

Cyclophilins in rheumatoid arthritis—stepping into an undiscovered country?

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Recent years have seen enormous progress in understanding the pathogenesis of rheumatoid arthritis (RA), an autoimmunological disorder that primarily affects the joints and leads to their progressive destruction. Advances in molecular biology techniques such as the use of gene transfer and gene silencing technology, the utilization of novel animal models of destructive arthritis—particularly in conjunction with newly established transgenic and knockout mice—and the observation of very early stages of human disease have provided exciting novel insights into key mechanisms that ultimately lead to the development of established RA in humans and that contribute to joint destruction. It has become increasingly clear that the mechanisms of rheumatoid joint destruction are linked closely to changes that occur predominantly at sites of interaction between the rheumatoid synovium and articular cartilage and bone [1]. Numerous data have shown that cells of the inflamed synovium constitute a highly interdependent network, in which the interaction of stromal cells—specifically synovial fibroblasts—with infiltrating inflammatory cells such as lymphocytes and macrophages creates a unique environment that results in the development of chronic destructive synovitis [2].

Thickening of the RA synovium is largely due to a hyperplasia of the most superficial lining layer that in the course of disease grows from 2–4 layers of cells to more than 10 layers. About two-thirds of the cells in the lining layer express macrophage markers such as CD11b, CD14, CD33, and CD68 as well as major histocompatibility complex (MHC) class II molecules and can thus be

identified as macrophages [3,4]. These macrophages constitute a major source of inflammatory factors in the RA synovium, and it is now evident that macrophage-derived cytokines such as TNF- α contribute prominently to the activation of synovial cells in RA. Thus, mice transgenic for human TNF- α (hTNFtg), a major inflammatory cytokine in RA, constitutively develop a chronic inflammatory polyarthritis that is highly destructive [5]. Although treatment of arthritic hTNFtg mice with monoclonal antibodies against hTNF prevents development of disease, there is only a narrow window of time in which anti-TNF- α treatment can inhibit the onset of arthritis entirely, suggesting that chronic exposure of cells to inflammatory mediators may induce a stable activation and alterations in tissue homeostasis that can become independent of the specific trigger. Furthermore, most recent data suggest that activation pathways of synovial cells differ between the various animal models of arthritis and that each of these models reflects only part of human pathology [6]. This notion is supported by clinical data using inhibitors of individual cytokines such as anti-TNF- α agents. Although there has been a breakthrough in the treatment of RA using these novel biologics, response is still limited with respect to both the number of patients going into remission and the degree to which remission can be achieved in individual patients.

As a consequence, there are continuous efforts to identify new molecular targets for anti-inflammatory and anti-resorptive intervention as well as to better understand the mechanisms of known mediators of inflammation and joint destruction. In this context, interest has focused, not only on well-established molecules, such as inflammatory cytokines, chemokines, and transcription factors, but also on molecules that are involved in more basic biologic processes such as

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expression, folding, and degradation of proteins. The investigation of cyclophilins constitutes a prominent example of such approaches.

Cyclophilins constitute a family of evolutionarily conserved proteins that are expressed ubiquitously in eukaryotic cells [7]. They are found mainly in the cytoplasm and have peptidyl-prolyl isomerase activity catalyzing the *cis*–*trans* interconversion of peptide bonds N-terminal to proline [8]. The entire spectrum of their functions has not been clarified, but it appears that cyclophilins are involved mainly in the folding of nascent proteins [9]. Cyclophilin A is the prototype of this family and is expressed most widely in mammals. There are at least 15 different cyclophilin genes in the mouse genome, which differ from cyclophilin A mainly by terminal extensions that appear responsible for subcellular localization and protein–protein interactions. Initial interest in cyclophilin A in immunity has come mainly from the fact that cyclophilin A can bind the immunosuppressive agent cyclosporine A with very high affinity [10,11]. Most recent data have supported the functional involvement of cyclophilin A in mediating the effects of cyclosporine A by demonstrating that *Ppia*^{−/−} (the gene encoding cyclophilin A) mice are resistant to cyclosporine A-mediated immunosuppression and that cyclophilin A is the primary mediator of the immunosuppressive effects of cyclosporine A [12]. However, a growing body of evidence suggests that cyclophilin A is involved also in the pathogenesis of immune-mediated disorders [13–16]. Although *Ppia*^{−/−} mice show only a slight loss in viability and adult *Ppia*^{−/−} animals have no obvious decrease in their life spans, they spontaneously develop an allergic condition that is similar to that of IL-4 overexpressing animals and is linked most likely to elevated levels of IL-4 [17]. In addition, CD4⁺ cells of *Ppia*^{−/−} mice produce significantly higher amounts of IL-2, and Th2 cells of these animals are hypersensitive to T cell receptor (TCR) stimulation. These data suggest that cyclophilin A is involved in the regulation of Th1–Th2 balance and that overexpres-

sion of cyclophilin A is linked to autoimmune diseases. In line with this concept, cyclophilin A has been identified as an inflammatory mediator in different conditions. It has been shown to be produced by macrophages following LPS stimulation and more recently linked to atherosclerosis and endothelial dysfunction [14,15].

Of interest, RA has been the first condition in which a secreted form of cyclophilin A has been demonstrated in extracellular fluids. As shown by Billich and colleagues, cyclophilin A was increased in the synovial fluids of RA patients compared to OA patients and correlated with the total cell counts [13]. However, the precise origin of cyclophilin A in RA, as well as the functional consequences of increased cyclophilin A expression, has remained largely undefined (Fig. 1).

In this issue of *Clinical Immunology*, Won-Ha Lee and colleagues extend the findings on cyclophilin A in RA by identifying the source of cyclophilin A in the rheumatoid synovium and by demonstrating pro-inflammatory functions of cyclophilin A in macrophage-like cells [18]. As demonstrated in their study, macrophages of the synovial lining layer constitute the major source of cyclophilin A in the RA synovium. In addition, they show that stimulation of monocytes with cyclophilin A results in increased production of inflammatory cytokines, specifically TNF- α , interleukin (IL)-1 β , IL-8, and monocyte chemoattractant protein (MCP)-1. Won-Ha Lee and his colleagues suggest further that cyclophilin A is involved in the expression of gelatinase B (matrix metalloproteinase 9, MMP-9) by monocytes and that these stimulatory effects of cyclophilin A on MMP-9 production are mediated through the nuclear factor kappa B (NF κ B) transcription factor. These data are of interest because they tighten the links between cyclophilins and key pathological mechanisms of RA and in the light of what is known about cyclophilins have a number of possible implications. At the same time, the major caveat is given by the authors themselves when they state in the title of their

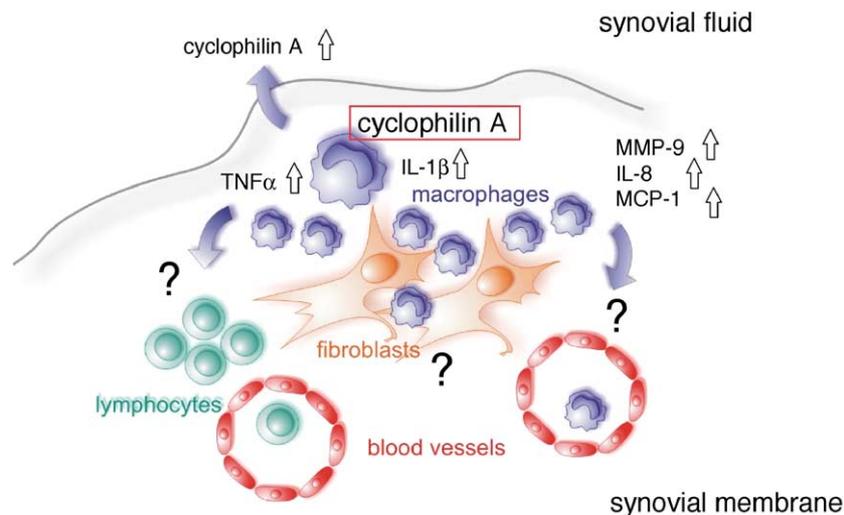


Fig. 1. Potential effects of elevated cyclophilin A in the rheumatoid synovium (for details see text).

work that “Cyclophilin A may contribute to the inflammatory process in rheumatoid arthritis...” Certainly, their data will encourage further research on the functional relevance of increased cyclophilin A in RA. Such research should take advantage of the availability of the *Ppia*^{-/-} mice and investigate if these animals—along with their allergic phenotype—exhibit altered susceptibility to models of chronic destructive arthritis. Furthermore, such research will have to clarify whether *Ppia*^{-/-} macrophages have a decreased ability of autocrine and paracrine stimulation, as well as (due to potentially reduced levels of MMP-9) altered homing into inflamed synovial tissue. Given the aforementioned data on changes in TCR signaling in the *Ppia*^{-/-} mice, it will also be of interest to see whether cyclophilin A affects the composition of the T cell compartment in the chronically inflamed synovium and/or modulates the reactivity of arthritis-derived versus normal T cells.

Collectively, these data provide further evidence of the complexity of synovial inflammation and point to novel, as yet undiscovered pathways in the interactions of different cells in the RA synovial membrane.

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Cyclophilin A may contribute to the inflammatory processes in rheumatoid arthritis through induction of matrix degrading enzymes and inflammatory cytokines from macrophages

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Abstract

Cyclophilin A (CypA) levels increase in the sera and synovial fluids of rheumatoid arthritis (RA) patients, but the cell types expressing CypA and the function of CypA in the pathogenesis of RA are not known yet. Immunohistochemistry analyses revealed high level CypA staining in the macrophages in the lining layers of human RA and osteoarthritis synovium. Low level CypA staining was also detected in endothelial cells, lymphocytes, and smooth muscle cells in RA synovium. Further investigation of the CypA function using monocyte/macrophage cell lines revealed that CypA induced expression of cytokine/chemokines such as TNF- α , IL-8, MCP-1, and IL-1 β and matrix metalloproteinase (MMP)-9 through a pathway that is dependent on NF κ B activation. Furthermore, MMP-9 staining pattern overlapped with that of CypA in both RA and OA synovium. Our data suggest that CypA may stimulate macrophages to degrade joint cartilage via MMP-9 expression and promote inflammation via pro-inflammatory cytokine secretion.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by the synovial inflammation that leads to the destruction of the cartilage and bone. Synovial inflammation involves lining layer thickening and infiltration of inflammatory cells into the sublining area [1,2]. In normal joints, macrophages are resident cells and cover the synovial lining layer. The number of macrophages in the joint greatly increases in both the lining and sublining areas of RA synovium [3] and the degree of increase well correlates with the severity of cartilage destruction [4–6]. Furthermore, selective depletion of the macrophages from the synovial lining before the induction of experimental arthritis resulted

in prevention of both joint inflammation and cartilage destruction [7–9]. Cartilage destruction is mediated by the extracellular matrix (ECM) degrading enzymes including serine proteases, MMPs, and the cathepsins [1]. MMP-9 levels have been shown to be elevated in the sera and synovial fluids of RA patients [10,11].

Cyclophilin A (CypA) is a soluble ubiquitously distributed intracellular protein belonging to the immunophilin family [12]. Additional experiments, however, have implicated CypA in inflammation since it is secreted from smooth muscle cells and macrophages in response to oxidative stress and lipopolysaccharide (LPS) [13–15]. Platelets also secrete CypA upon activation [16]. Furthermore, CypA has been detected to be elevated in the serum of sepsis patients and the synovial fluids of RA patients [17,18]. In inflammatory diseases such as atherosclerosis, CypA works as a proinflammatory cytokine and activates

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endothelial cells to produce inflammatory mediators and undergo apoptosis [14,19]. Recently, CypA was reported to be related to the growth and differentiation of other cells, such as human embryonic nerve cells [20].

Although the pro-inflammatory activities of CypA are well known, the role of CypA in the pathogenesis of RA is not specifically known. We analyzed synovial tissues from human RA patients to reveal the cell types expressing CypA. We also tested the possible role of CypA during the development of RA using human and murine monocytic cell lines.

Materials and methods

Monoclonal antibodies, cell lines, and reagents

Monoclonal antibodies (mAbs) to CD68 (KP1), CD3 (F7.2.38), and α -actin (1A4) and rabbit polyclonal antibody to von Willebrand factor (vWF) (N1505) were purchased from DAKO (Glostrup, Denmark); mouse polyclonal antibody to CypA from BIOMOL International (USA); rabbit polyclonal antibody to I κ B and mAb to phospho-I κ B (Ser32/36) (5A5) from Cell Signaling (USA); mAb to MMP-3 (SL-1 IID4) and rabbit polyclonal antibody to MMP-9 from Chemicon (USA). Human monocytic leukemia cell line THP-1 [21] and mouse monocytic cell line RAW264.7 were obtained from the American Type Culture Collection (Rockville, MD, USA). Recombinant human CypA was purchased from BIOMOL International (USA).

Histological analysis

For the immunohistochemical analysis, synovial tissue samples were collected from RA patients who were undergoing joint replacement therapy and processed to make paraffin blocks. RA was diagnosed according to the criteria of the American College of Rheumatology. The study was approved by an institutional review committee and the subjects gave informed consent. Standard 5- μ m sections were stained using an LSAB kit (DAKO, Copenhagen, Denmark) according to the manual provided by the manufacturer. The sections were then counterstained with Hematoxylin which stains the nucleus in blue color. For the double staining of CD68 and CypA, the specimens were sequentially treated with anti-CypA mAb; biotin-linked secondary antibody; streptavidin-alkaline phosphatase; fuchsin for the visualization of CypA (red color); and the slides were mounted in Faramount aqueous mounting medium (DAKO) to take pictures. The sections were then incubated in distilled water to wash away the mounting solution and sequentially treated with anti-CD68 mAb. The anti-CD68 mAb was pre-conjugated with biotin using an Animal Research Kit (DAKO, Copenhagen, Denmark) according to the manufacturer's manual. The sections were then treated with streptavidin–horseradish peroxidase followed by diaminobenzidine

(DAB) for the visualization of CD68 (brown color). Finally, the sections were counterstained with hematoxylin (blue color) for the visualization of nuclei. Finally, the slides were mounted in 1:1 mixture of Xylene and Malinol (Muto Pure Chemicals, Tokyo, Japan). During this final step, the previous red color staining was partially bleached.

Immunofluorescence assay

THP-1 cells were activated with either 1 μ g/ml LPS or 0.1 μ M CypA for up to 3 h in RPMI medium with 0.1% fetal bovine serum. For the detection of NF- κ B, THP-1 cells (2×10^5) were washed in PBS and resuspended in 10 μ l of 4% formaldehyde in distilled water and put onto the slide glass. The fixed cells were dried for 1 h at RT and washed with PBS for 5 min. Cells were then permeabilized with 1% Triton in PBS for 10 min at RT, washed with 0.02% Tween-20 in PBS for 20 min, and 0.02% Tween-20/1% BSA in PBS for 5 min. Cells were then treated with anti-p50 polyclonal antibody (SC-1191, Santa Cruz, USA) (1:100 dilution in PBS containing 3% BSA) for 45 min at 37°C, washed with 0.02% Tween-20/1% BSA in PBS for 5 min, treated with Alexa Fluor 488-labeled rabbit anti-goat antibody (A-11078, Molecular Probes) (1:100 dilution) for 45 min at 37°C, and washed with 0.02% Tween-20 in PBS for 5 min, and PBS for 5 min. Cells were then dried in 37°C oven for 45 min and the slides were mounted in 1:1 mixture of Xylene and Malinol.

Western blot analysis, ELISA, and gelatin zymogram

For the detection of I κ B or phosphorylated I κ B, THP-1 cells (1×10^7 /well in 6 well plate containing 1 ml RPMI medium with 0.1% fetal bovine serum) were either mock-treated or stimulated with 100 ng/ml LPS or 0.1 μ M CypA. Cell lysates were prepared at 15, 30, 60, and 120 min after activation in 150 μ l of triple-detergent lysis buffer and used for Western blot analysis as described previously [22]. For the detection of MMP-9 and cytokines, THP-1 cells (1×10^5 /well in 96 well plate containing 100 μ l RPMI medium with 0.1% fetal bovine serum) were either mock-treated or stimulated with 1 μ g/ml LPS or 0.1–1 μ M CypA. Culture supernatants were collected 24 h after activation. Cytokine levels in the culture supernatant were measured using sandwich ELISA (R and D Systems). The detection limits were <10 pg/ml for all the cytokines. The MMP-9 activity in the culture supernatant was determined by substrate gel electrophoresis [23].

Results and discussion

CypA expression is concentrated in areas enriched in macrophages and endothelial cells in RA synovium

Synovial tissues were obtained from RA and osteoarthritis (OA) patients and CypA, CD68 (a macrophage

marker), and von Willebrand factor (vWF) (an endothelial cell marker) expression patterns were analyzed using immunohistochemistry (Fig. 1). In RA synovium, CypA staining was detected in the macrophages in the lining layer and the microvessel endothelial cells in the sublining area (compare panels A with B and C). CypA expression was also detected in the macrophages in the sublining area in certain cases (data not shown). In OA synovium, the number of macrophages and endothelial cells decreased greatly (compare panels B and C with D and F, respectively). Although CypA expression was detected in most of the macrophages, endothelial cells expressed relatively less CypA (Figs. 1D, E, and F). We analyzed 8 different RA synovial tissues and 7 different OA synovial tissues with essentially the same results.

We focused on the CypA expressing cells in the lining layer, since these cells are in the invasive front of synovium and thus could be responsible for the cartilage destruction. To demonstrate that the cells expressing CypA in the lining layers of RA and OA synovium are the macrophages, we performed double immunohistochemistry. As shown in Fig. 2, the CypA staining pattern overlapped with CD68 staining pattern in the lining layers indicating that macrophages do express CypA in the RA and OA synovium. We also analyzed consecutive sections of other RA and OA tissues with antibodies for CypA and CD68 and confirmed that macrophages do express CypA in the synovial tissues (data not shown).

We further compared staining patterns for CD3 (a lymphocytes marker), α -actin (a smooth muscle cell marker), CypA, CD68, and vWF in consecutive sections from 4 different RA and 3 different OA synovium and summarized the results in Table 1. CypA expression was detected in more than 70% of the macrophages in both RA and OA synovium. Since the absolute number of macrophages in RA synovium was much bigger than that in OA synovium, the absolute amount CypA in a RA synovial tissue is expected to be much greater than in an OA synovial tissue. In case of the endothelial cells, more than 50% of RA synovial endothelial cells expressed CypA while less than 30% of OA synovial endothelial cells expressed CypA. Expression of CypA in the smooth muscle cells (SMCs) was detected in 3 out of 4 cases of RA synovium and tended to be higher in RA than in OA tissues. Lymphocytes were not present in OA tissues while more than 50% of the RA lymphocytes expressed CypA. These results indicate that the CypA expression levels increase in regions with heavy inflammation even though it is known to be expressed in virtually all cell types.

It has been demonstrated that CypA is expressed and secreted by activated macrophages [13] and endothelial cells [19]. Since high levels of pro-inflammatory cytokines and inflammatory mediators have been found to be present in RA synovium, these mediators could activate macrophages and endothelial cells to express CypA. It is interesting that both RA and OA macrophages express high levels of CypA while OA endothelial cells express less CypA than RA

endothelial cells. It has been known that the pathogenesis of RA involves auto-reactive T-cell generation and inflammation. As a result, heavy infiltration of inflammatory cells with formation of new microvessels is observed in RA synovium. In this condition, both macrophages and endothelial cells are expected to be activated and express CypA. In contrast, OA develops through cartilage erosion with relatively low level inflammation [24]. OA synovium contains little or no infiltration of inflammatory cells in the sublining area, which thus negate the necessity for new microvessel formation. This low level inflammation appears to induce the activation of the lining layer macrophages which then express CypA while endothelial cells are less activated and express less amount of CypA.

CypA induces cytokine and MMP-9 expression in monocytic cell lines via NF- κ B activation

Since CypA expression was observed in macrophage-rich areas, we investigated whether CypA is involved in the pathogenesis of RA through macrophage activation. The presence of CypA in the lining layer suggests that CypA may contribute to the cartilage destruction through induction of matrix degrading enzymes in the invasive front of the synovium. When the mouse monocytic RAW264.7 cells were treated with 0.1–1 μ M CypA and tested for the secretion of gelatinolytic enzymes, MMP-9 induction was detected in a dose dependent manner (Fig. 3A). Likewise, CypA treatment induced MMP-9 expression in the human monocytic cell line, THP-1 (Fig. 3B). Western blot analysis also confirmed induction of MMP-9 but not MMP-3, which is known to be expressed in activated synovial fibroblasts (data not shown) [25,26]. Kim et al. [19] reported that CypA induced conversion of pro-MMP-2 into active form in endothelial cells. We also observed low level conversion of pro-MMP-2 into active form in both mouse and human monocytic cells when these cells were treated with the highest dose of CypA (data not shown). To demonstrate that the MMP-9 induction was not caused by any contaminating endotoxins, we stimulated the cells with CypA in the presence of the endotoxin blocker, polymyxin B. MMP-9 expression induced by CypA was not affected by polymyxin B, which effectively blocked LPS induced MMP-9 expression (Fig. 3C).

In resting cells, NF- κ B/I κ B complexes are present in the cytoplasm. Activation of cells under appropriate condition leads to phosphorylation and subsequent degradation of I κ B. The free NF- κ B then translocates into the nucleus to activate genes with NF- κ B binding sites. Since activation of NF- κ B is required for the expression of MMP-9, we tested whether CypA induces activation and nuclear translocation of NF- κ B. Immunofluorescence analyses (Fig. 4A) revealed that CypA induced nuclear translocation of NF- κ B in 2 h after treatment and these nuclear NF- κ B translocated back to cytoplasm by 3 h. Likewise, CypA induced I κ B phosphorylation and degradation and I κ B within 2 h (Figs. 4B and C).

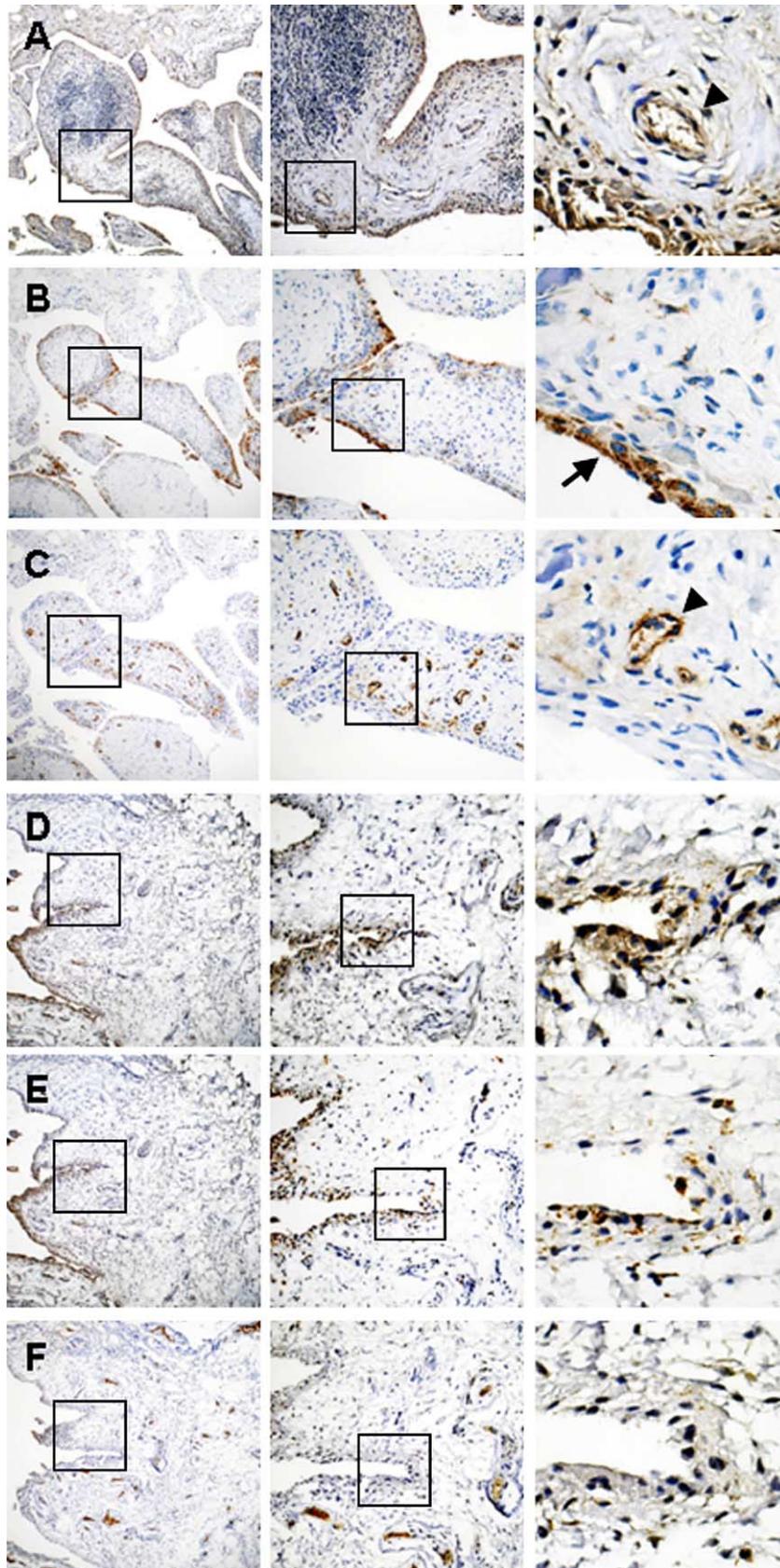


Fig. 1. Immunohistochemical analyses of synovial tissues from arthritis patients revealed CypA expression in macrophages and endothelial cells. Synovial tissues from RA (A–C) and OA patients (D–F) were stained with antibodies specific to CypA (A and D), CD68 (B and E), or vWF (C and F). The arrows indicate the lining layer and the arrow heads indicate the microvessel. Magnifications are 40 \times for the left, 100 \times for the middle, and 200 \times for the right panel. Squares in the pictures indicate the region magnified in higher magnifications.

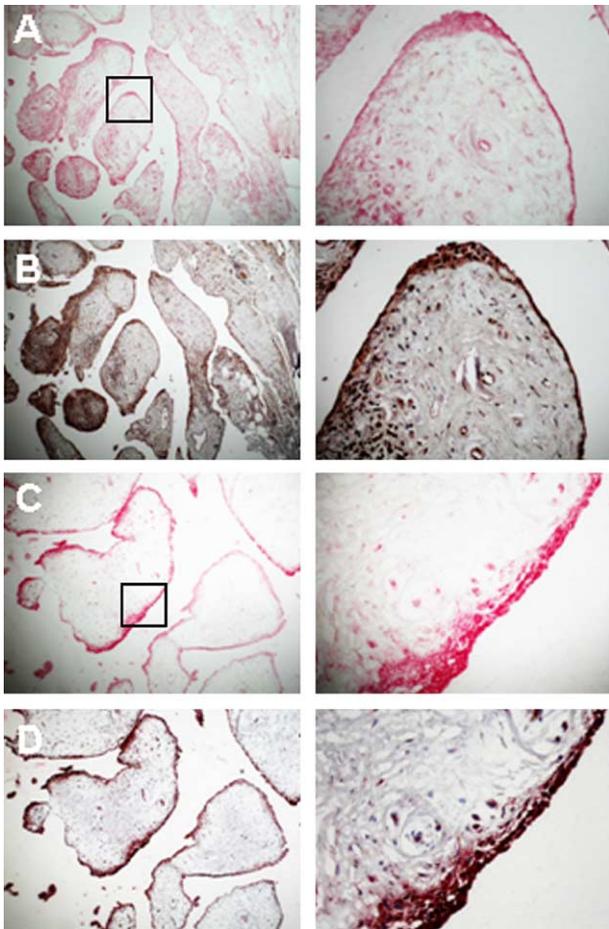


Fig. 2. Double immunohistochemical analyses demonstrate that macrophages express CypA in RA and OA synovium. Synovial tissues from RA (A and B) and OA patients (C and D) were first stained with anti-CypA monoclonal antibody (red color) (A and C) and the same sections were then re-stained with anti-CD68 monoclonal antibody (brown color) (B and D). Magnifications are 40× for the left and 200× for the right panel. Squares in the pictures indicate the region magnified in higher magnifications.

To demonstrate that CypA induced MMP-9 expression requires NF-κB activation, THP-1 cells were pretreated with the known NF-κB inhibitors, such as *N*-tosyl-L-phenylalanylchloromethyl ketone (TPCK) [27], ethyl pyruvate [28], and sulfasalazine [29] before CypA treatment. All

Table 1
Expression pattern of CypA in different cell types in RA and OA synovial tissues

Tissue	Macrophages	Endo. cells	SMCs	Lymphocytes
RA #1	+++ ^a	++	+	++
RA #2	+++	+++	++	n.a. ^b
RA #3	+++	++	–	++
RA #4	+++	+++	++	++
OA #1	+++	+	+	n.a.
OA #2	+++	+	+	n.a.
OA #3	+++	+	+	n.a.

^a –, no expression; +, < 30%; ++, 30–70%; +++, > 70% of each cell type expresses CypA.

^b Not available due to absence of lymphocytes in the tissue section.

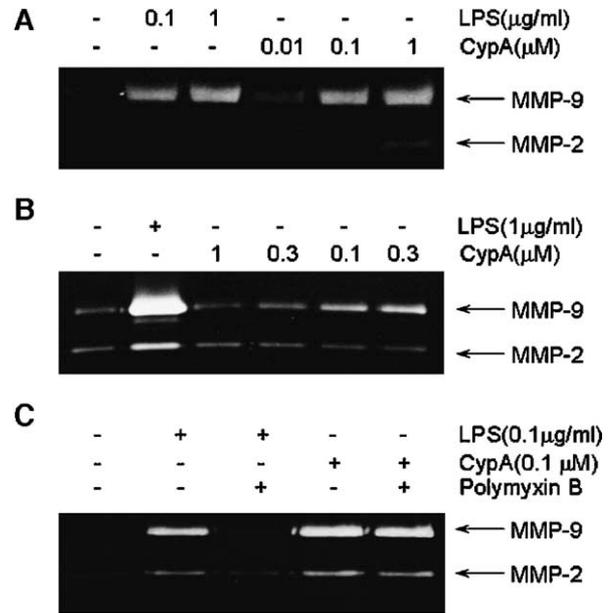


Fig. 3. CypA treatment induces MMP-9 secretion in monocyte/macrophage cell lines. RAW264.7 cells (A) and THP-1 (B) cells were activated with 0.01–1 μM CypA for 24 h and the culture supernatants were used for the detection of MMP-9 through gelatin zymogram. As a positive control, the cells were treated with 1 μg/ml LPS. (C) THP-1 cells were stimulated with either 0.1 μM CypA or 0.1 μg/ml LPS in the presence of 10 μg/ml polymyxin B. Culture supernatants were collected in 24 h for the measurement of MMP-9 levels.

these inhibitors blocked CypA induced MMP-9 expression in a dose dependent manner (Fig. 5).

We also tested whether CypA contribute to the inflammatory process by inducing pro-inflammatory cytokines. CypA treatment induced tumor necrosis factor (TNF)-α, interleukin (IL)-8, macrophage chemoattractant protein (MCP)-1, and IL-1β expression in a dose dependent manner (Fig. 6) in the THP-1 cells. When the RAW264.7 cells were used, CypA also induced TNF-α expression which reached the highest level when 0.1 μM CypA was used (data not shown). These pro-inflammatory cytokines are already known to be expressed in elevated levels in the RA synovium and enhance multiple inflammatory reactions in autocrine and paracrine manner [2,30,31]. These data further strengthen the role of CypA as one of the inflammatory mediators involved in the pathogenesis of RA.

CypA staining pattern overlaps with that of MMP-9

Our data indicate that CypA induces the matrix degrading enzyme, MMP-9. To find out whether the presence of CypA correlates with the expression of MMP-9, we analyzed staining patterns of these molecules in serial sections of both RA and OA synovial tissues. As shown in Fig. 7, CypA staining patterns in the lining layers overlapped with MMP-9 staining patterns in both RA and OA synovium. As a negative control, we stained the tissue sections for MMP-3, which is known to be elevated in RA patients' sera [31] and synovial fluid [32] and has shown to be expressed in activated synovial

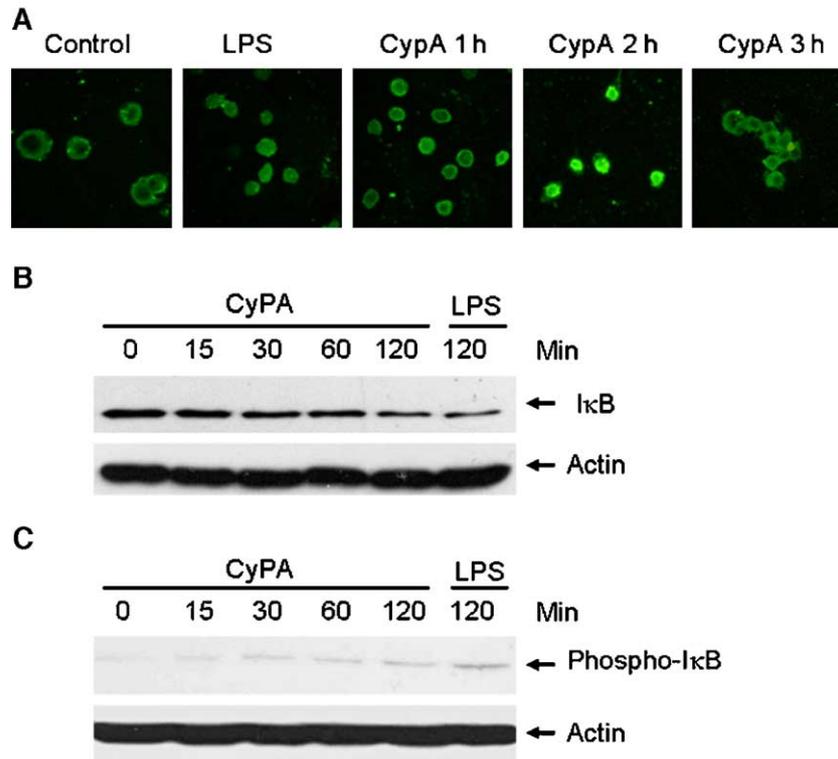


Fig. 4. CypA induces degradation of IκB and nuclear translocation of NF-κB in THP-1 cells. (A) THP-1 cells were stimulated with 0.1 μM CypA for 1, 2, and 3 h and subcellular locations of NF-κB were detected using immunofluorescence analysis as described in the Materials and methods section. As a negative control, cells incubated for 2 h without activation were used. As a positive control, cells stimulated with 1 μg/ml LPS for 2 h were used. Picture magnifications are 400×. (B and C) THP-1 cells were activated with 0.1 μM CypA and the levels of IκB (B) and phosphorylated IκB (C) were measured using Western blot analysis at indicated time points. Cell lysates obtained from THP-1 cells activated with 1 μg/ml LPS for 2 h were used as positive controls.

fibroblasts in vitro [25,26]. MMP-3 staining was neither detected nor correlated with the CypA/MMP-9 staining patterns in both RA and OA synovium.

Our data demonstrate that various cells including macrophages and endothelial cells are the source of CypA in RA synovial tissues. Further analyses using monocytic cell lines and immunohistochemical data have demonstrated that CypA induces pro-inflammatory cytokines and MMP-9

expression in macrophages. MMP-9 expressed in the invasive front of RA synovium will then contribute to the joint cartilage destruction. Furthermore, pro-inflammatory

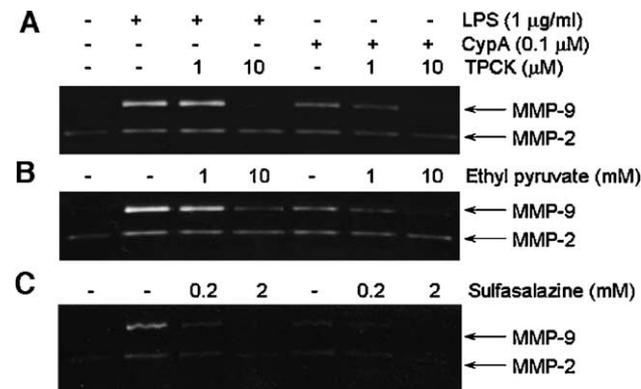


Fig. 5. CypA induced MMP-9 expression requires NF-κB activation. THP-1 cells were pretreated with indicated concentrations of NF-κB inhibitors and treated with either 1 μg/ml LPS or 0.1 μM CypA. TPCK (A), ethyl pyruvate (B), and Sulfasalazine (C) were used as NF-κB inhibitors at indicated concentrations. Culture supernatants were collected 24 h after activation and MMP-9 levels were measured using gelatin zymogram.

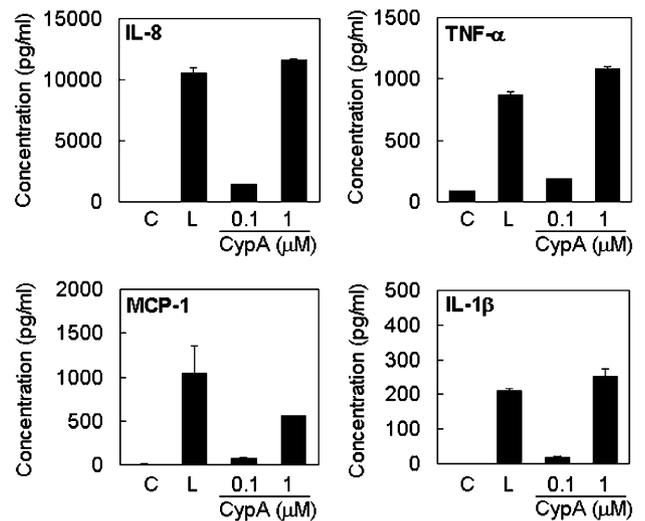


Fig. 6. CypA induces pro-inflammatory cytokine expression. THP-1 cells were stimulated with 0.1 and 1 μM CypA for 24 h and the culture supernatants were collected. As a positive control, cells stimulated with 1 μg/ml LPS. Concentrations of TNF-α (A), MCP-1 (B), IL-8 (C), and IL-1β (D) in the culture supernatants were measured via sandwich ELISA. C, no treatment; L, LPS treatment.

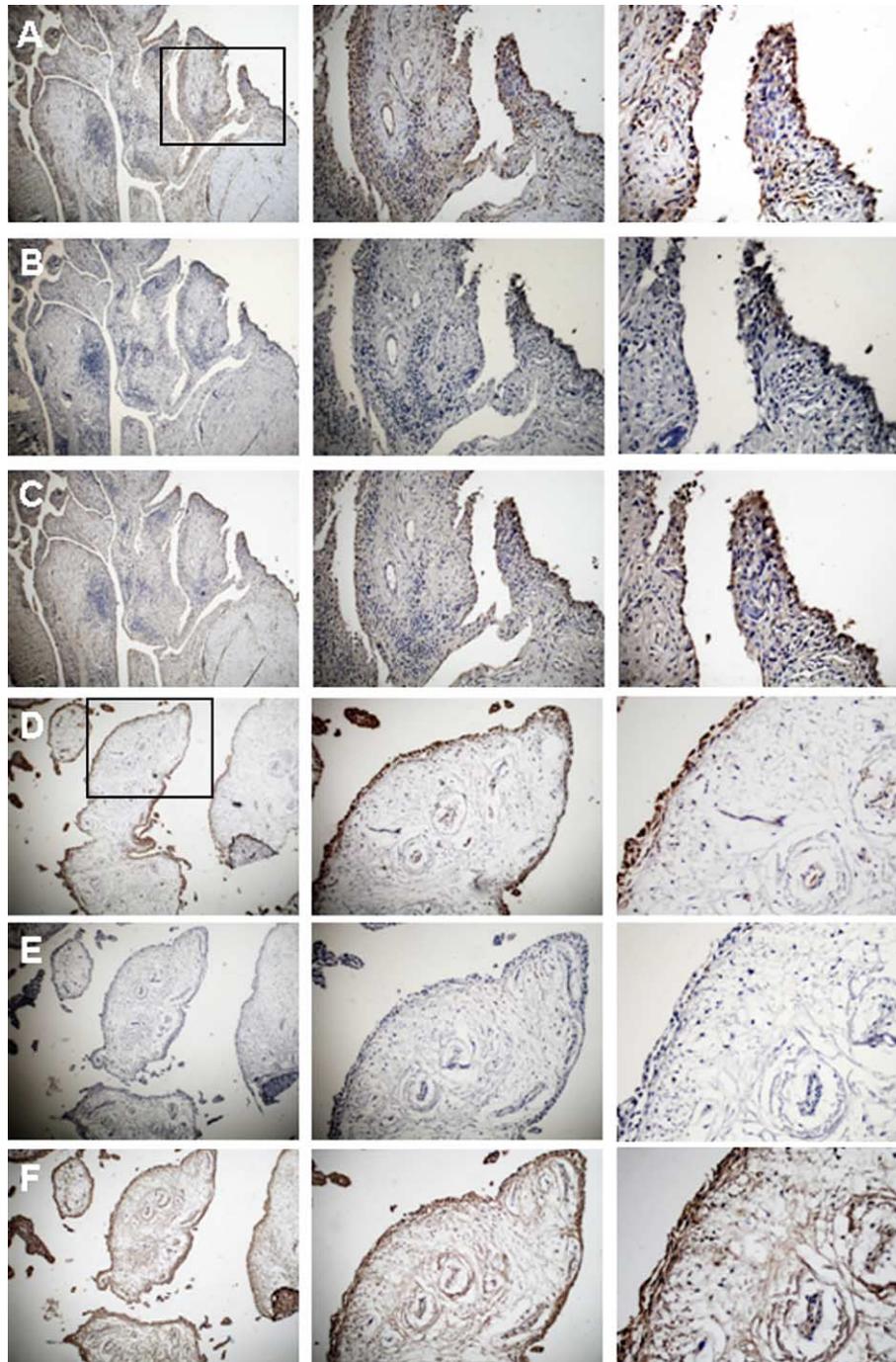


Fig. 7. CypA staining pattern correlates with that of MMP-9 in synovial tissues. Serial sections of RA (A–C) or OA (D–F) synovial tissues were stained for CypA (A and D), MMP-3 (B and E), and MMP-9 (C and F). Magnifications are 40 \times for the left, 100 \times for the middle, and 200 \times for the right panel. Squares in the pictures indicate the region magnified in higher magnifications.

cytokines will enhance the activation of inflammatory cells via autocrine and paracrine manner.

Although we investigated the effect of CypA only in macrophages, CypA may affect other cell types as well. Pro-inflammatory activities of CypA on the endothelial cells have been studied in inflammatory vascular diseases including atherosclerosis. CypA expression has been observed in murine atherosclerotic plaques and cultured endothelial cells responded to CypA treatment via apoptosis

and production of inflammatory markers including adhesion molecules, E-selectin, and VCAM-1. CypA also induced conversion of pro-MMP-2 into active forms [14,19]. CypA in RA synovium may have similar effects on endothelial cells. Recent analysis using cultured human aortic smooth muscle cells demonstrated the mitogenic effect of CypA [33]. Currently, the role of CypA in the functioning of lymphocytes with regard to inflammation is not known, and is the subject of further study.

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CORRIGENDUM

Corrigendum to “Cyclophilin A may contribute to the inflammatory processes in rheumatoid arthritis through induction of matrix degrading enzymes and inflammatory cytokines from macrophages” [Clin. Immunol. 116 (2005) 217–224]

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The author regrets that Fig. 3B on page 221 contains incorrect labels. The corrected figure is shown here.

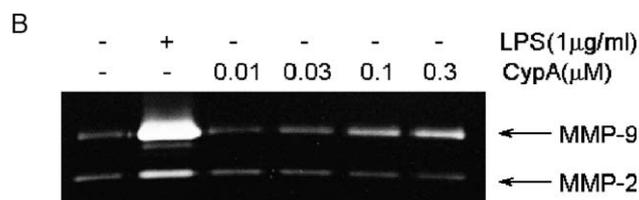


Figure 3.

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