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BRIEF REPORT

# Carboxypeptidase B Serves as a Protective Mediator in Osteoarthritis

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***Objective.*** **We previously demonstrated that carboxypeptidase B (CPB) protects against joint erosion in rheumatoid arthritis by inactivating complement component C5a. We also found that levels of CPB are abnormally high in the synovial fluid of individuals with another joint disease, osteoarthritis (OA). We undertook this study to investigate whether CPB plays a role in the pathogenesis of OA.**

***Methods.*** **We compared the development of OA in CPB-deficient (*Cpb2*$^{-/-}$) mice and wild-type mice by subjecting them to medial meniscectomy and histologically assessing cartilage damage, osteophyte formation, and synovitis in the stifle joints 4 months later. We measured levels of proCPB, proinflammatory cytokines, and complement components in synovial fluid samples from patients with symptomatic and radiographic knee OA. Finally, we used enzyme-linked immunosorbent assay, flow cytometry, and hemolytic assays to assess the effect of CPB on formation of membrane attack complex (MAC)–a complement effector critical to OA pathogenesis.**

***Results.*** ***Cpb2*$^{-/-}$ mice developed dramatically greater cartilage damage than did wild-type mice ($P < 0.01$) and had a greater number of osteophytes ($P < 0.05$) and a greater degree of synovitis ($P < 0.05$). In synovial fluid samples from OA patients, high levels of proCPB were associated with high levels of proinflammatory cytokines and complement components, and levels of proCPB correlated positively with those of MAC. In in vitro complement activation assays, activated CPB suppressed the formation of MAC as well as MAC-induced hemolysis.**

***Conclusion.*** **Our data suggest that CPB protects against inflammatory destruction of the joints in OA, at least in part by inhibiting complement activation.**

Carboxypeptidase B (CPB; also known as thrombin-activatable fibrinolysis inhibitor) is a basic carboxypeptidase that cleaves C-terminal basic residues (arginine or lysine) from protein and peptide substrates. It is encoded by the *CPB2* gene and produced primarily by the liver as a circulating plasma zymogen (proCPB) and becomes activated by the thrombin–thrombomodulin complex during thrombotic events. CPB was initially described as a fibrinolysis inhibitor because it can remove C-terminal lysines from partially digested fibrin and thereby reduce the binding of plasminogen and tissue plasminogen activator to the fibrin clot. CPB has since been shown to also cleave and inactivate several inflammatory proteins, namely, C5a, C3a, bradykinin, and thrombin-cleaved osteopontin (1). Its ability to modulate inflammatory substrates suggests that CPB may also function to suppress inflammation. Consistent with this notion, we previously found that CPB protects against rheumatoid arthritis by dampening C5a-mediated inflammation in synovial joints (2).

Although historically viewed as a noninflammatory degenerative disease, osteoarthritis (OA) is also accompanied by low-grade inflammation in the joints

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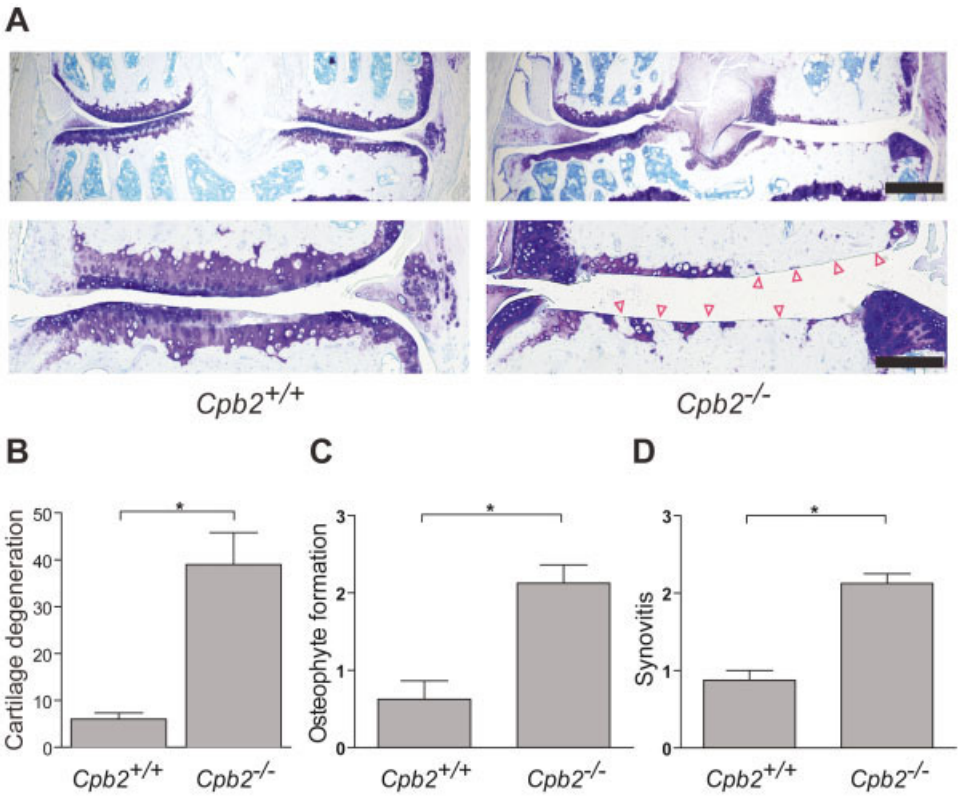


**Figure 1.** Pro–carboxypeptidase B deficiency exacerbates osteoarthritis (OA) in mice. **A,** Representative toluidine blue–stained sections of the stifle joints of *Cpb2*$^{-/-}$ mice (backcrossed >9 generations to the C57BL/6J background) (n = 4) and wild-type C57BL/6J control (*Cpb2*$^{+/+}$) mice (n = 5) that underwent medial meniscectomy to induce experimental OA. **Arrowheads** indicate areas of total cartilage loss. Top, Bar = 500 $\mu$m. Bottom, Bar = 200 $\mu$m. **B–D,** Histologic quantification of cartilage degeneration (**B**), osteophyte formation (**C**), and synovitis (**D**) in **A**. Values are the mean ± SEM. $* = P \leq 0.05$ by Mann-Whitney U test.

(3). Indeed, we recently discovered that the inflammatory complement system is critical to the pathogenesis of OA, with deficiency in the central complement component C5 or in a component of the downstream membrane attack complex (MAC; C5b–9) effector attenuating arthritis in mouse models of OA (4). In the present study, we investigated the role of CPB in OA by using mouse models of OA, in vitro analyses of human OA synovial fluid, and in vitro complement activation assays.

## MATERIALS AND METHODS

**Surgical induction of OA in mice.** We performed mouse studies under protocols approved by the Stanford Committee of Animal Research and in accordance with National Institutes of Health guidelines. Medial meniscectomy was performed as described (5) on 16-week-old, male CPB-deficient (*Cpb2*$^{-/-}$) mice or age-matched C57BL/6J control mice.

**Histologic assessment of OA development in mice.** Stifle joints from *Cpb2*$^{-/-}$ and control mice were stained with toluidine blue. Cartilage degeneration, osteophyte formation, and synovitis were evaluated as described previously (4).

**Measurement of proCPB, complement, and cytokines in OA synovial fluid.** Synovial fluid samples were obtained from knee OA patients who were older than 45 years and had radiographic Kellgren/Lawrence grade ≥3 changes (6) under protocols approved by the Stanford Institutional Review Board and with informed consent. ProCPB levels were measured using a Zymutest TAFI-Ag enzyme-linked immunosorbent assay (ELISA) kit (Aniara). For measurement of soluble C5b–9 (sMAC) and C3a, we used BD Biosciences OptEIA Human C5b–9 and Quidel Microvue C3a Plus EIA kits. Analysis of cytokines was performed as described elsewhere (7).

**Quantitation of sMAC by ELISA.** Ten percent normal human serum (NHS) was incubated with 20 n*M* of activated CPB (American Diagnostica) in Hanks' balanced salt solution with or without 25 $\mu M$ of potato carboxypeptidase inhibitor (Sigma), and sMAC was measured by ELISA.

**Immunofluorescence visualization of sMAC adsorption.** C5b–9 complexes were allowed to adsorb to glass coverslips following incubation of the glass with either 10% untreated NHS or NHS that had been pretreated with 35 n*M* of

activated CPB. Coverslips were fixed, washed, and then stained with anti-human C5b–9 (Dako) or isotype control, followed by staining with Alexa Fluor 488–conjugated anti-mouse IgG (Invitrogen). Thirty-six high-power fields per sample were imaged, and C5b–9 complexes with an area >0.1 $\mu m^2$ were counted using ImageJ software (National Institutes of Health; online at http://rsbweb.nih.gov/ij/).

**Flow cytometric analysis of C5b–9 deposition.** Huh7 hepatocarcinoma cells were incubated in either 25% NHS or NHS pretreated with 35 n*M* of activated CPB, and cells were stained with anti-human C5b–9 followed by Alexa Fluor 488–conjugated anti-mouse IgG.

**Hemolysis assay.** Unsensitized rabbit erythrocytes were resuspended in gelatin veronal buffer and incubated at 37°C for 60 minutes in 10% serum from 1-year-old *Cpb2*$^{-/-}$ or age-matched mice (n = 5 per group) with or without bovine cartilage extract (Sigma) in gelatin veronal buffer/Mg-EGTA buffer (CompTech). Positive (100% lysis; water) and negative (0% lysis; gelatin veronal buffer/Mg-EGTA buffer) controls were included. Absorbance of the supernatants was measured at 412 nm, and hemolysis was calculated relative to the positive control.

## RESULTS

**CPB protects mice against surgically induced OA.** We used the medial meniscectomy mouse model of OA (5) to investigate the role of CPB in the pathogenesis of OA. We performed medial meniscectomy on CPB-deficient (*Cpb2*$^{-/-}$) mice and their wild-type counterparts, and 16 weeks later we evaluated OA-like pathology in sections of their stifle joints. Compared to the operated joints of age-matched control mice, those of *Cpb2*$^{-/-}$ mice had a much greater degree of cartilage loss ($P = 0.0189$), osteophyte formation ($P = 0.0294$), and synovitis ($P = 0.0265$) (Figure 1). These findings suggest that CPB protects against the development and progression of OA in mice.

**Levels of proCPB correlate with levels of proinflammatory mediators in human OA synovial fluid.** Levels of proinflammatory mediators are abnormally high in the synovial fluid of individuals with OA (2). Because CPB negatively regulates local inflammatory responses by enzymatically cleaving and inactivating its proinflammatory substrates (1), we investigated the association between levels of proCPB and levels of proinflammatory mediators in OA synovial fluid. To this end, we performed ELISA and multiplexed bead-based immunoassays to quantitate proCPB, proinflammatory cytokines, chemokines, and complement components in OA synovial fluid. Using the mean level of proCPB across all OA synovial fluid samples, we stratified the samples into low-proCPB and high-proCPB groups. A heatmap display of the levels of proinflammatory mediators identified by Significance Analysis of Microarrays as differentially expressed between the high-proCPB and low-proCPB groups shows that levels of several proinflammatory mediators, including interleukin-1$\beta$ (IL-1$\beta$), interferon-$\gamma$, IL-8, and macrophage inflammatory protein 1$\beta$, were higher in the high-proCPB group (Figure 2A). Levels of IL-10 and IL-13, cytokines with antiinflammatory properties, were also higher in the

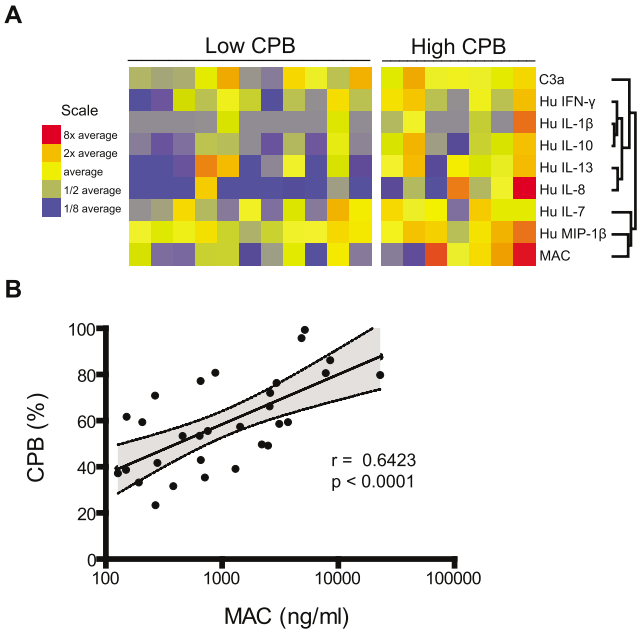


**Figure 2.** Levels of pro–carboxypeptidase B (proCPB) correlate positively with levels of inflammatory mediators in osteoarthritis (OA) synovial fluid. **A,** Heatmap display of inflammatory mediators whose levels differ significantly between OA synovial fluid samples from individuals with high levels of proCPB and those from individuals with low levels of proCPB (false discovery rate <4.1%; Significance Analysis of Microarrays was used for identifying statistically significant differences). Groups with high and low levels of proCPB were formed based on the mean proCPB level of all OA synovial fluid samples analyzed (64.8%). Blue represents a decrease relative to the mean value obtained in samples from OA patients, yellow represents no change, and red represents an increase. Cytokine and chemokine levels were measured with a multiplex bead-based immunoassay. Levels of proCPB, C3a, and membrane attack complex (MAC; C5b–9) were measured by enzyme-linked immunosorbent assay. Columns represent individual OA patients; rows represent individual inflammatory mediators. **B,** Pearson's correlation analysis of paired proCPB and MAC measurements in synovial fluid from each individual OA patient, with proCPB concentrations expressed as a percentage of that observed in pooled plasma from normal subjects. The solid line represents the best-fit line. The gray region demarcated by curved, dashed lines represents the 95% confidence interval for the best-fit line. Hu = human; IFN$\gamma$ = interferon-$\gamma$; IL-1$\beta$ = interleukin-1$\beta$; MIP-1$\beta$ = macrophage inflammatory protein 1$\beta$.

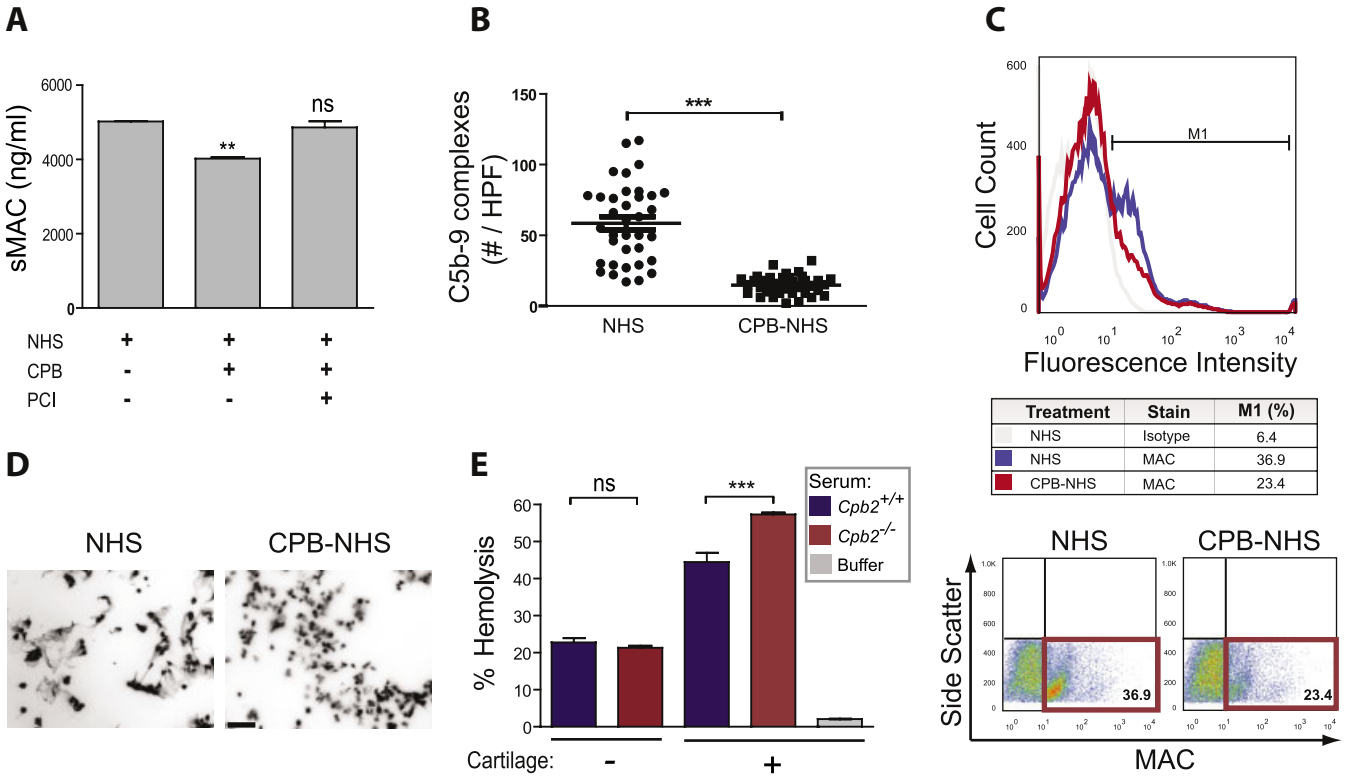


**Figure 3.** Carboxypeptidase B (CPB) inhibits complement activation in serum. **A,** Enzyme-linked immunosorbent assay analysis of soluble membrane attack complex (sMAC) formation in untreated normal human serum (NHS), in NHS treated with CPB, or in NHS treated with CPB that had been inactivated with potato carboxypeptidase inhibitor (PCI). Values are the mean ± SD of triplicates. ** = $P < 0.01$ versus untreated NHS, by one-way analysis of variance (ANOVA) with Bonferroni post hoc correction. NS = not significant. **B,** Immunostaining analysis of C5b–9 complexes (MAC) adsorbed to hydrophilic glass following incubation of glass coverslips with either untreated NHS or CPB-treated NHS. Shown is the number of complexes in each of 36 nonoverlapping high-power fields (hpf). Symbols represent individual hpf. Bars show the mean ± SD. *** = $P \leq 0.001$ by Mann-Whitney U Test. **C,** Top, Flow cytometric analysis of MAC deposition on the surface of Huh7 cells incubated with 25% untreated NHS or CPB-treated NHS. Cells were stained with anti–C5b–9 or mouse IgG2a isotype control followed by Alexa Fluor 488–conjugated goat anti-mouse IgG. Bottom, Representative dot plots and percentages of MAC+ cells. **D,** DAPI staining of nuclei in Huh7 cells incubated with untreated NHS or CPB-treated NHS. Cells incubated with CPB-treated NHS contain intact nuclei, while cells incubated with untreated NHS contain elongated nuclei with dispersed chromatin fibers. Bar = 100 $\mu$m. **E,** Hemolysis of rabbit red blood cells by 10% (volume/volume) sera from *Cpb2*$^{-/-}$ mice or C57BL/6J wild-type mice in the presence or absence of cartilage extract. Values are the mean ± SD of triplicates and are representative of ≥3 independent experiments. *** = $P \leq 0.001$ by one-way ANOVA with Bonferroni post hoc correction.

high-proCPB group (Figure 2A). In the setting of chronic inflammation, such as in the OA joint, CPB may act in concert with these regulatory cytokines to prevent the destructive effects incurred by sustained proinflammatory activity.

Two effector components of the complement cascade, namely, anaphylatoxin C3a and MAC (C5b–9), were significantly up-regulated in OA synovial fluid samples with high levels of proCPB. Indeed, by analyzing paired values of proCPB and MAC for each synovial fluid sample, we found that levels of proCPB correlated positively with levels of MAC (Pearson r = 0.64, $P < 0.0001$) (Figure 2B). This strong correlation suggests that CPB may control inflammation by regulating complement activation.

**CPB inhibits MAC formation and MAC-mediated cell lysis.** We previously reported a critical role of complement, in particular the MAC-mediated effector arm of the complement cascade, in the pathogenesis of OA (4). Mice genetically deficient in complement effectors C5 or C6 (2 components of the MAC) are protected against surgically induced OA, whereas mice deficient in the MAC inhibitor CD59 have greater cartilage loss and accelerated OA development. Given the importance of MAC in OA pathogenesis (4) and the strong correlation between levels of proCPB and MAC

in OA synovial fluid (Figure 2B), we investigated whether inhibition of MAC assembly might be one mechanism by which CPB exerts its protective effects in OA. To evaluate the role of CPB in complement activation, we preincubated NHS with activated CPB and assessed its ability to activate complement relative to that of NHS that had not been preincubated with CPB (Figure 3A). CPB treatment suppressed sMAC formation in NHS, an effect that was abolished by inactivation of CPB with potato carboxypeptidase inhibitor (Figure 3A). We validated the in vitro formation of sMAC by immunostaining for C5b–9 complexes bound to glass coverslips that were allowed to react with either CPB-treated NHS or untreated NHS. As predicted, markedly fewer C5b–9 complexes formed on glass coverslips incubated with CPB-treated NHS than on those incubated with untreated NHS (Figure 3B).

Upon activation of the complement cascade, MAC readily forms channels in the membranes of target cells, resulting in either cell signaling or cell lysis, depending on the concentration of MAC (8). We therefore evaluated the ability of CPB to regulate MAC assembly at the surface of Huh7 hepatocarcinoma cells. Consistent with our previous findings with sMAC (Figures 3A and B), MAC deposition was markedly lower on Huh7 cells incubated with CPB-treated NHS than on Huh7 cells incubated with untreated NHS (Figure 3C). Moreover, Huh7 cells incubated with CPB-treated NHS contained intact nuclei, in stark contrast to the disrupted appearance of nuclei from cells incubated with untreated NHS (Figure 3D), which suggests that CPB inhibits MAC-mediated cell lysis.

To further confirm our findings, we compared the ability of serum from *Cpb2*$^{-/-}$ mice and serum from wild-type C57BL/6J mice to activate complement in a modified alternative pathway (AP) hemolytic assay (AH50). In this assay, complement activity was determined by measuring the hemolysis of rabbit red blood cells (RBCs) after addition of CPB-deficient or wild-type mouse sera. There was no significant difference in hemolytic activity when RBCs reacted with CPB-deficient or wild-type mouse sera alone (Figure 3E). However, when we added cartilage extracellular matrix (ECM) components to the serum to increase complement activation above that attained spontaneously, sera from CPB-deficient mice lysed RBCs more efficiently than did sera from wild-type mice. A hallmark of cartilage degeneration in OA is the breakdown and progressive loss of ECM components into the synovial space, and some of these ECM components can induce formation of MAC from serum complement components (4). These data suggest that circulating CPB exerts its antiinflammatory or cytotoxic effects at least in part by suppressing or limiting complement activation triggered by cartilage ECM components.

## DISCUSSION

In the present study, we show that CPB is important in protecting against the development of OA and that it may do so, at least in part, by inhibiting complement activation in the synovial joints. We found that 1) genetic deficiency of CPB exacerbated OA in a mouse model, 2) high levels of CPB were associated with high levels of proinflammatory cytokines in the synovial fluid of individuals with OA, and levels of CPB correlated positively with levels of MAC in those samples, and 3) CPB inhibited the formation and activity of MAC in vitro.

While traditionally considered a plasma protein, proCPB is also produced and secreted by fibroblast-like synoviocytes (FLS) (9). Moreover, thrombomodulin, a critical cofactor for thrombin-mediated activation of proCPB, is expressed on FLS (9). These findings suggest that locally produced and activated CPB may act in concert with CPB from plasma exudates into the joint space to control inflammation in OA.

Several of the proinflammatory mediators that were associated with high levels of CPB are known to be secreted by multiple cell types in response to signaling by CPB substrates (e.g., C5a signaling through C5a receptor induces the production of IL-1$\beta$ and IL-8). Because CPB cleavage of its substrates reduces their proinflammatory activity, the observed association suggests that levels of CPB may increase in OA synovial fluid as part of a homeostatic counterregulatory mechanism to suppress inflammation in OA joints.

Our finding that levels of proCPB correlated positively with levels of MAC is particularly interesting in light of our previous finding that MAC plays a critical role in OA pathogenesis (4). We now show that treating human serum with activated CPB reduces the formation of soluble and cell surface MAC, resulting in a reduction in RBC death. We propose that CPB-mediated inhibition of MAC in synovial joints likewise attenuates MAC-induced killing of synovial lining and cartilage cells. In addition, sublytic levels of the MAC can stimulate production of cytokines and other proinflammatory mediators (8)–including many of those associated with high levels of proCPB in OA synovial fluid–and thus suppression of MAC-induced inflammation could be another way in which CPB protects against joint destruction in OA.

CPB could conceivably inhibit MAC formation in

several different ways. It could do so by suppressing activation of the alternative complement pathway, which is activated spontaneously when the highly reactive thioester bond of complement component C3b reacts with a hydroxyl or amine group on a protein's surface. By cleaving C-terminal lysines (1), CPB removes reactive primary amines from proteins and could in this way eliminate potential C3b-binding sites and thereby suppress activation of the alternative pathway. One candidate for such a target protein is fibrin, which contains many C-terminal lysines, is a known CPB substrate (1), and can activate complement (10). Indeed, fibrin deposition is a prominent finding in OA joint tissues (11), and complement, including MAC, colocalizes with fibrin deposits at sites of tissue injury (12). Another way in which CPB-mediated removal of C-terminal lysines could suppress activation of the alternative complement pathway is by generating a net negative charge at the protein surface; polyanions are known to strongly inhibit the alternative complement pathway (13). Additionally, C-terminal lysines of fibrin act as docking sites for plasminogen, a fibrinolytic protein recently identified as an inhibitor of both the alternative and the classical complement pathways (14). Cleavage of fibrin by CPB would preclude the binding of plasminogen to fibrin, leaving plasminogen free to interact with and inhibit complement components.

The mouse medial meniscectomy model is relevant to human OA because humans who have undergone medial meniscectomy have a significantly increased risk of developing OA (15). Further, arthroscopic partial meniscectomy in humans is associated with chemokine-driven inflammation (16).

Our findings suggest that CPB serves as an important antiinflammatory mediator that protects against inflammatory joint destruction following injury or iatrogenic removal of the meniscus. Thus, we propose that CPB down-regulates inflammation and cytotoxicity in synovial joints and that it does so in part by suppressing the formation of MAC.

## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Robinson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Lepus, Song, Wang, Robinson.

**Acquisition of data.** Lepus, Song, Wang, Wagner, Robinson.

**Analysis and interpretation of data.** Lepus, Song, Wang, Wagner, Lindstrom, Chu, Sokolove, Leung, Robinson.

## REFERENCES


1. Leung LL, Myles T, Nishimura T, Song JJ, Robinson WH. Regulation of tissue inflammation by thrombin-activatable carboxypeptidase B (or TAFI). Mol Immunol 2008;45:4080–3.
2. Song JJ, Hwang I, Cho KH, Garcia MA, Kim AJ, Wang TH, et al. Plasma carboxypeptidase B downregulates inflammatory responses in autoimmune arthritis. J Clin Invest 2011;121:3517–27.
3. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007; 213:626–34.
4. Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, et al. Identification of a central role for complement in osteoarthritis. Nat Med 2011;17:1674–9.
5. Kadri A, Funck-Brentano T, Lin H, Ea HK, Hannouche D, Marty C, et al. Inhibition of bone resorption blunts osteoarthritis in mice with high bone remodelling. Ann Rheum Dis 2010;69:1533–8.
6. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502.
7. Sohn DH, Sokolove J, Sharpe O, Erhart JC, Chandra PE, Lahey LJ, et al. Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. Arthritis Res Ther 2012;14:R7.
8. Bohana-Kashtan O, Ziporen L, Donin N, Kraus S, Fishelson Z. Cell signals transduced by complement. Mol Immunol 2004;41: 583–97.
9. Sharif SA, Du X, Myles T, Song JJ, Price E, Lee DM, et al. Thrombin-activatable carboxypeptidase B cleavage of osteopontin regulates neutrophil survival and synoviocyte binding in rheumatoid arthritis. Arthritis Rheum 2009;60:2902–12.
10. Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement—their role in inflammation. Semin Immunopathol 2012;34:151–65.
11. Weinberg JB, Pippen AM, Greenberg CS. Extravascular fibrin formation and dissolution in synovial tissue of patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 1991;34: 996–1005.
12. Rampersad R, Barton A, Sadovsky Y, Nelson DM. The C5b-9 membrane attack complex of complement activation localizes to villous trophoblast injury in vivo and modulates human trophoblast function in vitro. Placenta 2008;29:855–61.
13. Weiler JM, Linhardt RJ. Comparison of the activity of polyanions and polycations on the classical and alternative pathways of complement. Immunopharmacology 1989;17:65–72.
14. Barthel D, Schindler S, Zipfel PF. Plasminogen is a complement inhibitor. J Biol Chem 2012;287:18831–42.
15. Roos H, Lauren M, Adalberth T, Roos EM, Jonsson K, Lohmander LS. Knee osteoarthritis after meniscectomy: prevalence of radiographic changes after twenty-one years, compared with matched controls. Arthritis Rheum 1998;41:687–93.
16. Scanzello CR, McKeon B, Swaim BH, DiCarlo E, Asomugha EU, Kanda V, et al. Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. Arthritis Rheum 2011;63:391–400.