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Identification of a central role for complement in osteoarthritis

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Osteoarthritis, characterized by the breakdown of articular cartilage in synovial joints, has long been viewed as the result of 'wear and tear'[1]. Although low-grade inflammation is detected in osteoarthritis, its role is unclear[2–4]. Here we identify a central role for the inflammatory complement system in the pathogenesis of osteoarthritis. Through proteomic and transcriptomic analyses of synovial fluids and membranes from individuals with osteoarthritis, we find that expression and activation of complement is abnormally high in human osteoarthritic joints. Using mice genetically deficient in complement component 5 (C5), C6 or the complement regulatory protein CD59a, we show that complement, specifically, the membrane attack complex (MAC)-mediated arm of complement, is crucial to the development of arthritis in three different mouse models of osteoarthritis. Pharmacological modulation of complement in wild-type mice confirmed the results obtained with genetically deficient mice. Expression of inflammatory and degradative molecules was lower in chondrocytes from destabilized joints from C5-deficient mice than C5-sufficient mice, and MAC induced production of these molecules in cultured chondrocytes. Further, MAC colocalized with matrix metalloprotease 13 (MMP13) and with activated extracellular signal-regulated kinase (ERK) around chondrocytes in human osteoarthritic cartilage. Our findings indicate that dysregulation of complement in synovial joints has a key role in the pathogenesis of osteoarthritis.

The pathogenesis of osteoarthritis is unclear, and there are currently no treatments that prevent the development of osteoarthritis. Seeking to gain insight into osteoarthritis, we used mass spectrometry to identify proteins aberrantly expressed in the synovial fluid—the fluid that bathes the synovial joints—of individuals with osteoarthritis. We found that proteins of the complement system are differentially expressed in osteoarthritic compared to healthy synovial fluids (**Supplementary Table 1**). Using less sensitive proteomic techniques, we previously detected 10 of these 12 differentially expressed complement proteins in osteoarthritic synovial fluids[5]. The complement system consists of three distinct pathways that converge at the formation of the C3 and C5 convertases, which are enzymes that mediate activation of the C5a anaphylatoxin and formation of MAC (comprising the complement effector C5b-9) (**Fig. 1a**)[6]. Components of the classical (C1s and C4A) and alternative (factor B) pathways, the central components C3 and C5, and the C5, C7 and C9 components of MAC were all aberrantly expressed in synovial fluids from individuals with osteoarthritis (**Fig. 1a** and **Supplementary Table 1**).

Validating our proteomic results, an ELISA analysis showed that the concentrations of C3a (**Fig. 1b**) and C5b-9 (**Fig. 1c**) were significantly higher in synovial fluids from individuals with early-stage osteoarthritis than in synovial fluids from healthy individuals. Thus, complement activation occurs in synovial joints early in the course of osteoarthritis and persists, albeit at a lower level, during the late phases of osteoarthritis (**Fig. 1c**). Additionally, an immunohistochemical analysis revealed the presence of MAC in the synovium (data not shown) and around the chondrocytes in cartilage (**Fig. 1d**) from individuals with end-stage osteoarthritis, consistent with previous findings[7–9].

To determine whether the synovium is a source of complement components, we analyzed the expression of genes encoding complement-related proteins (those identified in synovial fluid;

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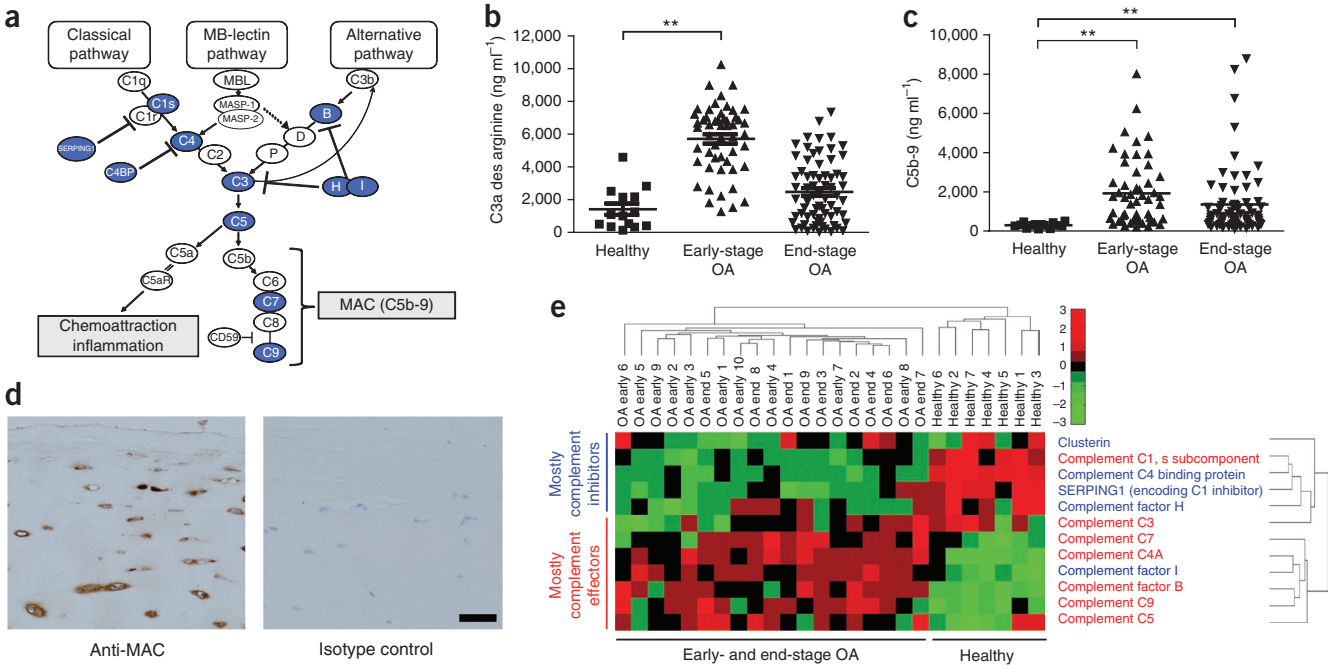

Figure 1 Complement components are aberrantly expressed and activated in human osteoarthritic joints. (**a**) Schematic of the complement cascade; blue-filled circles denote the complement effectors and inhibitors identified as aberrantly expressed in osteoarthritic synovial fluid. MBL, mannose-binding lectin; MASP, mannose-binding lectin-associated serine protease; SERPING1, serpin peptidase inhibitor, clade G (C1 inhibitor), member 1; C4BP, C4-binding protein; B, D, P, H, I: complement factors B, D, P, H and I; C5aR, C5a receptor; MAC, membrane attack complex. (**b**,**c**) ELISA quantification of C3a des arginine (**b**) and the soluble form of MAC (complement effector C5b-9) (**c**) in synovial fluids from healthy individuals ($n = 14$) and from individuals with early-stage osteoarthritis ($n = 52$) or end-stage osteoarthritis ($n = 69$). C3a des arginine is a carboxypeptidase-cleaved, stable form of C3a that is generated from C3 during activation of the complement cascade. $^{**}P \leq 0.01$ by one-way analysis of variance (ANOVA) and Dunnett's *post hoc* test. (**d**) Immunohistochemical staining of MAC in cartilage from individuals with end-stage osteoarthritis. We used isotype-matched antibodies as negative controls. Staining is representative of that seen in samples from four different individuals with osteoarthritis. Scale bar, 100 μm. (**e**) Cluster analysis of gene-expression profiles in microarray data sets from synovial membranes from healthy individuals (downloaded from the NCBI Gene Expression Omnibus) and from individuals with early- or end-stage osteoarthritis (experimentally determined). Our analysis was limited to the set of genes encoding the complement-related proteins differentially expressed in osteoarthritic synovial fluid compared to healthy synovial fluid (**Supplementary Table 1**). The scale bar represents the fold change in gene expression compared to the reference control. Complement effectors are shown in red text, and complement inhibitors are shown in blue text. MB, mannose binding; OA, osteoarthritis.

Supplementary Table 1) in synovial membranes from individuals with osteoarthritis and from healthy individuals. Analysis by unsupervised hierarchical clustering revealed two major clusters: one cluster containing all the expression profiles from individuals with osteoarthritis (both early and end stage) and one cluster containing all the profiles from healthy individuals (**Fig. 1e** and **Supplementary Fig. 1**). Notably, expression of transcripts encoding the complement effectors C7, C4A, factor B, C9 and C5 was markedly higher, and expression of transcripts encoding the complement inhibitors clusterin, factor H, C4-binding protein and C1 inhibitor was markedly lower, in osteoarthritic compared to healthy synovial membranes (**Fig. 1e** and **Supplementary Fig. 1**). Our results suggest that the synovial membrane may take on a pathogenic role in osteoarthritis by contributing to excessive complement activation.

To investigate the role of complement in the pathogenesis of osteoarthritis, we used a mouse model of osteoarthritis induced by medial meniscectomy[10]. In humans, tearing of the meniscus often requires meniscectomy, which is a risk factor for knee osteoarthritis[11]. Because the C5 effector is located at the nexus of the complement cascade (**Fig. 1a**), we surgically induced osteoarthritis in C5-deficient ($C5^-$, lacking the ability to secrete the C5 proprotein from cells)[12] and C5-sufficient ($C5^+$) wild-type mice. Sixteen weeks after surgery, $C5^-$ mice showed substantially less cartilage loss, osteophyte formation and synovitis than $C5^+$ mice (**Fig. 2a**,**b** and **Supplementary Fig. 2d**). By contrast, osteoarthritis in this model was not affected by genetic deficiency in *C3* (data not shown). The fact that $C3^{-/-}$ mice were not protected against osteoarthritis can be explained by the observation that compensatory mechanisms operate in $C3^{-/-}$ mice: coagulation factors compensate for the lack of C3, allowing C5 activation to proceed even in the absence of C3 (ref. 13). Corroborating our findings in the $C5^-$ congenic mouse strain, treatment with a neutralizing monoclonal antibody to C5 (ref. 14) attenuated osteoarthritis in wild-type mice (**Fig. 2c**). We also tested the effect of CR2-fH, a fusion protein that inhibits activation of C3 and C5 (ref. 15), on osteoarthritis. Administration of CR2-fH attenuated the development of osteoarthritis in wild-type mice (**Fig. 2d**).

We next determined whether the MAC-mediated effector arm of the complement cascade is important in osteoarthritis. We found that mice deficient in C6, an integral component of the MAC (**Fig. 1a**), were protected against the development of both osteoarthritis and synovitis induced by medial meniscectomy (**Fig. 2e**,**f** and **Supplementary Fig. 2d**). Conversely, mice deficient in CD59a, an inhibitor of MAC[6] (**Fig. 1a**), developed more severe osteoarthritis and synovitis than their wild-type littermates (**Fig. 2g**,**h** and **Supplementary Fig. 2a**,**d**).

Not only meniscectomy but also meniscal tearing can lead to the development of osteoarthritis in humans[11]. We therefore also examined

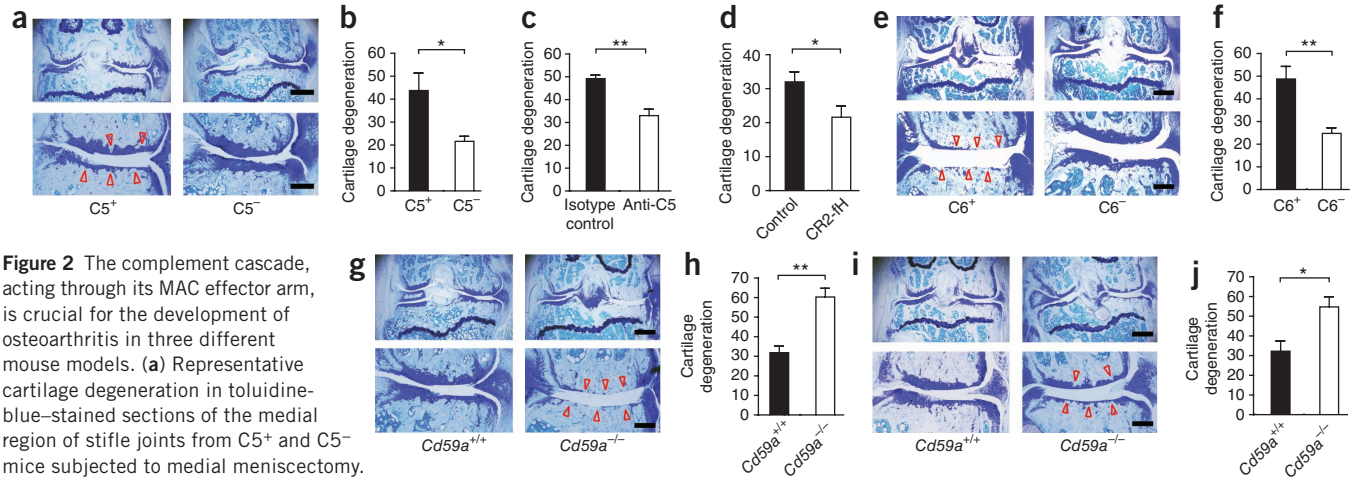

Figure 2 The complement cascade, acting through its MAC effector arm, is crucial for the development of osteoarthritis in three different mouse models. (**a**) Representative cartilage degeneration in toluidine-blue–stained sections of the medial region of stifle joints from C5+ and C5− mice subjected to medial meniscectomy. (**b**) Quantification of the cartilage degeneration shown in **a** ($n = 5$ mice per group). (**c**) Quantification of the cartilage degeneration in wild-type mice subjected to medial meniscectomy and then treated intraperitoneally with 750 μg of either the monoclonal antibody BB5.1 specific to C5 or an isotype-control antibody ($n = 5$ mice per group). (**d**) Quantification of the cartilage degeneration in wild-type mice subjected to medial meniscectomy and then treated intravenously with 250 μg of CR2-fH or PBS ($n = 5$ mice per group). (**e**) Representative cartilage degeneration in toluidine-blue–stained sections of the medial region of stifle joints from C6+ and C6− mice subjected to medial meniscectomy. (**f**) Quantification of the cartilage degeneration shown in **e** ($n = 13$ mice per group). (**g**) Representative cartilage degeneration in toluidine-blue–stained sections of the medial region of stifle joints from *Cd59a*$^{+/+}$ and *Cd59a*$^{-/-}$ mice subjected to medial meniscectomy. (**h**) Quantification of the cartilage degeneration shown in **g** ($n = 10$ mice per group). (**i**) Representative cartilage degeneration in toluidine-blue–stained sections of the medial region of stifle joints from *Cd59a*$^{+/+}$ and *Cd59a*$^{-/-}$ mice subjected to DMM. (**j**) Quantification of the cartilage degeneration shown in **i** ($n = 5$ mice per group). Arrowheads indicate areas of cartilage degeneration. Scale bars in the low-magnification (top) images, 500 μm; scale bars in the high-magnification (bottom) images, 200 μm. Error bars, mean ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$ by *t* test.

the role of complement in the destabilization of the medial meniscus (DMM) model of osteoarthritis[16–18]. We found that deficiency in CD59a accentuated the osteoarthritic phenotype in mice subjected to DMM (**Fig. 2i,j** and **Supplementary Fig. 2b–d**). Deficiency in CD59a also accentuated the milder osteoarthritis that developed spontaneously in aged mice (**Supplementary Fig. 3**). These findings suggest that MAC-mediated complement activity has a pathogenic role in osteoarthritis of disparate etiologies.

Resistance to the development of histological osteoarthritis in complement-deficient mice translated to functional benefit.

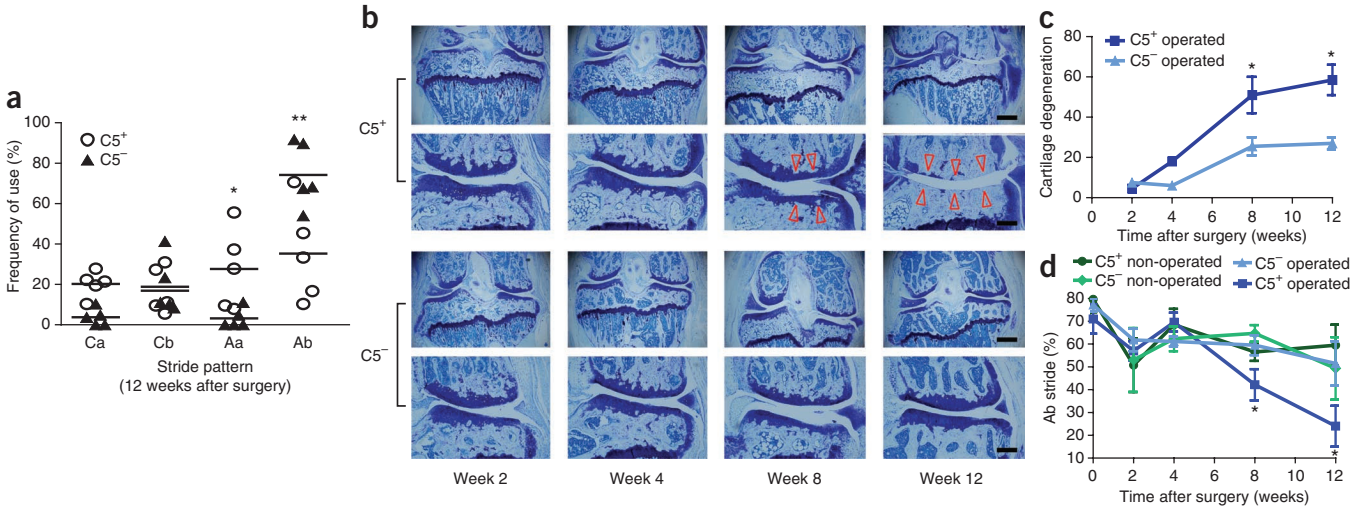

Figure 3 C5 deficiency protects against the progressive development of osteoarthritic joint pathology and gait dysfunction. (**a**) Gait analysis of C5+ and C5− mice 12 weeks after medial meniscectomy ($n = 5$ mice per group). Ab stride pattern means the sequence of paw strides was left front, right hind, right front, left hind; Aa stride pattern: right front, right hind, left front, left hind; Ca stride pattern: right front, left front, right hind, left hind; and Cb stride pattern: left front, right front, left hind, right hind. $^*P < 0.05$, $^{**}P < 0.01$ by *t* test. Results are representative of two independent experiments. (**b**) A histological analysis of articular cartilage at serial time points after medial meniscectomy. Representative toluidine-blue–stained sections of the medial region of stifle joints are shown; arrowheads indicate areas of cartilage degeneration. Scale bars in the low-magnification (top) images, 500 μm; scale bars in the high-magnification (bottom) images, 200 μm. (**c**) Quantification of the cartilage degeneration in C5+ and C5− mice subjected to medial meniscectomy (C5+ and C5− operated). $^*P \leq 0.05$ by *t* test comparing C5+ and C5− operated mice. Data are the mean ± s.e.m. (**d**) Gait analysis of C5+ and C5− operated mice and of C5+ and C5− non-operated mice at serial time points after medial meniscectomy. $^*P \leq 0.05$ by *t* test comparing C5+ operated mice and C5− operated mice. At week 8, $n = 6$ for C5+ operated mice and $n = 6$ for C5− operated mice; at week 12, $n = 4$ for C5+ operated mice and $n = 4$ for C5− operated mice; and at all time points, $n \geq 4$ for C5+ non-operated mice and $n = 3$ for C5− non-operated mice.

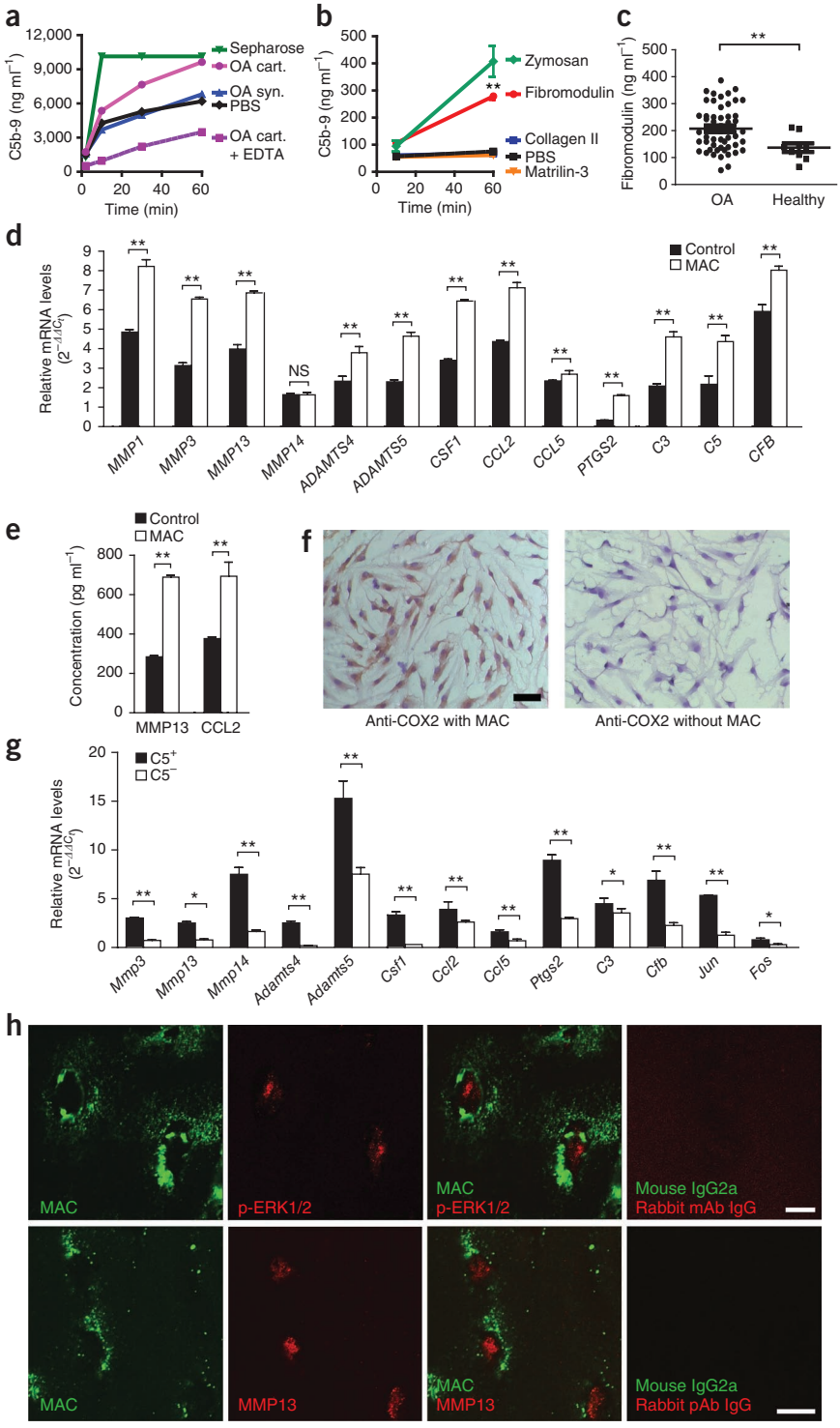

Figure 4 Cartilage ECM components can induce MAC formation, and MAC induces chondrocyte expression of inflammatory and catabolic molecules. (**a**,**b**) ELISA quantification of C5b-9 (soluble MAC) in 67% human serum incubated with 20 μg ml^{-1} of pulverized human osteoarthritic cartilage (OA cart.) or synovium (OA syn.) (**a**) or in 10% human serum incubated with 20 μg ml^{-1} of recombinant cartilage ECM components (**b**). Sepharose and zymosan were the positive controls; PBS and EDTA were the negative controls. $^{**}P \leq 0.01$ by one-way ANOVA with a Dunnett's *post hoc* test comparing each cartilage component with PBS. Data are the mean of triplicate values ± s.d. and are representative of three independent experiments. (**c**) ELISA quantification of fibromodulin in synovial fluids from individuals with osteoarthritis ($n = 50$) and healthy individuals ($n = 9$). $^{**}P \leq 0.01$ by *t* test. (**d**–**f**) Quantitative PCR analysis of relative mRNA expression (**d**), ELISA analysis of protein expression (**e**) and immunocytochemical analysis of COX2 expression (**f**) in human chondrocytes incubated with or without MAC for 72 h. Scale bar, 50 μm. $^{**}P \leq 0.01$ by *t* test. Data are the mean ± s.d. of triplicates and are representative of three independent experiments. (**g**) mRNA expression in chondrocytes from C5^{+} and C5^{-} mice ($n = 4$ mice per group) subjected to DMM. Data are the mean + s.d. of triplicates and are representative of results from four mice from two independent experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ by fixed-effect ANOVA taking into account both destabilized and non-destabilized joints. (**h**) Immunofluorescent analysis of phospho-ERK1 and phospho-ERK2 (p-ERK1/2), MMP13 and MAC colocalization in human osteoarthritic cartilage. Scale bars, 10 μm. NS, not significant; mAb, monoclonal antibody; pAb, polyclonal antibody.

Twelve weeks after medial meniscectomy, C5^{-} mice had a normal gait, whereas C5^{+} mice developed an abnormal gait (**Fig. 3**). Time-course studies revealed that neither C5^{+} nor C5^{-} mice had proteoglycan loss or cartilage degeneration at 2 and 4 weeks after medial meniscectomy (**Fig. 3b**,**c**). This period of latency is similar to that observed in the DMM model[16] and in humans who have undergone medial meniscectomy[11]. Eight and 12 weeks after surgery, however, C5^{+} mice had significant proteoglycan and cartilage loss and synovitis, whereas C5^{-} mice did not (**Fig. 3b**,**c** and **Supplementary Fig. 4**). The osteoarthritic phenotype was pronounced in these mice, probably because of their genetic background and age at the time of surgery, which are both factors that influence the severity of mouse osteoarthritis[16].

Products of dysregulated cartilage remodeling and repair may contribute to joint inflammation in osteoarthritis[19–22]. We examined the ability of osteoarthritic cartilage or specific components of the extracellular matrix (ECM) of cartilage to activate complement *in vitro*. Pulverized osteoarthritic cartilage induced the formation of C5b-9 (MAC), as did the ECM components fibromodulin and aggrecan, but the ECM components type II collagen and matrilin-3 did not (**Fig. 4** and **Supplementary Fig. 5**). Fibromodulin, which can bind C1q and activate the complement cascade[23], was present at higher concentrations in osteoarthritic compared to healthy synovial fluid (**Fig. 4c**). Other cartilage ECM components, such as cartilage oligomeric matrix protein, are also present at abnormally high levels in osteoarthritic synovial fluid and can activate complement[19,20]. Together, these results suggest that the release or exposure of

cartilage ECM components may contribute to the pathophysiology of osteoarthritis by activating complement.

Our *in situ* (**Fig. 1d**) and *in vivo* (**Fig. 2e–j** and **Supplementary Figs. 2**,**3**) findings indicate that MAC is important in mediating complement-related cartilage damage in osteoarthritis. But how does MAC damage cartilage? Extensive deposition of MAC induces cell lysis and necrotic cell death, whereas sublytic MAC can activate signaling pathways that drive the expression of proinflammatory and catabolic molecules[24]. Because many of the MAC-encircled chondrocytes in osteoarthritic cartilage seem to be morphologically intact (**Figs. 1d**, **4h** and **Supplementary Fig. 6**), we examined whether MAC induces the expression of proinflammatory and degradative enzymes in osteoarthritis. We first examined the expression of genes encoding these molecules in cultured chondrocytes coated with sublytic concentrations of MAC. Sublytic MAC increased the chondrocytes' expression of multiple genes implicated in osteoarthritis: genes encoding cartilage-degrading enzymes[17,18,22] (MMPs and disintegrin-like and metallopeptidase with thrombospondin type 1 motif (ADAMTSs)), inflammatory cytokines[25] (chemokine (C-C motif) ligand 2 (CCL2), colony-stimulating factor 1 (CSF1) and CCL5) and cyclo-oxygenase 2 (ref. 26) (**Fig. 4d–f**). Sublytic MAC also induced the expression of complement effectors (**Fig. 4d**); chondrocyte production of complement may thus synergize with complement derived from the synovial membrane to amplify pathogenic complement signaling in osteoarthritis.

We next examined the effect of complement deficiency on the *in vivo* expression of these genes in destabilized mouse joints. Twenty weeks after DMM surgery, chondrocytes from the destabilized joints of C5-deficient mice, which are deficient in MAC and are protected against osteoarthritis (**Figs. 2a**,**b** and **3**), expressed lower concentrations of these inflammatory and degradative mediators than did chondrocytes from the destabilized joints of C5-sufficient mice (**Fig. 4g**). mRNA levels of Jun and Fos, proinflammatory transcription factors whose expression is induced by MAC[27,28], were also lower (**Fig. 4g**). In addition, in human osteoarthritic cartilage, MAC colocalized with MMP13 and with activated ERK1 and ERK2 (**Fig. 4h** and **Supplementary Fig. 6**), kinases that mediate resistance to MAC-mediated cell lysis[29] and stimulate the expression of MMP13 by inducing the expression of Fos[30].

Here we have found that the complement cascade is crucial for the pathogenesis of osteoarthritis. Cartilage ECM components released by or exposed in osteoarthritic cartilage may trigger the complement cascade. Additionally, dysregulation of gene expression in joint tissues may contribute to a local preponderance of complement effectors over inhibitors in osteoarthritis, permitting complement activation to proceed unchecked. Complement activation in turn results in the formation of MAC on chondrocytes, which either kills the cells or causes them to produce matrix-degrading enzymes, inflammatory mediators and other complement effectors, all of which promote joint pathology.

Recent findings suggest that low-grade complement activation contributes to the development of degenerative diseases, such as age-related macular degeneration[31] and Alzheimer's disease[32]. We propose that osteoarthritis can be added to this list of diseases. Our findings provide a rationale for targeting the complement system as a disease-modifying therapy for osteoarthritis.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Accession codes. Experimentally determined microarray data are available in the Gene Expression Omnibus under the accession code GSE32317.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

A.L.R. and W.H.R. initiated the investigation of complement in osteoarthritis, and Q.W. conducted key studies. Q.W., A.L.R., D.M. Larsen, H.H.W. and W.H.R. conducted the studies of osteoarthritis in mouse models. A.E., A.L.R. and M.S. performed the gait analysis studies. Q.W. and H.H.W. performed the *in vitro* MAC deposition experiments. C.M.L. and J.J.S. performed the immunohistochemical analyses of cartilage. J.F.C., G.B., S.Y.R., L.P., S.R.G., R.G. and D.M. Lee conducted or contributed to the proteomic analysis of osteoarthritic synovial fluid. S.Y.R. and D.M. Lee performed the ELISA analysis of osteoarthritic and healthy synovial fluids. C.R.S. and M.K.C. performed the gene expression analysis of the synovium, and G.B., R.G. and D.M. Lee contributed to the analysis of these data sets. A.L.R., C.M.L., J.J.S. and I.H. performed the *in vitro* complement activation and stimulation assays using samples provided by S.B.G. V.M.H., J.M.T. and N.K.B. provided the antibody specific to C5 and the CR2-fH fusion protein. T.W.-C. provided the $C6^{-}$ and $Cd59a^{-/-}$ mice. V.M.H., T.M.L. and D.M. Lee provided scientific input. T.M.L., A.L.R. and W.H.R. wrote the manuscript, and Q.W., C.R.S., T.W.-C., S.R.G., M.K.C., V.M.H. and D.M. Lee edited the manuscript. All authors analyzed the data and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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1. Felson, D.T. Clinical practice. Osteoarthritis of the knee. *N. Engl. J. Med.* **354**, 841–848 (2006).
2. Goldring, M.B. & Goldring, S.R. Osteoarthritis. *J. Cell. Physiol.* **213**, 626–634 (2007).
3. Pelletier, J.P., Martel-Pelletier, J. & Abramson, S.B. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum.* **44**, 1237–1247 (2001).
4. Hill, C.L. *et al.* Synovitis detected on magnetic resonance imaging and its relation to pain and cartilage loss in knee osteoarthritis. *Ann. Rheum. Dis.* **66**, 1599–1603 (2007).
5. Gobezie, R. *et al.* High abundance synovial fluid proteome: distinct profiles in health and osteoarthritis. *Arthritis Res. Ther.* **9**, R36 (2007).
6. Kemper, C. & Atkinson, J.P. T-cell regulation: with complements from innate immunity. *Nat. Rev. Immunol.* **7**, 9–18 (2007).
7. Cooke, T.D., Bennett, E.L. & Ohno, O. The deposition of immunoglobulins and complement in osteoarthritic cartilage. *Int. Orthop.* **4**, 211–217 (1980).
8. Corvetta, A. *et al.* Terminal complement complex in synovial tissue from patients affected by rheumatoid arthritis, osteoarthritis and acute joint trauma. *Clin. Exp. Rheumatol.* **10**, 433–438 (1992).
9. Cooke, T.D. Significance of immune complex deposits in osteoarthritic cartilage. *J. Rheumatol.* **14** (Spec No), 77–79 (1987).
10. Kamekura, S. *et al.* Osteoarthritis development in novel experimental mouse models induced by knee joint instability. *Osteoarthritis Cartilage* **13**, 632–641 (2005).
11. Englund, M. & Lohmander, L.S. Risk factors for symptomatic knee osteoarthritis fifteen to twenty-two years after meniscectomy. *Arthritis Rheum.* **50**, 2811–2819 (2004).

12. Ooi, Y.M. & Colten, H.R. Genetic defect in secretion of complement C5 in mice. *Nature* **232**, 207–208 (1979).
13. Huber-Lang, M. *et al.* Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* **12**, 682–687 (2006).
14. Banda, N.K. *et al.* Mechanisms of effects of complement inhibition in murine collagen-induced arthritis. *Arthritis Rheum.* **46**, 3065–3075 (2002).
15. Banda, N.K. *et al.* Targeted inhibition of the complement alternative pathway with complement receptor 2 and factor H attenuates collagen antibody-induced arthritis in mice. *J. Immunol.* **183**, 5928–5937 (2009).
16. Glasson, S.S. *In vivo* osteoarthritis target validation utilizing genetically-modified mice. *Curr. Drug Targets* **8**, 367–376 (2007).
17. Glasson, S.S. *et al.* Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* **434**, 644–648 (2005).
18. Stanton, H. *et al.* ADAMTS5 is the major aggrecanase in mouse cartilage *in vivo* and *in vitro*. *Nature* **434**, 648–652 (2005).
19. Happonen, K.E. *et al.* Regulation of complement by cartilage oligomeric matrix protein allows for a novel molecular diagnostic principle in rheumatoid arthritis. *Arthritis Rheum.* **62**, 3574–3583 (2010).
20. Neidhart, M. *et al.* Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation. *Br. J. Rheumatol.* **36**, 1151–1160 (1997).
21. Scanzello, C.R., Plaas, A. & Crow, M.K. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? *Curr. Opin. Rheumatol.* **20**, 565–572 (2008).
22. Sofat, N. Analysing the role of endogenous matrix molecules in the development of osteoarthritis. *Int. J. Exp. Pathol.* **90**, 463–479 (2009).
23. Sjöberg, A., Onnerfjord, P., Morgelin, M., Heinegard, D. & Blom, A.M. The extracellular matrix and inflammation: fibromodulin activates the classical pathway of complement by directly binding C1q. *J. Biol. Chem.* **280**, 32301–32308 (2005).
24. Bohana-Kashtan, O., Ziporen, L., Donin, N., Kraus, S. & Fishelson, Z. Cell signals transduced by complement. *Mol. Immunol.* **41**, 583–597 (2004).
25. Scanzello, C.R. *et al.* Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. *Arthritis Rheum.* **63**, 391–400 (2011).
26. Attur, M. *et al.* Prostaglandin E2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the EP4 receptor. *J. Immunol.* **181**, 5082–5088 (2008).
27. Badea, T.D. *et al.* Sublytic terminal complement attack induces c-fos transcriptional activation in myotubes. *J. Neuroimmunol.* **142**, 58–66 (2003).
28. Rus, H.G., Niculescu, F. & Shin, M.L. Sublytic complement attack induces cell cycle in oligodendrocytes. *J. Immunol.* **156**, 4892–4900 (1996).
29. Kraus, S., Seger, R. & Fishelson, Z. Involvement of the ERK mitogen-activated protein kinase in cell resistance to complement-mediated lysis. *Clin. Exp. Immunol.* **123**, 366–374 (2001).
30. Litherland, G.J. *et al.* Protein kinase C isoforms ζ and ι mediate collagenase expression and cartilage destruction via STAT3- and ERK-dependent c-fos induction. *J. Biol. Chem.* **285**, 22414–22425 (2010).
31. Daiger, S.P. Genetics. Was the Human Genome Project worth the effort? *Science* **308**, 362–364 (2005).
32. Rogers, J. *et al.* Complement activation by β-amyloid in Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **89**, 10016–10020 (1992).

ONLINE METHODS

Human samples. We studied human samples under protocols that were approved by the Stanford University and the Hospital for Special Surgery Institutional Review Boards and that included obtaining the subjects' informed consent as well as under protocols that were approved by the Partners Healthcare Institutional Review Board and that did not require obtaining the subjects' informed consent. For the ELISA analysis, we obtained synovial fluids from healthy individuals with no symptoms of knee osteoarthritis, individuals with early-stage knee osteoarthritis with symptoms lasting less than 1 year, as assessed by the presence of arthroscopically visible cartilage lesions or by radiographic imaging, and patients with end-stage knee osteoarthritis undergoing knee joint replacement surgery. For transcriptional profiling, we obtained osteoarthritic synovial membranes from patients undergoing knee joint replacement and from patients with early-stage knee osteoarthritis undergoing arthroscopy for meniscal tears with cartilage degeneration but no full-thickness cartilage loss[25].

Complement in synovial fluids. We used OptEIA ELISA kits (BD Biosciences) to detect C3a des arginine and C5b-9 in synovial fluids.

Transcriptional profiling. We analyzed RNA from osteoarthritic synovial membranes using Affymetrix Human U133 Plus 2.0 chips (GEO GSE32317). We downloaded data from healthy synovial membranes (analyzed on the same platform and array) from the NCBI Gene Expression Omnibus (GEO GSE12021)[33]. We merged these data sets[34] and computed the robust multichip average expression for the genes. We organized transcript expression profiles by unsupervised hierarchical clustering (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm).

Immunohistochemistry and immunofluorescence. Primary antibodies were as follows: mouse antibody to C5b-9 (clone aE11; Dako), rabbit antibody to MMP13 (Abcam), rabbit antibody to ERK1 and ERK2 phosphorylated on Thr202 or Tyr204 (20G11, Cell Signaling) and the corresponding isotype-control antibodies. For the immunohistochemical analysis, we used VECTASTAIN Elite ABC Kits (Vector Labs). For the immunofluorescent analysis, we used Alexa Fluor 555– or Alexa Fluor 488–conjugated secondary antibodies (Invitrogen).

Surgical mouse models of osteoarthritis. We performed the mouse studies under protocols approved by the Stanford Committee of Animal Research and in accordance with the guidelines of the US National Institutes of Health. We generated the medial meniscectomy[10,16,35] and DMM models[10,16–18]. In the medial meniscectomy studies, the age of the mice at the time of surgery and the duration of the experiment after surgery were as follows: C5 deficiency: 20-week-old mice and 16 weeks duration; time course: 20-week-old mice; C6 deficiency: 20-week-old mice and 20 weeks duration; and Cd59a deficiency: 12-week-old mice and 16 weeks duration. We used 15-month-old mice and continued the experiment for 12 weeks after surgery in the Cd59a DMM studies; we used 20-week-old mice and continued the experiment for 20 weeks after surgery in the C5 DMM studies. A/J congenic C5-sufficient (B10.D2-Hc1H2dH2-T18c/nSnJ) and C5-deficient (B10.D2-Hc0H2d-T18c/oSnJ) mice were from Jackson Laboratories. C6-deficient mice were backcrossed with C3H/He mice for ten generations[36]; the controls, C3H/He mice, were from Jackson Laboratories. *Cd59a*$^{-/-}$ mice were generated as described[37].

Treatment of mouse osteoarthritis. We treated 21-week-old wild-type C57Bl/6 mice intraperitoneally with 750 μg of either the monoclonal antibody BB5.1 specific to C5 (ref. 38) or an isotype-control antibody twice per week until the end of the experiment (18 weeks) starting 1 week after medial meniscectomy. We treated 21-week-old wild-type C57Bl/6 mice intravenously with 250 μg of CR2-fH[15] or with PBS once a week until the end of the experiment (12 weeks) starting 1 week after medial meniscectomy.

Scoring of cartilage degeneration in mouse osteoarthritis. We stained sections of mouse joints with toluidine blue. We evaluated cartilage degeneration using a modified version of a previously described scoring system[10]: depth of cartilage degeneration (score of 0–4) × width of cartilage degeneration (with a score of 1 meaning one-third of the surface area, a score of 2 meaning two-thirds of the surface area and a score of 3 meaning the whole surface area) in each third of the femoral-medial and tibial-medial condyles. The scores for the six regions were summed.

Gait analysis. We analyzed mouse gait with the video-based Noldus CatWalk System[39].

***In vitro* complement activation assays.** Using previously described methods[23], we prepared suspensions of pulverized human osteoarthritic cartilage to which we added sepharose 4B (Pharmacia), zymosan A (Sigma), collagen II (Sigma or Chondrex), aggrecan (Sigma), matrilin-3 (R&D Systems) or fibromodulin (Novus Biologicals). We then added normal human serum (Quidel, Inc.), incubated the samples at 37 °C, stopped the reactions by adding EDTA and measured C5b-9 concentrations using ELISA (Quidel).

Generation of sublytic MAC on chondrocytes. We isolated chondrocytes from human osteoarthritic cartilage by digesting it with trypsin and type II collagenase. We formed sublytic MAC on the surface of the chondrocytes by mixing 0.8 μg ml^{-1} of C5b6 (EMD Chemicals) with 10 μg ml^{-1} of each of the remaining complement components of MAC (C7 (Quidel), C8 (EMD Chemicals) and C9 (EMD Chemicals)) as described[40]. After 72 h, we harvested the culture supernatants for a bead-array (Millipore) analysis of protein amounts, isolated the RNA for quantitative PCR analysis and performed immunocytochemistry with a goat antibody to COX2 (Santa Cruz Biotechnology).

Quantitative PCR. We isolated RNA from cultured chondrocytes derived from human osteoarthritic cartilage or from chondrocytes isolated from the knee joint of C5-deficient and C5-sufficient mice. We measured mRNA levels by quantitative PCR and normalized them to those of 18s RNA or β-actin ($2^{-\Delta\Delta Ct}$). The primers and probes used were from Applied Biosystems.

Statistical analyses. We analyzed cartilage degeneration scores using a two-tailed unpaired *t* test and multiple comparisons by one-way ANOVA followed by a Dunnett's *post hoc* test or by a fixed-effect ANOVA.

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

33. Huber, R. *et al.* Identification of intra-group, inter-individual, and gene-specific variances in mRNA expression profiles in the rheumatoid arthritis synovial membrane. *Arthritis Res. Ther.* **10**, R98 (2008).
34. Irizarry, R.A. *et al.* Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res.* **31**, e15 (2003).
35. Lin, A.C. *et al.* Modulating hedgehog signaling can attenuate the severity of osteoarthritis. *Nat. Med.* **15**, 1421–1425 (2009).
36. Zhou, W. *et al.* Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J. Clin. Invest.* **105**, 1363–1371 (2000).
37. Holt, D.S. *et al.* Targeted deletion of the CD59 gene causes spontaneous intravascular hemolysis and hemoglobinuria. *Blood* **98**, 442–449 (2001).
38. Frei, Y., Lambris, J.D. & Stockinger, B. Generation of a monoclonal antibody to mouse C5 application in an ELISA assay for detection of anti-C5 antibodies. *Mol. Cell. Probes* **1**, 141–149 (1987).
39. Gabriel, A.F., Marcus, M.A., Honig, W.M., Walenkamp, G.H. & Joosten, E.A. The CatWalk method: a detailed analysis of behavioral changes after acute inflammatory pain in the rat. *J. Neurosci. Methods* **163**, 9–16 (2007).
40. Chen, Y. *et al.* Terminal complement complex C5b-9–treated human monocyte-derived dendritic cells undergo maturation and induce Th1 polarization. *Eur. J. Immunol.* **37**, 167–176 (2007).