Effect of Vitamin D on Peripheral Blood Mononuclear Cells from Patients with Psoriasis Vulgaris and Psoriatic Arthritis

Susana Cubillos*, Nadine Krieg, Johannes Norgauer

Department of Dermatology, Jena University Hospital, Jena, Thüringen, Germany

*s_cubillos@yahoo.com

Abstract

Background
Psoriasis, a chronic skin disease with or without joint inflammation, has increased circulating proinflammatory cytokine levels. Vitamin D is involved in calcium homeostasis, bone formation, osteoclastogenesis and osteoclast activity, as well as regulation of immune response. We aimed to study osteoclast differentiation and cytokine secretion of peripheral blood mononuclear cells (PBMCs) from patients with psoriasis vulgaris and psoriatic arthritis, in response to 1,25(OH)2D3.

Methods
Serum levels of bone turnover markers were measured by ELISA in patients with psoriasis vulgaris and psoriatic arthritis, and healthy controls. PBMCs were isolated and cultured with or without RANKL/M-CSF and 1,25(OH)2D3. Osteoclast differentiation and cytokine secretion were assessed.

Results
Psoriatic arthritis patients had lower osteocalcin, as well as higher C-telopeptide of type I collagen and cathepsin K serum levels compared with psoriasis vulgaris patients and controls. PBMCs were isolated and cultured with or without RANKL/M-CSF and 1,25(OH)2D3. Osteoclast differentiation and cytokine secretion were assessed.

Conclusions
Our data confirmed altered bone turnover in psoriatic arthritis patients, and demonstrated increased osteoclastogenic potential and proinflammatory cytokine secretion capacity of these PBMCs compared with psoriasis vulgaris and controls. 1,25(OH)2D3 abrogated these effects.
Introduction

Psoriasis is a chronic inflammatory skin disease with or without joint inflammation. Phototherapy and topical application of vitamin D analogs are widely used in the treatment of psoriasis vulgaris. Topical vitamin D regulates serum calcium levels and phototherapy alters systemic levels of vitamin D, a crucial factor in the regulation of extracellular calcium homeostasis and bone metabolism [1,2]. Osteoclasts resorb mineralized bone and osteoblasts are responsible for new bone formation. Osteoclasts are multinucleated cells derived from the monocyte/macrophage lineage [3]. Osteoclast differentiation is supported by osteoblasts through cell-to-cell interactions and two major cytokines—receptor activator of NF-κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF) [4-6]. Activation of the receptors RANK and c-Fms on osteoclast precursors by these ligands induces calcium signalling pathways linked to activation of the nuclear factor of activated T cells cytoplasmic 1 (NFATc1) [7,8], which regulates the expression of osteoclast-specific markers such as the type I collagen degrading cathepsin K (CTSK), the osteopontin dephosphorylating tartrate-resistant acid phosphatase (TRAP) and calcitonin receptor [9-11]. This receptor binds to calcitonin (CT), a hormone produced primarily by thyroid C-cells in response to elevated serum calcium levels [10]. CT reduces blood calcium, through inhibition of bone resorption [12] and regulation of 1,25(OH)₂D₃ production in the kidney [13]. In addition to the hormonal control of calcium homeostasis, the vitamin D active form 1,25(OH)₂D₃ functions on cellular growth, proliferation and differentiation. Locally produced 1,25(OH)₂D₃ by osteoblasts is involved in the regulation of osteoclastogenesis and osteoclast activity [14] increasing the expression of RANKL as well as decreasing the expression of its antagonist osteoprotegerin in osteoblasts [15]. Cells of the monocyte/macrophage lineage hydroxylate 25(OH)D₃ into 1,25(OH)₂D₃ [16,17]. In particular, PBMCs-derived osteoclasts also respond to vitamin D through vitamin D receptor with increased NFATc1 expression [18].

Bone and immune cells share bone marrow progenitors and are affected by the same cytokines and metabolites such as 1,25(OH)₂D₃. In fact, 1,25(OH)₂D₃ inhibits the expression of cytokines such as IL-1, IL-2, IL-6, IL-12, IL-23, interferon γ (IFN-γ), tumour necrosis factor α (TNF-α) and chemokines such as IL-8 and chemokine (C-C motif) ligand 5 (CCL5 or RANTES) by monocytes, T and B cells [19-22]. Conversely, 1,25(OH)₂D₃ increases production of IL-10 of activated T and B cells, and interferon β in osteoclast precursors [23,24]. Besides high levels of circulating proinflammatory cytokines [25,26], patients with psoriatic arthritis have higher circulating bone and cartilage degradation products [27,28] and higher number of osteoclast precursors than healthy individuals [29]. Cytokine effects on the osteoclast differentiation and activity are well studied, but knowledge about cytokine secretion pattern of PBMCs derived from patients with psoriasis vulgaris and psoriatic arthritis and their capacity to differentiate into mature osteoclasts is limited. Therefore we aimed to study the osteoclast differentiation and cytokine secretion capacity of PBMCs from psoriasis vulgaris and psoriatic arthritis patients in response to 1,25(OH)₂D₃. We found increased osteoclastogenic potential and proinflammatory cytokine secretion capacity of PBMCs from patients with psoriatic arthritis compared with psoriasis vulgaris and controls. In addition, 1,25(OH)₂D₃ abrogated these effects.

Materials and Methods

Subject characteristics

This study, approved by the Ethics Committee at the Medical Faculty of the Friedrich-Schiller University Jena (Project 1940-01/07), was conducted according to the principles of the
Declaration of Helsinki. Written consent was obtained from all participants prior to enrolment. Patients were diagnosed based on clinical and pathological findings at the Department of Dermatology of the Jena University Hospital. All patients fulfilled the CASPAR criteria [30]. The presence of joint manifestations was confirmed with power doppler ultrasonography (Esaote, Italy) and Rheumascan Xeralite (Mivenion GmbH, Germany). Twenty one patients with psoriasis vulgaris and fifteen healthy controls (HC) were included. Nine patients with psoriasis vulgaris had no clinical signs of joint inflammation (PsV) and twelve were diagnosed with psoriatic arthritis (PsA). Demographic data are shown in S1 Table. Patients with other types of psoriasis (guttate, inverse, pustular, erythrodermic), other skin diseases, allergy, autoimmune diseases, any topical or systemic treatment, including vitamin D supplementation or phototherapy 5 months before or at the time of recruitment were excluded.

Serum levels of bone turnover markers

Human calcitonin (CT) and 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] (Shanghai Sunred Biological Technology Co., Ltd, China), osteocalcin (OCN) (ALPCO Diagnostics, USA), C-telopeptide of type I collagen (CTX-1) and of type II collagen (CTX-2) (CUSABIO Biotech Co., Ltd, China), and cathepsin K (CTSK) (Uscn Life Science Inc, China) ELISA kits were used according to the manufacturer instructions to assess the respective patient and control serum levels. Detection ranges of ELISA kits were: 0.7–200 mmol/L for CT, 0.7–150 ng/ml for 1,25-(OH)2D3, 0.31–1250 ng/ml for OCN, 25–800 ng/ml for CTX-1, 0.312–20 ng/ml for CTX-2, and 15.6–1000 pg/ml for CTSK.

Serum calcium levels

Serum calcium levels were determined using the QuantiChrom calcium assay kit (BioAssay Systems, USA). The assay was used according to the manufacturer instructions to assess the respective patient and control serum levels. The linear detection range was 0.08 to 20 mg/dl.

Isolation and culture conditions of PBMCs

PBMCs were isolated from blood samples by density gradient centrifugation. In 24-well plates, 1 x 10⁶ cells/ml/well (0.5 x 10⁶ cells per cm²) containing alpha MEM medium with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin were placed. Cells were cultured with or without 30 ng/ml RANKL and 25 ng/ml M-CSF (Promokine GmbH, Germany), in the presence or absence of 10 nM calcitriol (1,25(OH)2D3, Cayman Chemical, USA). The medium was replenished at 4, 8, 11 and 14 days, and after 14 days cells were fixed for tartrate-resistant acid phosphatase (TRAP) staining and 200 µl/well supernatant six wells of each condition were pooled and stored at -20°C until cytokine levels and TRAP enzymatic activity were measured.

TRAP activity assay

At 14 days in culture, mature osteoclasts were identified as TRAP+ multinucleated cells and counted per microscope field after incubation with TRAP stain (Sigma-Aldrich, USA) at 37°C for 5 to 10 minutes. In addition, TRAP enzymatic activity in PBMCs culture supernatants was measured at 14 days as follows: a standard curve was made by using known concentrations naphthol (Sigma-Aldrich, USA) as substrate and the supernatant from the sample with the highest amount TRAP+ cells. Samples and the standard curve with TRAP stain solution were added to microtiter plates, incubated at 37°C for 10 hours, and absorbance at 540 nm was measured. The amount of dephosphorylated substrate (nmoles) in the samples was calculated by
comparing their absorbance with a 4-parameters-logistic substrate standard curve. Data was normalized against total protein and results were expressed as nmoles/mg protein/hour.

Cytokine levels in PBMCs culture supernatants
Levels of TNF-α, IL-1b, IFN-γ, IL-17, IL-23, IL-2, RANTES and IL-10 in 14 days culture supernatants were simultaneously and concurrently assessed with Q-Plex multiplex arrays according to manufacturer instructions. At least five samples from each patient and control were tested. Each cytokine concentration in the samples was calculated by comparing the sample chemiluminescence intensities with the chemiluminescence intensities of the standard curves with the Q-View software (QUANSYS Biosciences, USA).

Statistical analyses
Statistical analyses were performed using the GraphPad Prism software (GraphPad Software, Inc, USA). Differences between groups were analyzed by Mann-Whitney or t-test in the case of two groups, and further one-way analysis of variance (ANOVA) with Dunnett's multiple comparison tests in the case of more than two groups. Additionally of Mann-Whitney for two groups, cytokine profiling grouped data were analyzed by two-way ANOVA with post hoc Bonferroni test. Spearman’s non-parametric correlation test was also applied. P<0.05 was considered statistically significant.

Results
Subject demographics
We did not find significant differences in age between HC, PsV and PsA groups (after 1 way ANOVA analysis with Bonferroni’s multiple comparison test). Furthermore, although we found a significant age effect (P<0.05) from females in the PsV group (significant older than control females), we did not observe any significant interaction between age and gender after two way ANOVA with Bonferroni post test analysis (S1 Table).

Serum bone turnover markers
Product of bone degradation such as CTX-1 and the hormone OCN involved in bone formation are useful markers to assess bone turnover and already reported as bone remodelling markers in patients with PsA [28,31]. Here we assessed serum levels of these markers to confirm bone turnover status in the patient and control samples evaluated. We found no significant differences in CTX-1 and OCN serum levels between patients with PsV and controls; however, higher levels of CTX-1 (P = 0.002) and lower levels of OCN (P = 0.011) were observed in the serum of patients with PsA compared with controls (Fig 1A and 1B). The CTX-1/OCN ratios were also highly significant in patients with PsA compared with controls (P = 0.0004, Fig 1C). In contrast to CTX-1, no significant differences in CTX-2 serum levels were found in any of the analyzed groups (Fig 1D). It is well known that osteoclasts use CTSK to degrade collagen type I, and CTX-1 is a product of this degradation [32]. Here we investigate for the first time CTSK serum levels to assess bone degradation status in patients with psoriasis. In addition to higher CTX-1/OCN ratios in patients with PsA compared with controls, we found also higher levels of CTSK in patients with PsA compared with PsV (P = 0.028, Fig 1E). Also, levels of degradation product per enzyme CTSK (CTX-1/CTSK ratios) were highly significant in patients with PsA compared with controls (P = 0.002, Fig 1F). Furthermore, we found a significant inverse Spearman’s coefficient correlation between CTSK and CT levels in patients with PsA.
(r = -0.711, P = 0.014) indicating the reciprocal relationship between both parameters (Table 1).

Serum levels of 1,25(OH)2D3, calcium and calcitonin

Next, 1,25(OH)2D3, calcium and CT levels in serum from patients with PsV and PsA, and controls were analysed (Fig 2). No significant differences between these groups were observed as expected. However, we found a significant positive Spearman’s coefficient correlation between CT and 1,25(OH)2D3 in patients and controls, indicating a direct and very strong relationship between increase or decrease of both variables in controls and a less strong relationship in case of psoriasis patients (HC, r = 0.947, P < 0.0001; PsV, r = 0.714, P = 0.047; PsA, r = 0.711, P = 0.014; Table 1).

Comparison of TRAP enzymatic activity between cultured PBMCs

PBMCs from controls and patients with PsV and PsA were incubated with or without RANKL/M-CSF (RM) in the presence or absence of 1,25(OH)2D3 (Vit D). Thereafter, TRAP enzymatic activity was measured in order to assess osteoclast activity. No differences were observed between PBMCs from patients and controls under culture control conditions, but in the presence of RM, PBMCs of PsA showed higher TRAP enzymatic activity compared with PBMCs from PsV and controls (P < 0.05, P = 0.041, respectively; Fig 3A–3C). However, a significant higher number of PsA multinucleated TRAP+ cells per field was observed in the absence or presence of RM compared with PBMCs of PsV and controls (P < 0.001, Fig 4A–4C). Next the effect of 1,25(OH)2D3 on TRAP activity was analysed in PBMCs of HC, PsV and PsA.
We observed no effect of 1,25(OH)\(_2\)D\(_3\) in the case of control PBMCs while 1,25(OH)\(_2\)D\(_3\) slightly inhibited TRAP activity from PBMCs of PsV compared with culture control conditions \((P < 0.05)\). Furthermore, 1,25(OH)\(_2\)D\(_3\) diminished TRAP activity in PBMCs of PsA, even in the presence of RM compared with culture control conditions \((P = 0.008, P = 0.040, \text{ respectively})\) and with RM alone \((P < 0.05)\) (Fig 3A–3C). However, 1,25(OH)\(_2\)D\(_3\) diminished the number of multinucleated TRAP+ cells from all groups in the presence of RM, but in a greater proportion and even in the absence of RM in PBMCs of PsA \((P < 0.001, \text{ Fig 4A–4C})\).

Differential cytokine secretion during osteoclastogenesis in vitro

Vitamin D plays an important role in the modulation of the immune system [19–24]. Therefore, we assessed the effect of vitamin D on cytokine secretion profile from psoriatic PBMCs upon osteoclastogenesis. Data was normalized due to baseline secretion differences between individual samples from the same group and treatment, as follows: For each sample, culture condition and cytokine, a fold change was calculated; equal to the cytokine concentration in the stimulated (RM, Vit D and RM+VitD) divided by the culture control condition (without RM, Vit D and RM+VitD) (Fig 5).

Table 1. Spearman coefficient correlation analysis of serum parameters in healthy controls and patients with psoriasis vulgaris and psoriatic arthritis.

| Serum parameters | HC \((n = 14)\) | PsV \((n = 8)\) | PsA \((n = 11)\) |
|------------------|----------------|----------------|----------------|
| CTSK vs CT       | -0.16 (0.57)   | -0.41 (0.32)   | -0.71 (0.01)   |
| CTSK vs Ca       | -0.09 (0.75)   | -0.14 (0.74)   | -0.09 (0.79)   |
| CTSK vs Vit D    | -0.17 (0.55)   | -0.48 (0.23)   | -0.53 (0.10)   |
| CTSK vs CTX-1    | 0.42 (0.14)    | 0.55 (0.16)    | -0.22 (0.52)   |
| CTSK vs OCN      | 0.09 (0.77)    | -0.48 (0.23)   | 0.09 (0.79)    |
| CTSK vs CTX-1/OCN| 0.35 (0.22)    | 0.52 (0.18)    | -0.19 (0.57)   |
| CTSK vs CTX-2    | 0.09 (0.76)    | 0.50 (0.20)    | -0.09 (0.79)   |
| CT vs Ca         | -0.35 (0.22)   | 0.38 (0.35)    | -0.10 (0.77)   |
| CT vs Vit D      | **0.95 (<0.0001)** | **0.71 (<0.05)** | **0.71 (0.01)** |
| Ca vs Vit D      | -0.21 (0.47)   | 0.38 (0.35)    | 0.05 (0.89)    |
| CT vs CTX-1      | 0.29 (0.31)    | -0.43 (0.30)   | 0.12 (0.72)    |
| CT vs OCN        | 0.11 (0.70)    | 0.36 (0.39)    | 0.11 (0.76)    |
| CT vs CTX-2      | -0.12 (0.71)   | -0.14 (0.75)   | -0.32 (0.34)   |
| Ca vs CTX-1      | -0.28 (0.33)   | -0.10 (0.82)   | 0.00 (1.00)    |
| Ca vs OCN        | -0.15 (0.62)   | 0.57 (0.14)    | -0.23 (0.50)   |
| Ca vs CTX-2      | -0.39 (0.19)   | -0.10 (0.82)   | 0.46 (0.15)    |
| Vit D vs CTX-1   | 0.32 (0.26)    | -0.02 (0.96)   | -0.01 (0.98)   |
| Vit D vs OCN     | 0.01 (0.98)    | 0.52 (0.18)    | 0.35 (0.30)    |
| Vit D vs CTX-2   | -0.23 (0.45)   | -0.71 (0.05)   | 0.05 (0.89)    |
| CTX-1 vs OCN     | -0.17 (0.56)   | -0.52 (0.18)   | -0.10 (0.77)   |
| CTX-1 vs CTX-2   | -0.09 (0.78)   | -0.37 (0.37)   | -0.04 (0.92)   |
| OCN vs CTX-2     | -0.01 (0.99)   | -0.13 (0.76)   | 0.36 (0.29)    |

Abbreviations: HC, healthy controls; PsV, psoriasis vulgaris; PsA, psoriatic arthritis; CTSK, cathepsin K; CT, calcitonin; Ca, calcium; Vit D, 1,25(OH)\(_2\)D\(_3\); CTX-1, C-telopeptide of type I collagen; OCN, osteocalcin; CTX-2, C-telopeptide of type II collagen.

\(^{a}\)n is the number of participants. \(P < 0.05\).

doi:10.1371/journal.pone.0153094.T001

We observed no effect of 1,25(OH)\(_2\)D\(_3\) in the case of control PBMCs while 1,25(OH)\(_2\)D\(_3\) slightly inhibited TRAP activity from PBMCs of PsV compared with culture control conditions \((P < 0.05)\). Furthermore, 1,25(OH)\(_2\)D\(_3\) diminished TRAP activity in PBMCs of PsA, even in the presence of RM compared with culture control conditions \((P = 0.008, P = 0.040, \text{ respectively})\) and with RM alone \((P < 0.05)\) (Fig 3A–3C). However, 1,25(OH)\(_2\)D\(_3\) diminished the number of multinucleated TRAP+ cells from all groups in the presence of RM, but in a greater proportion and even in the absence of RM in PBMCs of PsA \((P < 0.001, \text{ Fig 4A–4C})\).
PBMCs of PsA in the presence of RM showed an increased secretion of cytokines such as TNF-α, IL-1β, IFN-γ, IL-17, IL-23, IL-2 and RANTES (91.83 fold, \( P < 0.05 \); 1.62 fold, \( P < 0.05 \); 2.64 fold, \( P < 0.001 \); 8.59 fold, \( P = 0.032 \); 5.89 fold, \( P < 0.01 \); 2.52 fold, \( P = 0.008 \); 3.63 fold.

Fig 2. Levels of 1,25(OH)\(_2\)D\(_3\), calcium and calcitonin in serum from psoriasis patients and controls. Box and whisker plots show serum concentrations of A) 1,25(OH)\(_2\)D\(_3\), B) calcium and C) calcitonin, in healthy controls (HC, \( n = 14 \)), and patients with psoriasis vulgaris (PsV, \( n = 8 \)) and psoriatic arthritis (PsA, \( n = 11 \)) measured by ELISA kits. Box and whisker plots represent median with minimum to maximum values.

doi:10.1371/journal.pone.0153094.g002
Fig 3. Effect of 1,25(OH)2D3 on TRAP activity from psoriasis and control PBMCs during osteoclastogenesis. PBMCs from A) healthy controls (HC, n = 14), and patients with B) psoriasis vulgaris (PsV, n = 9) and C) psoriatic arthritis (PsA, n = 11) were cultured with or without RANKL/M-CSF (RM) in the presence or in the absence of 1,25(OH)2D3 (Vit D); where n is the number of participants. TRAP enzymatic activity was measured in culture supernatants after 14 days. Box and whisker plots represent median with minimum to maximum values. *\( P < 0.05 \) indicate statistically significant differences obtained by one-way ANOVA with Dunnetts’s multiple comparison test, and, #\( P < 0.05 \) and ##\( P < 0.01 \) only by Mann-Whitney or t-test. a\( P < 0.05 \) indicate statistically significant differences between PsA and PsV cultured with RM obtained by one-way ANOVA with Dunnetts’s multiple comparison test, and, b\( P < 0.05 \) between PsA and HC cultured with RM only by Mann-Whitney or t-test.

doi:10.1371/journal.pone.0153094.g003
Fig 4. Effect of 1,25(OH)2D3 on TRAP+ osteoclast number from psoriasis and control PBMCs during osteoclastogenesis. PBMCs from A) healthy controls (HC, n = 4), and patients with B) psoriasis vulgaris (PsV, n = 4) and C) psoriatic arthritis (PsA, n = 4) were cultured with or without RANKL/M-CSF (RM) in the presence or in the absence of 1,25(OH)2D3 (Vit D) where n represents the number of samples. TRAP+ multinucleated cells (≥ 3 nuclei) per visual field, identified as mature osteoclasts, were counted after 14 days. Columns represent mean ± SD. *P<0.05, **P<0.01 and ***P<0.001 indicate statistically significant differences obtained by one-way ANOVA with Dunnetts’s multiple comparison test. aP<0.001 indicate statistically significant differences between PsA and PsV, and, PsA and HC cultured with and without RM, obtained by one-way ANOVA with Dunnetts’s multiple comparison test.
P<0.01, respectively) compared with PBMCs of HC (Fig 5A–5G). Furthermore, PBMCs of PsA showed higher secretion of TNF-α, IL-1β, IFN-γ, IL-23, IL-10 (71.49 fold, P<0.05; 1.69 fold, P<0.01; 1.76 fold, P<0.05; 5.68 fold, P<0.001; 2.16 fold, P<0.05, respectively) compared with PBMCs of PsV (Fig 5A–5C, 5E and 5H).

Addition of 1,25(OH)2D3 decreased levels of IL-1β (2.09 fold, P<0.0001; 9.85 fold, P<0.0001; 3.74 fold, P<0.01, respectively), IL-17 (71.49 fold, P=0.008; 51.54 fold, P=0.007; 11.93 fold, P=0.02, respectively) and IL-2 (20.0 fold, P<0.0001; 18.51 fold, P=0.035; 8.11 fold, P=0.008, respectively) by PBMCs of PsA, PsV and HC; IFN-γ by PsA and PsV (3.87 fold, P<0.0001; 2.79 fold, P<0.05, respectively); and TNF-α, IL-23 and RANTES by PsA (22.83 fold, P<0.05; 6.69 fold, P<0.01; 3.12 fold, P<0.05, respectively) compared with RM alone (Fig 5A–5G). On the other hand, PBMCs of PsA secreted higher levels of IL-1β and IL-10 in the
presence of RM+Vit D (7.95 fold, \( P < 0.01 \) and 1.58 fold, \( P = 0.048 \), respectively) compared with PsV (Fig 5B and 5H). However, the effect of 1,25(OH)\(_2\)D\(_3\) in the presence of RM showed no differences in any of the cytokines compared with the effect of 1,25(OH)\(_2\)D\(_3\) alone (Fig 5).

Discussion

For decades the beneficial effects of sunlight in the treatment of skin diseases such as psoriasis vulgaris, and immune system modulation are known. After sunlight exposure of the skin, 7-dehydrocholesterol converts to pre-vitamin D\(_3\) [33], which is hydroxylated in the liver into 25-hydroxyvitamin D\(_3\) [25-(OH)D\(_3\)] [34] and in the kidney into 1,25-dihydroxyvitamin D\(_3\) [1,25(OH)\(_2\)D\(_3\)], the active vitamin D metabolite involved in extracellular calcium homeostasis and bone metabolism. Furthermore, lack of sun exposure and adequate vitamin D supply results in vitamin D deficiency and musculoskeletal pathologies [35,36]. Epidemiological studies show that vitamin D deficiency is frequent in patients with psoriasis vulgaris and psoriatic arthritis [37–40]. Although phototherapy and topical application of vitamin D analogs have been used as first-line treatment with satisfactory results in the treatment of psoriasis vulgaris [1,2], therapies including vitamin D in patients with psoriatic arthritis are currently not available. Pilot studies with oral vitamin D analogs supplementation have demonstrated efficacy and safety in the treatment of psoriatic arthritis [41,42] and psoriasis vulgaris [43,44]. However, there have been only few randomized controlled trials on vitamin D supplementation, which include patients with psoriasis vulgaris but none with psoriatic arthritis [45–48]. Moreover, the only finished randomized controlled trial did not show any vitamin D benefit [45] while the other three trials have not yet been completed [46–48]. More studies are needed to confirm if correction of deficiency would result in a statistically significant clinical improvement. On the other hand, to date, many studies have focused on the evaluation of the effects of cytokines and immune cells on osteoclast differentiation, but few on the reciprocal effects of osteoclasts on immune cells under physiological and pathological conditions. We aimed to study the osteoclastogenic potential and cytokine profile of PBMCs from patients with psoriasis vulgaris and psoriatic arthritis in response to 1,25(OH)\(_2\)D\(_3\).

Osteoclasts are important players in calcium homeostasis, and calcitonin is a hormone which reduces blood calcium inhibiting bone resorption as well as osteoclast formation [12]. We assessed the calcium and bone resorption marker levels in patients with psoriasis vulgaris and psoriatic arthritis. No differences in serum levels of 1,25(OH)\(_2\)D\(_3\), calcium and CT between patients and controls were observed. However, our results showed a strong direct positive Spearman’s correlation between CT and 1,25(OH)\(_2\)D\(_3\) in HC, and a less strong Spearman’s coefficient of about 0.7 in all psoriasis patients.

CTSK, highly expressed in osteoclasts, is involved in the degradation of type I collagen [11], and OCN secreted by osteoblasts, is involved in bone formation [49]. Besides CTX-1/OCN ratios, we determined also the CTX-1/CTSK ratios in psoriasis patients and controls as CTX-1 is a product of collagen type I degradation by CTSK [32]. Such a ratio has not been reported before. We confirmed an altered bone remodelling in PsA patients demonstrated by higher CTX-1, lower OCN serum levels, and also higher CTX-1/OCN and CTX-1/CTSK ratios compared with controls. As CTX-1 is present in scar tissue as well as in dermis, tendons and ligaments, it was not surprising that also PsV patients with an impaired skin barrier and altered wound healing had slightly higher serum levels of CTX-1/OCN and CTX-1/CTSK ratios as controls. However, values were still lower compared with those found in patients with PsA. Although, recently CTSK has been found highly expressed in psoriasis patient skin and involved in development of psoriasis-like skin lesions, inflammation and bone erosion in mouse models [50,51], we found only significant higher CTSK levels in patients with PsA.
compared with PsV. Furthermore, our results showed a negative high coefficient correlation between CT and CTSK serum levels in patients with PsA but no correlation in case of PsV and HC. It is known that CT inhibits bone resorption in response to high serum calcium levels [52]. Our results suggest an altered osteoclast resorption response to hypercalcemic serum conditions which might explain partially an increase in the degradation of type I collagen by CTSK in the case of patients with PsA.

Psoriatic arthritis has been associated with high numbers of circulating osteoclast precursors; and osteoclasts, differentiated from those precursors have increased resorptive activity [29]. 1,25(OH)₂D₃ promotes osteoclast differentiation by increasing expression of mature osteoclast-associated genes [53] and stimulating bone resorption [54,55], but also it decreases resorptive activity in osteoclasts matured and maintained in the presence of 1,25(OH)₂D₃ [56]. Moreover, 1,25(OH)₂D₃ inhibits RANKL-induced osteoclastic differentiation in the absence of osteoblasts in vitro [57]. Our results showed high numbers per field of multinucleated TRAP+ cells in the presence of RM by PBMCs of PsA, as already reported [58]. In addition, we observed high TRAP activity in the presence of RM by PBMCs of PsA, and also high multinucleated TRAP+ cell numbers in the absence of RM. Moreover, to our knowledge, it has not been reported before that 1,25(OH)₂D₃ inhibited TRAP activity by PBMCs of PsV and PsA, and reduced TRAP+ cell numbers in a greater extent by PsA. No increases in TRAP activity in the presence of RM, but high multinucleated TRAP+ cell numbers by all PBMCs were observed. These discrepancies could be explained by the heterogeneity of the PBMCs population [59] with osteoclast precursors which in turn comprise several subpopulations, even with different proliferative capacities [60,61]. In addition, TRAP is expressed and secreted also by other cells than mature osteoclasts such as macrophages and dendritic cells [62].

Immune cells play an important role in the pathology of psoriasis, and together with osteoclasts in the pathology of joint inflammation in patients with PsA. Osteoclasts can present antigens to T cells [63], secrete chemokines such as IL-8 and RANTES [64] and recruit T cells in vitro [65]. Osteoclasts may express IL-6, TNF-α, IL-1α, IL-1β and macrophage inflammatory protein-1α in vivo [66–68]. Anti-CD3-stimulated PBMCs of PsA secreted higher IL-2 IFN-γ and IL-10, lower IL-17, and showed no differences in TNF-α secretion [69]. Our results showed that RM-stimulated PBMCs of PsA secreted higher levels of proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, IL-23, IL-2 and RANTES compared with control PBMCs, and additionally higher TNF-α, IL-1β, IFN-γ, IL-23 and IL-10 compared with PBMCs of PsV, suggesting that these cytokines may contribute to the joint inflammation scenery in patients with PsA. No differences in any cytokine secretion were observed between RM-stimulated PBMCs of PsV and HC; which correspond to those authors who reported no differences in IL-10, IL-1β and IL-23 levels of LPS-stimulated PBMCs from cutaneous psoriasis [70]. Here we show that PBMCs from patients with PsV and PsA have differential cytokine secretion in response to 1,25(OH)₂D₃. In the presence of RM, 1,25(OH)₂D₃ decreased levels of IL-1β, IL-17 and IL-2 by PsA, PsV and HC; IFN-γ by PsA and PsV; and TNF-α, IL-23 and RANTES by PsA after 14 days compared with RM-stimulated PBMCs. However, 1,25(OH)₂D₃ inhibited secretion of any tested cytokine regardless of the presence or absence of RM.

Taken together, our study confirmed an altered bone remodelling in patients with PsA characterized by lower serum OCN, and higher CTSK, CTX-1, CTX-1/OCN and CTX-1/CTSK ratios compared with patients with PsV and controls. Additionally, patients with PsA have a negative correlation between CT and CTSK. PBMCs from patients with PsA have higher TRAP enzymatic activity in the presence of RM, but definitely showed increased sensitivity to the inhibition by 1,25(OH)₂D₃. Under RM stimulation, PBMCs of PsA produced higher levels of proinflammatory cytokines compared with PBMCs of HC, but also TNF-α, IL-1β, IFN-γ,
IL-23 and the anti-inflammatory cytokine IL-10 compared with PBMCs of PsV; with differences in response to 1,25(OH)2D3.

Our data provides new insight into the different cytokine secretion profiles of PBMCs in the circulation of patients with PsA and PsV, and differences in their capacity to differentiate into osteoclasts and respond to 1,25(OH)2D3. Therefore, these data also suggest the development of therapeutic strategies including vitamin D for patients with psoriatic arthritis.

Supporting Information
S1 Table. Subjects demographics from patients with PsV and PsA, and healthy controls.
Data from age variable are shown as mean ± SD. *One-way ANOVA with Bonferroni’s multiple comparison test. **Two-way ANOVA with Bonferroni post test analysis.

Acknowledgments
We would like to thank the Department of Dermatology from the Jena University Hospital for their help in collecting blood samples and data from psoriasis patients specially the laboratory of Experimental Dermatology III.

Author Contributions
Conceived and designed the experiments: SC JN. Performed the experiments: SC. Analyzed the data: SC JN. Contributed reagents/materials/analysis tools: SC NK. Wrote the paper: SC JN.

References
1. Soleymani T, Hung T, Soung J. The role of vitamin D in psoriasis: a review. Int J Dermatol. 2015; 54:383–392. doi:10.1111/ijd.12790 PMID: 25601579
2. Lim HW, Silpa-archa N, Amadi U, Menter A, Van Voorhees AS, Lebwohl M. Phototherapy in dermatology: A call for action. J Am Acad Dermatol. 2015; 72: 1078–1080. doi:10.1016/j.jaad.2015.03.017 PMID: 25981004
3. Ash P, Loutit JF, Townsend KM. Osteoclasts derived from haematopoietic stem cells. Nature 1980; 283: 669–670. PMID:7354855
4. Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM, et al. Osteoblastic cells are involved in osteoclast formation. Endocrinology 1988; 123: 2600–2602. PMID:2844518
5. Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med. 1998; 188: 997–1001. PMID:9730902
6. Tanaka S, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stanley ER, et al. Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. J Clin Invest. 1993; 91: 257–263. PMID:8423223
7. Ishida N, Hayashi K, Hoshijima M, Ogawa T, Koga S, Miyatake Y, et al. Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator. J Biol Chem. 2002; 277: 41147–41156. PMID:12171919
8. Takayanagi H, Kim S, Koga T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrating RANKL signalling in terminal differentiation of osteoclasts. Dev Cell. 2002; 3: 889–901. PMID:12478613
9. Saftig P, Hunziker E, Wehmeyer O, Jones S, Boylea A, Rommerskirch W, et al. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci USA. 1998; 95: 13453–13458. PMID:9811821
10. Ek-Rylander B, Flores M, Wendel M, Heinegård D, Andersson G. Dephosphorylation of osteopontin and bone sialoprotein by osteoclastic tartrate-resistant acid phosphatase. Modulation of osteoclast adhesion in vitro. J Biol Chem. 1994; 269: 14853–14856. PMID:8195113
Nicholson GC, Moseley JM, Sexton PM, Mendelsohn FA, Martin TJ. Abundant calcitonin receptors in isolated rat osteoclasts. Biochemical and autoradiographic characterization. J Clin Invest. 1986; 78: 355–360. PMID: 3016026

Wallach S, Rousseau G, Martin L, Azria M. Effects of calcitonin on animal and in vitro models of skeletal metabolism. Bone 1999; 25: 509–516. PMID: 10574570

Shinki T, Ueno Y, DeLuca HF, Suda T. Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D3-1alpha-hydroxylase gene in normocalcemic rats. Proc Natl Acad Sci USA. 1999; 96: 8253–8258. PMID: 10393981

Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino AC, et al. Metabolism of vitamin D(3) in human osteoblasts: evidence for autocrine and paracrine activities of 1α,25-dihydroxyvitamin D(3). Bone 2007; 40: 1517–1528. PMID: 17395559

Horwood NJ, Elliott J, Martin TJ, Gillespie MT. Osteotropic agents regulate the expression of osteoclast differentiation factor and osteoprotegerin in osteoblastic stromal cells. Endocrinology 1998; 139: 4743–4746. PMID: 9794488

Adams JS, Beeker TG, Hongo T, Clemens TL. Constitutive expression of a vitamin D 1-hydroxylase in a myelomonocytic cell line: a model for studying 1,25-dihydroxyvitamin D production in vitro. J Bone Miner Res. 1990; 5: 1265–1269. PMID: 1963733

Reichel H, Koefler HP, Norman AW. Synthesis in vitro of 1,25-dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 by interferon-γ-stimulated normal human bone marrow and alveolar macrophages. J Biol Chem. 1987; 262: 10931–10937. PMID: 3112152

Kogawa M, Anderson PH, Findlay DM, Morris HA, Atkins GJ. The metabolism of 25-(OH)-vitamin D(3) by osteoclasts and their precursors regulates the differentiation of osteoclasts. J Steroid Biochem Mol Biol. 2010; 121: 277–280. doi: 10.1016/j.jsbmb.2010.03.048 PMID: 20304055

D’Ambrosio D, Cippitelli M, Cuccio MG, Mazzeo D, Di Lucia P, Lang R, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappa B downregulation in transcriptional repression of the p40 gene. J Clin Invest. 1998: 101: 252–262. PMID: 9421488

Lemire JM, Archer DC, Beck J, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th 1 functions. J Nutr. 1995; 125: 1704S–1708S. PMID: 7782931

Muller K, Diamant M, Bendtzen K. Inhibition of production and function of interleukin-6 by 1,25-dihydroxyvitamin D3. Bone 2007; 40: 1517–1528. PMID: 17395559

Raychaudhuri S, Mitra A, Datta-Mitra A. Immunomodulatory mechanisms of action of calcitriol in Psoriasis. Indian J Dermatol. 2014; 59: 116–122. doi: 10.4103/0019-5154.127668 PMID: 24700927

Heine G, Niesner U, Chang HD, Steinmeyer A, Zügel U, Zuberbier T, et al. 1,25-Dihydroxyvitamin D3 promotes IL-10 production in human B cells. Eur J Immunol. 2008; 38: 2210–2218. doi: 10.1002/eji.200838216 PMID: 18651709

Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. J Clin Invest. 2006; 116: 146–155. PMID: 16341266

Cho YB, Hwang YJ, Hahn HJ, Jung JW, Jung HJ, Lee YW, et al. A comparison of serum inflammatory cytokines according to phenotype in patients with psoriasis. Br J Dermatol. 2012; 167: 762–767. doi: 10.1111/j.1365-2133.2012.11038.x PMID: 22564054

Nakajima H, Nakajima K, Tarutani M, Morishige R, Sano S. Kinetics of circulating Th17 cytokines and adipokines in psoriasis patients. Arch Dermatol Res. 2011; 303: 451–455. doi: 10.1007/s00403-011-1159-3 PMID: 21681565

Dalbeth N, Pool B, Smith T, Callon KE, Lobo M, Taylor WJ, et al. circulating mediators of bone remodeling in psoriatic arthritis: implications for disordered osteoclastogenesis and bone erosion. Arthritis Res Ther. 2010; 12: R164. doi: 10.1186/ar3123 PMID: 20796300

Szentpetery A, McKenna MJ, Murray BF, Ng CT, Brady JJ, Morrin M, et al. Periarticular bone gain at proximal interphalangeal joints and changes in bone turnover markers in response to tumor necrosis factor inhibitors in rheumatoid and psoriatic arthritis. J Rheumatol. 2013; 40: 653–662. doi: 10.3899/jrheum.120397 PMID: 23457381

Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM. Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest. 2003; 111: 821–831. PMID: 12639988

Taylor W, Gladman D, Hellwell P, Marchesoni A, Mease P, Mielants H, et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheumat. 2006; 54: 2665–2673. PMID: 16871531
31. Magarò M, Altomonte L, Mirone L, Zoli A, Tricerri A. Serum osteocalcin as an index of bone turnover in active rheumatoid arthritis and in active psoriatic arthritis. Clin Rheumatol. 1989; 8: 494–498. PMID: 2612118
32. Garnero P, Ferreras M, Karsdal MA, Nicamhlaoibh R, Risteli J, Borel O, et al. The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. J Bone Miner Res. 2003; 18: 859–867. PMID: 12733725
33. DeLuca HF, Schnoes HK. Metabolism and mechanism of action of vitamin D. Ann Rev Biochem. 1976; 45: 631–666. PMID: 183601
34. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA. 2004; 101: 7711–7715. PMID: 15128933
35. Chen TC, Chimeh F, Lu Z, Mathieu J, Person KS, Zhang A, et al. Factors that influence the cutaneous synthesis and dietary sources of vitamin D. Arch Biochem Biophys. 2007; 460: 213–217. PMID: 17254541
36. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008; 87: 1080S–1086S. PMID: 18400738
37. Gisondi P, Rossini M, Di Cesare A, Idolazzi L, Farina S, Beltrami G, et al. Vitamin D status in patients with chronic plaque psoriasis. Br J Dermatol. 2012; 166: 505–510. doi: 10.1111/j.1365-2133.2011.10699.x PMID: 22013980
38. Grazio S, Naglić DB, Anić B, Grubišić F, Bobek D, Bakula M, et al. Vitamin D serum level, disease activity and functional ability in different rheumatic patients. Am J Med Sci. 2015; 349: 46–49. doi: 10.1097/MAJ.0000000000000340 PMID: 25310509
39. Suárez-Varela MM, Reguera-Leal P, Grant WB, Rubio-López N, Llopis-González A. Vitamin D and psoriasis pathology in the Mediterranean region, Valencia (Spain). Int J Environ Res Public Health. 2014; 11: 12108–12117. doi: 10.3390/ijerph111212108 PMID: 25429679
40. Touna Z, Eder L, Zisman D, Feld J, Chandran V, Rosen CF, et al. Seasonal variation in vitamin D levels in psoriatic arthritis patients from different latitudes and its association with clinical outcomes. Arthritis Care Res (Hoboken). 2011; 63: 1440–1447.
41. Gaál J, Lakos G, Szodoray P, Kiss J, Horváth I, Horkay E, et al. Immunological and clinical effects of alphocalcidol in patients with psoriatic arthropathy: results of an open, follow-up pilot study. Acta Derm Venereol. 2009; 89: 140–144. doi: 10.2340/00015555-0555 PMID: 19325997
42. Huckins D, Felson DT, Holick M. Treatment of psoriatic arthritis with oral 1,25-dihydroxyvitamin D3: a pilot study. Arthritis Rheum. 1990; 33: 1723–1727. PMID: 2242069
43. Finanmor DC, Sinigaglia-Coimbra R, Neves LC, Gutierrez M, Silva JJ, Torres LD, et al. A pilot study assessing the effect of prolonged administration of high daily doses of vitamin D on the clinical course of vitiligo and psoriasis. Dermatoendocrinol. 2013; 5: 222–234. doi: 10.4161/derm.24808 PMID: 24494059
44. Takamoto S, Onishi T, Morimoto S, Imanaka S, Yukawa S, Kozuka T, et al. Effect of 1 alpha-hydroxycholecalciferol on psoriasis vulgaris: a pilot study. Calcif Tissue Int. 1986; 39: 360–364. PMID: 3100000
45. Hata TR, Audish D, Kotol P, Coda A, Cabibgting F, Miller J, et al. A randomized controlled double-blind investigation of the effects of vitamin D dietary supplementation in subjects with atopic dermatitis. J Eur Acad Dermatol Venereol. 2014; 28: 781–789. doi: 10.1111/jdv.12176 PMID: 23638978
46. Scragg R, Waayer D, Stewart AW, Lawes CM, Toop L, Murphy J, et al. The Vitamin D Assessment (ViDA) Study: design of a randomized controlled trial of vitamin D supplementation for the prevention of cardiovascular disease, acute respiratory infection, falls and non-vertebral fractures. J Steroid Biochem Mol Biol. 2015. http://dx.doi.org/10.1016/j.jsbmb.2015.09.010.
47. Chulalongkorn University (Sponsor). The efficacy of vitamin D3 for the treatment of chronic plaque type psoriatic patients with vitamin D deficiency and insufficiency: a randomized controlled trial. Available: https://clinicaltrials.gov/ct2/show/NCT01339741
48. Pontificia Universidad Catolica de Chile (Sponsor). Effect of vitamin D supplementation on metabolic parameters of patients with moderate to severe psoriasis. Available: https://clinicaltrials.gov/ct2/show/NCT02271971
49. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev. 1989; 69: 990–1047. PMID: 2664828
50. Hao L, Zhu G, Lu Y, Wang M, Jules J, Zhou X, et al. Deficiency of cathepsin K prevents inflammation and bone erosion in rheumatoid arthritis and periodontitis and reveals its shared osteoimmune role. FEBS Lett. 2015; 589: 1331–1339. doi: 10.1016/j.febslet.2015.04.008 PMID: 25896020
51. Hirai T, Kanda T, Sato K, Takaishi M, Nakajima K, Yamamoto M, et al. Cathepsin K is involved in development of psoriasis-like skin lesions through TLR-dependent Th17 activation. J Immunol. 2013; 190: 4805–4811. doi: 10.4049/jimmunol.1200901 PMID: 23543761

52. Friedman J, Raisz LG. Thryocalcitonin: inhibitor of bone resorption in tissue culture. Science 1965; 150: 1465–1467. PMID: 5892553

53. Gu J, Tong XS, Chen GH, Wang D, Chen Y, Yuan Y, et al. Effects of 1α,25-(OH)2D3 on the formation and activity of osteoclasts in RAW264.7 cells. J Steroid Biochem Mol Biol. 2015; 152: 25–33. doi: 10.1016/j.jsbmb.2015.04.003 PMID: 25864627

54. Holtrop ME, Cox KA, Clark MB, Holick HF, Anast CS. 1,25-dihydroxycholecalciferol stimulates osteoclasts in rat bones in the absence of parathyroid hormone. Endocrinology 1981; 108: 2293–2301. PMID: 6894424

55. Raisz LG, Trummel CL, Holick MF, DeLuca HF. 1,25-dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. Science 1972; 175: 768–769. PMID: 4333999

56. Kogawa M, Findlay DM, Anderson H, Ormsby R, Vincent C, Morris HA, et al. Osteoclastic metabolism of 25(OH)-vitamin D3: A potential mechanism for optimization of bone resorption. Endocrinology 2010; 161: 4613–4625. doi: 10.1210/en.2010-0334 PMID: 20739402

57. Lee SK, Kalinowski J, Jastrzebski S, Lorenzo JA. 1,25(OH)2 vitamin D3-stimulated osteoclast formation in spleen-osteoblast cocultures is mediated in part by enhanced IL-1 alpha and receptor activator of NF-kappa B ligand production in osteoblasts. J Immunol. 2002; 169: 2374–2380. PMID: 12193704

58. Ikä M, Jäyä-Läitić, Ivićević S, Grubišić F, Marušić A, et al. Association of systemic and intra-articular osteoarticular potential, pro-inflammatory mediators and disease activity with the form of inflammatory arthritis. Int Orthop. 2014; 38: 183–192. doi: 10.1007/s00264-013-2121-0 PMID: 24100919

59. Grage-Griebenow E, Flad HD, Ernst M. Heterogeneity of human peripheral blood monocyte subsets. J Leukoc Biol. 2001; 69: 11–20. PMID: 11200054

60. Komano Y, Nanki T, Hayashida K, Taniguchi K, Miyasaka N. Identification of a human peripheral blood monocyte subset that differentiates into osteoclasts. Arthritis Res Ther. 2006; 8: R152. PMID: 16987426

61. Lari R, Kitchener PD, Hamilton JA. The proliferative human monocyte subpopulation contains osteoclast precursors. Arthritis Res Ther. 2009; 11: R23. doi: 10.1186/ar2616 PMID: 1922861

62. Janckila AJ, Parthasarathy RN, Parthasarathy JK, Seelan RS, Hsueh YC, Rissanen J, et al. Properties and expression of human tartrate-resistant acid phosphatase isoform 5a by monocyte-derived cells. J Leukoc Biol. 2005; 77: 209–218. PMID: 15542543

63. Li H, Hong S, Qian J, Zheng Y, Yi Q. Cross talk between the bone and immune systems: osteoclasts function as antigen-presenting cells and activate CD4+ and CD8+ T cells. Blood 2010; 116: 210–217. doi: 10.1182/blood-2009-11-255026 PMID: 20304810

64. Pappalardo A, Thompson K. Novel immunostimulatory effects of osteoclasts and macrophages on human yG T cells. Bone 2015; 71: 180–188. doi: 10.1016/j.bone.2014.10.019 PMID: 2544546

65. Grassi F, Manferdini C, Cattini L, Piacentini A, Gabusi E, Facchini A, et al. T cell suppression by osteoclasts in vitro. J Cell Physiol. 2011; 226: 982–990. doi: 10.1002/jcp.22411 PMID: 20857429

66. O’Keefe R, Teot L, Singh D, Puzas JE, Rosier RN, Hicks DG. Osteoclasts constitutively express regulators of bone resorption: an immunohistochemical and in situ hybridization study. Lab Invest. 1997; 76: 457–465. PMID: 9111508

67. Rahimi P, Wang C, Stashenko P, Lee SK, Lorenzo JA, Graves DT. Monocyte chemoattractant protein-1 expression and monocyte recruitment in osseous inflammation in the mouse. Endocrinology 1995; 136: 2725–2759. PMID: 7750500

68. Rothe L, Collin-Osdoby P, Chen Y, Sunyer T, Chaudhary L, Tsay A, et al. Human osteoclasts and osteoclast-like cells synthesize and release high basal and inflammatory stimulated levels of the potent chemokine interleukin-8. Endocrinology 1998; 139: 4353–4363. PMID: 9751519

69. Bosé F, Capsoni F, Molteni S, Raeli L, Diani M, Altomare A, et al. Differential expression of interleukin-2 by anti-CD3-stimulated peripheral blood mononuclear cells in patients with psoriatic arthritis and patients with cutaneous psoriasis. Clin Exp Med. 2014; 39: 385–390.

70. Ekman AK, Sigurdardottir G, Carlström M, Kartul N, Jenmalm MC, Enerbäck C. Systemic elevated Th1, Th2, and Th17-associated chemokines in psoriasis vulgaris before and after ultraviolet B treatment. Acta Derm Venereol. 2013; 93: 525–531.