

Contribution of Cyclophilin A to the Regulation of Inflammatory Processes in Rheumatoid Arthritis

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Abstract

Introduction Previous studies show that cyclophilin A (CypA) acts as a strong chemotactic cytokine to neutrophils, eosinophils, and monocytes in rheumatoid arthritis (RA).

Methods In this study, monocytes were stimulated by purified CypA and the production of matrix metalloproteinase (MMPs), the cell invasion and the release of inflammatory cytokines were detected respectively by gelatin zymography, invasion assay, and cytometric bead array FCM.

Results The elevated level of inflammatory cytokine IL-8 was also detected. Results showed that CypA significantly promoted the invasion of THP-1 cells and increased the production of MMP-2 and MMP-9, which displayed a biphasic concentration dependency. In vivo experiments found that the cartilage erosion scores in CypA injection group were significantly higher than those in control group ($P < 0.05$).

Conclusion Our findings suggest that CypA significantly enhances the secretion of MMP-2 and MMP-9, the cell invasion, and the inflammatory cytokines production of monocytes. Our findings may shed some new light on the inflammatory process and the degradation of cartilage and bone in RA.

Keywords Cyclophilin A · rheumatoid arthritis · monocytes

List of abbreviations

CypA	cyclophilin A
CsA	cyclosporine A
MMP	matrix metalloproteinase
PCR	polymerase chain reaction
IPTG	Isopropyl- β -D-thiogalactopyranoside
Ni-NTA	Ni-nitrilotriacetic acid
FMLP	<i>N</i> -Formyl-Met-Leu-Phe
CBA	cytometric bead array
LPS	lipopolysaccharide
PMB	polymyxin B
TNF- α	tumor necrosis factor α
IL	interleukin
RA	rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is the most common chronic autoimmune disorder characterized by the vasculature infiltration of the leukocytes into the synovial membrane and the exudation in the synovial fluid, which lead to synovial hyperplasia, pannus formation, and the destruction of articular cartilage. Chemotactic cytokines play an important role in the recruitment of leukocytes into the inflamed synovium [1]. In addition to the chemokines detected in the synovial fluid of RA patients such as CXCL8, CCL2, CCL3, and CCL5 [2], other factors have attracted researchers' attention. Cyclophilin A (CypA) is a recently reported cytokine that acts as a chemoattractant to human monocytes, neutrophils, eosinophils, and T cells [3].

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Cyclophilins belong to the family of immunophilins [4], which are discovered from humans as a cytosolic binding protein for the immunosuppressive drug cyclosporine A (CsA) in 1984 [5]. CypA, the most abundant cyclophilin protein, constitutes 0.1–0.4% of the total cellular protein [6]. Although CypA is widely distributed in cytoplasm, it can also be secreted into the extracellular environment as a pro-inflammatory product of lipopolysaccharide activated monocytes/macrophages [7] and endothelial cells [8, 9]. The high levels of CypA were detected in the serum and the synovial fluids of RA patients [10], and the concentration of CypA was closely related to the disease severity [11]. Moreover, CypA was observed to have induced the expression of matrix metalloproteinase (MMP)-9 to promote the cartilage destruction [12]. However, the role of high-level CypA in the development of RA remains unclear.

This study was designed to explore the role of CypA in the inflammatory process and the degradation of cartilage and bone in RA, using purified CypA and SCID-HuRAG models.

Materials and Methods

Cells and Animal

Human monocyte THP-1 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin and 2% L-glutamine at 37°C in a humidified atmosphere of 5% CO₂.

Male NOD/SCID mice (purchased from SLAC, Shanghai Laboratory Animal Co. Ltd., China) aged 6 to 8 weeks were maintained in independent ventilated cages (Suzhou Feng's Animal Experiment Equipment Co. Ltd., China) under specific pathogen-free conditions, and were given food and water sterilized.

Recombinant CypA Expression Vector Construction and Expression

Escherichia coli JM109 (DE3) strain (Novagen, USA) was transformed with the pET-32a+ expression vector (Novagen, USA) containing the gene encoding the human CypA. The gene was generated by amplifying the cDNA encoding human CypA from the human hepatic cell line HHCC-97 by polymerase chain reaction (PCR) using the forward primer (5'CGGCATATGGTCAACCCACCGTGTCTTCGAC3') containing the NdeI site and the reverse primer (5'ATGCAAGCTTTTCGAGTTGTCCACAGTCAGC3') containing the Hind III site. PCR assays were performed under the following conditions: a round of denaturation (94°C for 3 min), followed by 30 cycles of amplification

(94°C for 30 s, 56°C for 30 s, and 72°C for 30 s), and by a final extension at 72°C for 5 min. The PCR product was double digested with Nde I and Hind III and ligated into the expression vector pET-32a+, which was encoded for a (His)₆-tag at the C terminal. The ligation product was transformed into JM109 (DE3) and the potential clone was confirmed by DNA sequencing. The transformed cells with correct inserts were grown on a LB-agar plate with 100 mg/L ampicillin overnight at 37°C. Several clones were inoculated with 3 ml LB medium containing 100 mg/L ampicillin and were grown overnight at 30°C. About 1 ml of the overnight cultures was inoculated into a flask containing 100 ml of LB medium with 100 mg/L ampicillin and was grown at 30°C, 250 rpm until the OD₆₀₀ reached 0.6. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mmol/L and the cultures were induced overnight, shaking at 250 rpm at 30°C. Bacterial cells were harvested by centrifugation at 4,000×g for 10 min and pellets were used for protein extractions.

Purification and Endotoxin Removal

Bacterial pellets were resuspended in lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, pH8.0), 3 ml/g of cell pellets, and lysozyme was added to a final concentration of 1 g/L. After incubation on ice for 30 min, the induced products of these recombinants were extracted on ice by ultrasonication (10 cycles, 15 s each, with a 30-s interval). The lysates were centrifuged at 25,000×g for 15 min at 4°C and the supernatants were collected. The recovered pellets were resuspended in denaturing buffer (8 M urea, 0.1 M NaH₂PO₄, 0.01 M Tris-Cl, pH8.0), 3 ml/g of cell pellet, and were incubated for 2 h at room temperature. Denatured lysates were centrifuged at 25,000×g for 15 min at 4°C and the supernatants were collected. Both supernatants were loaded on a 10% SDS-PAGE gel to find the position of the expression protein. Since the constructed CypA-pET32a+ express vector had a 6His-Tag at its C terminus, the soluble supernatants were conducted by loading onto a Ni-nitrilotriacetic acid (Ni-NTA) agarose column, to which the 6His-tag of the recombinant protein would bind. The column was pre-equilibrated with B buffer (25 mM NaH₂PO₄, 500 mM NaCl, pH4.0) and C buffer (50 mM NaH₂PO₄, 200 mM NaCl, 40 mM imidazole, pH8.0). After washings with C buffer, soluble CypA was eluted by elution buffer (50 mM NaH₂PO₄, 150 mM NaCl, and 200 mM imidazole, pH8.0). Then the AKTA purifier-100 (Amersham Biosciences, Sweden) system was used to remove the endotoxin. The elution buffer was dialyzed thoroughly in 20 mmol/L citrate buffer, pH4.5 (A buffer) and loaded onto a DEAE SepharoseTM column pre-equilibrated with A buffer. After that, the effluent was collected. SDS-PAGE and Western blotting were performed

according to the standard procedures. Eluted protein was run on SDS-PAGE gels and either stained with Coomassie blue or Western blotted, stained by a goat anti-rabbit IgG horseradish peroxidase conjugate (Bio-Rad, USA) and detected by ECL plus detection system (Amersham Biosciences, UK). Concentrations of purified samples were determined with the BCA Protein assay kit (Pierce, USA) using BSA as a standard.

Chemotaxis Assay

All chemotaxis assays [3] were conducted using monocytes, THP-1 cells. The assays were performed using 48-well modified Boyden chamber (Neuro Probe), with the two compartments separated by a polyvinyl pyrrolidone-free poly-carbonate filter with a 5- μ m pore size (Neuro Probe). The monocytes (1×10^6 cells/ml) in serum-free RPMI-1640 supplemented with 2% BSA were added to the upper compartments, whereas the media containing different dilutions of recombinant CypA (10, 100, 200, 500, and 1,000 ng/ml) or *N*-Formyl-Met-Leu-Phe (FMLP, to provide a positive control for chemotactic responses) or medium alone were added to the lower wells. The concentration of FMLP used was 10^{-7} M, which induced optimal monocyte migration. The chambers were then incubated in the incubator at 37°C with 5% CO₂ for 90 min. The filter was then removed, the non-migrated cells were scraped off, and the filter was recovered, fixed, and stained with crystal violet reagent (Sigma) to discriminate monocytes. The number of cells appearing on the lower face of the filter was counted under microscope for each well, and each experimental condition was assayed in triplicate wells. A chemotactic index was calculated for each well by dividing the cell number of that well by the number of cells counted in negative control wells.

Gelatin Zymogram

For analysis of MMPs expression and their proteolytic capacity, gelatin zymogram was conducted [13]. Lipopolysaccharide (LPS), polymyxin (PMB), and different dilutions of CypA (10, 100, 200, 500, and 1,000 ng/ml) were added separately to THP-1 cells (3×10^5 cells/well in 96-well plate with incomplete RPMI-1640 medium). After stimulation for 24 h, culture supernatant of each well was collected and centrifuged to remove the cellular debris. Each sample supernatant (30 μ l) mixed with SDS sample buffer was then loaded onto a 10% polyacrylamide gel containing 0.1% gelatin under non-reducing conditions. After electrophoresis, gels were washed twice with 2.5% *v/v* Triton X-100 for 30 min and incubated for 16 h in low salt collagenase buffer containing 50 mmol/L Tris-Cl (pH 7.5), 0.2 mol/L NaCl, and 0.01 mmol/L CaCl₂. The gels were

subsequently stained with 0.5% Coomassie blue (G-250) and were destained with buffer consisting of 20% methanol, 10% acetic acid, and 70% distilled water for 30 min to visualize the zymogen bands. The zones of gelatinolytic activity were shown by negative staining. The zymography gels were scanned and analyzed using GeneSnap from SynGene Tools.

Invasion Assay

The THP-1 cell invasion assay was performed using Boyden Chamber, equipped with an 8- μ m pore size polycarbonate filter coated with diluted matrigel 80 μ l (1:3 dilution in RPMI-1640) to form a continuous thin layer. The cells (1×10^5 cells/well) suspension with different concentrations of CypA were added into the chamber and cultured for 24 h at 37°C in a humidified atmosphere of 5% CO₂. The cells remaining in the upper compartment were completely removed with gentle swabbing. The filter was fixed and stained with crystal violet reagent. The cells invading the lower surface of the filter in five microscopic fields were counted. Triplicate samples were conducted and the data were expressed as the average cell number of 15 fields.

Inflammatory Cytokines Detection by CBA FCM

THP-1 cell culture media in different concentrations of CypA for 24 h were collected and assessed by cytometric bead array (CBA) (BD Biosciences Corp., San Jose, CA, USA), and the levels of human inflammatory cytokine (tumor necrosis factor α (TNF- α), interleukin (IL)-1 β , IL-6, IL-8, IL-10, and IL-12p70) were measured simultaneously following the experimental instructions. Briefly, 50 μ l of samples or known concentrations of standard samples (0–5,000 pg/ml) were added to a mixture of 50 μ l each of capture Ab-bead reagent and detector Ab-phycoerythrin (PE) reagent. The mixture was subsequently incubated for 3 h at room temperature away from light and washed to remove unbound detector Ab-PE reagent. Data were acquired by flow cytometry (FACS Calibur, BD Bioscience) and analyzed on computer (CBA software 1.1; BD Bioscience).

Effects of CypA on SCID-HuRAg Model

SCID-HuRAg models established by subcutaneous implantation were used to investigate the effects of CypA on synovitis. Briefly, the SCID mice were anesthetized intraperitoneally with ketamine (2 mg) and xylazine (0.4 mg) in 0.2 ml PBS per mouse. The tissue implants were grafted subcutaneously on the back of the mice. After the subcutaneous tissue had been exposed, a 0.5 cm incision was made in the oblique paraspinal muscle. The RA

synovium followed with normal cartilage were co-implanted in the chamber in the muscle. The implanted synovial samples were of comparable volume and consisted of macroscopically vascularized synovial membranes. All surgical procedures were performed under sterile conditions [14]. Animal studies were approved by the local regulatory agency (Laboratory Animal Research Centre of the Fourth Military Medical University). Four weeks after implantation, 12 SCID-HuRAG mice were divided into two groups randomly. CypA was administered by single subcutaneous injections into the implanted tissue at a dose of 400 ng/g weight in six SCID-HuRAG mice and the other six SCID-HuRAG mice were injected with normal saline in the same way as controls. The injections were repeated twice a week for a total of five times. The mice were sacrificed 1 week after the final injection and the implanted tissues from each mouse were removed, fixed in 4% paraformaldehyde, and decalcified with EDTA. After paraffin embedding, tissue sections (2–3 μ m) were stained with hematoxylin and eosin (HE) for morphological evaluation. Invasion into the cartilage was quantified according to a semi-quantitative scores ranging from 0 to IV, referring to the number of invading cell layers and the number of affected cartilage sites [15, 16]. Finally, the data reported by each researcher were compiled for analysis of results. Animal researchers and pathologists in this study recorded the data on separate case record forms without exchanging any information until the conclusions were made.

Statistical Analysis

All values were expressed as the means \pm standard deviation. Statistical analyses were performed with Student's test using SPSS software, and $P<0.05$ was considered significant.

Results

Expression, Purification, and Activity of Recombinant CypA

Under our optimized induction conditions, 40% of the protein could be recovered in the soluble form (Fig. 1a). The purity of the protein reached above 95% by SDS-PAGE analysis (Fig. 1b). Western-blot results indicated that the rabbit anti-CypA polyclonal antibody bound specifically to the expressed CypA protein (Fig. 1c). The LPS level in the CypA preparation was lower than 5 EU/ml, which was quantified by a limulus amoebocyte lysate assay.

Migration of THP-1 Cells in Response to CypA

The response of THP-1 cells to CypA was dose-dependent and exhibited a typical bell-shaped curve. The chemotactic

cell number was 36.33 ± 4.04 in the control group without CypA stimulation. The optimum chemotaxis dose of CypA was 200 ng/ml and the chemotactic index ($423\pm 32\%$ control, $P<0.05$) was higher than that in the FMLP positive control group ($263\pm 28\%$ control, $P<0.05$; Fig. 2).

MMPs Expression of THP-1 Cells with CypA Stimulation

The gel zymography results showed that MMP-9 and MMP-2 secretion increased significantly in THP-1 cells under CypA stimulation ($P<0.05$) and the optimum dose of CypA was 100 ng/ml (Fig. 3). The density of MMP-9 in the CypA stimulation (10, 100, 200, 500, and 1,000 ng/ml) groups increased significantly by $28\pm 6\%$, $149\pm 13\%$, $138\pm 14\%$, $114\pm 16\%$, and $86\pm 5\%$, compared respectively with that in the blank control group ($P<0.05$). The density of MMP-2 in CypA (100, 200, 500, and 1,000 ng/ml) groups increased respectively by $80\pm 12\%$, $47\pm 3\%$, $43\pm 7\%$, and $12\pm 28\%$ ($P<0.05$). However, no significant difference was found in the density of MMP-2 between CypA group (10 ng/ml) and the blank control group (Fig. 3). CypA-induced MMPs production was not influenced by PMB while PMB effectively blocked LPS-induced MMP-9 and MMP-2 expression (Fig. 4).

Invasive Ability of THP-1 Cells with CypA Stimulation

Cell invasion assay showed that the invasive cell number was 427.20 ± 9.72 without CypA stimulation but with CypA stimulation (100, 200, 500, and 1,000 ng/ml), the number significantly increased by $170\pm 21\%$, $106\pm 27\%$, $90\pm 53\%$, and $57\pm 20\%$, respectively ($P<0.05$). The optimum CypA concentration for THP-1 cells was 100 ng/ml and low concentration such as 10 ng/ml had little effect on the number of cells that invaded into the matrigel (Fig. 5).

Effect of CypA on the Levels of IL-8 in THP-1 Medium

Compared with that in the control group, the concentration of IL-8 increased when CypA was added in different dilutions (Fig. 6). In the control group, the concentration of IL-8 was 285.60 ± 9.64 pg/ml. After CypA treatment (10, 100, 200, 500, and 1,000 ng/ml), the expression of IL-8 increased respectively by $40\pm 9\%$, $50\pm 4\%$, $41\pm 6\%$, $57\pm 8\%$, and $63\pm 9\%$ ($P<0.05$). However, the levels of TNF- α , IL-1 β , IL-6, IL-10, and IL-12p70 were undetectable.

Effects of CypA on Cartilage Invasion on SCID-HuRAG Model

Histological evaluation 1 week after the final CypA injection revealed a significant increase of synovium invasiveness into the cartilage (Fig. 7). Deep invasion (an

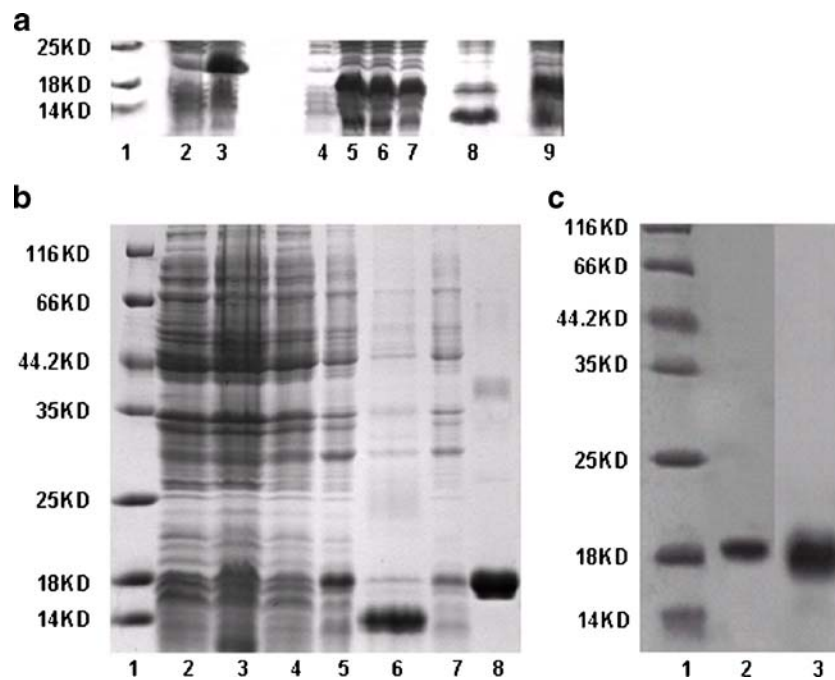


Fig. 1 Expression, purification, and characterization of recombinant CypA. **a** SDS-PAGE analysis of the recombinant CypA. *Lane 1* protein marker, *lanes 2–3* total lysates of JM109 (DE3) bacteria culture transformed with pET32a+ vector before and after induction with IPTG, respectively, *lane 4* total lysates of JM109 (DE3) culture transformed with pET32a+–CypA before induction with IPTG, *lanes 5–7* total lysates of three JM109 (DE3) clone cultures transformed with pET32a+–CypA induced with IPTG, *lanes 8–9* the soluble supernatants and the denatured supernatants. **b** Purification of

recombinant protein by Ni–NTA chromatography. The eluted protein was analyzed by SDS-PAGE. *Lane 1* protein marker, *lanes 2–4* total lysates of JM109 (DE3) culture, *lane 5* the soluble supernatants of the crude extract containing IPTG-induced recombinant cyclophilin A, *lanes 6–7* flow-through from the Ni–NTA column, *lane 8* the elution buffer containing purified protein. **c** Western-blot assay. *Lanes 1–2* were the SDS-PAGE results of protein marker and purified CypA respectively, *lane 3* was the Western-blot results detected by rabbit anti-CypA polyclonal antibody

invasion score of ≥ 2.5) was observed in five of the six cartilage sections in the CypA group. In contrast, deep invasion was observed only in one of the six sections in the control group. The cartilage erosion score in CypA group

was significantly higher than that in the control group ($P < 0.05$), indicating that CypA may aggravate the cartilage erosion in SCID–HuRAg model.

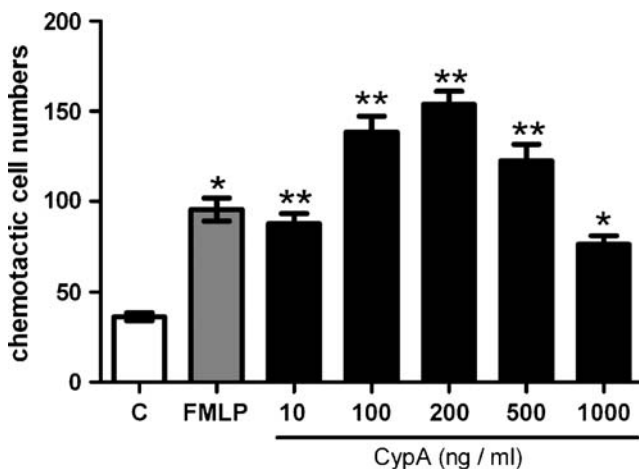
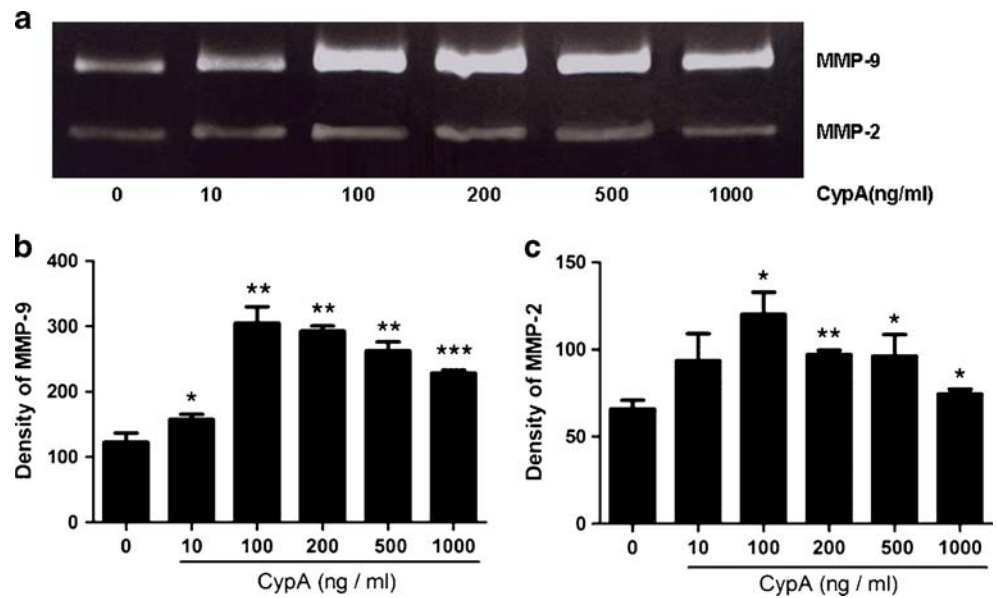


Fig. 2 The chemotaxis of CypA for THP-1 cells. The chemotactic cell numbers increased significantly when CypA was added in different dilutions. Graphs show mean (\pm SD) chemotactic cell numbers for each group, and $n=3$ wells per group. Significant differences ($*P < 0.05$; $**P < 0.01$) compared to control group are indicated. *C* not treated, negative control; *FMLP* *n*-Formyl–Met–Leu–Phe, positive control

Discussion

Recent studies have uncovered many important properties and functions of CypA, such as protein folding and repair [17, 18], involvement in apoptosis [19], pathogenesis of vascular diseases, and human immunodeficiency virus infection [6, 20], and interaction with CD147 [21]. The presence of elevated levels of extracellular cyclophilins has been reported in several different inflammatory diseases, including severe sepsis, and vascular smooth muscle cell diseases. High levels of CypA were also detected in the lining layers of human RA synovium. Immunohistochemistry analysis revealed that macrophages, lymphocytes, endothelial cells, and smooth muscle cells in RA synovium were the sources of CypA [22]. The results of these studies suggest that CypA may be involved in the pathogenesis of RA. The current study was conducted to explore the role of CypA in the regulation of inflammatory process in RA. We studied the effects of CypA stimulation on chemotaxis,

Fig. 3 CypA treatment induces MMPs secretion in THP-1 cells. **a** THP-1 cells (three wells per group) were treated with 10–1,000 ng/ml CypA for 24 h and the culture supernatants were used for the detection of MMPs through gelatin zymogram. **b** and **c** are the values of MMP-9 and MMP-2 enzyme activity of THP-1 cells, respectively. The data are shown as the mean±SD of three independent experiments. Significant differences (* $P<0.05$; ** $P<0.01$; *** $P<0.001$) compared to control group are indicated



MMPs production, invasion, and cytokine secretion of THP-1 cells. More important, the functions of CypA on cartilage invasion were observed using SCID-HuRAG models and the CypA used in this study was purified in our lab, with LPS concentration lower than 5 EU/ml.

As the infiltration of leukocytes from periphery into the inflamed synovium was a key step in the development of an inflammatory process in RA, we examined the CypA's capacity of inducing the migration of a subset of

leukocytes—monocytes. Our findings demonstrated that CypA significantly promoted the chemotaxis of THP-1 cells, which was consistent with results reported by Khromykh et al. [23]. We also found that the chemoatctic ability of THP-1 was the highest when the concentration of CypA was 200 ng/ml and further increase of CypA concentration decreased the chemotactic index. Our findings and the results of other study [23] all indicate that CypA acts as a strong chemoattractant for monocytes.

Fig. 4 CypA treatment induces MMPs secretion in THP-1 cells without any influence of endotoxins contamination. **a** THP-1 cells (three wells per group) were stimulated with either 100 ng/ml LPS or 100 ng/ml CypA in the presence of 10 µg/ml polymyxin B. Culture supernatants were collected in 24 h for the measurement of MMP-9 and MMP-2 levels. **b** and **c** are the values of MMP-9 and MMP-2 enzyme activity of THP-1 cells, respectively. Values are expressed as means±SD ($n=3$). * Indicates $P<0.05$, ** indicates $P<0.01$

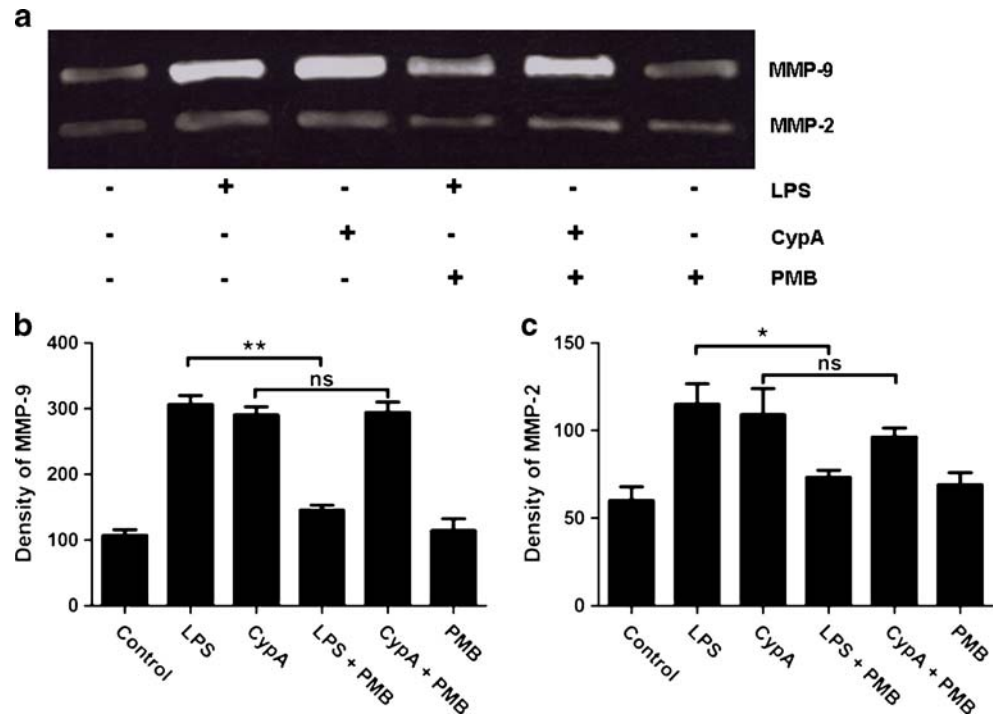
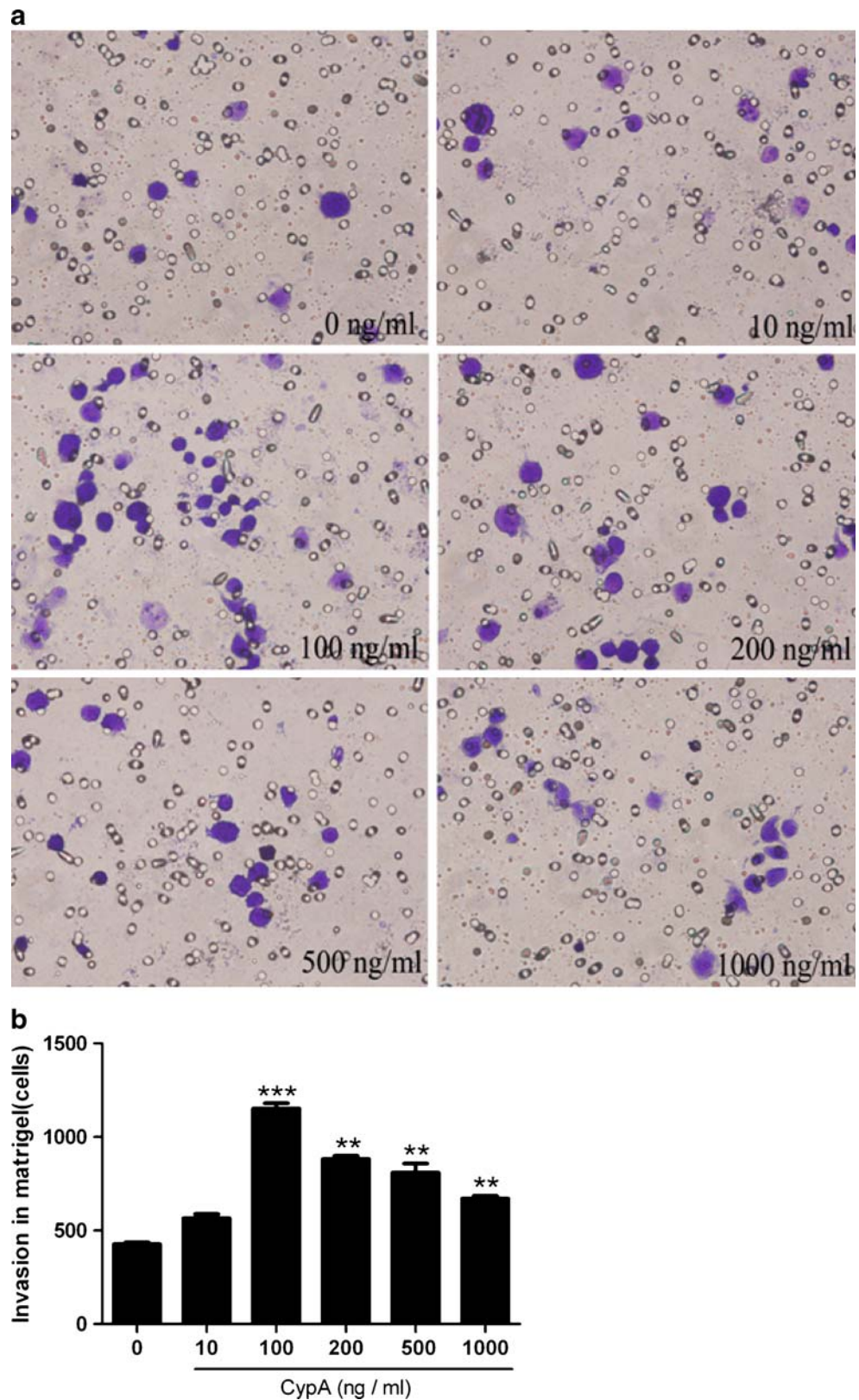


Fig. 5 CypA treatment induces invasion of THP-1 cells. **a** The crystal violet staining results of invading cells. After stimulation with different concentrations of CypA (10, 100, 200, 500, and 1,000 ng/ml), THP-1 cells on the lower surface filters were stained with crystal violet to show that the cells had invaded through and attached to the lower side of the filter (original magnification $\times 400$). The number of cells increased compared with that in the culture of THP-1 cells alone. **b** Graphs show mean (\pm SD) invading cell numbers for each group, and $n=3$ chambers per group. Significant differences (** $P<0.01$; *** $P<0.001$) compared to control group are indicated



Degradation of articular cartilage is one of the early features of RA and is mediated by an increased activity of proteolytic systems [24]. Normally, there will be a tight balance between MMPs and TIMPs. However, in patho-

logical conditions, such as RA, an MMP/TIMP imbalance ensues. This kind of imbalance will lead to the excess of MMPs, resulting in cartilage destruction. In our study, we used a blocker, polymyxin B, to eliminate the influence of

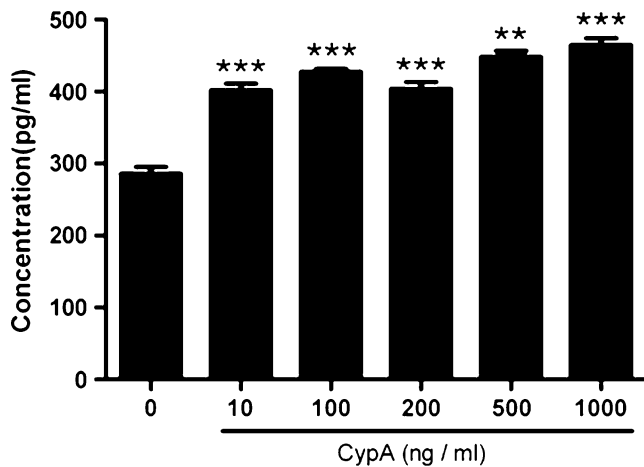


Fig. 6 CypA induces IL-8 expression of THP-1 cell. THP-1 cells (three wells per group) were treated with 10–1,000 ng/ml CypA for 24 h and the culture supernatants were determined by human inflammatory cytokine CBA kit using flow cytometry. Data are shown as the means \pm SD of three dependent experiments. Significant differences (** $P < 0.01$; *** $P < 0.001$) compared to control group are indicated

endotoxins and found that CypA significantly stimulated the secretion of MMP-2 and MMP-9, as well as the cell invasion of THP-1 cells. The secretion and the invasion peaked at CypA concentration of 100 ng/ml, which were similar to Billich's clinical report that the concentrations of CypA in synovial fluids from RA patients were in the range of 11 to 705 nM, based on the enzyme activity [10]. These results suggest that CypA may contribute to the cartilage destruction of RA through induction of MMP-9 and MMP-2 in the invasive front of the synovium. Another interesting finding was that the effects of CypA displayed a biphasic concentration dependency, which may relate to the cytotoxicity caused by high concentrations of CypA.

In our study, CBA analysis of cytokines release of THP-1 cells treated with CypA showed that the level of IL-8 was significantly elevated while other cytokines like TNF- α and IL-1 β were not affected. IL-8/CXCL8 was reported abundantly present in both synovial tissue and fluid [25, 26] and significantly higher in clinically inflamed RA joints than that in clinically uninvolved joints, indicating a possibly crucial role in promoting synovial inflammation [27]. One suggested reason is that IL-8 induces the migration of inflammatory cells, especially neutrophils by generating a chemical gradient within the site of inflammation [28–31] and another is that IL-8 participates in synovioblasts activation [32]. We presume that CypA may promote the inflammatory response through inducing the secretion of pro-inflammatory cytokine IL-8. Our findings further strengthen the role of CypA as an inflammatory mediator participating in the development of RA.

In studies of RA, various kinds of animal models for arthritis have been reported, such as mutation-, knockin-,

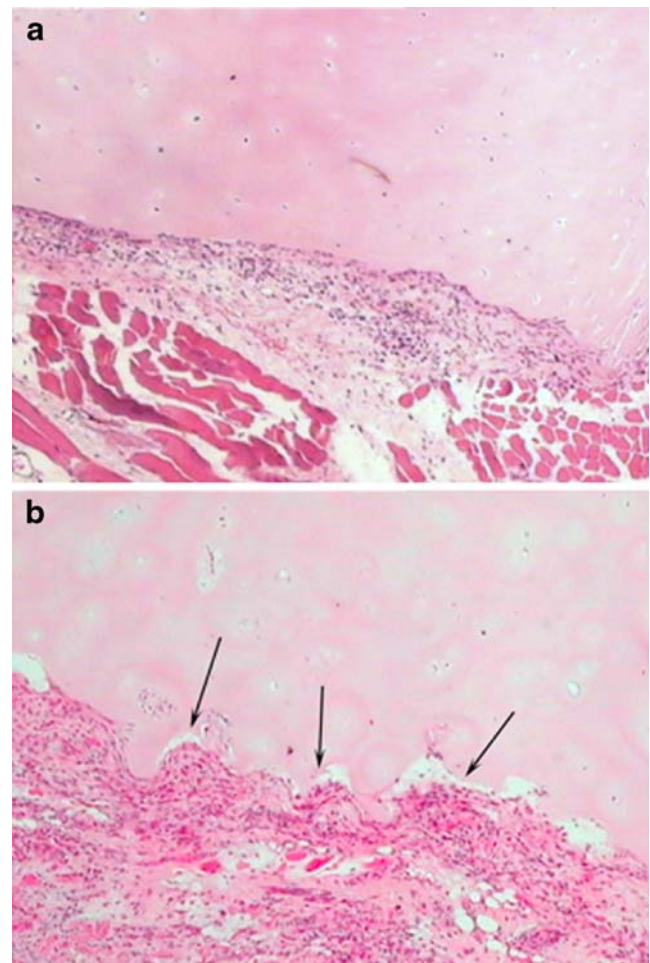


Fig. 7 Light microscopic features of cartilage erosion in SCID-HuRAg model. **a** In blank control group, synovial fibroblasts accumulate at the edge of the cartilage, but the invasion is hardly seen ($n=1/6$). **b** In CypA-treated group, the deep invasion of the cartilage by synovial fibroblasts are observed ($n=5/6$). Arrows show the invasive front of the synovial tissue (original magnification $\times 400$)

and knockout-induced arthritics. However, these models all have some clinical distance from human diseases. In our study, we employed a SCID-HuRAg model, in which human engrafted tissue was applied, to investigate the effects of CypA on inflammation. Our in vivo data showed that the cartilage erosion scores in CypA group were higher than those in control group, which may be the result of CypA inducing MMP-9 and MMP-2 production. But further investigation is needed.

Conclusion

Our findings show that pathogenically CypA significantly promotes the THP-1 cells migration, the MMP-2 and MMP-9 secretion, and cell invasion, and induces the production of pro-inflammatory cytokines IL-8 at the

concentration of 100–200 ng/ml. The concentrations of CypA used in this study are pathophysiologically relevant. CypA is also involved in cartilage erosion of RA. Our results suggest that CypA may be a possible therapeutic target in RA treatment.

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Competing interests The authors declare that they have no competing interests.

Authors' contributions LW participated in the design of the study and drafted the manuscript. CW, one of the co-first authors, conducted the chemotaxis, invasion, gel zymography, and CBA assays; performed the statistical analysis and helped to draft the manuscript. JJ constructed the animal model and performed in vivo assays. XM and YL participated in the construction of the expression vector and the induction of the CypA protein. HZ and HT performed the purification of the CypA. ZC and PZ, the corresponding authors, participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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