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Differential Expression of Chemokines, Chemokine Receptors and Proteinases by Foreign Body Giant Cells (FBGCs) and Osteoclasts

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ABSTRACT

Osteoclasts and foreign body giant cells (FBGCs) are both derived from the fusion of macropahges. These cells are seen in close proximity during foreign body reactions, therefore it was assumed that they might interact with each other. The aim was to identify important genes that are expressed by osteoclasts and FBGCs which can be used to understand peri-implantitis and predict the relationship of these cells during foreign body reactions. Bone marrow macrophages (BMM) were treated with receptor activator of nuclear factor kappa B ligand (RANKL) to produce osteoclasts. Quantitative PCR (qPCR) was used to identify the genes that were expressed by osteoclasts and FBGCs compared to macrophage controls. TRAP staining was used to visualise the cells while gelatine zymography and western blots were used for protein expression. Tartrate-resistant acid phosphatase (TRAP), matrix metallo proteinase 9 (MMP9), nuclear factor of activated T cells 1 (NFATc1), cathepsin K (CTSK) and RANK were significantly lower in FBGCs compared to osteoclasts. Inflammation specific chemokines such as monocyte chemotactic protein (MCP1 also called CCL2), macrophage inflammatory protein 1 alpha (MIP1α), MIP1β and MIP1γ, and their receptors CCR1, CCR3 and CCR5, were highly expressed by FBGCs. FBGCs were negative for osteoclast specific markers (RANK, NFATc1, CTSK). FBGCs expressed chemokines such as CCL2, 3, 5 and 9 while osteoclasts expressed the receptors for these chemokines i.e. CCR1, 2 and 3. Our findings show that osteoclast specific genes are not expressed by FBGCs and that FBGCs interact with osteoclasts during foreign body reaction through chemokines. J. Cell. Biochem. 115: 1290–1298, 2014.

KEY WORDS: FBGCs; OSTEOCLASTS; TRAP; MMP9; CTSK; MCP1; CCR2; RANKL; M-CSF; IL-4; GM-CSF

Osteoclasts are multinucleated giant cells that are derived from monocyte/macrophage lineage cells and are positive for tartrate-resistant acid phosphatase (TRAP). Osteoclast generation from BMMs is mediated by macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL), which are necessary and sufficient for the formation of osteoclasts [Quinn et al., 2002]. FBGCs are formed when monocytes come in contact with a foreign body, leading to cell fusion and multinuclear cell formation. FBGC formation can also be mediated by IL-4 [McNally and Anderson, 1995]. Recently, we and others showed that osteoclasts and FBGCs share similar molecular receptors during fusion [Yagi et al., 2005; Khan et al., 2013]. Peri-implantitis, a type of foreign body reaction, produces a great challenge to the success of implants [Mombelli, 2002]. Peri-implantitis results in a significant destruction of bone around the implant that prevents attachment of bone to implants [Klinge et al., 2005]. During peri-implantitis foreign body giant cells are seen at the surface of the implant while osteoclasts are seen near the bone [Konttinen et al., 2006].

TRAP is a purple metalloproteinase common to bone and the immune system. In cell culture, osteoclasts are identified by positive staining for this enzyme and differentiated from monocytes [Burstone, 1959]. TRAP, along with other proteinases such as cathepsin K and matrix metallopreintases (MMPs) degrade the organic components of bone.

Osteoclasts and FBGCs are formed from the fusion of macrophages/monocytes on the surface of bone and foreign bodies, respectively. Osteoclast activity activates osteoblasts to form

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TABLE I. Primer Sequence for Genes and Their Accession Number That Were Used for PCR Expression

Primer	Forward	Reverse	Accession
CCR1	GAACGGTTCTGGAAGTACCT	TGGTTGACACCTATGGTCTG	NT_039483
RANK	CAGACTTCACTCCATACCCA	GCCAAACTGAATGATGCCAG	NM_009399
MMP9	ATGAGGTGAACAAGGTGGAC	TCAATTCTCAGACGCCACAC	NT_039207
DCSTAMP	CTCAGTGTGTCTGAGACTTG	GCAGCCTTGCAAACTCAAAC	NT_039621
CCR2	GTTTCTCAAGCCACAGTTCC	CCTGGAAAGCAGAAGGAAAG	NM_009915
CCR3	CAGTCACAATGACCTCTAGC	GAATTGTCAACTGGCCAGCA	NM_009914
CCR5	CTGGACTCCCTACAACATTG	ACACTGAGAGATAACTCCGG	NM_009917
GM-CSFRa	TGAAGCGATGCTGATAGACG	GTCGTTAAACATGCCCTTCC	NT_039687
CCL3	GATTCCACGCCAATTCATCG	TCTCAGGCATTCAGTTCCAG	NM_011337
CCL4	CAGACAGATCTGTGCTAACC	ATCTGAACGTGAGGAGCAAG	NM_013652.2
TRAP	GCCTACCTGTGTGTGGACATGA	GACCTTTCGTTGATGTCGCAC	NM_001102405
NFATc1	TAGTGTCACCTCGACCCT	GGGATGATTGGCTGAAGGAAC	NM_001164111
CTSK	TGTATAACGCCACGGCAAAGG	CATGGTTCACATTATCACGGTCA	NM_007802
MCP1	TGAGTAGGCTGGAGAGCTACAAG	TGTATGTCTGGACCCATTCCTTC	NM_011333
CCL5	CATGAAGATCTCTGCAGCTGCC	GCACTTGCTGCTGGTGTAGA	NM_013653
CCL9	CAGAGCAGTCTGAAGGCACAG	CGTGAGTTATAGGACAGGCAGC	NM_011338
18 S	CTTAGAGGGACAAGTGGCG	ACGCTGAGCCAGTCAGTGTA	X00686

new bone, while FBGCs activate fibroblasts to secrete collagen to encapsulate the foreign body [Miller and Anderson, 1989]. After differentiation of macrophages into osteoclasts in vivo, there is turnover of osteoclast nuclei and they undergo apoptosis within 16 days [Parfitt et al., 1996]. However, the mechanism of osteoclast apoptosis is poorly understood. By contrast, FBGCs remain attached to the foreign body for longer periods. Although these cells share the same apparent fusion mechanism, they differentiate into completely different cells; the similarities and differences between these cells are not clear.

In the current study we sought to identify the molecular mechanism involved in formation and contrasting functions of osteoclasts and FBGCs. Understanding these mechanisms will open the possibility of creating targeted therapeutics for controlling the formation of these multi-nucleated cells in disease. With the recent emphasis on targeting chemokines for therapeutic reasons, it is imperative to identify specific differences in gene expression underlying these mechanisms.

MATERIAL AND METHODS

CELL CULTURE

Six week old C57/BL6 mice were obtained from the Animal Resources Center (Perth, Australia). Experiments were approved by the Animal Ethics Committee of Griffith University. At 8 weeks of age, cells from the bone marrow were obtained from humeri and tibiae after euthanasia. Bone marrow cells were flushed with α-MEM media (Invitrogen, USA) and filtered through a 600 μm cell strainer (Invitrogen). The cells were then seeded in 150 × 25 mm round cell culture dishes at a density of $1.5 \times 10^4/cm^2$. To differentiate these cells, M-CSF (30 ng/ml) was added for macrophages, M-CSF and RANKL (35 ng/ml) for osteoclasts and GM-CSF and IL-4 (both 50 ng/ml) for FBGCs. All cytokines were purchased from PeproTech Asia (Rehovot, Israel). Cells were cultured with α-MEM along with 10% FCS and 1% Penicillin and incubated in a humidified atmosphere of 5% CO_2 at 37°C for 4 days. Cells were fixed with acetone/citrate solution for TRAP staining (Sigma-Aldrich MO, USA).

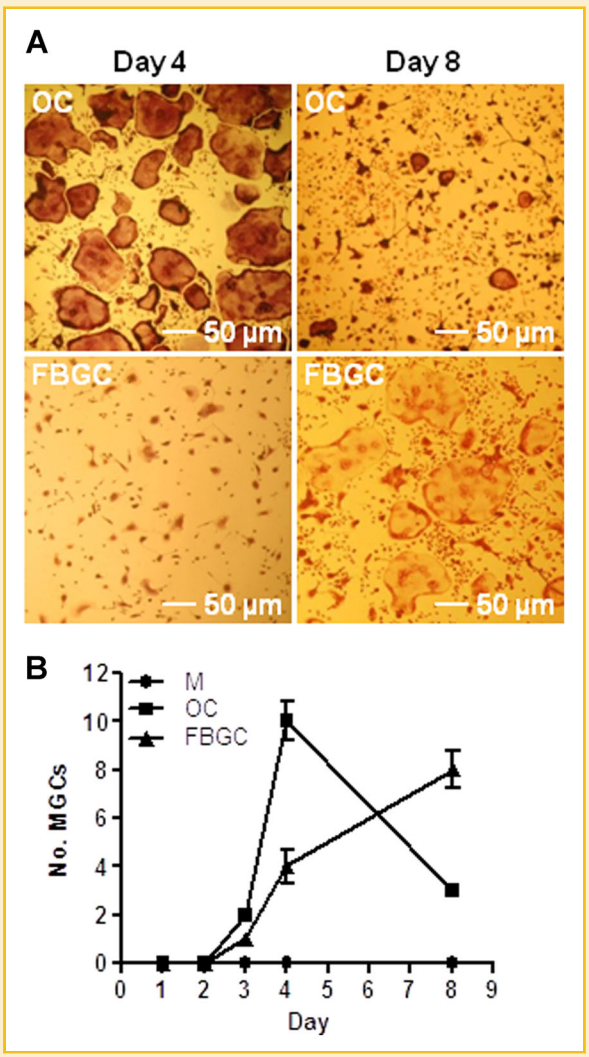

Fig. 1. TRAP staining of multinucleated giant cells (MGCs). A. TRAP positive osteoclasts were observed on day 4 while FBGCs were TRAP negative. On Day 8, however, TRAP positive FGBCs were noted. B. There was significant increase in the number of MGCs in osteoclast cultures compared to FBGCs on day 4 while on day 8 the increase was observed in FBGCs.

QUANTITATIVE PCR

The Nucleospin RNA II (Macherey-Nagel PA, USA) kit was used to extract mRNA from the cell on each day from day 0 to day 4, following the manufacturer's instructions. RNA was converted to cDNA using ImProm-II reverse transcriptase (RT, Promega) and oligo dT primer according to the manufacturer's protocol. Analysis was undertaken with Bio-Rad iCycler in the presence of Bio-Rad SYBR II green supermix according to the manufacturer's instructions. Day 0 was used as a reference. Primers used in this study are listed in Table I.

WESTERN BLOT

All the cells were first washed with phosphate buffer saline (PBS) three times and lysed in RIPA buffer (Thermo Scientific, Sydney). Protein concentrations were measured with DC protein assay (Biorad) using BSA standards. SDS-PAGE gel electrophoresis was used to separate equal amounts of proteins before transferring to polyvinylindene fluoride membranes. 5% skimmed milk was used to block the membrane for 2 h and subsequently probed with primary anti-mouse NFATc1 or calnexin antibody at 1:500 dilutions overnight. Membranes were then washed five times with tris-buffer saline with 0.5% Tween 20 (TBST) and exposed to secondary horseradish peroxidise conjugated antibody for 2 h. Subsequently, the membranes were washed five times with TBST and visualised using peroxidase chemiluminescence assay according the manufacturer's protocols (Bio-Rad).

GELATINE ZYMOGRAPHY

Cell culture media was collected from the different treatments. Equal amount of protein were diluted with non reducing zymograph buffer (62 mM Tris-HCl, pH 6.8 with 4% SDS, 25% glycerol and 0.01% bromophenol blue) and incubated for 5 min at room temperature. Samples were then loaded on to denaturing SDS polyacrylaminde gel with gelatine (Bio-Rad, USA) and after running the gel at 100 V, gels were washed with renaturing buffer (2.5% Triton X-100) for 30 min at room temperature and then incubated in a development solution (50 mM Tris, 200 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij 35) at room temperature for 2 days. Finally, gels were stained with coomassie blue R-250 for 1 h and subsequently destained in menthanol/acetic acid until clear bands were visualised against blue background.

STATISTICAL ANALYSIS

Analysis of Variance (ANOVA) was used to test the difference in cell count and gene expression of the four phenotypic cells; Scheffe's test was used as a post Hoc test. Significance was accepted at $P < 0.05$.

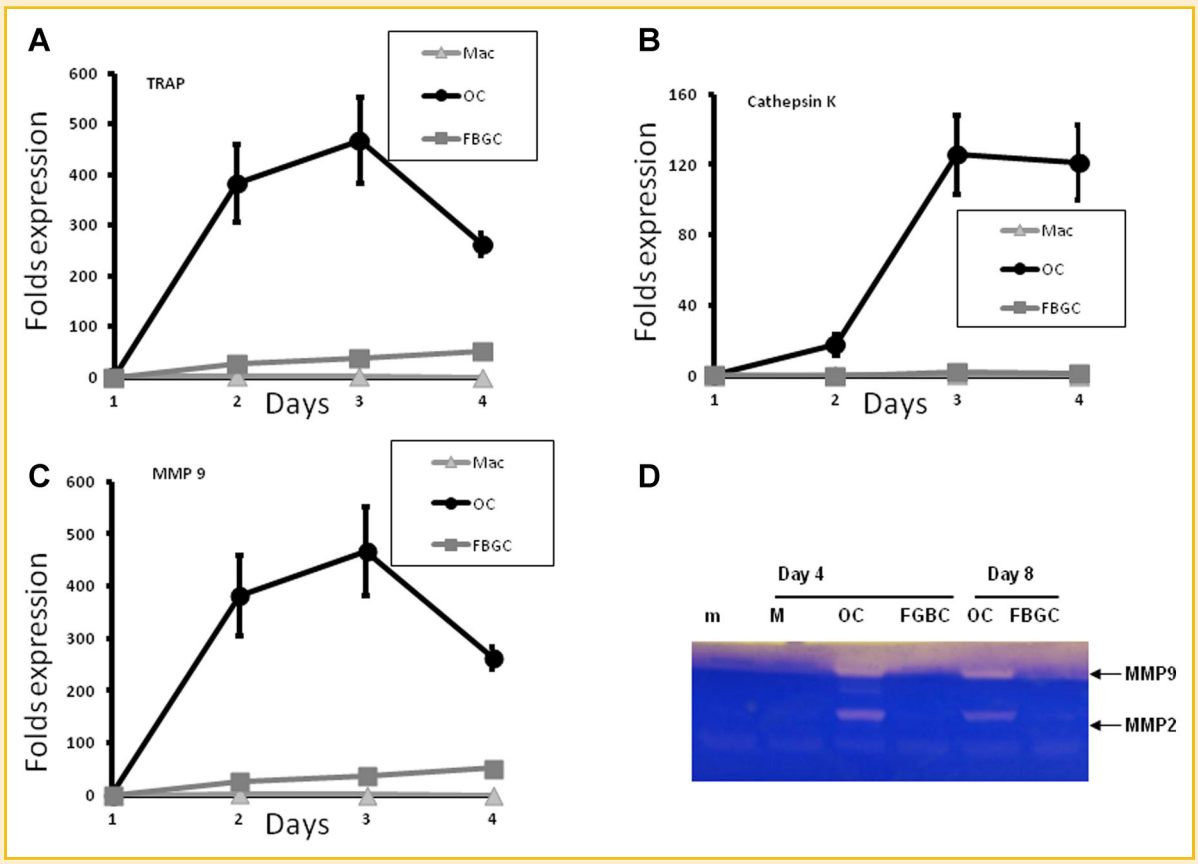

Fig. 2. Proteinase expression by osteoclasts and FBGCs. A. Osteoclasts expressed TRAP significantly more than FBGCs. B. Cathepsin K was highly expressed by osteoclast while it was not detected in FBGCs. C. Osteoclasts expressed (1000-fold) MMP 9 significantly more than FBGCs. D. Zymograph of MMP 2 and MMP 9 clearly shows a band for osteoclasts (OC) while no bands were seen for FBGCs, macrophages (M) and media (m).

RESULTS

OSTEOCLASTS HAVE A SHORTER LIFE-SPAN COMPARED TO FBGCs

TRAP staining was used to determine the size and number of giant cells (Figure 1A). Mature osteoclasts were seen on day 4, but when cultures were extended to day 8 fewer osteoclasts were observed ($P < 0.05$). In contrast, FBGCs were recorded in low numbers on day 4, but increased their number and number of nuclei when cultures were extended to 8 days ($P < 0.05$) (Figure 1B). Furthermore, microscopic analysis showed significantly greater intensity for TRAP staining in osteoclasts compared to FBGCs on day 4 (Figure 1A). This was also supported by gene expression analysis. FBGCs showed low levels of TRAP gene expression compared to osteoclasts which demonstrated sustained expression over 4 days. FBGCs were TRAP positive on day 8 (Figure 2A).

FBGCs ARE NEGATIVE FOR MMP2, MMP9, TRAP AND CATHEPSIN K

qPCR expression and zymography was used to compare the expression of proteinases in macrophages, osteoclasts and FBGCs (Figure 2). Gene expression analysis showed a significant difference in MMP9 ($P = 2.8 \times 10^{-35}$), CTSK ($P = 1.15 \times 10^{-19}$) and TRAP transcripts ($P = 1.0 \times 10^{-13}$) (Figure 2). TRAP expression was undetectable in macrophages, while osteoclasts showed a 450-fold increase in TRAP mRNA. In comparison, FBGCs showed a 15-fold change in TRAP expression (Figure 2A). Osteoclasts showed 1000-fold more CTSK expression compared to macrophages and FBGCs (Figure 2B). Furthermore, osteoclasts showed 1000-fold more expression of MMP9 compared to macrophages and FBGCs (Figure 2C). Gelatin zymography supported the gene expression data, showing no bands for MMP9 by macrophages and FBGCs, while osteoclast showed a clear band for MMP9 and MMP2 (Figure 2D).

OSTEOCLAST MARKERS ARE NOT INDUCED IN FBGCs

There was a significant difference in NFATc1 ($P = 6.8 \times 10^{-11}$) between osteoclasts, macrophages and FBGCs (Figure 3A). On day 4 osteoclasts expressed more than 43-fold NFATc1 compared to macrophages, while osteoclasts expressed more than 100-fold NFATc1 compared to FBGCs. RANK expression was also significantly ($P = 8.4 \times 10^{-10}$) different between the three cell types. Osteoclasts expressed RANK twofold more than macrophages and eightfold more than FBGCs (Figure 3B). Western blot confirmed the gene expression data of NFATc1 as FBGCs showed no bands (Figure 3C). We also observed light band of NFATc1 on day 8 compared to day 4 that supports our earlier assumption that osteoclasts are short lived (Figure 1).

CCL2, 3 AND 9 CHEMOKINES ARE HIGHLY EXPRESSED BY FBGCs COMPARED TO OSTEOCLASTS

Gene expression was used to compare important chemokines and their relevant receptors in macrophages, osteoclasts and FBGCs. There was a significant difference in the level of CCL2 ($P = 1.2 \times 10^{-12}$), CCL3 ($P = 1.4 \times 10^{-4}$), CCL4 ($P = 2.3 \times 10^{-16}$) and CCL9 ($P = 2.7 \times 10^{-8}$) expression in the three types of cells. CCL2 was expressed by FBGCs 10-fold more than osteoclasts (Figure 4A). FBGCs expressed CCL3 17-fold more compared to macrophages and

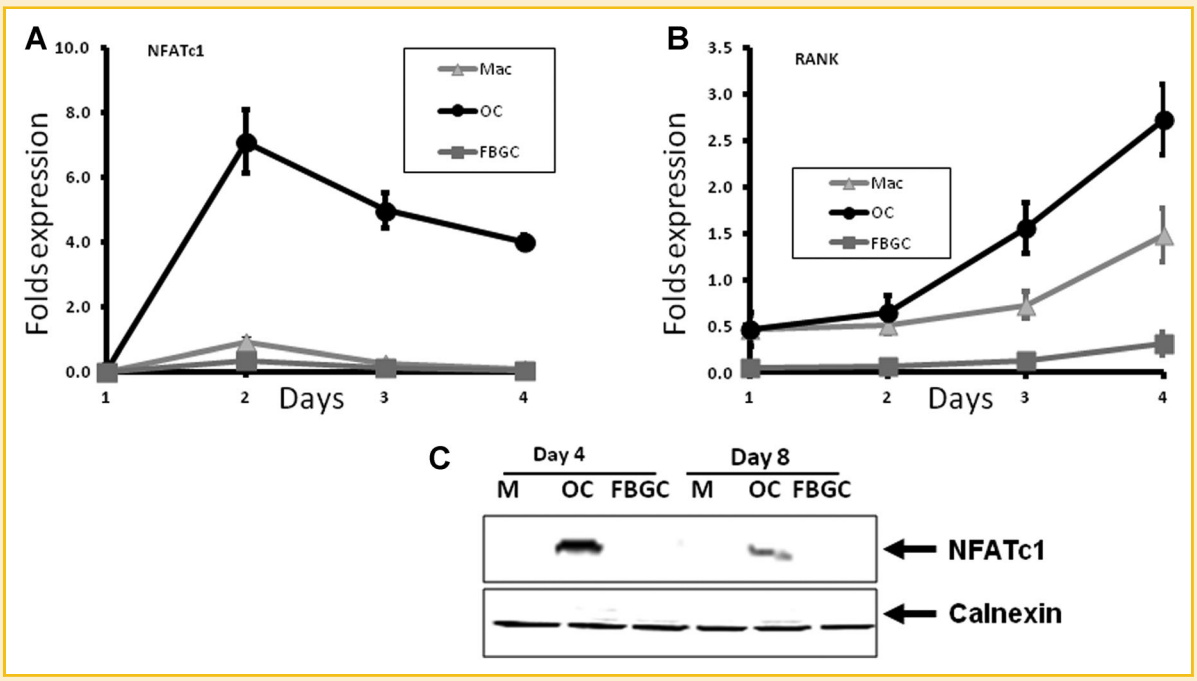

Fig. 3. RANK and NFATc1 was expressed by osteoclasts significantly more than FBGCs. A. Western blot of NFATc1 shows a clear band for osteoclasts (OC) while macrophages (M) and FBGCs did not show a band for NFATc1. B. RANK expression was significantly inhibited in FBGCs compared to osteoclasts. C. Gene expression supported by Western blots: NFATc1 was expressed significantly more (100-fold) by osteoclasts compared to FBGCs.

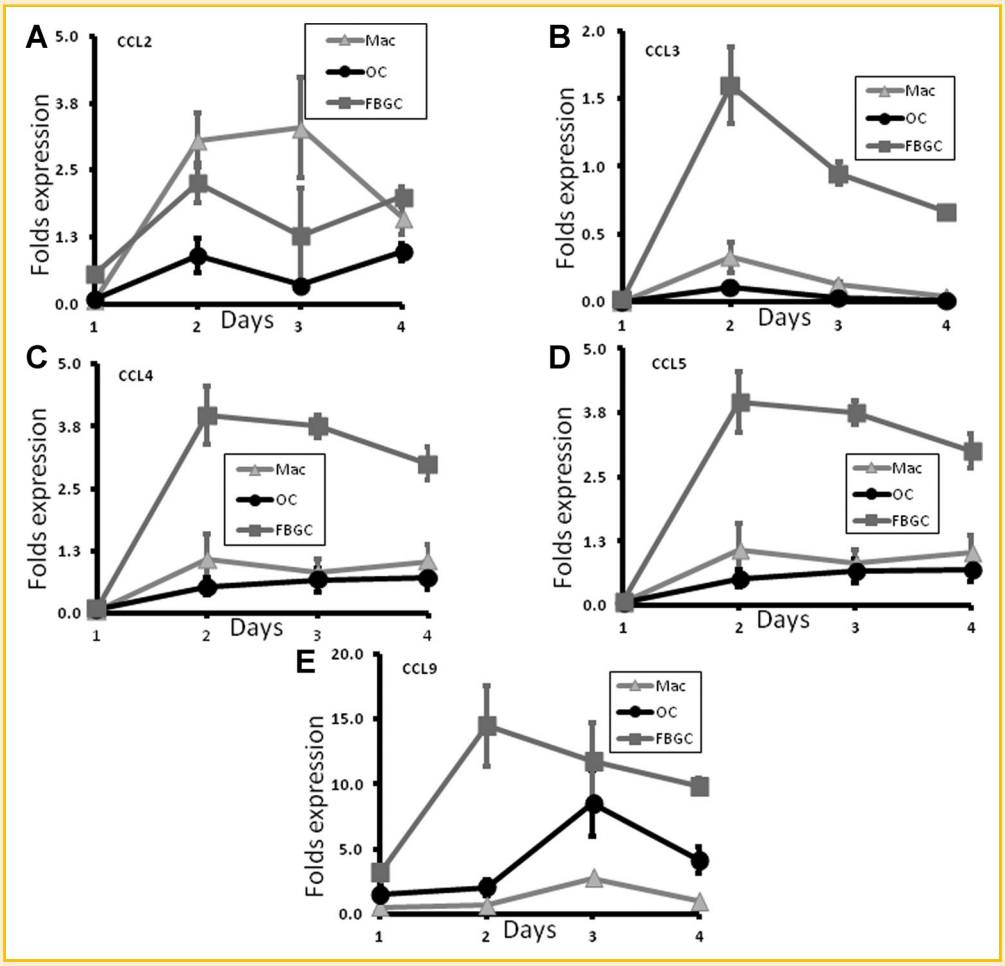

Fig. 4. Expression of chemokines by osteoclasts and FBGCs. A. FBGCs expressed CCL2 significantly more than osteoclasts but the difference between macrophage and FBGCs were not significant. B. CCL3 (MIP1α) was expressed significantly more by FBGCs than osteoclasts and macrophages. C. FBGCs expressed CCL4 (MIP1β) significantly more than osteoclasts and macrophages. D. CCL5 (RANTES) was expressed by FBGCs significantly more than osteoclasts and macrophages. E. CCL9 (MIP1γ) was expressed significantly more by FBGCs on day 2 compared to osteoclasts while on day three the difference was not significant, on day 4 however, CCL9 was again expressed more by FBGCs than osteoclasts.

54-fold more expression compared to osteoclasts (Figure 4B). FBGCs expressed CCL4 fivefold more than macrophages and 40-fold more compared to osteoclasts (Figure 4C). CCL5 was expressed by FBGCs eightfold more than osteoclasts (Figure 4D). FBGCs expressed CCL9 twofold more than osteoclasts and 10-fold more compared to macrophages (Figure 4E).

OSTEOCLASTS EXPRESSED CCR1, 2 AND 3 HIGHER THAN FBGCs

There was a significant difference in the expression of CCR1 ($P = 1.7 \times 10^{-8}$), CCR2 ($P = 1.4 \times 10^{-5}$) and CCR3 ($P = 3 \times 10^{-7}$) between macrophages, osteoclasts and FBGCs (Figure 4). Osteoclasts expressed CCR1 more than fivefold compared to FBGCs while 20-fold more compared to macrophages (Figure 5A). Osteoclasts expressed CCR2 16-fold more than macrophages and 40-fold compared to FBGCs (Figure 5B). Osteoclasts expressed CCR3 sixfold more than macrophages and 17-fold more compared to FBGCs (Figure 5C). Macrophages expressed CCR5 about eightfold more than osteoclasts and FBGCs (Figure 5D). There was also a significant difference in the GM-CSFRα ($P = 0.006$) expression. FBGCs expressed 10-fold more GM-CSFRα compared to osteoclasts and 16-fold more compared to macrophages (Figure 5E).

CD44 AND INTEGRIN $\alpha_V\beta_3$

Expression analysis of adhesion genes showed significant differences between macrophages, osteoclasts and FBGCs (Figure 6). Gene expression analysis showed a significant difference in CD44 expression ($P = 0.008$). Osteoclasts showed fourfold more expression of CD44 compared to FBGCs, while in comparison to macrophages a twofold increase was observed (Figure 6A). Furthermore, there was a significant difference in the $\alpha_V\beta_3$ expression ($P = 1.9 \times 10^{-7}$). Osteoclasts expressed $\alpha_V\beta_3$ more than 10-fold compared to macrophages while osteoclasts expressed 16-fold more $\alpha_V\beta_3$ compared to FBGCs (Figure 6B).

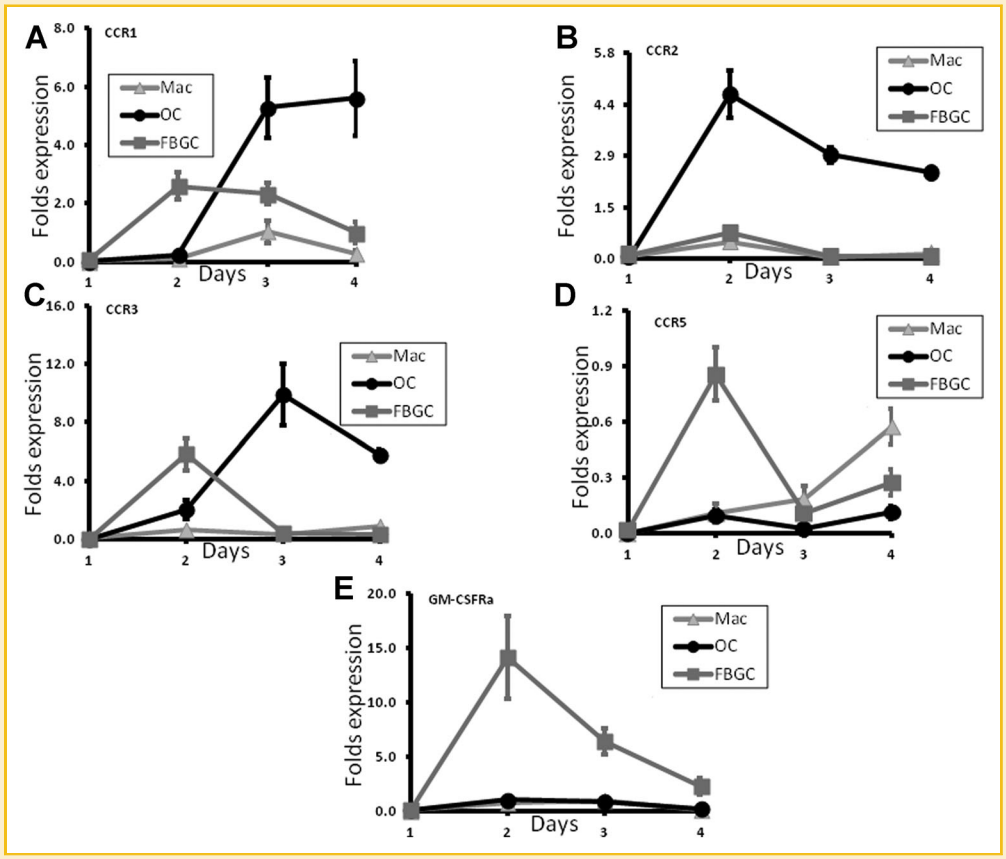

Fig. 5. Chemokine receptor expression in different osteoclasts and FBGCs. A. Osteoclasts expressed CCR1 significantly more than FBGCs. B. CCR2 was expressed by osteoclasts significantly more than FBGCs and macrophages. C. CCR3 expression was significantly higher in osteoclasts compared to FBGCs. D. CCR5 expression was significantly higher in FBGCs on day 2, while on day 4 macrophages expressed CCR5 higher than osteoclasts. E. GMCSF receptor (GM-CSFRa) was expressed significantly higher by FBGCs on day 2 and 3 while the difference was not significant on day 4.

DISCUSSION

RANK is a member of the tumor necrosis factor family of proteins and is expressed in osteoclasts and their precursors. Its expression in monocytes is increased by the presence of MCSF [Cappellen et al., 2002], and it is essential for osteoclast development [Dougall et al., 1999]. Activation of RANK selectively induces NFATc1 expression [Takayanagi et al., 2002], and its inactivation results in decreased osteoclast formation [Takayanagi et al., 2002]. Alternatively, over-expression of NFATc1 increases the resorptive activity of osteoclasts on bone slices [Song et al., 2009]. We observed that gene expression for RANK and NFATc1 was significantly higher in osteoclasts compared to FBGCs, the RANK pathway in osteoclasts is inhibited by IL-4 [Moreno et al., 2003; Stein et al., 2008] and GM-CSF [Hodge et al., 2004] and both IL4 and GM-CSF direct monocytes to the FBGC lineage [McNally and Anderson, 1995].

MMP9 is expressed at high levels during osteoclast development [Reponen et al., 1994], an observation supported by our study. NFATc1 signalling is a downstream regulator of RANK and RANK mediates MMP9 expression during osteoclast function [Sundaram et al., 2007]. However, IL4 attenuates MMP9 expression [MacLauchlan et al., 2009; Hyc et al., 2011], and we observed that MMP9 was not expressed by FBGCs. CD44 and Integrin αVβ3 are important for osteoclasts because both CD44 and Integrin αVβ3 control the motility of osteoclasts by downregulation of MMP9 [Spessotto et al., 2002; Chellaiah and Hruska, 2003]. We also found that osteoclasts expressed significantly higher levels of Integrin $\alpha V\beta_3$ than any other multinucleated giant cell, consistent with osteoclast attachment to bone. Integrin $\alpha V\beta_3$ facilitates the attachment process, while FBGCs function by phagocytosis and secrete enzymes on to the surface of implants [Boissy et al., 1998]. Cathepsin K is a proteolytic enzyme expressed by osteoclasts [Drake et al., 1996] and is also mediated by RANK signaling through NFATc1 [Matsumoto et al., 2004]. Inhibition of Cathepsin K causes impaired bone resorption [Saftig et al., 1998], exemplifying its importance for osteoclast function. Importantly, high expression of IL4 in synovial tissue reduces the mRNA expression of Cathepsin K, producing a concomitant reduction in the number of osteoclasts [Lubberts et al., 2000]. We observed that osteoclasts express Cathepsin K 64-fold more than FBGCs, which is consistent with

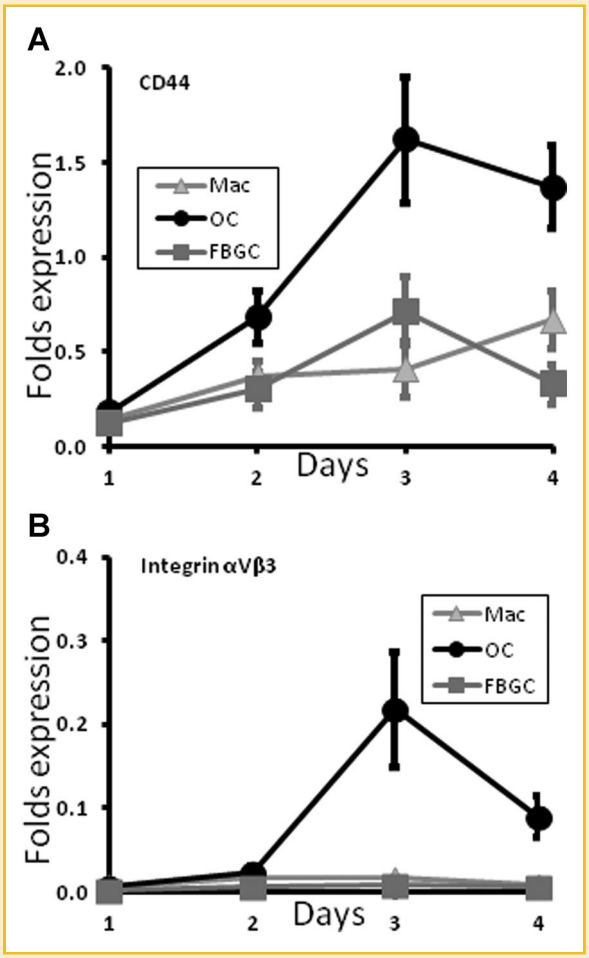

Fig. 6. Integrin alpha v beta 3 (Iαvβ3) and CD 44 expression by osteoclasts and FBGCs. A. Iαvβ3 was expressed by osteoclasts significantly more than macrophages and FBGCs. B. Osteoclasts expressed CD 44 significantly more than FBGCs.

other reports that IL4 inhibits Cathepsin K expression [Lubberts et al., 2000].

Collection of fluids around newly inserted implants correlates positively with CCL3 (MIP1α) concentration and subsequent inflammation around the implant [Petkovic et al., 2010]. Furthermore, CCL3 stimulates osteoclast formation in human bone marrow culture [Choi et al., 2000]. Inflammatory bone resorption is associated with CCL3, CCL4 (MIP1β) and CCL5 (RANTES) and its receptors CCR1 and CCR5 [Repeke et al., 2010]. CCL9 (MIP1γ), another member of CC chemokine family [Hara et al., 1995; Poltorak et al., 1995], promotes osteoclast formation and survival [Okamatsu et al., 2004]. We found that CCL3, CCL4 and CCL9 are expressed at significantly higher level by FBGCs compared to osteoclasts. In addition, CCL9 and its receptor CCR1 are highly expressed by osteoclasts [Lean et al., 2002], and CCR1 is upregulated after RANK activation [Yu et al., 2004]. We also observed that osteoclasts express CCR1 fivefold more than FBGCs. CCR5 and its ligands CCL3, CCL4 and CLL5 (RANTES) [Schall et al., 1990; Qin et al., 1996] are implicated in bone resorption during periodontal disease [Ferreira et al., 2011]. In contrast, we found that FBGCs expressed CCL5 and CCR5 significantly higher than osteoclasts. CCL2, CCL3 and CCL5 chemokines are very strong factors involved in chemotaxis in macrophages [Volin et al., 1998; Hancock et al., 2000; Ono et al., 2003; Maurer and von Stebut, 2004], which are highly expressed during foreign body reactions [Jones et al., 2007]. Our results confirm the high expression of CCL2, CCL3 and CCL5 in FBGCs. CCR2 is upregulated in preosteoclasts and CCR2 KO mice are resistant to bone loss after ovariectomy [Binder et al., 2009]. Our data also showed that osteoclasts expressed CCR2 40-fold more than FBGCs, while CCR1 was expressed fivefold more by osteoclasts compared to FBGCs. CCL2 has been shown to be important for FBGCs formation [Kyriakides et al., 2004], and high levels of CCL2 are observed in the exudates from foreign body reactions [Kyriakides et al., 2004]. This is consistent with our data showing that foreign body giant cells excrete twice as much CCL2 (also called MCP1) compared to osteoclasts.

The effect of GM-CSF on osteoclasts occurs in a time dependent manner. A short term addition of GM-CSF results in stimulation of osteoclasts while long term exposure attenuates osteoclast formation [Hodge et al., 2004]. GM-CSF receptor alpha (GM-CSFRa) is said to be up regulated in the presence of GM-CSF [Miyamoto et al., 2001]. In addition, GM-CSF along with IL4 also increase the number and size of FBGCs [McNally and Anderson, 1995] which supports our results that FBGCs expressed GM-CSF receptor 10-fold more than osteoclasts.

In conclusion, although osteoclasts and FBGCs share some phenotypic resemblances, they display markedly different gene expression profiles consistent with their functions as bone resorbing cells and tissue phagocytes, respectively. The typical osteoclast markers (TRAP, F-actin and MMP9) are highly osteoclast specific, and expressed at very low levels, or not at all, by FBGCs. In contrast chemokines such as CCL2, CCL3, CCL4, CCL5, CCL9 and GM-CSFRa were highly expressed by FBGCs compared to osteoclasts while their receptors CCR1, CCR2 and CCR3, were highly expressed by osteoclasts except CCR5. Thus, the relative differential expression of chemokines and their receptors on FBGC and osteoclasts may explain clinically relevant cell–cell interactions in inflammatory implantitis. During peri-implantitis various cytokines and chemokines are released [Petkovic-Curcin et al., 2011]. These chemokines are reported to stimulate osteoclasts during various inflammatory conditions [Oba et al., 2005; Fransson et al., 2009; Miyamoto et al., 2009; Maruotti et al., 2011]. The chemokines detected in our model of FBGCs are known to stimulate osteoclasts (Figure 3 and 4). Furthermore, we observed the expression of the chemokine receptors (CCR1, 2 and 3) on osteoclasts (Figure 3 and 4). As osteoclasts and FBGCs are observed in close proximity during foreign body reactions [Nuss and von Rechenberg, 2008], it is possible that chemokines produced by FBGCs during peri-implantitis [Anderson et al., 2008; Petkovic et al., 2010; Venza et al., 2010] work on the receptors expressed by osteoclasts (CCR1, 2 and 3) resulting in increased bone resorption [Oba et al., 2005; Fransson et al., 2009; Miyamoto et al., 2009; Maruotti et al., 2011] leading to implant loosening [Al-Nawas et al., 2012].

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