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Monocyte activation is elevated in women with knee-osteoarthritis and associated with inflammation, BMI and pain

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SUMMARY

Objective: Monocytes contribute to synovitis and disease pathogenesis in osteoarthritis (OA). Low-grade inflammation occurs in OA and correlates with disease severity and progression. Since monocyte development and function is altered by systemic inflammation, we analyzed monocyte numbers and function between individuals with knee OA and healthy age- and sex-matched controls.
Design: We analyzed markers of soluble and cellular inflammation in peripheral blood of women with knee OA and compared them to healthy age- and sex-matched controls. Soluble inflammatory mediators (TNF, IL-6, IL-10 and CRP) in the serum were measured by high-sensitivity ELISA. Leukocyte numbers, surface expression of monocyte activation markers, and monocyte production of pro-inflammatory mediators (TNF and IL-1β) following stimulation were measured by flow cytometry.
Results: Women with knee OA ($n = 15$) had elevated levels of serum c-reactive protein (CRP) and a lower proportion of circulating monocytes. Monocytes from OA participants had elevated expression of the activation markers CD16, CCR2, and HLA-DR and induced greater production of tumor necrosis factor (TNF) and IL-1β compared to healthy controls. Higher serum TNF and BMI were correlated with increased monocyte expression of CCR2. Additionally monocyte CCR2 expression and serum TNF were correlated with worse pain on a validated questionnaire.
Conclusions: Our findings suggest monocytes are activated prior to their entry into the synovium. Modulating systemic inflammation and monocyte recruitment to the synovium could be of therapeutic benefit.

Introduction

Although the root cause of osteoarthritis (OA) remains unknown, there are multiple factors that contribute to disease risk, progression and severity (i.e., genetics, age, obesity, smoking, joint injury, metabolic dysfunction)[1]. The immune system plays a pivotal role in the pathogenic mechanisms of OA. Both soluble (e.g., cytokines, chemokines, complement) and cellular (e.g., monocytes, macrophages) mediators of immunity contribute to cartilage destruction, abnormal bone remodeling, synovitis and joint effusion[2]. Cartilage destruction results from chondrocyte activation, which stimulates the release of matrix metalloproteinases (MMPs), disintegrin and metalloproteinases with thrombospondin motifs (ADAMTSs), and pro-inflammatory cytokines (tumor necrosis factor (TNF), IL-1β, IL-6). Activated chondrocytes undergo hypertrophy and cease to produce new cartilage matrix[3]. The loss of cartilage, concurrent with other pathological joint tissue changes such as abnormal subchondral bone remodeling, subchondral cysts and osteophytes, destabilizes the joint.

Mechanical injury is thought to trigger the disease by activating joint tissues to release proinflammatory mediators[4]. While the temporal sequence of OA initiation remains unclear, some animal models suggest that damage to subchondral bone and articular cartilage initiates a cascade of events, including the

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origination of inflammatory signals that incite synovitis[2,5,6]. Inflammatory signals in the subchondral bone influence the development and activation of osteoclasts, which contribute to bone resorption and joint destruction in OA[7]. Osteoclasts can be derived from recently recruited monocytes in the appropriate microenvironment[8], and can contribute to cartilage degradation[9]. Synovitis is propagated by synovial macrophages and results in an inflammatory edema and joint effusion[4]. Synovial macrophages are activated by the pro-inflammatory cytokines and danger-associated molecular patterns (DAMPs) released by cartilage and bone breakdown (i.e., proteoglycans, glycosaminoglycan, hyaluronan, calcium pyrophosphate, sodium urate)[10,11]. They respond by releasing vascular endothelial growth factor (VEGF), TNF, IL-1β, IL-6, and chemokines (i.e., CCL2), stimulating the vascularization of the synovium and recruitment of circulating leukocytes[10,11]. The thickening of the synovial membrane is driven by an influx of monocytes, which differentiate into synovial macrophages[10,11]. Inflammatory mediators produced in the synovial membrane diffuse into the joint via the synovial fluid, and bathe the cartilage, increasing chondrocyte apoptosis[5]. Synovial fluid may additionally access and activate the subchondral bone through osteochondral lesions[5]. Although the relative importance and temporal sequence of subchondral bone and synovium activation in OA pathogenesis remains to be determined, monocyte involvement is central to both.

Monocyte chemokines and cytokines are found at increased concentrations in the synovial fluid of osteoarthritic joints[12]. In humans, soluble monocyte and macrophage markers (CD14, CD163) in the serum and synovial fluid are correlated with the number of activated synovial macrophages, joint-space narrowing and osteophytes[13]. Depletion of synovial macrophages in animal models decreases osteophyte formation[14,15] and cartilage destruction[16]. Many depletion studies additionally deplete monocytes, and thus likely reduce osteoclast numbers and bone resorption. Subchondral bone remodeling and innate immune infiltration are highest during early OA[17]. This body of data suggests that monocytes and their downstream progeny (i.e., osteoclasts and synovial macrophages) drive OA disease pathogenesis. Thus we believe reducing monocyte activation and recruitment to the joint may be of therapeutic benefit to individuals with OA.

Monocytes can be subdivided into three subsets based on their expression of two surface markers (CD14 and CD16): classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical monocytes ($CD14^{+}CD16^{++}$). Intermediate monocytes, also referred to as inflammatory monocytes, produce more pro-inflammatory cytokines on a per cell basis and are recruited to sites of inflammation. These cells have been used as biomarkers in cancer, atherosclerosis and colitis[18]. However there is a paucity of data on circulating monocyte numbers, phenotype or cytokine production in individuals with OA compared to healthy controls.

Hematopoietic stem cells (HSCs) are finely tuned to even subtle changes in systemic levels of inflammation. Slight increases in inflammation trigger HSCs to produce monocytes to provide a rapid innate response to infection or injury[19]. People with OA have elevated circulating inflammatory mediators including c-reactive protein (CRP), TNF, IL-6[20–22]. The magnitude of systemic inflammation strongly correlates with OA disease severity and progression[21,22]. We have shown that age-associated increases in systemic inflammation can remodel the monocyte compartment by increasing their output and activating them to produce higher levels of pro-inflammatory cytokines[23]. There is some evidence of innate immune activation in OA, as circulating monocytes from individuals with OA have greater osteoclastogenic potential and resorptive activity compared to healthy controls[24]. Thus we hypothesized that low-grade inflammation in OA, similar to what occurs with age or other chronic inflammatory conditions, activates circulating monocytes, increasing their surface expression of trafficking and activation markers and their production of pro-inflammatory cytokines.

Materials and methods

Participants and ethics

The study population included 22 community-dwelling women with clinical (symptomatic) knee OA over the age of 50 who were enrolled in a larger randomized controlled trial (clinical trial number NCT02370667). Women were included if they answered yes to three or more of the following American College of Rheumatology clinical criteria[25]: (a) Have knee pain in most days of the week (b) Have fewer than 30 min of morning stiffness (c) Have crepitus with active range of motion (d) Have a bony enlargement (e) Have bony tenderness with palpation (f) Have signs of inflammation and/or have been diagnosed with radiographic osteoarthritis. Participants were excluded if they had any other forms of arthritis (e.g., rheumatoid, psoriatic), active non-arthritic knee disease (e.g., bursitis), patellofemoral symptoms, knee surgery (e.g., high tibial osteotomy, joint replacement, ligament repair), history of an osteoporotic fracture, planned surgery in the next 6 months, lower extremity trauma in the last 3 months, an unstable heart condition or neurological conditions (e.g., stroke), recent or current exposure to radiation, or other health conditions that might be exacerbated by the protocol. Following consent, weight-bearing radiographs of the affected knee in a fixed flexion posture were obtained to confirm disease (i.e., Kellgren–Lawrence grade $\geq$2). Each OA participant was age-matched to a control, of the same sex, within 3–4 years of their age. Control participants without OA were recruited from the community and submitted to the same exclusion criteria listed above and did not answer yes to any of the American College of Rheumatology clinical criteria cited above.

Self-reported knee pain intensity was reported using the Knee injury and Osteoarthritis Outcome Score (KOOS) pain subscale, which is a five-point Likert scale (0 = no pain, 4 = intense pain) rating pain during nine different activities[26]. The score was normalized to a score out of 100, where higher scores represent less pain[27]. The 6 min walk test (6MWT) was used to capture mobility, by measuring the furthest distance a participant can walk in 6 min in a well-lit, rectangular hallway[28]. This study was approved by the Hamilton Integrated Research Ethics Board. All participants provided written, informed consent (Controls: REB# 1949; OA

Table I
Participant description

	Control		OA		*t*-test *P*-value
	n	Mean (Min–Max)	*n*	Mean (Min–Max)	
Age	15	65.40 (51–84)	22	66.9 (51–84)	0.683
BMI(kg/m^2)	11	27.35 (19.16–36.35)	22	30.00 (20.7–44.6)	0.211

OA patient characteristics	*n*	Mean (SD) or Median (Min–Max)*
Radiographic Score	20	3 (1–4)
KOOS pain score	22	54.14 (15.03)
KOOS Symptom	22	51 (15.50)
KOOS ADL	22	61.05 (17.52)
KOOS Sport/Rec	22	30 (0–75)
KOOS QOL	22	31 (13–75)
6 min walk test distance (m)	22	461.92 (65.41)

* Median shown where variable does not follow a Gaussian distribution.

Table II
Soluble inflammatory mediators in serum

	Control		OA		Mann–Whitney *U* test *P* value
	n	Median (Min–Max)	*n*	Median (Min–Max)	
IL-10 (pg/mL)	8	3.49 (0.18–21.57)	19	7.63 (0.09–31.08)	0.0697
IL-6 (pg/mL)	8	1.56 (0.49–2.38)	20	1.67 (0.74–4.63)	0.2425
TNF (pg/mL)	8	7.98 (2.89–10.07)	20	8.27 (4.33–16.78)	0.4448
CRP (mg/L)	5	3.468 (2.794–6.599)	18	5.552 (2.971–12.12)	**0.0352**

Bold value indicates statistical significance.

participants: REB #15-021). Characteristics of the OA and control participants are summarized in Table I.

Immunophenotyping

Venous blood was collected in heparinized tubes and 100 μL was stained for 30 min with monoclonal antibodies of the following specificities: CD45-BV510, CCR2-PE, CD15-BV610, CD14-BV421, CD56-AF700 (BioLegend); CD16-PE-Cy7, HLA-DR-PerCP-Cy5.5, CD19-AF700 (eBioscience); CD11b-APC, CD3-AF700 (BD Pharmingen) CX3CR1-FITC (MBL Life Sciences). Samples were then incubated with 1X Fix/Lyse Buffer (eBioscience) for 10 min with frequent inversion and centrifuged at room temperature, washed and resuspended in FACS Wash (5 mM EDTA, 0.5% BSA in PBS). Samples were then run on an LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo 10 software (Treestar). Total cell counts were determined with 123 count eBeads (eBioscience). For gating strategy, see Supplemental Figures 2 and 3. Fluorescence intensities of the proteins were detected by flow cytometry and follow a logarithmic-normal distribution in the cell populations analyzed, thus geometric means were calculated using the FlowJo 10 software to quantify the mean fluorescence intensity (intra-assay CV: 2%

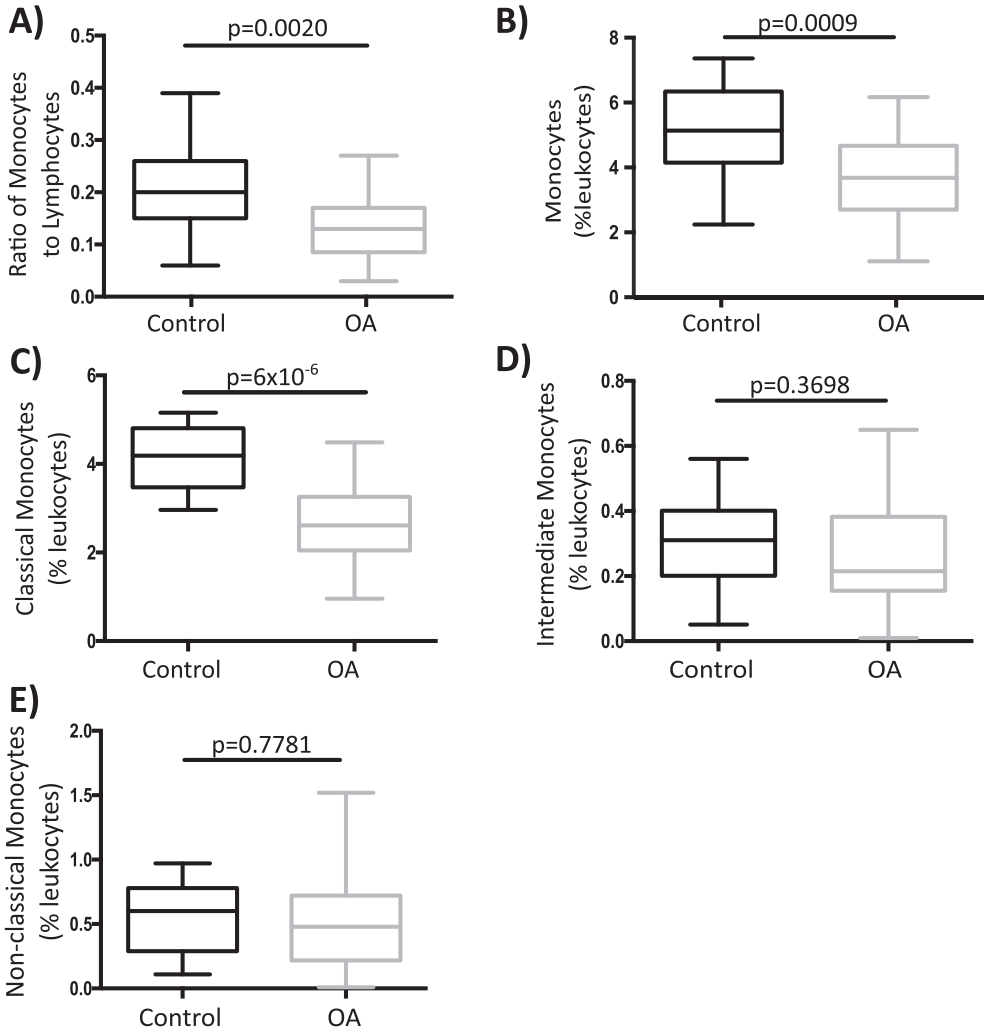
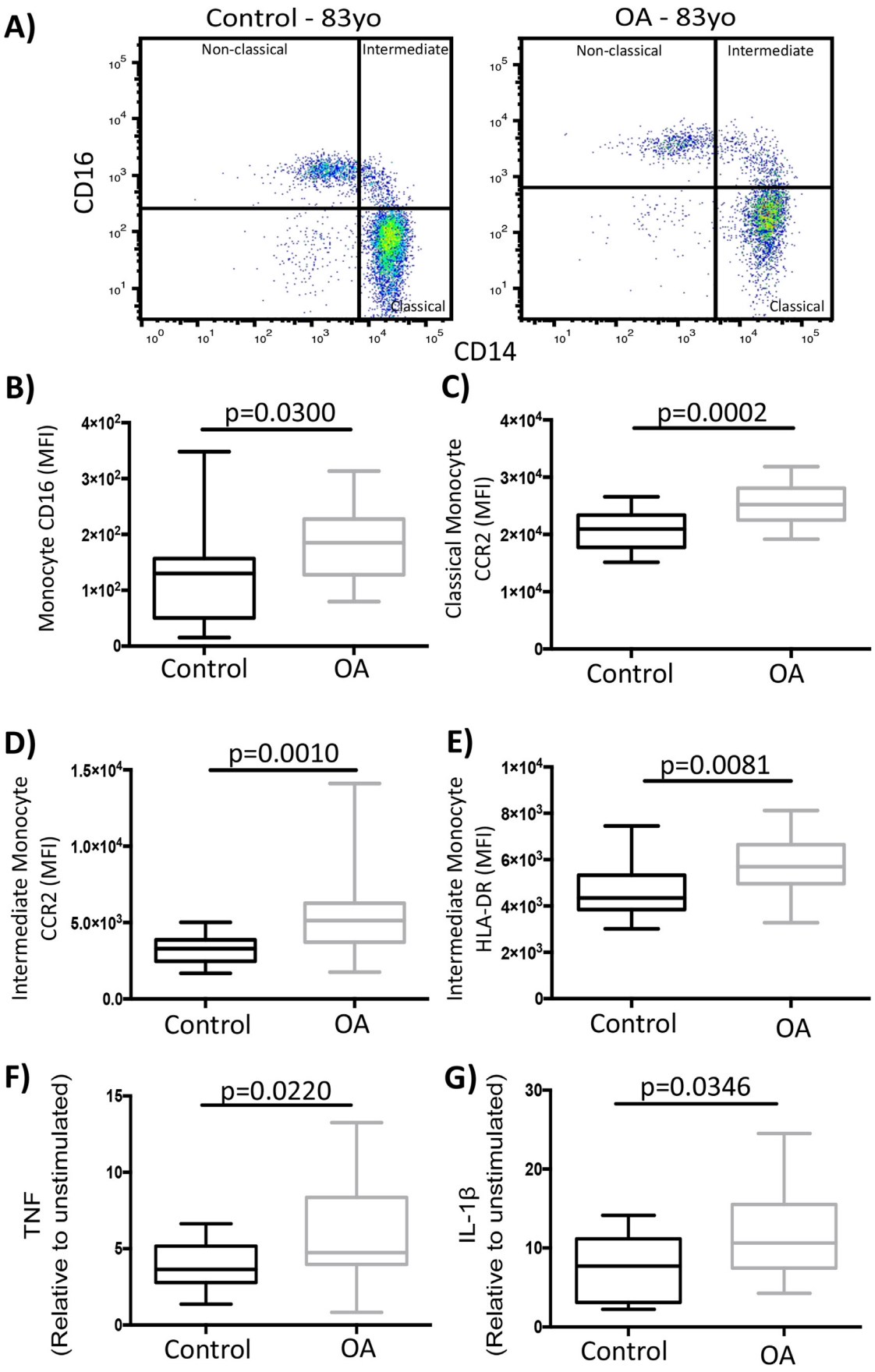


Fig. 1. Women with OA have altered proportions of circulating leukocyte compared to healthy controls. A) The proportion of circulating monocytes to leukocytes was lower in individuals with OA ($n = 15$) compared to controls ($n = 22$). B–E) Total monocytes and classical monocytes as a percentage of circulating leukocytes, were lower in individuals with OA, but there was no difference in intermediate or non-classical monocytes. Two-tailed Mann–Whitney test used except in A & C) where an Student's *t*-test was applied.


A)
Control - 83yo
OA - 83yo
Non-classical
Intermediate
Classical
CD16
CD14
B)
p=0.0300
Monocyte CD16 (MFI)
Control
OA
C)
p=0.0002
Classical Monocyte CCR2 (MFI)
Control
OA
D)
p=0.0010
Intermediate Monocyte CCR2 (MFI)
Control
OA
E)
p=0.0081
Intermediate Monocyte HLA-DR (MFI)
Control
OA
F)
p=0.0220
TNF (Relative to unstimulated)
Control
OA
G)
p=0.0346
IL-1β (Relative to unstimulated)
Control
OA

inter-assay CV: 12%). Background fluorescence intensity was measured in isotype controls and was subtracted from antibody specific fluorescence intensity.

Intracellular cytokine staining

Intracellular cytokine staining was performed on cryopreserved peripheral blood mononuclear cells (PBMCs) isolated from whole blood by Ficoll density-gradient centrifugation and Leucosep tubes (Grenier Bio-one) stored at −150°C in human AB serum (Lonza) with 10% dimethyl sulfoxide until use. Briefly, PBMCs from participants were later cultured in two conditions (1×10^6 PBMCs per condition) for 4 h at 37°C/5% CO_2: complete media (RPMI, Invitrogen, ON, CA) supplemented with 10% FBS and 1X Protein Transport Inhibitor (eBioscience) and complete media with 50 ng/mL lipopolysaccharide (LPS) (Invivogen). Surface staining was performed for 30 min at room temperature with the conjugated antibodies CD14-PE-Dazzle, CD3-APC-Cy7 fixed with 1X Fix/Lyse buffer (eBioscience) for 10 min. Cells were permeabilized with 1X Permeabilization Buffer (eBioscience) at room temperature for 30 min. Cells were then stained at room temperature for 30 min with the following antibodies diluted in 1X Permeabilization Buffer: TNF-Alexa Fluor 700, IL-1β-PE (eBioscience), IL-6-FITC (BioLegend). Cells were washed and resuspended in FACS Wash prior to analysis. Monocytes were defined by size, granularity and expression of CD14(+) and CD3(−). Flow cytometry analysis was performed as described above.

Serum cytokines

Venous blood was collected and centrifuged at 1.5 × g for 10 min at 25°C and serum was stored at −140°C until processed. Serum cytokines IL-6, IL-10 and TNF were measured using Milliplex MAP 60K cytokine panel as per manufacturer's protocol (EMD Millipore, HSTCMAG-28SK; Intra-assay CV: <5%, Inter-assay CV: <15%). CRP was analyzed by an in-house ELISA. In short, the wells were coated with capture antibody (1 ug/mL, ab8279, Abcam) overnight, blocked with blocking buffer (PBS with 10% FBS) for 2 h. Samples were diluted 1/10,000 and added to the wells, incubated for 1 h before being washed with washing buffer (PBS with 0.05% Tween-20). Plate was incubated for 1 h with HRP-conjugated detection antibody (1ug/mL, ab24462, Abcam) diluted in blocking buffer for 1 h before being washed. Plates were developed with TMB as per manufacturer's protocol. The serum values were measured in duplicate and an average value was reported (intra-assay CV: 1.5%, inter-assay CV: 7.2%, 90.1% recovery).

Statistical analysis

The Student *t* test was used to evaluate differences between OA and Controls for normally distributed variables, tested using the Kolmogorov–Smirnov Test. The Mann–Whitney *U* test was used to evaluate the difference between OA and controls for non-normally distributed variables. Correlation analyses between monocyte activation markers, inflammation, age, BMI, pain and mobility were performed using Spearman's rank correlation rho or Pearson correlation depending on normality of variables. Statistical analysis was performed using SPSS (Version 21; IBM, Armonk, NY, USA), Prism (Version 6; GraphPad, San Diego, CA, USA).

Results

Women with OA have higher serum CRP and altered circulating leukocyte proportions compared to healthy controls

Serum CRP was elevated in the OA group (median: 5.55 mg/L) compared to controls (median: 3.468 mg/L) (Cohen's *d* [95%CI]: 0.925 [−0.101–1.952], $P = 0.0352$, Table II); however, levels of serum cytokines did not differ between the two groups (Table II). To evaluate differences in cellular inflammation, we quantitated leukocyte populations using flow cytometry. The ratio of circulating monocytes to lymphocytes was lower in women with OA compared to healthy controls (mean difference [95%CI]: −0.07533 [−0.1211 to −0.02958], Cohen's *d* [95%CI]: −1.119 [−1.823 to −0.415], $P = 0.002$; Fig. 1(A)). This was due to both a lower number of circulating monocytes and a higher number of circulating lymphocytes in the OA group (Fig. 1; Supplementary Table 1). The lower number of circulating monocytes was primarily driven by the decrease in classical monocytes (mean difference [95%CI]: −1.52 [−2.099 to −0.9477] % leukocytes, Cohen's *d* [95%CI]: −1.84 [−2.634 to −1.047], $P = 6 \times 10^{-6}$; Fig. 1(B)–(E)).

Circulating monocytes express higher levels of activation markers in OA

We measured surface expression of monocyte trafficking (CCR2) and activation (CD16 and HLA-DR) markers with flow cytometry by analyzing mean fluorescence intensity (MFI). The median expression of CD16 on all monocytes was higher in OA group compared to controls (median difference [95%CI]: 55.08 [7.790–105.8] MFI, Cohen's *d* [95%CI]: 0.712 [0.029–1.395], $P = 0.03$, Fig. 2(A),(B)). This occurred on all monocytes, rather than a specific subset, as can be seen by the shift of the total monocyte population in a representative flow cytometry plot of an individual with OA compared to their age-matched control [Fig. 2(A)]. Expression of CCR2 was elevated in classical and intermediate monocytes in women with OA compared to controls (mean difference [95%CI]: 4863 [2451–7275]MFI, Cohen's *d* [95%CI]: 1.371 [0.681–2.12], $P = 0.0002$; median difference [95%CI]: 1830 [885–3045]MFI, Cohen's *d* [95%CI]: 1.049 [0.344–1.755], $P = 0.001$; Fig. 2(C)–(D)), as was the expression of HLA-DR on intermediate monocytes (mean difference [95%CI]: 1350 [324–1965] MFI, Cohen's *d* [95%CI]: 0.887 [0.194–1.581], $P = 0.0081$; Fig. 2(E)).

Monocytes from women with OA produce more inflammatory cytokines following stimulation

It has been reported that peripheral blood mononuclear cells (e.g., lymphocytes and monocytes) from individuals with OA produce higher levels of IL-6, TNF and IL-1β compared to healthy controls, however the particular cell type producing these cytokines was not identified[29]. We performed intracellular staining to identify whether monocytes were responsible for the increased production of IL-6, TNF and IL-1β, in unstimulated conditions and following stimulation with lipopolysaccharide (LPS). LPS, a TLR-4 agonist, was chosen as many of the DAMPs released in OA have been shown to activate macrophages through TLR-4[12]. There was no difference in basal production of TNF, IL-1β or IL-6 by monocytes (Supplemental Fig. 1), however monocytes from women with OA had a higher induction of TNF and IL-1β production following

Fig. 2. Circulating Monocytes are activated in women with OA compared to controls. Age-matched control and OA participant flow cytometry plots show a global increase in CD16 expression on monocytes in the individual with OA. (B). Expression of CCR2 on classical and intermediate monocytes (C,D) and HLA-DR on intermediate monocytes were higher in women with OA (E). Monocyte production of tumor necrosis factor (TNF) and IL-1β following stimulation with LPS relative to unstimulated was greater in the OA group (F,G). Two-tailed Mann–Whitney test used except in C where an Student's *t*-test was applied. $n = 15, 22$ (A–E); $n = 13, 17$ (F–G); MFI-Geometric Mean fluorescence intensity.

stimulation with LPS compared to healthy controls (median difference [95%CI]: 1.102 [0.222–3.712] TNF production (relative to unstimulated), Cohen's *d* [95%CI]: 0.843 [0.09–1.596], $P = 0.022$; median difference [95%CI]: 2.925 [0.1064–8.143] IL-1β production (relative to unstimulated), Cohen's *d* [95%CI]: 0.834 [0.081–1.586], $P = 0.0346$; Fig. 2(F)–(G)). However there was no difference in the production of IL-6 basally or in response LPS (data not shown).

Age and inflammation alter monocyte characteristics and function. We performed correlation analysis to determine if these factors were related to monocyte activation in OA. Age did not correlate with any of the monocyte activation markers (Supplementary Table 2), however TNF was positively associated with the expression of CCR2 on intermediate monocytes (Spearman's rho [95%CI] = 0.5092 [0.07201–0.7823], $P = 0.0218$, $n = 20$; Fig. 3(A)). Additionally, BMI was positively associated with the expression of CCR2 on classical monocytes (Pearson coefficient [95%CI] = 0.5064 [0.1077–0.7648], $P = 0.0162$, $n = 22$; Fig. 3(B)).

We next determined whether the monocyte activation markers correlated with pain or mobility. Intermediate monocyte CCR2 expression and TNF were both inversely correlated with the KOOS Pain Score (Spearman's rho [95%CI] = −0.4884 [−0.7603 to −0.07077], $P = 0.0211$, $n = 22$; Pearson coefficient [95% CI] = −0.4926 [−0.7678 to −0.6391], $P = 0.0273$, $n = 20$; Fig. 3(C)–(D)). Mobility, measured by the 6MWT distance, did not correlate with monocyte activation markers (Supplementary Table 2), but inversely correlated with BMI (Pearson coefficient [95%CI] = −0.511 [−0.7728 to −0.2007], $P = 0.0151$, $n = 22$; Fig. 3(E)).

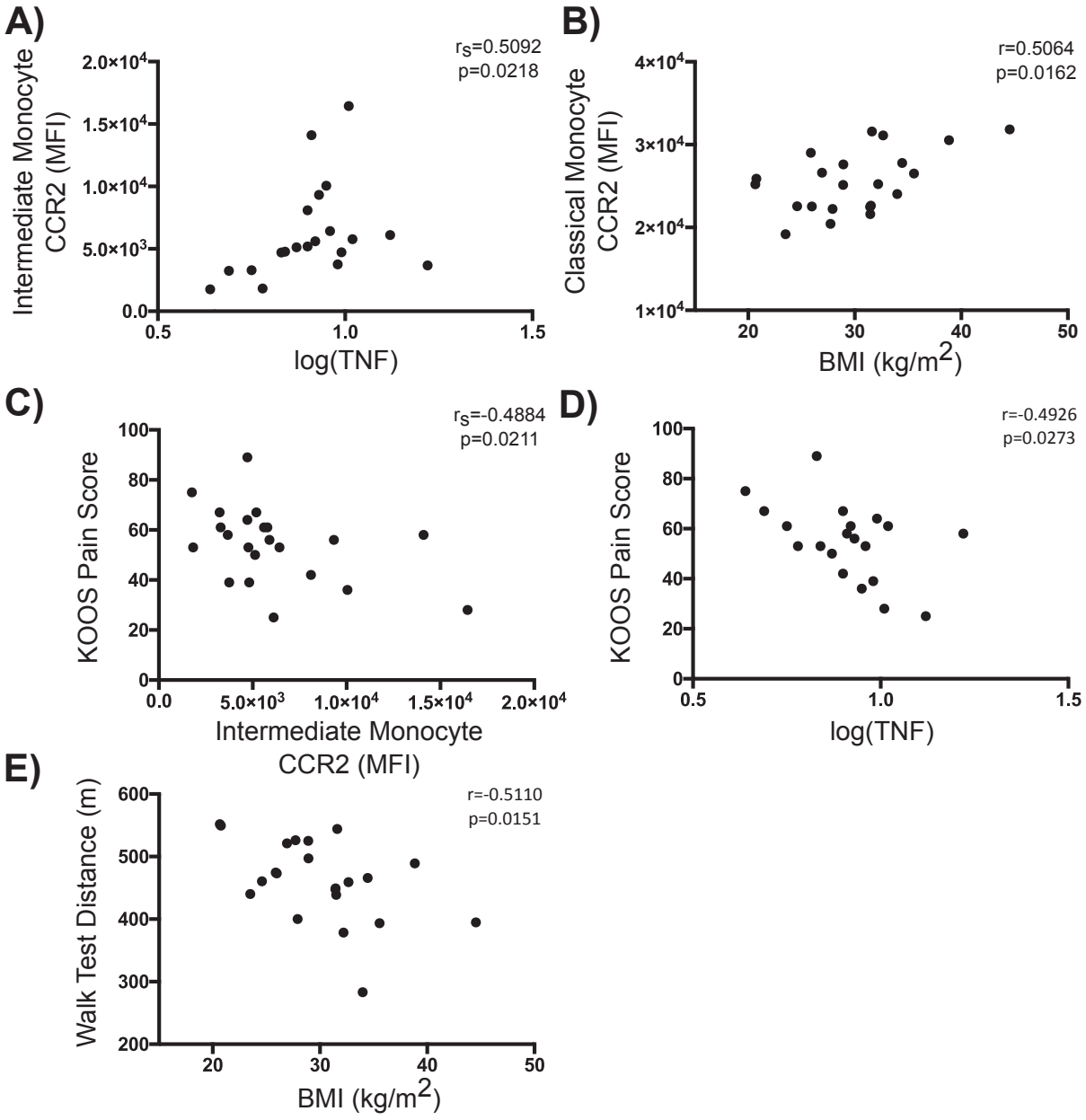


Fig. 3. Monocyte activation correlates with inflammation, BMI and pain in OA. A) Log transformed serum TNF(pg/mL) correlates with intermediate monocyte CCR2 expression ($n = 20$). B) BMI correlates with classical monocyte CCR2 expression ($n = 22$). Intermediate monocyte CCR2 expression (C, $n = 22$) and log transformed serum TNF(pg/mL) (D, $n = 20$) are inversely correlated to the Knee injury and Osteoarthritis Outcome Score (KOOS) Pain Score. E) BMI is inversely correlated to the 6 min walk test distance ($n = 22$). Spearman's correlation coefficient was reported for A & C, while Pearson's correlation coefficient was reported for B, D and E. BMI-Body Mass Index; MFI-Geometric Mean fluorescence intensity.

Discussion

Soluble inflammation has been well characterized in both the synovial fluid and serum of people with OA, however the changes to monocyte populations in OA have not. Consistent with previous reports[30], we found elevated levels of circulating CRP in women with OA compared to their age- and sex-matched controls. The median and maximum values of other cytokines measured (IL-6, TNF and IL-10) were elevated in the OA group compared to controls, however due to the low sample size and large variation, they were not statistically different. To our knowledge this is the first report demonstrating lower numbers of circulating monocytes relative to leukocytes in individuals with OA compared to healthy controls. Our quantitation of monocytes (mean (SD): 3.02 (0.39) $\times$ 10^5 cells/mL; 3.66 (0.28)%leukocytes) was similar to a previous report (mean [SD]: 3.79 [0.37] $\times$ 10^5 cells/mL; 3.7 (0.4)%leukocytes[31]), however this group did not include healthy controls in order to demonstrate that these counts were lower in OA (mean [SD]: 4.97 (1.02) $\times$ 10^5 cells/mL; 5.25 (0.34)%leukocytes; Fig. 1; Supplementary Table 1). This decrease in circulating monocytes may be due to increased infiltration into synovial tissue since a key trafficking receptor, CCR2, was elevated on classical and inflammatory monocytes from individuals with OA. The decrease in circulating monocytes was most pronounced in the classical monocytes, which express the highest levels of CCR2 of the three monocyte subsets. The ligand for CCR2, CCL2/MCP-1, is elevated in the intimal lining of individuals with joint disease[32]. Furthermore, CCR2 has been shown to be critical to monocyte trafficking and infiltration of the synovium as CCR2 knockout animals have less synovial macrophages and experience less cartilage damage and osteophyte formation[33]. Whether the elevated expression of CCR2 on monocytes of individuals with OA increases the capacity to traffic to and invade synovial tissues warrants further investigation.

In addition to the increased invasive potential, circulating monocytes from individuals with OA were more activated. We found elevated expression of CD16 and HLA-DR on monocytes and intermediate monocytes, respectively, in women with OA. The expression of CD16 increases during the differentiation of monocytes into osteoclasts[7], which aligns with the increased resorptive activity of monocyte-derived osteoclasts from individuals with OA[24]. Additionally, the increased expression of CD16 can increase the sensitivity of monocytes to stimulation by immune complexes, which are found at elevated concentrations in OA joints and in circulation[34]. The expression of HLA-DR is elevated on inflammatory monocytes, which allows them to present antigen and activate T cells more efficiently. The elevated expression of CD16 and HLA-DR on monocytes in individuals with OA did not correlate with serum cytokines or BMI (data not shown). This suggests these differences are unique to the OA disease process and independent of systemic inflammation and BMI.

We have previously shown that CCR2 levels on monocytes are increased during chronic inflammation and specifically in the presence of TNF[35]. Levels of serum TNF in individuals with OA correlated with increased expression of CCR2 on intermediate monocytes. Furthermore, serum TNF and intermediate monocyte CCR2 expression correlated with increased pain in women with knee OA. This supports previous findings that serum and synovial fluid levels of the CCR2 ligand, CCL2/MCP-1, correlate with pain and physical disability in OA[36]. Not only will monocytes expressing CCR2 traffic to areas with high CCL2/MCP-1 levels, but they are also major producers of CCL2 and may thus increase cellular recruitment and pain. Since pain is one of the most significant causes of immobility and health care utilization in people with OA, reducing monocyte recruitment to the joint may be of therapeutic benefit.

We found monocytes in individuals with OA produce more cytokines when they encounter a TLR4 agonist (e.g., LPS). Although basal production of IL-1β and TNF by monocytes were not different between the OA and control group, the induction of both cytokines following stimulation with LPS was greater in women with OA. This indicates that monocytes in OA may produce more inflammatory cytokines on a per cell basis following stimulation with DAMPs, which are found in high abundance in the synovial fluid[12]. Together these findings confirm circulating monocytes are more activated and have greater inflammatory potential in individuals with OA compared to controls.

Obesity increases the risk of developing OA[37,38]. This association is not solely due to increased mechanical load, as the risk also exists for non-weight-bearing joints[39]. Adipose tissue and altered lipid metabolism contribute to increased systemic inflammation[40]. We found BMI was positively correlated with the expression of CCR2 on classical monocytes, which is consistent with previous data showing BMI and fat mass correlate with increased expression of CCR2 on monocytes[41]. Thus, adiposity may accelerate the CCR2-mediated infiltration of monocytes to the synovium where they contribute to pathogenesis (i.e., pain, cartilage erosion). In animal models of OA, blockade of CCL2/CCR2 leads to decreased inflammation, macrophage accumulation in the joint and cartilage damage[33]. This, in conjunction with controlling weight and adiposity, may be able to slow the progression of OA.

Although our study is limited by a small sample size and cross-sectional design, it is the first study, to our knowledge, to specifically characterize changes in circulating monocytes in individuals with OA compared to healthy controls. We have shown that monocytes in OA display an activated phenotype with increased expression of activation markers as well as increased production of pro-inflammatory cytokines. Monocyte activation may contribute to the development of multiple comorbidities that commonly co-exist with OA, such as depression, obesity, metabolic syndrome and dyslipidemia[4,39,42]. Activation and homing of inflammatory monocytes has been associated with many chronic inflammatory conditions including dementia, atherosclerosis and type II diabetes[18,43]. As in OA, these conditions are characterized by slight but measurable changes in soluble and cellular inflammation and similar changes in leukocyte phenotype and function[23]. Since having one chronic inflammatory condition is associated with a higher chance of developing multiple co-morbidities[44], it is believed that there are common immunological changes that predispose to multiple diseases. As an example, in mice, OA increases neuroinflammation and accelerates Alzheimer's disease pathology[45]. Changes in monocytes contribute to the development of chronic inflammatory conditions[46]. Monocyte trafficking mediated by CCR2/MCP-1 is essential for the development of atherosclerotic plaques[47] and has been shown to contribute to inflammation in the brain[48] and adipose tissue[49]. Further studies are warranted to explore the contribution of monocyte activation and trafficking to the risk of developing comorbidities in OA. Therapeutically targeting monocytes in OA may slow disease progression or decrease the risk of other chronic inflammatory diseases with monocyte involvement.

Contributions

All authors were involved in conception and design. Participants with OA were recruited by SK and MRM. DL performed experiments, DM, DMEB, and MRM analyzed and interpreted data, and drafted the article. DMEB and MRM obtained funding.

Competing interest statement

The authors do not have competing interests to declare.

Role of the funding source

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Supplementary data

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