integrate available information on release and function of extracellular cyclophilins into a unified model, focusing on outstanding issues that need to be clarified.

Major conclusions: Extracellular cyclophilins are critical players in pathogenesis of a number of inflammatory diseases. Their mechanism of action involves interaction with the receptor, CD147, and initiation of a poorly characterized signal transduction process culminating in chemotaxis and production of pro-inflammatory factors.

General significance: Extracellular cyclophilins present an attractive target for therapeutic interventions that can be used to alleviate symptoms and consequences of acute and chronic inflammation. This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

1. Introduction

Cyclophilins are ubiquitously expressed intracellular proteins first recognized as the host cell receptors for the potent immunosuppressive drug, cyclosporine A [1]. Later, it was established that cyclophilins and previously identified peptidyl–prolyl isomerases, which are characterized by their ability to catalyze the interconversion of *cis* and *trans* isomers of proline, are the same proteins [2,3]. The family of human cyclophilins includes seventeen identified members [4]. Cyclophilins are highly abundant proteins (for example, CyPA is present in cells in micromolar concentrations) that are localized to a variety of cellular organelles and subcellular compartments, suggesting that they may perform important cellular functions. It was therefore surprising that knockout of CyPA had no impact on cell viability or the life span of knock out animals [5]. Intracellular functions, in most cases related to the isomerase activity, have been characterized for only a small number of cyclophilins (see a recent review by Hoffmann and Schiene-Fischer [6]). This deficiency is due to our very limited knowledge of proteins that interact with cyclophilins, mostly because of the transitory nature of such interactions characteristic to enzymatic reactions. Interestingly, several members of the human cyclophilin family (RanBP2, SDCCAG-10, and cyclophilin-like proteins PPIL2 and PPIL6) were found to lack isomerase activity and did not bind CsA [4], probably reflecting redundancy within this family of molecules and their long evolutionary history.

The first cyclophilin to be found in extracellular fluids was cyclophilin B (CyPB), an abundant protein localized to ER due to the N-terminal signal sequence, which was identified in human milk and was initially described as a secreted protein [7]. A later study from the same group suggested that secreted CyPB is a C-terminal truncation of the protein that lacks a portion of the unconventional C-terminal ER retention signal [8]; however, the protease responsible for this cleavage or the mechanisms regulating this process under physiological or pathological conditions have not been reported. Moreover, a recent report characterized the ER retention signal in CyPB as overlapping with the CsA-binding site, which is located away from the C-terminus, and demonstrated that CsA treatment or mutation of the ^{128}Tryptophan in the CsA-binding site released CyPB into the extracellular milieu [9]. Given that CyPB has been found as a component of an extensive network of ER chaperones and folding catalysts that include BiP, Grp94, Grp170, co-chaperone ERdj3, and members of the protein disulfide isomerase (PDI) complex ERp72, P5, PDI, calnexin and calreticulin [10,11],

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interaction with these ER resident proteins via the CsA-binding site may determine the ER localization of CyPB and explain the secretion of this protein after CsA treatment. It remains unclear how secretion of CyPB is regulated when no CsA is present.

In 1992 a surprising and initially underappreciated finding was reported that cyclophilin A, an abundant cytosolic protein, is secreted by macrophages in response to LPS stimulation [12]. The mechanisms of CyPA secretion have remained mysterious for a long time, as this protein lacks any known export signal. Later studies demonstrated that CyPA secretion is regulated by a vesicular mechanism dependent on Rho activation [13], consistent with previous reports of CyPA release from cells subjected to different types of stress [12,14,15]. Recent proteomics analysis of CyPA released from irradiated breast cancer cells suggested post-translational modifications of the secreted cyclophilin [16], a finding confirmed in studies performed on smooth muscle cells treated with oxidation stress-inducing agent angiotensin II [17]. Here, specific acetylation on lysine residues 82 and 125 was found necessary for secretion and further activity of CyPA on endothelial cells. Consistent with previous studies showing Rho-dependent secretion of CyPA, acetylation of CyPA was also dependent on Rho, supporting the essential role of acetylation in the process of CyPA secretion, at least in the case of smooth muscle cells subjected to oxidative stress. It remains to be determined how widespread this pattern of secretion is and whether it involves other secreted cyclophilins (CyPB and CyPC). Given that constitutive secretion of CyPA and CyPB has been described for certain cell types (CyPA secretion from fibroblast-like synoviocytes [18], head and neck/oral squamous carcinoma cells [19], and adipocytes during differentiation [20], and CyPB secretion from chondrocytes [21] and pancreatic cancer cells [22]) it will be also important to determine the ratio of acetylated and non-acetylated cyclophilins, in particular CyPA, in various cell types and under various activation stimuli. Indeed, if acetylation marks the CyPA molecules destined for secretion, knowledge of the proportion of these molecules in the whole pool of intracellular CyPA in a particular cell type under specific treatment conditions (activation, infection, etc.) would provide an estimate of potential contribution of such cells to the inflammatory response and may identify them as a target for therapeutic interventions.

The third member of the cyclophilin family that can be secreted from cells is CyPC. Secretion of this protein was detected by proteomics approaches in pre-adipocytes undergoing differentiation to adipocytes [20] and unstimulated rat leptomeningeal cells [23]. Recent study demonstrated that, similar to CyPB, CyPC localizes to the ER and is released to the extracellular space by CsA treatment [24]. It remains to be determined whether extracellular CyPC plays any specific physiological or pathological role.

While initial studies have focused on the intracellular activities of cyclophilins, accumulating evidence suggests a role for these proteins as mediators of intercellular communications [25]. Two excellent recent reviews provided a comprehensive description of extracellular cyclophilins, their functions and activities [6,26]. In this article, we attempted to integrate available information on these medically important molecules into a unified model while focusing on the unresolved issues and controversies remaining in the field (Table 1).

2. Functions of secreted cyclophilins

Secretion of cyclophilins has been identified after stimulation with pro-inflammatory or oxidative agents, or by proteomics approaches that did not include functional analysis. Therefore, very little experimental information is available regarding the physiological role of extracellular cyclophilins, and this issue remains open to speculations. Some conjectures regarding potential functions of extracellular cyclophilins can be made from studies performed with knockout mice, although this model does not allow definitive discrimination between the effects of intracellular and extracellular cyclophilins. Initial analysis of CyPA knockout mice did not reveal any major defects, and the life span of the animals was the same as wild-type littermates, leading to a conclusion that CyPA is not essential in mammals [5]. This result is quite surprising given the high level expression of CyPA in all tissues [27], and suggests that the role of CyPA, including the extracellular

Table 1
Main unresolved issues related to extracellular cyclophilins.

Issue	What is known?	What is unknown?	References
Receptor for eCyPs	CD147 is essential for most known effects of eCyPs; CyPA catalyzes *cis–trans* isomerization of the Trp^{210}–Pro^{211} bond of CD147; CD147 interacts with signal transducing receptors, such as CD98 and integrin β1.	Is there any alternative receptor to eCyPs? Is there any difference between eCyPA and eCyPB in their interaction with CD147? Is isomerization of CD147 responsible for signal transduction and how are signals generated? What CD147-interacting molecules are involved in signal transduction initiated by eCyPs?	[47,6,54,55,28]
Signaling induced by eCyPs	eCyPs initiate CD147-dependent Ca^{2+} flux, ERK1/2 and JNK activation.	What are signal transduction pathways activated by eCyPs? Are there differences in signaling initiated by eCyPA, eCyPB and eCyPC?	[6,65–70]
Post-translational modifications of eCyPs	Acetylation of CyPA promotes its secretion and functional activity on endothelial cells.	Are acetylation or other post-translational modifications necessary for secretion of all eCyPs from all cell types? What is the ratio of modified and unmodified cyclophilins in various cell types? Do post-translational modifications alter interaction with CD147 and functional activity of eCyPs?	[16,17]
Mechanism of eCyP secretion	CyPA is secreted via a vesicular mechanism dependent on Rho activation.	How is secretion of eCyPs regulated? What is the mechanism of secretion of eCyPB and eCyPC?	[13,16]
Functional activities of eCyPs	eCyPA is a chemotactic agent for leukocytes; eCyPB stimulates adhesion of memory T cells to extracellular matrix; eCyPA stimulates production of ROS by VSMC and MMP and pro-inflammatory cytokines by myeloid cells	What are the physiological functions of eCyPs? What is the role of eCyPs in pathology of inflammatory and other diseases?	[37,49,40,41,75,25]
eCyPs as a therapeutic target	Blockade of eCyPs or CD147 ameliorates inflammation and disease in models of rheumatoid arthritis, asthma, biliary atresia; Atherosclerosis is reduced in $CyPA^{-/-}$ mice.	What other diseases can be treated by targeting eCyPs? How does treatment targeting eCyPs or CD147 compare to currently used treatments of inflammatory diseases (e.g., anti-TNFα)? Can non-permeable CsA derivative with extended half-life time be created?	[28,77,64,78,79]

pool of these molecules, is focused on responses to injury or infection. The main deficiencies noted in CyPA$^{-/-}$ mice were allergic pathology associated with increased IL-4 production, splenomegaly, and overexpression of Th2 cytokines. These defects were linked to elimination of the modulatory effect of CyPA on nonreceptor tyrosine kinase Itk [5], an activity performed by intracellular cyclophilin molecules. In contrast, most activities associated with extracellular cyclophilins are of activating, rather than suppressive, nature, justifying designation of the eCyPA as a pro-inflammatory factor [28]. Overall, targeted studies are necessary to extract information related to extracellular CyPA from CyPA knockout model, and conclusions from such studies will be compounded by the effects of intracellular CyPA. The same considerations can be applied to CyPB knockout mice, which show severe defects in collagen glycosylation, collagen crosslinking and fibrillogenesis, contributing to bone defects [29,30]. These pathologies are likely caused by elimination of intracellular activities of CyPB in assisting collagen folding, but lack of extracellular activities of CyPB may also contribute to this defect. Not much more helpful in this respect are knockout mice for the extracellular cyclophilin receptor, CD147, which have multiple defects in lymphocyte responsiveness [31], spermatogenesis [32,33], retinal [34,35] and neurological [36] functions at the early stages of development. While some of these defects can be explained by the loss of signaling from extracellular cyclophilins, reliable conclusions are compounded by a plethora of functional activities that have been lost in these mice (reviewed in [28]).

The pro-inflammatory activity assigned to eCyPA, and to some extent to eCyPB, through in vitro studies (reviewed in [37]) may contribute to both localized, protective responses associated with acute inflammation, and to damaging consequences of the chronic inflammation. Example of the protective effect of eCyPA is attenuation of both blood–brain barrier permeability and tissue damage after injection of recombinant CyPA in a stab wound model of injury [38]. Chemotactic properties of eCyPA [25], its ability to potentiate activity of classical chemokines to promote leukocyte recruitment [39], and stimulation of production of pro-inflammatory cytokines IL-6, IL-8, IL-1β, MCP-1, TNFα [40,41] and CXCL16, CXCL12 (SDF-1) (unpublished observation) by monocytes and THP-1 cells provide a plausible framework for beneficial activities of eCyPA during acute inflammation. In addition, several groups reported the ability of eCyPA to attenuate oxidative stress and prevent apoptosis, thus protecting neurons and vascular smooth muscle cells [42–44]. However, best characterized are the damaging effects of eCyPA related to its role in chronic inflammation (see below). This side of the two-face nature of eCyPA is also consistent with proposed function of eCyPA as a molecule related to damage-associated molecular pattern (DAMP) [45].

3. Receptor for extracellular cyclophilins

Extracellular activities of cyclophilins imply existence of a receptor on target cells, and in 2001 our group identified integral plasma membrane glycoprotein CD147 as a primary signaling receptor for eCyPB [46], and then demonstrated that CD147 is necessary for cell responses to eCyPA [47]. In both cases, peptidyl–prolyl isomerase activity of cyclophilins was required for signaling, and could be inhibited by CsA [47–50]. Receptor for extracellular CyPC remains to be identified, but, given the conservation of the active site of cyclophilins, it appears likely that CyPC can isomerize CD147 and use it as a signaling receptor. Using HeLa cells with artificially modulated CD147 expression, we demonstrated a strong correlation between the magnitude of chemotactic response to eCyPA and the level of CD147 expression [51]. One group reported that CyPA mutants that lacked enzymatic activity but preserved binding to CD147 still induced a strong chemotaxis of neutrophil-like cell line, HL-60 [52]. They also described three residues in CyPA (Arg^{69}, His^{70}, and Thr^{107}) mutation of which abrogated CD147 binding and chemotactic activity while preserving isomerase activity. Taken together, these results, which await further confirmation, suggest that chemotactic effect of CyPA can be directly mediated via binding to CD147 without involvement of the *cis–trans* isomerization. Such mode of action, however, is not supported by a very low affinity of CyPA–CD147 interaction [47] and the absence of CD147 downregulation after treatment with eCyPA (unpublished observation). Both these features suggest that eCyPs induce signaling by a mechanism different from a classical ligand–receptor interaction.

The essential role of CD147 in the effects of extracellular cyclophilins has been demonstrated in many studies (reviewed in [6]). However, a recent study on monocytic U937 cells showed that IL-8 induction by eCyPA was not affected by partial knockdown of CD147 using shRNA [53]. This finding, which awaits further confirmation, suggests that eCyPA may use alternative receptor for specific activities in some cell types. The signaling events and the exact mechanisms responsible for signal initiation at CD147 after interaction with eCyPA remain poorly characterized. Early studies suggested that peptide bonds formed by Pro^{180} in the second extracellular immunoglobulin-like domain of CD147 were the targets of CyPA [47] and CyPB [54] (Fig. 1). However, later analysis of CyPA interaction with the extracellular domain of CD147 (amino acids 94–214) using advanced NMR technology demonstrated that CyPA catalyzes *cis–trans* isomerization exclusively of the Trp^{210}–Pro^{211} bond [55]. Whether Pro^{211} is also a target for CyPB, or these cyclophilins isomerize different bonds on CD147, remains to be determined. The latter possibility is supported by differences between CyPA and CyPB in the effects on target cells: in contrast to CyPA, which has been shown to stimulate production of pro-inflammatory cytokines IL-6, IL-8, IL-1β, MCP-1 and TNFα by monocytes [40,41], no such activity was found for eCyPB [56]. Also, whereas eCyPB promotes integrin-mediated adhesion of CD4$^+$/CD45RO$^+$ memory T cells to the extracellular matrix, eCyPA does not exert this activity [49]. In fact, CD147 inhibits homotypic aggregation of CD4$^+$ T cells during eCyPA-induced chemotaxis [57], although eCyPA can promote adhesion of monocytes to extracellular matrix [58]. The differences between the effects of these cyclophilins targeting the same receptor (CD147) may also be due to peculiarities of their interactions with target cells. Binding of both eCyPA and eCyPB to most cells depends on heparan sulfate proteoglycans, and heparinase treatment abrogates signaling by both cyclophilins [47,59]. The requirement for heparan sulfates is likely explained by insufficient abundance of CD147 and very low affinity of cyclophilin–CD147 interaction, as upregulation of CD147 expression on activated T cells removes the heparinase sensitivity of eCyPA-induced signaling [60]. However, the mode of interaction with heparan sulfates and the affinity of this interaction differ between eCyPA and eCyPB: eCyPB binds to oligosaccharides via the N-terminal stretch of lysines (^{3}LysLysLys^5) [61], whereas eCyPA relies for this purpose on basic amino acids (Arg^{148}, Lys^{151}, Lys^{154}, and Lys^{155}) in the C-terminal part of the protein [62]. In addition, eCyPB has been shown to specifically interact with proteoglycan syndecan-1, which functions as a co-receptor for eCyPB [49], whereas no such affinity was detected for eCyPA (unpublished observation).

The major remaining issue is the actual mechanism that links isomerization of proline bonds in the extracellular domain of CD147 to intracellular signals culminating in calcium flux, ERK1/2 activation, and other signaling events induced by extracellular cyclophilins (reviewed in [6]). No signal-transducing molecules have been shown to associate with the cytoplasmic tail of CD147, suggesting that signal transduction may be mediated by interactions involving extracellular or/and transmembrane domains of the protein. In fact, the cytoplasmic tail of CD147 was shown to exert suppressive rather than stimulating activity, at least for NF-AT during T cell activation, by impairing Vav1 and Rac1 signaling [63]. The requirement of additional molecules (co-receptors or signal transducing entities) in CD147-dependent cell responses to eCyPA is illustrated by eosinophils that do not respond to eCyPA despite expressing CD147 [64]. Consistent with the idea of signaling via neighboring molecules, CD147 tightly associates with a

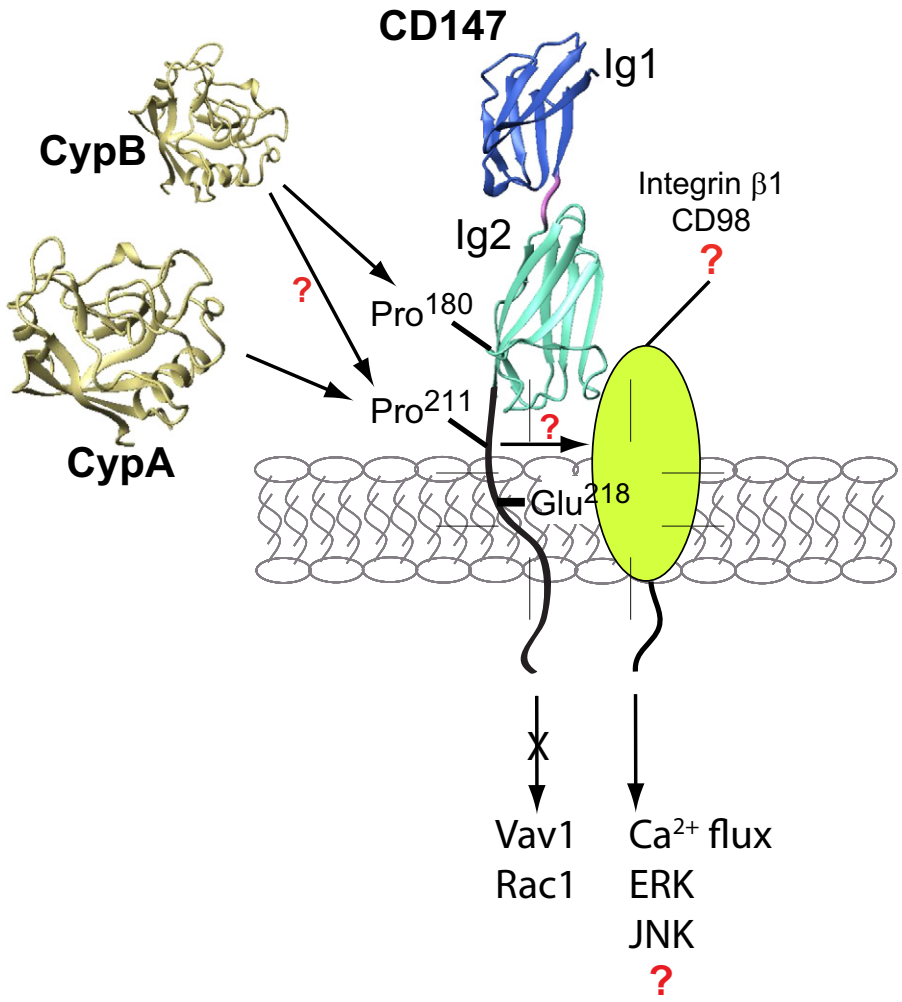

Fig. 1. Signaling interactions between extracellular cyclophilins and CD147. Interactions with CD147 are shown for eCyPA and eCyPB, and receptor for eCyPC remains unknown. Signaling is induced by isomerization of Pro^{211} bonds by eCyPA and Pro^{180} bond by eCyPB, although it remains possible that eCyPB also targets Pro^{211}. Isomerization alters the CD147 conformation inducing or changing its interaction with neighboring signal transducing molecules, such as CD98 and integrin β1 (the actual signaling molecule may be different in different cell types). Signals induced as a result of this process remain only partially characterized. Red question marks show the key gaps in our knowledge. More details are in the text.

number of signal transducing molecules, such as CD98 and integrin β1, and silencing or inhibition of these molecules inhibited CD147-mediated MMP induction and activation of ERK and JNK [65–70]. Such interaction may involve the transmembrane domain of CD147 which has an unusual feature: charged amino acid (Glu^{218}) in the hydrophobic region (Fig. 1). Thus, the plausible mechanism by which extracellular cyclophilins signal through CD147 is by isomerization of Pro^{180} or Pro^{211} bonds in the extracellular domain, which modifies CD147 interaction with a neighboring protein (co-receptor), changes the conformation of the complex and induces the signaling events originating from the co-receptor. It remains to be determined which proteins interact with CD147 in a fashion sensitive to isomerization of these proline residues. Structural studies on CD147 complexes, although highly challenging, will be invaluable in deciphering the mechanistic details of signaling initiated by extracellular cyclophilins.

Another important question awaiting resolution is the post-translational modifications of extracellular cyclophilins. Recent report from Berk's group suggested that acetylation is required for the release of CyPA from endothelial cells and its functional activity on smooth muscle cells [17]. However, most published in vitro studies describing activities of eCyPA relied on recombinant CyPA expressed in *Escherichia coli* and thus lacking acetylation. It is possible that differences in functional activity between acetylated and non-acetylated eCyPA are quantitative, rather than qualitative, and acetylated forms are just more efficient in inducing CD147-mediated responses. However, it is also possible that acetylation alters the interaction between eCyPA and CD147 and induces a different type of signaling response.

4. Role of secreted cyclophilins in disease

Increased levels of extracellular cyclophilins (mostly eCyPA) have been documented in practically all inflammatory diseases, and their role in disease pathogenesis has been verified in a variety of animal models of human diseases, including rheumatoid arthritis, sepsis, asthma, atherosclerosis and a number of viral infections (reviewed in [6, 26]). The main mechanism behind the pathogenic effect of extracellular cyclophilins is their chemotactic activity towards several types of leukocytes, including neutrophils, monocytes and T lymphocytes (reviewed in [28]). Attraction of excessive numbers of these cells to the site of inflammation exacerbates and extends the inflammatory response, thus contributing to conversion of the acute inflammation into the damaging chronic response. In this respect, it is interesting to follow the kinetics of release of extracellular cyclophilins. Such analysis was performed in the mouse model of allergic lung inflammation [71] and demonstrated that the level of eCyPA in the BAL fluid was elevated at day 6 after ovalbumin priming, further increased after challenge on day 7, started to decline on day 10 despite daily challenge, and returned to normal levels on day 12 (Fig. 2). The levels of eCyPB were slightly elevated on day 6 but rose

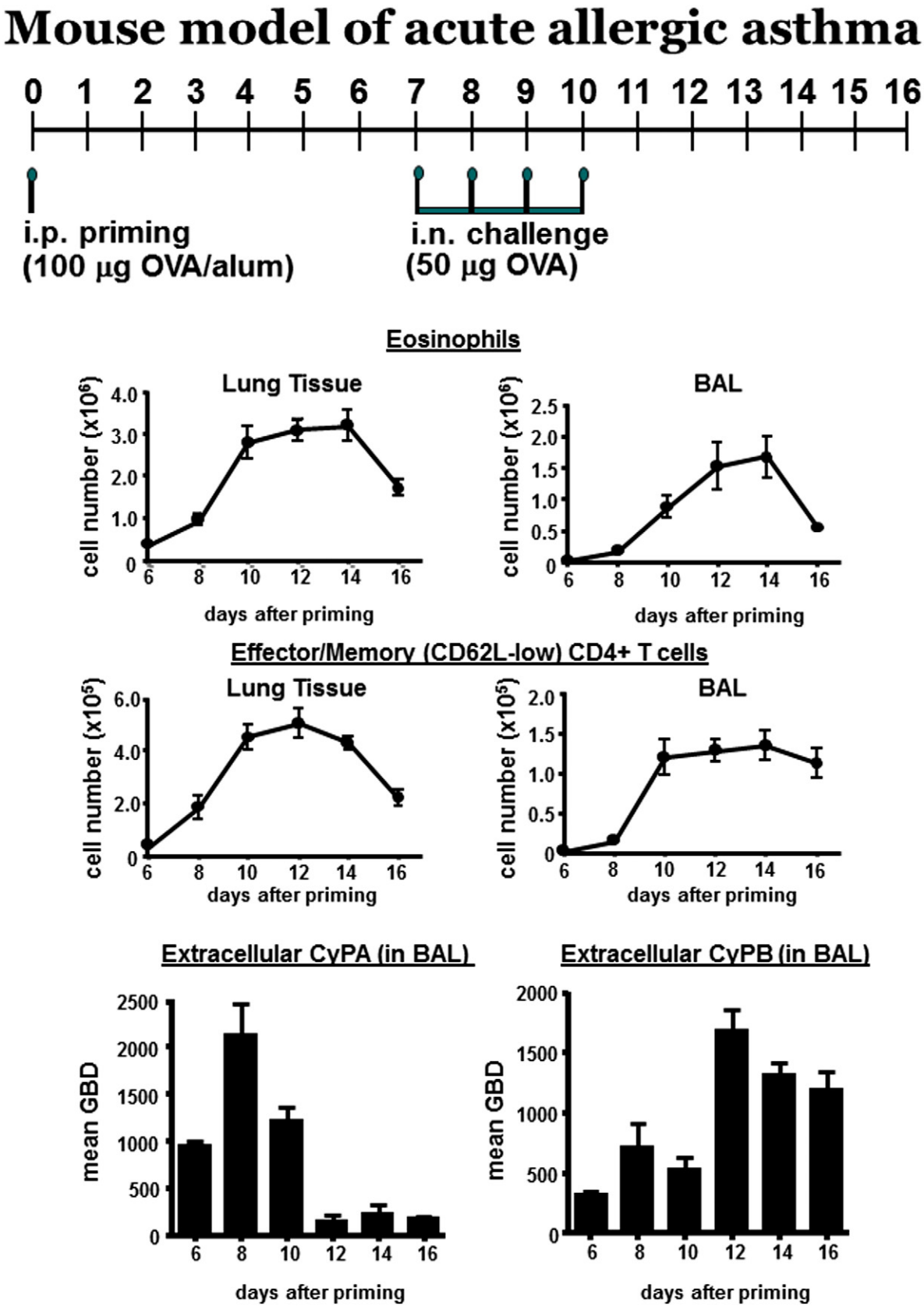

Fig. 2. Extracellular cyclophilins in allergic lung inflammation. Mice were primed by intraperitoneal (i.p.) injection of ovalbumin (OVA) in alum, and challenged with OVA intranasally (i.n.). Groups of OVA-primed/challenged mice were sacrificed every other day starting at day 6 through day 16 of the inflammatory response. Numbers of effector/memory $CD4^+$ T cells and eosinophils were determined in BAL fluid and lung tissue of individual mice by flow cytometric analysis, and are presented as mean ± SE, with $n = 10$ mice per time point. CyPA and CyPB analysis was performed by Western blot on cleared fluid from individual BAL samples, and results are presented as mean ± SE gel band density (GBD) for each time point as determined by densitometric analysis.
Modified from [71].

substantially on day 12 and stayed elevated until the end of the experiment on day 16. The inflammatory response measured by the number of effector/memory $CD4^+$ T cells and eosinophils in the BAL fluid and lung tissue elevated on day 8 (1 day after the challenge), reached the steady state on day 10 and continued until the end of the experiment (Fig. 2). These results suggest that eCyPA contributes to the initiation of the allergic response whereas eCyPB is responsible for the maintenance of inflammation. Of note, IP injection of cell-permeable non-immunosuppressive CsA derivative NIM811 or cell-impermeable derivative MM218 on days 7, 9 and 11 significantly reduced leukocyte numbers in lung tissues and airways of allergic mice [71]. Importantly, bronchial biopsies performed during disease remission in human patients with asthma showed an absence of classic leukocyte chemokines, despite the presence of persisting proinflammatory leukocytes [72]. It is likely that eCyPB or/and eCyPA is responsible for infiltration of these cells and persistent inflammation, so it will be important to test for the presence of cyclophilins in bronchial tissue. It would be very informative to perform similar analysis in other disease models and in samples obtained from patients. Availability of commercial ELISA kits that discriminate between CyPA, CyPB and CyPC makes it feasible to measure levels of these extracellular cyclophilins in plasma and tissues in clinical investigations. Such analysis would correlate the levels of eCyPs with the disease stage, and would provide the time frame for the most effective application of therapeutic approaches targeting eCyPs.

However, the chemotactic capacity of extracellular cyclophilins is not the only mechanism of their pro-inflammatory activity. Extracellular CyPA has been shown to upregulate production of pro-inflammatory cytokines IL-8, IL-1β, MCP-1 and TNFα by monocytes [40,41]; activate monocytes, neutrophils and VSMCs to secrete MMP-2 and MMP-9 thus contributing to tissue damage in rheumatoid arthritis, abdominal aortic aneurysm and atherosclerosis [41, 73–75]; stimulate VSMC proliferation and intimal thickening, an early step of atherosclerosis [76]; activate human umbilical vein endothelial cells and induce their apoptosis [75]; induce Smad2/3 phosphorylation in human hepatic stellate cells and promote liver fibrosis in a mouse model of biliary atresia (unpublished result). Given that most, if not all, of these effects are mediated through CD147, their breadth may be explained by the differences in responses between the cell types, but also suggests activation of a signaling pathway with broad activity.

5. Extracellular cyclophilins as therapeutic target

Given such broad pro-inflammatory activities of eCyPA and its contribution to pathogenesis of a large number of diseases, targeting extracellular cyclophilins seems like an attractive strategy for therapeutic interventions. The attractiveness of this approach is further increased by the lack of physiological functions of extracellular cyclophilins known to be essential for health, thus minimizing potential adverse effects of such treatment. Indeed, our experiments with treating mice for extended periods of time (up to 12 weeks) with cell-impermeable modification of CsA (see below) that neutralizes exclusively extracellular cyclophilins demonstrated lack of any visible toxicity (unpublished result).

Several approaches have been tested to neutralize the effects of extracellular cyclophilins in various disease models. The first one described was targeting the receptor for these factors, CD147, using monoclonal antibody (reviewed in [28]). This approach proved very effective in disease models of acute and chronic lung inflammation and rheumatoid arthritis, where treatment of mice with anti-CD147 antibody reduced by over 50% the inflammation (accumulation of immune cells and inflammatory cytokines in the affected organ) and tissue damage [64,77–79]. The only study where a direct comparison of treatments with anti-TNFα (an approved treatment for rheumatoid arthritis) and anti-CD147 antibodies was made, reported a stronger anti-erosion and anti-synovitis effects of the anti-CD147 antibody in SCID mice engrafted with human cartilage and rheumatoid synovium tissue (a rheumatoid arthritis model) [79]. Comparison of treatments targeting eCyPs and their receptor CD147 to currently used treatments needs to be reproduced in other models of rheumatoid arthritis (e.g., collagen-induced arthritis model), and extended to other inflammatory disease models. Confirmation of the advantage of targeting eCyPs would justify accelerated clinical development of this promising therapeutic modality.

In a mouse model of myocardial infarction after ischemia/reperfusion (I/R), the infarct size was reduced by about 50% in mice treated with anti-CD147 antibody, preserving left ventricular systolic function damaged in control animals [80]. Importantly, no additional protective effect of anti-CD147 antibody was detected in CyPA knockout mice subjected to I/R, suggesting that CyPA and CD147 act through a common pathway in mediating I/R injury. Consistent with this interpretation, recruitment of monocytes and neutrophils in I/R injury was inhibited by the anti-CD147 treatment, and the magnitude of inhibition correlated with reduction of infarct size [80]. Using anti-CD147 antibodies as anti-inflammatory treatment is an attractive proposition given available technologies for humanization of murine monoclonal antibodies and a long half-life of antibodies in the blood.

Another approach to inhibit eCyP-induced CD147 activities is by using antagonistic peptides derived from extracellular domains of CD147. Such 12-amino acid peptides were shown to inhibit CD147-dependent MMP production and invasiveness of synoviocytes from RA patients [81], reduce MMP-2 and MMP-9 production and invasive potential of monocytic THP-1 cells differentiated into macrophage-like cells by PMA treatment [58,82], and decrease infection of host cells by SARS coronavirus [83]. However, no in vivo results with CD147-derived peptides have been published.

The most promising from the clinical perspective approach to target extracellular cyclophilins is by small molecule drugs that specifically bind and inactivate these molecules. Feasibility of this approach was demonstrated by the studies using non-immunosuppressive analogs of CsA. Although these drugs target both intracellular and extracellular cyclophilins, they can be used as a proof-of-principle for the protective effects of anti-cyclophilin treatments. One such non-immunosuppressive CsA analog, NIM811, has been shown to significantly diminish inflammation in a mouse model of acute lung injury [77], prevent disease reactivation in a model of chronic allergic asthma [84], reduce myocardial fibrosis in coxsackievirus B3 induced myocarditis in mice [85], and prevent weight loss and reduce liver fibrosis in a mouse model of biliary atresia (unpublished result). These results support potential application of non-immunosuppressive cyclosporines, which are being developed for treatment of HCV infection, to anti-inflammation treatment modalities. However, the ability of these drugs to inhibit a variety of intracellular cyclophilins, many of which exert physiologically relevant functions, introduces a serious caveat to their clinical usage, especially when extended treatment times are expected as is the case with chronic inflammation. In addition, high intracellular concentrations of cyclophilins create a sink that takes most of the added drug out of circulation. A significant advance in dealing with these problems was achieved by synthesis of modified CsA analogs unable to enter cells and thus inhibiting only extracellular cyclophilins. Given that these compounds do not get into the cells, they can be used at lower concentrations and are non-immunosuppressive. The first example of such cell-impermeable cyclosporine was polyethyleneglycol-modified CsA synthesized from 8-amino-CsA, an analog of CsA that retains the immunosuppressive function and ability to inhibit *cis–trans* isomerase activity of CyPA [86]. The bulky PEG group prevented the compound from entering the cells. This compound was shown to partially inhibit HIV-1 entry into target cells [86], but its further development was not pursued due to a complicated synthesis procedure not appropriate for large scale production. This problem was partially resolved when MM218 was synthesized in 2010. This drug contains a

6-mer D-glutamic acid moiety and 5(6)-carboxytetramethylrhodamine fluorescent probe attached to side chain-modified [D-Ser]^8-CsA [87]. The presence of the fluorescent probe allowed clear-cut demonstration of the extracellular localization of the drug, which was shown to potently inhibit leukocyte recruitment, reduce airway mucus, suppress Th2 cytokine levels and improve lung function in a murine model of acute allergic asthma [71]. Importantly, MM218 significantly outperformed NIM811 in all these activities. Recently, a cell-impermeable benzimidazole derivative of CsA has been reported [50]. This compound was highly effective at inhibiting the recruitment of leukocytes during inflammation in a mouse model of experimentally induced peritonitis and delayed-type hypersensitivity reaction. Our studies in the mouse model of biliary atresia demonstrated that treatment with cell-impermeable CsA prevented weight loss, decreased Smad2 phosphorylation and reduced expression of fibrosis mediator TIMP-4, MMP-7 and IL-6 in the liver, and inhibited development of liver fibrosis (unpublished results).

Taken together, these results support the great potential of therapeutic targeting of extracellular cyclophilins for treatment of inflammatory diseases.

6. Conclusions

Studies discussed in this review provide a framework for evaluating the activities of extracellular cyclophilins under physiological and pathophysiological conditions. However, our understanding of the mechanisms of action of eCyPs is far from complete, and several important questions await their resolution (Fig. 1 and Table 1). In particular, it would be extremely important to refine our understanding of cyclophilin-induced signaling through CD147. How does isomerization of Pro211 on CD147 initiate intracellular signals? What other sites on CD147, besides Pro211, are involved in signaling response to eCyPs? What other proteins interact with CD147 to transduce signals in response to eCyPs? What signal transduction pathways are initiated by eCyPs? How do post-translational modifications (phosphorylation and acetylation) of cyclophilins affect their secretion, and their ability to interact with CD147 and induce signaling? Answers to these and related questions would be essential for future translational efforts aimed at targeting cyclophilin/CD147 pathway. Available data support the view that the role of eCyPs is most prominent as enhancers and extenders of inflammatory response, which often is the basis of disease pathology. In some diseases, such as chronic asthma, extracellular cyclophilins appear to be the primary chemoattractants for the inflammatory cells [84]. Therefore, drugs targeting eCyPs and their receptor can potentially alleviate symptoms of many diseases involving systemic and local inflammation.

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