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Mutant monocyte chemoattractant protein 1 protein attenuates migration of and inflammatory cytokine release by macrophages exposed to orthopedic implant wear particles

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Abstract: Wear particles generated from total joint replacements can stimulate macrophages to release chemokines, such as monocyte chemoattractant protein 1 (MCP-1), which is the most important chemokine regulating systemic and local cell trafficking and infiltration of monocyte/macrophages in chronic inflammation. One possible strategy to curtail the adverse events associated with wear particles is to mitigate migration and activation of monocyte/macrophages. The purpose of this study is to modulate the adverse effects of particulate biomaterials and inflammatory stimuli such as endotoxin by interfering with the biological effects of the chemokine MCP-1. In the current study, the function of MCP-1 was inhibited by the mutant MCP-1 protein called 7ND, which blocks its receptor, the C–C chemokine receptor type 2 (CCR2) on macrophages. Addition of 7ND decreased MCP-1-induced migration of THP-1 cells in cell migration experiments in a dose-dependent manner. Conditioned media from murine macrophages exposed to clinically relevant polymethylmethacrylate (PMMA) particles with/without endotoxin [lipopolysaccharide (LPS)] had a chemotactic effect on human macrophages, which was decreased dramatically by 7ND. 7ND demonstrated no adverse effects on the viability of macrophages, and the capability of mesenchymal stem cells (MSCs) to form bone at the doses tested. Finally, proinflammatory cytokine production was mitigated when macrophages were exposed to PMMA particles with/without LPS in the presence of 7ND. Our studies confirm that the MCP-1 mutant protein 7ND can decrease macrophage migration and inflammatory cytokine release without adverse effects at the doses tested. Local delivery of 7ND at the implant site may provide a therapeutic strategy to diminish particle-associated periprosthetic inflammation and osteolysis. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A:000–000, 2013.

Key Words: macrophages, chemotaxis, inflammation, particles, cytokines

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INTRODUCTION

Total joint replacement (TJR) is a highly successful surgical procedure for end-stage arthritis. However, wear particles are inevitable byproducts of all joint replacements.[1] Wear begins during the initial "bedding in" phase and continues during use of the TJR. Wear particles stimulate chronic inflammation that delays osseointegration, and leads to periprosthetic bone loss (osteolysis) and implant loosening.

Cells of the monocyte/macrophage lineage (macrophages, foreign body giant cells, and osteoclasts) are among the key cells that perpetuate the inflammatory reaction to orthopedic wear particles generated from TJRs. Wear particles stimulate macrophages to release chemokines, such as monocyte chemoattractant protein 1 (MCP-1), which is the most important chemokine regulating systemic and local cell trafficking and infiltration of monocyte/macrophages in chronic inflammation.[2,3] *In vitro*, *in vivo*, and tissue retrieval studies by our group and others have demonstrated a critical role for MCP-1 in particle-induced inflammation.[4–9] Thus, one possible strategy to curtail the adverse events associated with wear particles is to mitigate systemic and local migration and activation of monocyte/macrophages to the location of particle generation. The purpose of this study is to modulate the adverse effects of polymer particles and inflammatory stimuli such as endotoxin by interfering with the biological effects of the chemokine MCP-1. This

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was accomplished by the use of a mutant MCP-1 protein called 7ND in which the 2–8 N-terminal amino acids of MCP-1 are deleted. Functionally deficient 7ND competes with MCP-1 to attach to its receptor, the C–C chemokine receptor type 2 (CCR2) on macrophages.

In the current study, we examine the migration of macrophages to MCP-1, and to conditioned media from macrophages challenged with polymethylmethacrylate (PMMA) particles with/without endotoxin. We then blocked the CCR2 receptors on the macrophages by adding 7ND and examined any changes in macrophage migration. Cytokine production by macrophages exposed to PMMA particles with/without lipopolysaccharide (LPS) in the presence or absence of 7ND was also evaluated. Finally, to determine whether 7ND treatment had any adverse effects on the ability of mesenchymal stem cells (MSCs) to form bone, we determined the production of bone nodules (using Von Kossa staining) in the presence/absence of 7ND. We hypothesized that the mutant MCP-1 protein 7ND would mitigate cytokine production by LPS and particle-exposed macrophages, decrease macrophage chemotaxis, yet have no effect on the ability of MSCs to form bone.

MATERIALS AND METHODS

Mutant MCP-1 protein (7ND)

7ND recombinant protein is a mutant MCP-1 protein which lacks the N-terminal amino acids 2 through 8, and has been shown to function as a dominant negative inhibitor of MCP-1.[10–12] The 7ND in the current study was supplied by Dr. Egashira in Kyushu University (Japan).

Preparation of PMMA particles

We used phagocytosable PMMA particles as the challenging agent because these particles are relatively uniform in size and shape, relatively cheap, and have been used extensively by our group and others for *in vitro* and *in vivo* studies of particle-induced inflammation.[13–20] PMMA particles (Polysciences, Warrington, PA) 1–10 μm in diameter, were washed with 70% ethanol and incubated overnight with shaking at 4°C. The particles were then washed extensively with phosphate-buffered saline (PBS) and resuspended to obtain a concentrated 5% (v/v) stock solution. The particles were shown to be free of endotoxin according to the Limulus Amebocyte Lysate Assay (BioWhittaker) which is sensitive to 1.0–0.1 EU/mL.

Migration conditioned media

RAW 264.7 cells (Cat #: TIB-71; ATCC, Manassas, VA), a mouse macrophage cell line, were used for the production of inflammatory cytokines through stimulation with endotoxin and/or PMMA particles. RAW cells were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. RAW cells were seeded at a density of 0.1×10^6 cells/well in 24-well plates in the presence of PMMA particles (0.05%, vol/vol) in serum-free DMEM or LPS (1 μg/mL). Cell-seeded culture plates without particles or LPS were used as a negative control. The cell culture supernatants were collected after 48 h and used for the chemotaxis assay.

Chemotaxis assay

The methodology for the chemotaxis experiments reported herein were originally developed and reported by Huang et al[14] from our laboratory. In the migration experiments, human macrophages were used as the migrating cells due to their high sensitivity to chemotactic cytokines.[14] THP-1 cells (Cat#: TIB-202; ATCC), a human macrophage cell line were suspended in ATCC-formulated RPMI-1640 medium with 10% FBS and 50 n*M* 2-mercaptoethanol (2-ME) at a concentration of 1.1×10^6 cells/mL. 7ND (0–500 ng/mL) was added to the cells 1 h before beginning the chemotaxis assay. For studies using conditioned media from RAW cell culture, THP-1 cells were preincubated with 1000 ng/mL 7ND. Chemotaxis assays were performed using a ChemoTx® Disposable Chemotaxis System (NeuroProbe, Gaithersburg, MD) containing 8 μm pores.[14] MCP-1 of 29 μL (10 ng/mL) or conditioned media from RAW cell culture was placed in the bottom chamber and 6×10^4 THP-1 cells were placed in the upper chamber of the chemotaxis system. After a 2-h incubation period, cells in the upper chamber were removed by aspiration and the porous membrane was removed. Cells in the bottom chamber were transferred to a 96-well plate. The bottom chamber was washed with 30 μL of H_2O which was also transferred to the 96-well plate (total volume 59 μL). The plate was transferred to −80°C freezer for 1 h and returned to room temperature until fully thawed. The freeze–thaw cycle was repeated two more times. To quantify the number of migrated cells, 100 μL of Picogreen dye (Life Technologies, Grand Island, NY) was added to each well and fluorescence was read at 480/520 nm using a plate reader (Spectramax M2e; Molecular Devices, Sunnyvale, CA). A linear dilution of THP-1 cells served as a reference to quantify cell number.

THP-1 cell viability

To determine whether 7ND affects the viability of THP-1 cells, a CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) was performed. THP-1 cells were suspended in complete RPMI 1640 medium with 2-ME at a concentration of 1.1×10^6 cells/mL. THP-1 cells (100 μL) were incubated in the presence of 7ND (0–500 ng/mL) for 3 h, which equals the time course of the chemotaxis assay. After 3 h, 20 μL of CellTiter 96® AQueous One Solution was added to each well containing THP-1 cells. The plate was returned to the incubator for a 1 h. Color change was detected by measuring absorbance at 490 nm using a plate reader (Spectramax M2e).

Inflammatory cytokine analysis

Different doses of 7ND (1, 10, 25, 50, and 100 ng/mL) were added into RAW cell-seeded cultures (0.1×10^6 cells/well) with PMMA particles (0.05%, vol/vol) and LPS (1 μg/mL). The supernatants were collected after 24 h and the concentration of tumor necrosis factor α (TNF-α) in the supernatant was assayed by enzyme-linked immunosorbent assay

(ELISA; R&D Systems, Minneapolis, MN). In addition, RAW 264.7 cells were exposed to PMMA particles for 24 h with/without 7ND, and the production of inflammatory cytokines including interleukin (IL) 1β (IL-1β), IL-10, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon γ (IFN-γ), IL-4, and IL-6 was determined by Luminex multiplex array (Life Technologies) following the manufacturer's recommended protocol. Supernatants from particle-challenged cell cultures were collected at 24 h. All collected supernatants were frozen at −80°C and stored. All Luminex assays were analyzed in triplicate using the manufacturer's provided buffers and protocols. Data were acquired on a Luminex 200 instrument and analyzed using the resident software (xPONENT 3.1; Luminex).

Osteogenic differentiation of MSCs and von Kossa staining

Murine MSCs harvested from long bones were cocultured with 7ND (250 ng/mL) in osteogenic medium, as described by Zhu et al.[21] After 28 days differentiation in osteogenic medium, cells were stained by von Kossa staining. Briefly, cell cultures were washed with PBS and incubated in 10% buffered formaldehyde solution for 30 min. Cell cultures were then washed 3 × 5 min with distilled water and incubated in 5% silver nitrate (Cat. No. 209139; Sigma) solution under ultraviolet light for 1 h. The stained cultures were imaged by light microscopy (100× magnification) and mineralized nodules and matrix were quantified using NIH ImageJ software (ver. 1.46r). Thresholds settings were adjusted to make all stained areas in the image black and all unstained areas white. The total area of positive stains corresponding to the black areas was measured using NIH ImageJ and expressed as a percentage of the culture well area.

Statistical analyses

Numerical data were analyzed using Graphpad Prism 5.0 software (San Diego, CA). The results are presented as mean and standard deviation. Statistical significance was tested by one-way ANOVA, followed by *post hoc* testing using Tukey's multiple comparison test. The level of significance was set to $p < 0.05$.

RESULTS

Mutant MCP-1 protein (7ND) inhibited macrophage migration

The 7ND protein blocked the CCR2 receptors, mitigating macrophage recruitment, even in the presence of exogenously added MCP-1. 7ND inhibited macrophage migration in a dose-dependent manner. The inhibition of cell migration showed statistical significance at a concentration of 500 ng/mL of 7ND group ($p < 0.01$; Fig. 1). At a concentration of 500 ng/mL of 7ND, more than 30% decrease in cell migration was observed ($p < 0.01$), which was below that observed when no 7ND (0 ng/mL) was added to the cultures.

The conditioned medium produced by RAW cells stimulated with PMMA particles only or PMMA particles plus LPS (which acted as an inflammatory and chemotactic stimulus for THP-1 cells) was placed in the bottom chamber of the chemotaxis assay system. As shown in Figure 2, this supernatant caused increased migration of THP-1 macrophages; preincubation of the THP-1 cells with 7ND dramatically decreased cell migration to a level comparable to the migration toward the nonstimulated RAW cells. A high level of migration was observed in the untreated groups as RAW cells produce a basal level of the cytokines MCP-1 and MIP-1α.[14] The presence of 7ND however mitigated the increased migration induced by stimulated cells, decreasing migration by 30–40% in all groups ($p < 0.01$; Fig. 2).

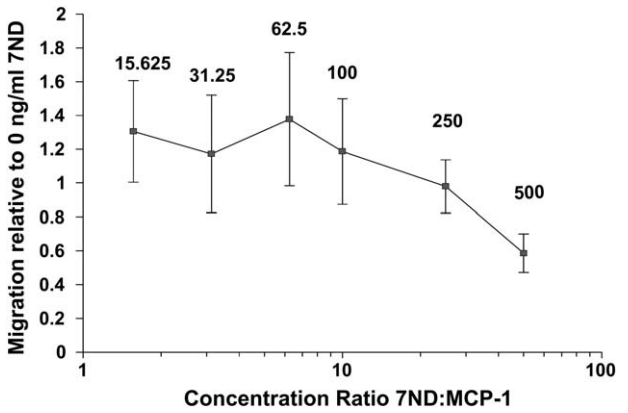

FIGURE 1. THP-1 cell migration is inhibited by preincubation with 7ND. THP-1 cells were preincubated with increasing concentrations of 7ND (0–500 ng/mL) for 1 h then placed in the upper well of a migration chamber. MCP-1 (10 ng/mL) was placed in the lower chamber and migration proceeded for 2 h. The ratio of migrated cells relative to no pretreatment are shown. Data are presented as mean ± standard deviation.

7ND does not affect cell viability of macrophages

The viability of THP-1 cells was quantified after 3 h of exposure to 7ND (0–500 ng/mL). There was no statistical difference in the viability of cells among all groups (Fig. 3). This

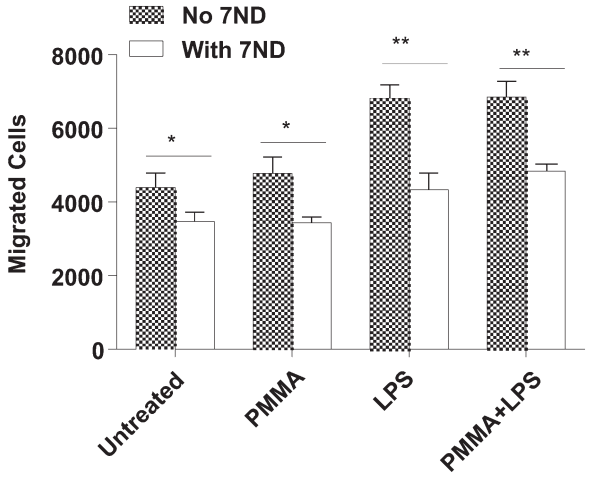

FIGURE 2. Migration of THP-1 cell pretreated with 7ND (1000 ng/mL) toward RAW cell supernatant. RAW cell supernatant was collected 48 h after stimulation with PMMA particles, LPS or a combination of both. Data are presented as mean ± standard deviation. *$p < 0.01$ and **$p < 0.001$.

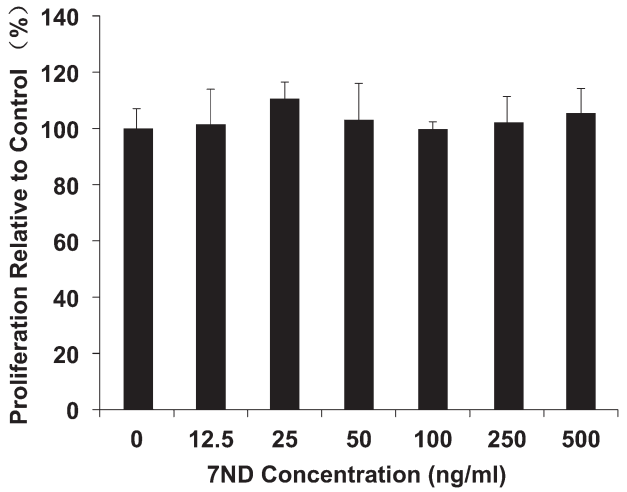

FIGURE 3. Viability of THP-1 cells treated with 7ND. THP-1 cells were incubated with increasing concentrations of 7ND (0–500 ng/mL) for 3 h. A CellTiter assay was then performed to determine cell viability. Data are presented as viability relative to untreated cells (0 ng/mL 7ND) and displayed as mean ± standard deviation.

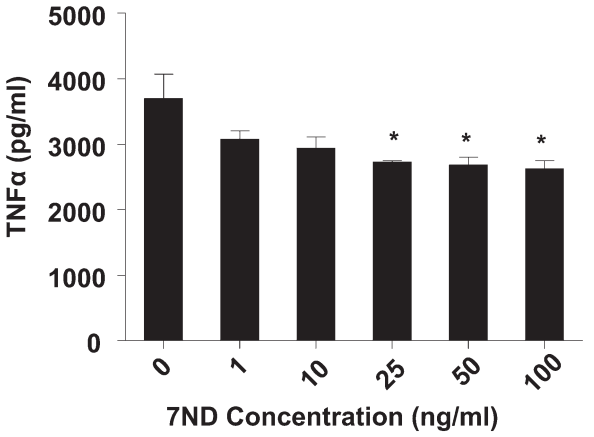

FIGURE 4. Inhibitory effects of 7ND on tumor necrosis factor-alpha release. Different dose of 7ND (1, 10, 25, 50, and 100 ng/mL) were added into RAW cell-seeded cultures containing LPS (10 μg/mL). The supernatants were collected after 24 h and analyzed by ELISA (R&D). Each datum plot represents the mean result of two separate cell preparations in which each experimental condition was tested in triplicate. *$p < 0.05$ versus control (0 ng/mL).

indicated that 7ND was not cytotoxic at the concentrations tested; therefore, any decrease in THP-1 cell migration could not be attributed to cell death. In addition, the viability assay was repeated after 24 h of 7ND exposure without effect (data not shown).

7ND mitigates inflammatory cytokine release from macrophages upon stimulation of PMMA particles or LPS *in vitro*

Exposure of RAW cells to PMMA particles and LPS simulates wear particle-induced inflammation. When RAW cells were stimulated with LPS *in vitro*, an increased production of the inflammatory cytokine TNF-α was observed. The addition of 7ND decreased the secretion of TNF-α significantly at the doses of 25, 50, and 100 ng/mL ($p < 0.05$). Even at lower doses, such as 1 and 10 ng/mL, the presence of 7ND demonstrated a trend to decrease TNF-α release (Fig. 4).

The production of important inflammatory cytokines by macrophages was determined using a Luminex assay after 24 h exposure to PMMA particles and LPS (a more clinically relevant scenario), with/without addition of 100 ng/mL 7ND. Particles and LPS together induced a significantly increased expression of TNF-α, IL-1β, GM-CSF, and IFN-γ (Figs. 4 and 5). For TNF-α and IL-1β, these values reached 3174.2 ± 18.1 and 45.2 ± 3.0 pg/mL, respectively (Figs. 4 and 5). All inflammatory cytokines were below the detectable limit in untreated controls. The addition of 7ND significantly decreased inflammatory cytokine production. For TNF-α and IL-1β, the inflammatory values were decreased by 31% and 30%, compared to their respective untreated controls ($p < 0.01$). GM-CSF production was also inhibited by 20% by 7ND, although this did not reach statistical significance. IFN-γ level was increased after particle plus LPS exposure, and was inhibited by 7ND by 20% ($p < 0.05$). Interestingly, no significant difference in IL-6 production could be shown in the 7ND treated group after particle exposure (665.1 ± 51 vs. 650 ± 43 pg/mL, $p > 0.05$). The expression of IL-10 and IL-4, which are anti-inflammatory cytokines, were reduced 15–20% by 7ND ($p < 0.05$).

7ND does not adversely affect the production of bone nodules by MSCS

To test whether 7ND had adverse effects on MSCs, we cultured mouse MSCs with 7ND in osteogenic medium. Osteogenic differentiation potential was evaluated by von Kossa staining after 28 days (Fig. 6A, 6B). MSCs with 7ND showed no difference in bone nodule formation compared to the group without 7ND.

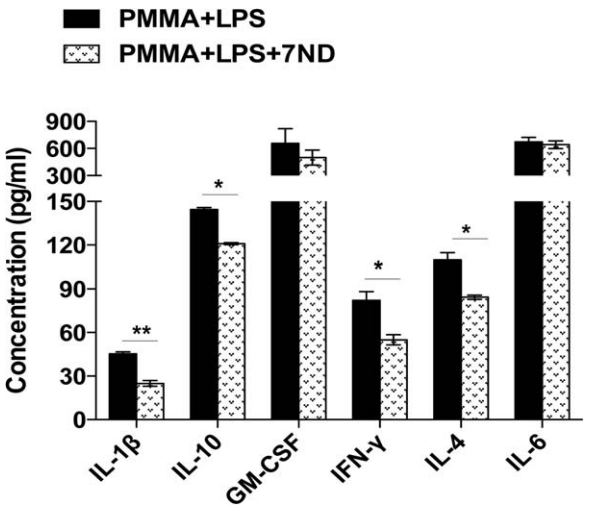

FIGURE 5. Inhibitory effects of 7ND on inflammatory cytokine release. 7ND (100 ng/mL) was added into RAW cell-seeded cultures containing LPS(1 μg/mL). The supernatants were collected after 24 h and analyzed by Luminex Assay (Invitrogen). Each datum plot represents the mean result of two separate cell preparations in which each experimental condition was tested in triplicate. *$p < 0.05$ and **$p < 0.01$.

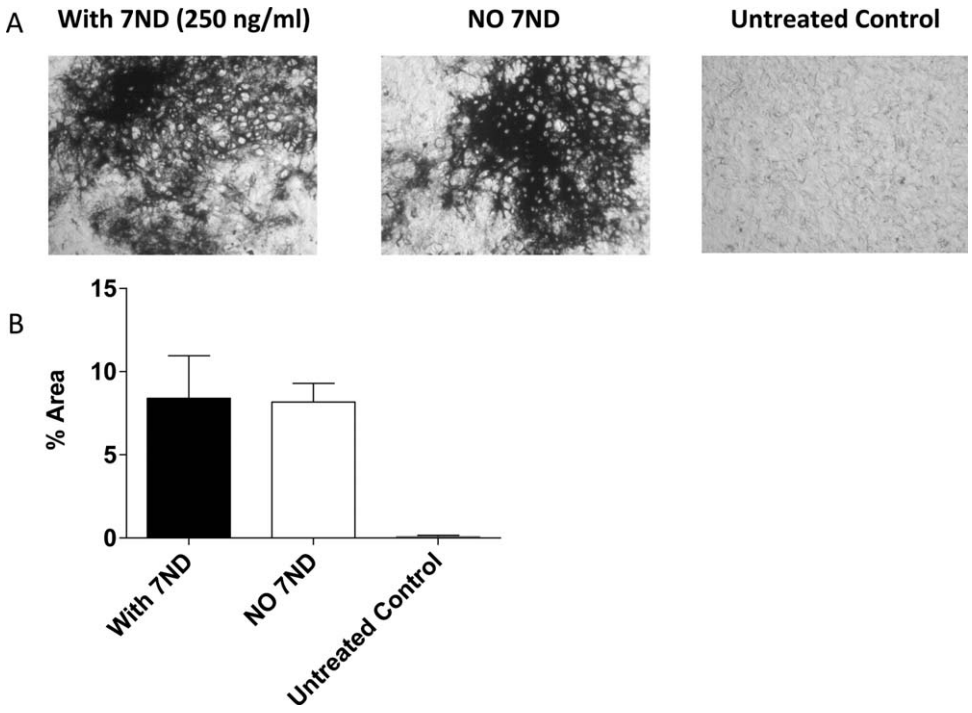

FIGURE 6. Effects of 7ND on the differentiation potential of primary murine MSCs. A, shows mineralization using Von Kossa staining, reflecting osteogenic differentiation potential and B, shows the quantification of mineralization graphically. Original magnification: ×100. MSCs cultured for 28 days in osteogenic medium exhibit a high degree of calcification; adding 7ND (250 ng/mL) to the culture does not affect osteogenesis. The control has virtually no calcification.

DISCUSSION

Currently, there are no clinically successful nonsurgical treatments for wear particle-induced periprosthetic osteolysis. Several unsuccessful treatments were far downstream in the inflammatory cascade, and directed toward a specific molecule or pathway, such as inhibition of prostaglandins or TNF, or osteoclast function.[22] These treatments were not clinically effective because there is much redundancy in the inflammatory cascade, and both bone resorption and formation are affected by wear particles.

The *in vivo* response to wear particles is complex, involving a range of cell types and cytokines including fibroblasts, osteoclast precursor cells, monocytes/macrophages, proinflammatory cytokines, chemokines, prostanoids, reactive oxygen intermediates, and other factors released locally into the synovial fluid and interfacial tissues. We recognize that the *in vitro* experiments described herein are a simplification of these complex cellular processes. Monocyte/macrophages and their derivatives (foreign body giant cells and osteoclasts) however are among the key cells that perpetuate the chronic inflammatory reaction to wear debris from TJRs. These cells migrate systemically to the area of particle generation and mediate bone destruction and adversely modulate bone formation by paracrine processes. MCP-1 is the primary chemotactic factor for monocytes/macrophages and has an important role in the pathogenesis of chronic inflammatory disorders.[23,24] MCP-1 is the most important chemokine that regulates migration and infiltration of monocytes/macrophages. Emerging evidence suggests that MCP-1-mediated inflammation is involved in wear particle-induced disorders.[4,6,8,25] Using a murine model, we showed that the majority of macrophages surrounding wear particles are of systemic rather than local origin and that MCP-1 plays a critical role in particle-induced chemotaxis and inflammation.[26] Furthermore, systemic interference with the MCP-1-CCR-2 ligand–receptor axis can decrease trafficking of exogenously injected macrophages and osteolysis associated with local polyethylene particle infusion.[26]

The purpose of this study was to develop a local intervention to downregulate the numerous proinflammatory and osteoclastogenic pathways in the inflammatory cascade which result in particle-induced osteolysis. It is anticipated that local delivery of 7ND will mitigate particle-induced inflammation during the "bedding-in" period after implantation of a joint replacement, and promote rapid osseointegration. Early osseointegration provides a protective seal around the implant thus mitigating the ingress of wear particles in the peri-implant space. In the current study, the addition of a mutant MCP-1 protein (7ND) which functions as a dominant negative inhibitor of MCP-1, decreased the migration of macrophages toward a chemotactic stimulus (exogenous MCP-1) in a dose-dependent manner. Similar inhibitory effects of 7ND were observed when conditioned medium from culture of macrophages exposed to clinically relevant PMMA particle with/without endotoxin. There was no difference in the viability of cells exposed to a wide range of doses of 7ND similar to those used in the migration assay. This confirmed that 7ND was not cytotoxic at the concentrations tested; therefore, any decreases in macrophages migration cannot be attributed to cell death.

Since MCP-1 plays a critical role in particle-related inflammatory pathways,[4,6,8,25] we further tested whether 7ND could inhibit the release of inflammatory cytokines. Addition of 7ND significantly decreased the release of inflammatory cytokines by particle and LPS exposure, including TNFα and IL-1β. To our knowledge, this finding has not been previously reported. When MCP-1 attaches to its receptor during inflammation and subsequent cell activation, this results in upregulation of many proinflammatory cytokines and chemokines. 7ND competes with MCP-1 for attachment to the same receptors but does not result in cell activation. This would result in a decrease in cytokine release in the presence of 7ND. Other important proinflammatory cytokines released by macrophages such as GM-CSF were similarly inhibited by 7ND. GM-CSF can amplify the inflammatory response by increasing the production of IL-1 and TNF by macrophages.[27] GM-CSF also produces other cytokines, such as IL-8 and MCP-1.[28] Although in the current study, the GM-CSF release in the presence or absence of 7ND did not reach statistical significance, we did observe a 20% suppression of GM-CSF by 7ND which suggests another potential control mechanism in the mitigation of the inflammatory cascade. IFN-γ can amplify the inflammatory response by increasing the production of IL-1 and TNF by macrophages. In our studies, we found that addition of 7ND also reduced the production of IFN-γ. Therefore, the addition of 7ND *in vitro* inhibited the production of important proinflammatory cytokines.

Interestingly, IL-6 was not affected by 7ND. The mechanism for this is unclear; however, IL-6 is not traditionally viewed as solely a proinflammatory cytokine in all biological environments and it can act as both a proinflammatory and anti-inflammatory cytokine.[29–31]

The levels of IL-10 and IL-4, two well-known anti-inflammatory cytokines, were significantly increased by PMMA particles exposed to macrophages. However, 7ND also attenuated the levels of both cytokines similar to that of the proinflammatory cytokines. The former finding may reflect a correcting mechanism to re-establish homeostasis in the face of an adverse stimulus.[32]

Although the 7ND data are promising with respect to inhibition of both migration of inflammatory macrophages and the subsequent release of proinflammatory factors, it is very important to establish a toxicity profile for adverse effects on other cell types, especially MSCs. Blocking antibody or reagents could unfavorably affect osseointegration by downregulating cells of the osteoblastic lineage. We found no adverse effects of 7ND on bone nodule formation at the same doses that inhibited inflammation. This is a key finding which adds to the safety profile of 7ND.

CONCLUSIONS

In vitro studies have confirmed that the MCP-1 mutant protein 7ND can decrease macrophage migration and inflammatory cytokine release without adverse effects at the doses tested. Local addition of 7ND may provide a therapeutic strategy to diminish particle-associated periprosthetic inflammation and osteolysis.

REFERENCES

1. Revell PA. The combined role of wear particles, macrophages and lymphocytes in the loosening of total joint prostheses. J R Soc Interface 2008;5:1263-1278.
2. Fuentes ME, Durham SK, Swerdel MR, Lewin AC, Barton DS, Megill JR, Bravo R, Lira SA. Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. J Immunol 1995;155: 5769-5776.
3. Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. J Exp Med 1998;187:601-608.
4. Trindade MC, Schurman DJ, Maloney WJ, Goodman SB, Smith RL. G-protein activity requirement for polymethylmethacrylate and titanium particle-induced fibroblast interleukin-6 and monocyte chemoattractant protein-1 release in vitro. J Biomed Mater Res 2000;51:360-368.
5. Shanbhag AS, Kaufman AM, Hayata K, Rubash HE. Assessing osteolysis with use of high-throughput protein chips. J Bone Joint Surg Am 2007;89:1081-1089.
6. Nakashima Y, Sun DH, Trindade MC, Chun LE, Song Y, Goodman SB, Schurman DJ, Maloney WJ, Smith RL. Induction of macrophage C–C chemokine expression by titanium alloy and bone cement particles. J Bone Joint Surg Br 1999;81:155-162.
7. Wang CT, Lin YT, Chiang BL, Lee SS, Hou SM. Over-expression of receptor activator of nuclear factor-kappaB ligand (RANKL), inflammatory cytokines, and chemokines in periprosthetic osteolysis of loosened total hip arthroplasty. Biomaterials 2010;31:77-82.
8. Epstein NJ, Bragg WE, Ma T, Spanogle J, Smith RL, Goodman SB. UHMWPE wear debris upregulates mononuclear cell proinflammatory gene expression in a novel murine model of intramedullary particle disease. Acta Orthop 2005;76:412-420.
9. Epstein NJ, Warme BA, Spanogle J, Ma T, Bragg B, Smith RL, Goodman SB. Interleukin-1 modulates periprosthetic tissue formation in an intramedullary model of particle-induced inflammation. J Orthop Res 2005;23:501-510.
10. Ni W, Egashira K, Kitamoto S, Kataoka C, Koyanagi M, Inoue S, Imaizumi K, Akiyama C, Nishida KI, Takeshita A. New anti-monocyte chemoattractant protein-1 gene therapy attenuates atherosclerosis in apolipoprotein E-knockout mice. Circulation 2001; 103:2096-2101.
11. Zhang Y, Rollins BJ. A dominant negative inhibitor indicates that monocyte chemoattractant protein 1 functions as a dimer. Mol Cell Biol 1995;15:4851-4855.
12. Zhang YJ, Rutledge BJ, Rollins BJ. Structure/activity analysis of human monocyte chemoattractant protein-1 (MCP-1) by mutagenesis. Identification of a mutated protein that inhibits MCP-1-mediated monocyte chemotaxis. J Biol Chem 1994;269:15918-15924.
13. Nich C, Rao AJ, Valladares RD, Li C, Christman JE, Antonios JK, Yao Z, Zwingenberger S, Petite H, Hamadouche M and others. Role of direct estrogen receptor signaling in wear particle-induced osteolysis. Biomaterials 2013;34:641-650.
14. Huang Z, Ma T, Ren PG, Smith RL, Goodman SB. Effects of orthopedic polymer particles on chemotaxis of macrophages and mesenchymal stem cells. J Biomed Mater Res A 2010;94: 1264-1269.
15. Ren PG, Lee SW, Biswal S, Goodman SB. Systemic trafficking of macrophages induced by bone cement particles in nude mice. Biomaterials 2008;29:4760-4765.
16. Burton L, Paget D, Binder NB, Bohnert K, Nestor BJ, Sculco TP, Santambrogio L, Ross FP, Goldring SR, Purdue PE. Orthopedic wear debris mediated inflammatory osteolysis is mediated in part by NALP3 inflammasome activation. J Orthop Res 2013;31: 73-80.
17. Ingham E, Green TR, Stone MH, Kowalski R, Watkins N, Fisher J. Production of TNF-alpha and bone resorbing activity by macrophages in response to different types of bone cement particles. Biomaterials 2000;21:1005-1013.
18. Lou PJ, Cheng WF, Chung YC, Cheng CY, Chiu LH, Young TH. PMMA particle-mediated DNA vaccine for cervical cancer. J Biomed Mater Res A 2009;88:849-857.

19. Zhang H, Ricciardi BF, Yang X, Shi Y, Camacho NP, Bostrom MG. Polymethylmethacrylate particles stimulate bone resorption of mature osteoclasts in vitro. Acta Orthop 2008;79:281-288.
20. Pearl JI, Ma T, Irani AR, Huang Z, Robinson WH, Smith RL, Goodman SB. Role of the Toll-like receptor pathway in the recognition of orthopedic implant wear-debris particles. Biomaterials 2011;32:5535-5542.
21. Zhu H, Guo ZK, Jiang XX, Li H, Wang XY, Yao HY, Zhang Y, Mao N. A protocol for isolation and culture of mesenchymal stem cells from mouse compact bone. Nat Protoc 2010;5:550-560.
22. Schwarz EM. What potential biologic treatments are available for osteolysis? J Am Acad Orthop Surg 2008;16 Suppl 1:S72-S75.
23. Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev 1998;9:9-23.
24. Rollins BJ. Chemokines. Blood 1997;90:909-928.
25. Wang CT, Lin YT, Chiang BL, Lee SS, Hou SM. Over-expression of receptor activator of nuclear factor-kappaB ligand (RANKL), inflammatory cytokines, and chemokines in periprosthetic osteolysis of loosened total hip arthroplasty. Biomaterials 2010;31:77-82.
26. Gibon E, Ma T, Ren PG, Fritton K, Biswal S, Yao Z, Smith L, Goodman SB. Selective inhibition of the MCP-1-CCR2 ligand-receptor axis decreases systemic trafficking of macrophages in the presence of UHMWPE particles. J Orthop Res 2012;30:547-553.
27. Baqui AA, Meiller TF, Chon JJ, Turng BF, Falkler WA Jr. Granulocyte-macrophage colony-stimulating factor amplification of interleukin-1 beta and tumor necrosis factor alpha production in THP-1 human monocytic cells stimulated with lipopolysaccharide of oral microorganisms. Clin Diagn Lab Immunol 1998;5:341-347.
28. Cavaillon JM, Haeffner-Cavaillon N. Cytokines and inflammation. Rev Prat 1993;43:547-552.
29. van der Poll T, Keogh CV, Guirao X, Buurman WA, Kopf M, Lowry SF. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. J Infect Dis 1997;176:439-444.
30. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, Achong MK. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 1998;101:311-320.
31. Zhao X, Boenisch O, Yeung M, Mfarrej B, Yang S, Turka LA, Sayegh MH, Iacomini J, Yuan X. Critical role of proinflammatory cytokine IL-6 in allograft rejection and tolerance. Am J Transplant 2012;12:90-101.
32. Duvel A, Frank C, Schnapper A, Schuberth HJ, Sipka A. Classically or alternatively activated bovine monocyte-derived macrophages in vitro do not resemble CD163/Calprotectin biased macrophage populations in the teat. Innate Immun 2012;18:886-896.