Odontoblast cell death induces NLRP3 inflammasome-dependent sterile inflammation and regulates dental pulp cell migration, proliferation and differentiation

doi:10.1111/iej.13483

Odontoblast cell death induces NLRP3 inflammasome-dependent sterile inflammation and regulates dental pulp cell migration, proliferation and differentiation

B. Al Natour[1,2], F. T. Lundy[1], P. N. Moynah[1,3], I. About[4], C. Jeanneau[4], C.R. Irwin[1], Y. Dombrowski[1] & I. A. El Karim[1]

[1]School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK; [2]Department of Oral Medicine and Oral Surgery, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan; [3]Department of Biology, The Kathleen Lonsdale Institute for Human Health Research, National University of Ireland Maynooth, Maynooth, Ireland; and [4]UMR 7287 CNRS, Faculté d'Odontologie, Université d'Aix-Marseille, Marseille, France

Abstract

Al Natour B, Lundy FT, Moynah PN, About I, Jeanneau C, Irwin C, Domberoski Y, El Karim IA. Odontoblast cell death induces NLRP3 inflammasome-dependent sterile inflammation and regulates dental pulp cell migration, proliferation and differentiation. *International Endodontic Journal*, **54**, 941–950, 2021.

Aim To investigate the ability of dead odontoblasts to initiate NLRP3 inflammasome-dependent sterile inflammation and to explore the effect on dental pulp cell (DPCs) migration, proliferation and odontogenic differentiation.

Methods Odontoblast-like cells were subjected to freezing-thawing cycles to produce odontoblast necrotic cell lysate (ONCL). DPCs were treated with ONCL to assess proliferation and migration. THP-1 differentiated macrophages stimulated with ONCL and live cell imaging and western blotting were used to assess NLRP3 inflammasome activation. Cytokines were measured with multiplex arrays and ELISA. qPCR, alkaline phosphatase and Alizarin red assays were used to assess odontogenic differentiation of DPCs. Data were analysed using the *t*-test or ANOVA followed by a Bonferroni *post hoc* test with the level of significance set at $P \leq 0.05$.

Results ONCL induced migration and proliferation of DPCs. Treatment of THP-1 macrophages with ONCL resulted in the release of the inflammatory cytokines IL-1β, IL-6, IL-8, TNFα, IFN-γ, CCL2 and angiogenic growth factors, angiogenin and angiopoietin. This inflammatory response was associated with activation of NFκB, p38MAPK and NLRP3 inflammasome. To confirm that ONCL induced inflammatory response is NLRP3 inflammasome-dependent, treatment with a caspase-1 inhibitor and a specific NLRP3 inhibitor significantly reduced IL-1β release in THP-1 macrophages ($P = 0.01$ and 0.001). Inflammasome activation product, IL-1β, induced odontogenic differentiation of DPCS as evident by the increase in odontogenic genes expression DMP-1, RUNX-2, DSPP and SPP, alkaline phosphatase activity and mineralization.

Conclusion Dead odontoblasts induced NLRP3 inflammasome-dependent sterile inflammation and activated the migration, proliferation and differentiation of DPCs.

Correspondence: Ikhlas A. El Karim, Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, The Wellcome-Wolfson Institute of Experimental Medicine, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7AE, Northern Ireland, UK (Tel.: +442890976026; e-mail: i.elkarim@qub.ac.uk).

Correction added on 02 March 2022 after first online publication the author name Dombrowski was corrected.

Keywords: cell death, danger associated molecular patterns, NLRP3 inflammasome, odontoblasts, reparative dentine, sterile inflammation.

Received 29 September 2020; accepted 25 January 2021

Introduction

Due to their peripheral location in the dental pulp, odontoblasts are the first cells likely to encounter mechanical, chemical or bacterial injury. Deep cavity preparation and iatrogenic mechanical pulp exposure usually result in localized odontoblast death associated with an acute inflammatory response. This inflammatory response is considered a prerequisite for pulpal wound healing and repair with many molecules, mediators and signalling pathways being implicated in the various stages of the repair process (Farges *et al.* 2015, Goldberg *et al.* 2015).

Although the inflammatory process and subsequent repair are preceded by injury and death of native odontoblasts, the role of odontoblast injury and death in this process is not fully understood. Injured cells release intracellular molecules known as damage-associated molecular patterns (DAMPs) that activate an innate immune response via signalling through pattern recognition receptors PRRs (Bianchi 2006). Some PRRs are located intracellularly and upon detecting danger signals can oligomerize into multiprotein complexes named Inflammasomes (Martinon *et al.* 2002). Canonical inflammasomes consist of a sensor component, an adaptor component and an effector component such as caspase 1. The upstream sensor component is typically a pattern recognition receptor, (nucleotide-binding oligomerization domain-like receptor (NLR) or absent in melanoma 2 (AIM2)-like receptor (Wang *et al.* 2002). Upon activation by pathogens or DAMPs, the sensor component oligomerizes and recruits the adaptor protein ASC [apoptosis-related speck-like protein containing a caspase recruitment domain (CARD)] and procaspase-1, leading to the activation of caspase-1. Active caspase-1 then cleaves the precursor pro-inflammatory cytokines pro-IL-1β and pro-IL-18 into their mature secreted forms (Martinon *et al.* 2002, Wang *et al.* 2002, Lamkanfi 2011).

Several inflammasomes have been described; amongst these NLRP3 has been characterized most extensively. NLRP3 belongs to the nucleotide-binding oligomerization domain-like receptor (NLR) protein family and is activated by multiple DAMPs (Gong *et al.* 2020). The activation of NLRP3 is highly regulated. It usually involves an initial priming signal, mediated via toll-like receptors (TLRs) inducing NFκB activation, which leads to transcription of genes encoding precursor forms of the cytokines IL-1β and IL-18 followed by a second signal required for NLRP3 assembly and subsequent release of mature IL1-β and IL-18. NLRP3 has been shown to play a central role in cell death induced inflammation, cellular homoeostasis and tissue repair (Wang *et al.* 2013, Anders & Schaefer 2014).

Although shown to be expressed in the dental pulp (Song *et al.* 2012), the role of NLRP3 in sterile pulpal inflammation and repair is yet to be elucidated. It was hypothesized that DAMPs released by dying odontoblasts upon dental trauma or injury activated NLRP3 inflammasome to initiate an inflammatory response and repair. Therefore, the aim of this study was to investigate the ability of DAMPs to initiate inflammation and to confirm if this inflammatory response is NLRP3 inflammasome-dependent. The effect of DAMPs and subsequent inflammation on dental pulp cell (DPC) migration, proliferation and differentiation was also studied.

Material and methods

Cell culture and generation of odontoblast-like cells

A mixed DPC population was derived by explant culture from immature permanent third molars extracted during normal treatment of 16- to 18-year-old patients and obtained in accordance with French ethics legislation and covered by the Office for Research Ethics Committees (Northern Ireland) ethical approval number 08/NIR03/15. Cells were grown in minimal essential medium with L-glutamine supplemented with 10% foetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 UI mL^{-1} penicillin and 100 mg mL^{-1} streptomycin (Gibco). To obtain odontoblast-like cells, DPCs at passage 4–6 were grown in odontogenic media containing 2 mmol L^{-1} β-glycerophosphate (βGP; Sigma-Aldrich, St. Louis, MI, USA) for 21 days, as previously described (El Karim *et al.* 2011). DPCs were grown 2 mmol L^{-1} βGP for 3 and 7 day to assess the expression of odontogenic genes DSPP, DMP-1, SPP-1, MSX-1 and RUNX2 (Thermo

Fisher Scientific). Alizarin Red assay and immunohistochemistry were carried out to assess mineralization and detect DSP protein at day 21 respectively. THP-1 cells were grown in Roswell Park Memorial Institute medium (RMPI-1640) media supplemented with 10% FBS and differentiated into macrophages by incubation in 100 nmol L^{-1} phorbol 12-myristate 13-acetate (PMA), (Sigma-Aldrich) for 72 h. THP1-ASC-GFP reporter Cells (InvivoGen, Toulouse, France) were cultured in RPMI-1640 media supplemented with 10% FBS, 2 mmol L^{-1} glutamine, 25 mmol L^{-1} HEPES, 100 μg mL^{-1} normocin and 50 UI mL^{-1} penicillin (Gibco).

Generation of odontoblast necrotic cell lysate (ONCL)

The odontoblastic phenotype of the cells was confirmed by upregulation of odontogenic genes DSPP, DMP-1, and RUNX2 at day 3 and SPP-1 and MSX-1 at day 7 (Figure S1 A). Immunohistochemistry showed, expression of dentine sialoprotein (DSP) in differentiated cells compared to untreated control (Figure S1B), and Alizarin red staining showed evidence mineralization (Figure S1C), further conforming odontoblast-like cell phenotype. Prior to generation of the necrotic cell lysate, odontoblast-like cells were detached from the flask using 0.25% Trypsin-EDTA (Gibco). The cell pellet obtained (2×10^5 cell mL^{-1}) was resuspended in either serum-free α-MEM for use in migration assay, or 10% α-MEM for proliferation assay or 10% RPMI for THP-1 stimulation experiments. The cell suspension was aliquoted at a volume of 1 mL per cryotube. To induce necrosis, cells were subjected to five freeze-thaw cycles, where the cryotubes were immersed in liquid nitrogen to induce snap freezing and then immediately placed in a bead bath at 37 °C to induce thawing as previously described (Liu *et al.* 2015). The lysate was then centrifuged at 120 ***g*** for 10 min and the supernatant was collected as ONCL. Trypan blue staining and lactate dehydrogenase assays confirmed complete cell necrosis using this method. For THP-1 macrophages stimulation experiments and DPCs proliferation assays, ONCL was diluted at a ratio of 1 : 4 in RPMI media or α-MEM containing 10% FBS respectively.

Cell migration assay

DPCs were seeded at a density of 1×10^4 per 100 μL in serum-free α-MEM onto the upper chamber of Transwell inserts with a pore size of 8 μm (Greiner Bio-One, Frickenhausen, Germany). The inserts were then placed into 24 well plates containing either; serum-free α-MEM or ONCL prepared in serum-free α-MEM. Cells were cultured in a 37 °C incubator with 5% CO_2 for 24 h to allow migration of cells through the membrane. After 24 h, nonmigrated cells were gently removed from the upper side of the membrane with a cotton swab. Migrated cells were fixed with ice-cold methanol for 15 min and stained with 0.5% crystal violet (Aquilant Scientific, Leicester, UK) for 20 min, followed by washing with distilled H_2O. The crystal violet staining was dissolved for 20 min in 33% acetic acid (diluted in distilled H_2O) at room temperature. The absorbance of the crystal violet/acetic acid solution was measured using a spectrophotometer at 600 nm using the Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific).

Cell proliferation assay

DPCs were seeded in 96-well plates at a density of 3×10^4 cell mL^{-1} and cultured to 70% confluence in α-MEM with 10% FBS. Cells were then treated with ONCL (prepared in α-MEM containing 10% FBS) for 1, 3 or 7 days. Control cells were treated with α-MEM containing 10% FBS for similar time points. After the relevant time period, 10 μL MTT solution was added to each well for 2 h at 37 °C. Media was aspirated and wells air-dried for 10 min. 200 μL DMSO was added and plates were incubated at 37 °C for a further 10 min. Samples were mixed and absorbance was measured at 510 nm using a Tecan GENios microplate reader (Tecan, Männedorf, Switzerland).

Cytokine measurements

To investigate the ability of ONCL to induce inflammation, differentiated THP-1 cells were incubated overnight with ONCL alone, purified *S. aureus* lipoteichoic acid (LTA; InvivoGen) alone or ONCL from odontoblasts pretreated with LTA overnight. The supernatant was collected for cytokine measurement. Human Magnetic Luminex Assay (R&D Systems, Minneapolis, MN, USA) was used to simultaneously measure IL-8, IL-1β, IL-6, TNFα, IFN-γ, CCL2, angiogenin and angiopoietin levels. Subsequent measurement of IL-1β was carried out using the Human IL-1β Duoset ELISA (R&D Systems).

Western blotting

THP-1 macrophages were lysed with 50 mmol L^{-1} Tris-HCL buffer (pH 7.4) containing 0.25 sodium dodecyl sulphate (SDS) and Halt protease inhibitor cocktail (Sigma-Aldrich). Cell lysates (10 μL) were fractioned by NuPAGE polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA). The blots were blocked in 5% nonfat milk for 1 h before probing overnight with rabbit primary Phospho-p38 MAPK antibody or mouse primary anti-Phospho-IκBα (Ser32/36) or primary rabbit anti-NLRP3 (D4D8T), all obtained from Cell Signal Technology (Hamilton House, London, UK) and used at 1 : 1000 dilution. Protein expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1 : 2000; Santacruz, Dallas, USA). The detection of the bound primary antibody was achieved using appropriate anti-species antibody conjugates (Merck Millipore, Darmstadt, Germany) and chemiluminescent substrate. Immunoreactive bands were visualized using a G: BOX Chemi System and GeneSys software (Syngene, Cambridge, UK).

ASC speck imaging in live cells

THP-1-ASC-GFP reporter cells were seeded onto black 96-well plates and then differentiated to macrophages with PMA as described above. THP-1/ASC-GFP macrophages were stimulated with ONCL for 4 h and ASC speck formation was visualized by an EVOS FL Auto microscope (Thermo Fisher Scientific) at 4× magnification. One distinct field of view (image) per well was defined and customized. Image J (Schneider *et al.*, 2012) plugins were used to assist quantifying activated positive ASC cells in all field of views of 6 wells representing 3 independent experiments.

Quantitative RT-PCR

RNA was extracted from DPCs using RNAeasy Plus (Qiagen, Manchester, UK) and cDNA was prepared using the VILO Transcriptor First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Quantitative PCR reactions were performed using TaqMan specific primers and probes, along with TaqMan Universal PCR Master Mix II with UNG (Thermo Fisher Scientific). Changes in DSPP, SPP-1, DMP-1, MSX-1 and RUNX2 expression at day 3, 7 and 14 were analysed using the Mx3005P qPCR System (Agilent Technologies, Cheadle, UK). Relative mRNA expression was normalized against the housekeeping genes, glucuronidase-beta (GUSB) and Beta 2-microglobulin (B2M). All primers were validated as obtained from Thermo Fisher Scientific.

Alkaline phosphatase (ALP) activity

DPCs were seeded in 12-well plates at a density of 5×10^4 cells mL^{-1} and grown to 70% confluence prior to treatment with IL-1β (product of NLRP3 activation) at 10 or 100 pg mL^{-1} for 21 days. Cells were harvested, lysed in 0.2% Triton x-100 on ice and collected for measuring ALP activity using a diethanolamine detection kit (Sigma-Aldrich) and substrate p-nitrophenylphosphate (pNPP) as previously described (Winning *et al.* 2019). Absorbance was measured over 20 consecutive reads at 405 nm. Values were normalized to total protein content, which was measured using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL) and expressed as ηmol pNPP/min/mg protein.

Alizarin red

DPCs were seeded in 12 well plates at 5×10^4 cells mL^{-1} and grown to 70% confluence prior to treatment with IL-1β or 2 mmol L^{-1} βGP as described above for 21 days. Cells were then fixed using 10% formaldehyde and stained with 40 mmol L^{-1} Alizarin red (Sigma-Aldrich) pH 4.1. To quantify the stain, 10% acetic acid was added and later neutralized with 10% ammonia hydroxide (Sigma-Aldrich). The supernatants were read in triplicate at 405 nm in 96-well format using opaque-walled, transparent-bottomed plates using a Tecan GENios microplate reader (Gregory *et al.* 2004).

Results

ONCL induces migration and proliferation of DPCs

To assess whether DAMPs released from dying odontoblasts contribute to the early stages of repair, the effect of ONCL on the migration and proliferation of DPCs was investigated. ONCL had chemotactic activity for DPCs ($P < 0.5$; Fig. 1a). Furthermore, treatment of DPCs with ONCL resulted in significant enhancement of the proliferation of DPCs at day 7 of ONCL treatment ($P < 0.001$; Fig. 1b).

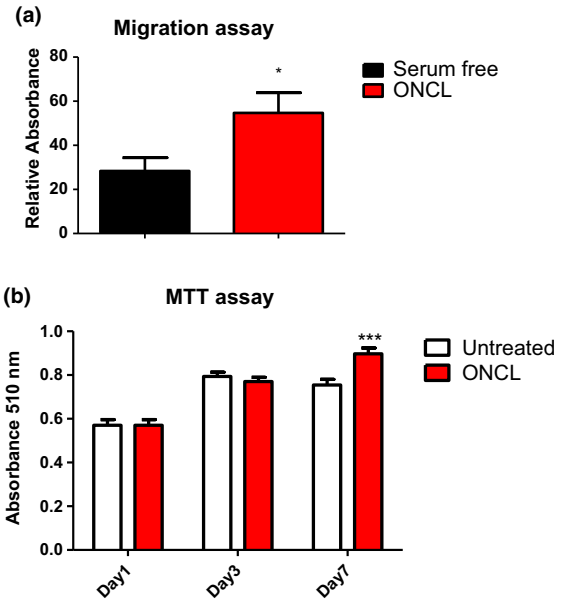

Figure 1 ONCL induces migration and proliferation of DPCs. Transwell cell migration assay and crystal violet staining were used to assess if ONCL is chemotactic to DPCs. Serum-free media is used as a control. Migrated DPCs were quantified by measuring the absorbance of solubilized crystal violet dye and percentage migration relative to the positive control was calculated. ONCL has significant chemotactic effect compared to serum-free media (a). MTT assay was performed to test the effect on ONCL on proliferation of DPCs, and as shown in (b), ONCL treatment resulted in significant proliferation at day 7. Data represent an average of three independent experiments and presented as mean ± SEM and analysed $*P < 0.05$. $***P < 0.001$, *t*-test and two way ANOVA.

ONCL induces an inflammatory response

Treatment of THP-1 macrophages with ONCL resulted in increased release of inflammatory cytokines; IL-8, ($P < 0.0001$) IL-1β, IL-6, ($P < 0.05$), TNF-α, IFN-γ, CCL2 and growth factors such as angiogenin and angiopoietin compared to untreated cells ($P < 0.001$) as shown in Fig. 2. The levels of cytokines release were also similar to that induced by treatment of THP-1 macrophages with bacterial LTA alone. To investigate if LTA can enhance the pro-inflammatory potential of damaged odontoblasts, odontoblast-like cells were treated with LTA overnight, before subjecting the cells to freeze/thaw cycles to produce ONCL. The results, however, revealed no difference in cytokines levels in THP-1 cells treated with sterile ONCL, or ONCL obtained from odontoblast-like cells pretreated with bacterial LTA (Fig. 2 black bars). This suggests that direct damage of odontoblasts, even in the absence of an overt inflammatory stimulus, is sufficient to trigger a strong inflammatory response.

ONCL inflammatory response is inflammasome-dependent

To probe the signalling pathways that underlie the ONCL induced expression of pro-inflammatory cytokines, the expression of phospho-IκBα, p38 MAPK and NLRP3 as measures of inflammasome activation pathways was assessed. ONCL induced the activation of phospho-IκBα, p38 MAPK and up-regulated NLRP3 (Fig. 3a–c). The efficacy of ONCL was comparable to that of LTA, which was previously been shown to induce NLRP3 expression via the NFkB pathway (Wang et al 2016).

Whilst upregulation of NLRP3 is associated with inflammasome activation, the ability of ONCL to directly activate inflammasomes via ASC speck formation, a key indicator of ASC oligomerization and activation of pathways downstream of NLRP3 was tested. As shown in Fig. 3e,f, THP-1-ASC-reporter macrophages stimulated with ONCL induced ASC expression in the cytoplasm ($P < 0.0001$). To further confirm whether ONCL induced inflammation was mediated via inflammasome activation; the inhibition of inflammasome activation and subsequent IL-1β release with a caspase 1 inhibitor Ac-YVAD-CHO (Santacruz) was investigated. As shown in Fig. 3g, pretreatment with Ac-YVAD-CHO significantly reduced IL-1β release in THP-1 macrophages treated with ONCL ($P < 0.01$). To delineate the specific inflammasome responsible for IL-1β, cells were incubated with the NLRP3 specific inhibitor MCC950 (InvivoGen) before treatment with ONCL and as shown in Figure 3H, this resulted in a significant reduction in IL-1β release ($P < 0.001$).

Inflammasome activation by-product IL-1β regulates odontogenic differentiation of DPCs

As inflammasome activation leads to the release of IL-1β, whether the product of inflammasome activation promoted odontogenic differentiation of DPCs was tested. DPCs were incubated with different concentrations of IL-1β. At low concentration, IL-1β induced the expression of osteo/odontogenic genes SPP1 ($P < 0.0001$), DSP ($P < 0.05$), DMP-1 ($P < 0.001$) and Runx2 ($P < 0.05$) at different time points and increased ALP activity and mineralization as evident from Alizarin red stain quantification ($P < 0.05$; Figure 4).

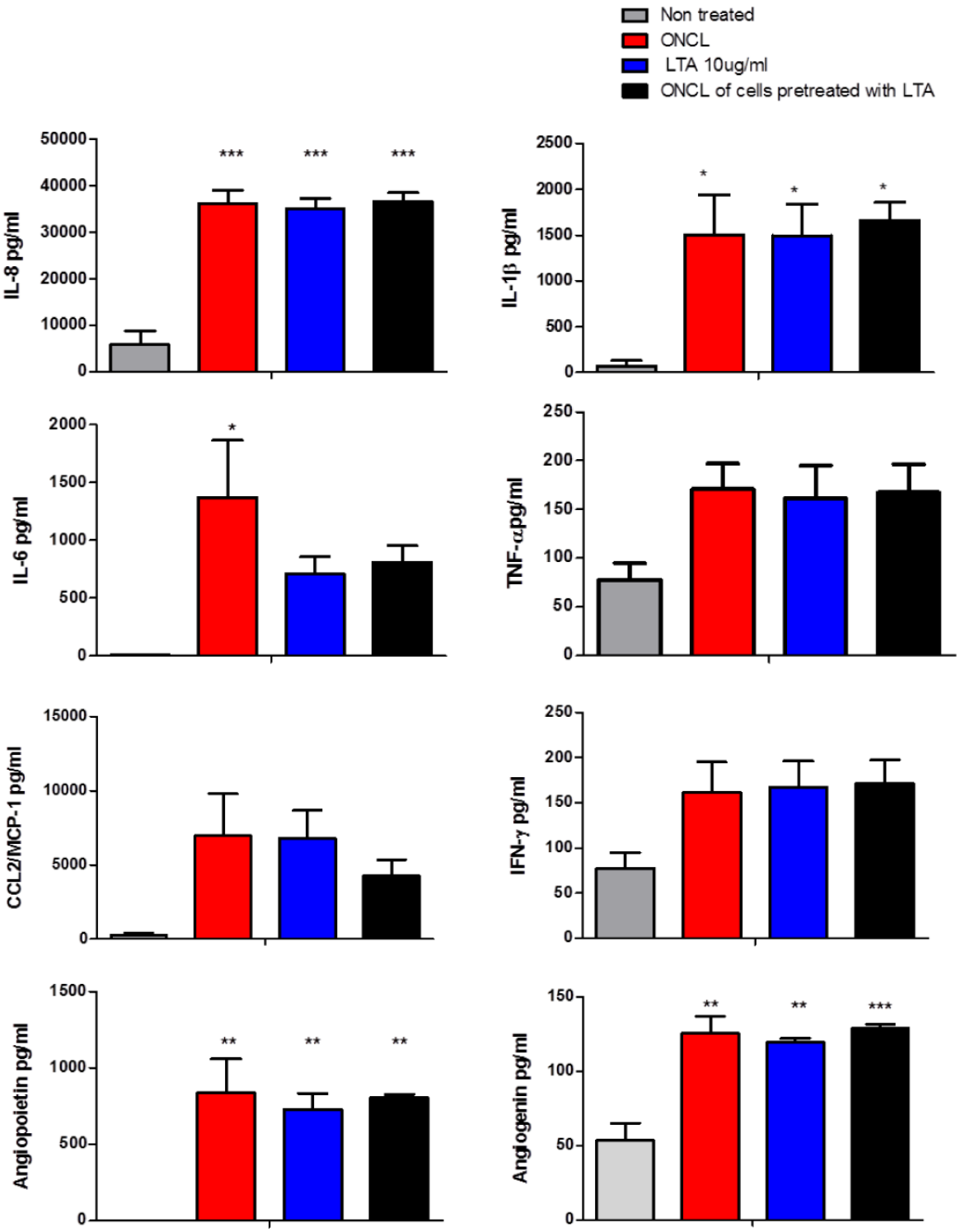

Figure 2 ONCL induces release of inflammatory cytokines in THP-1 macrophages. Cytokine levels in the supernatant of THP-1 macrophages treated with sterile ONCL (red bar) and cells treated with ONCL obtained from odontoblast pretreated with bacterial LTA (black bar). THP- cells treated with LTA (10 μg mL^{-1}; blue bar) served as positive control and untreated cells (white bar) as negative control. Data represent avenge of three independent experiments and presented as mean ± SEM. *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$, ANOVA with Bonferoni correction.

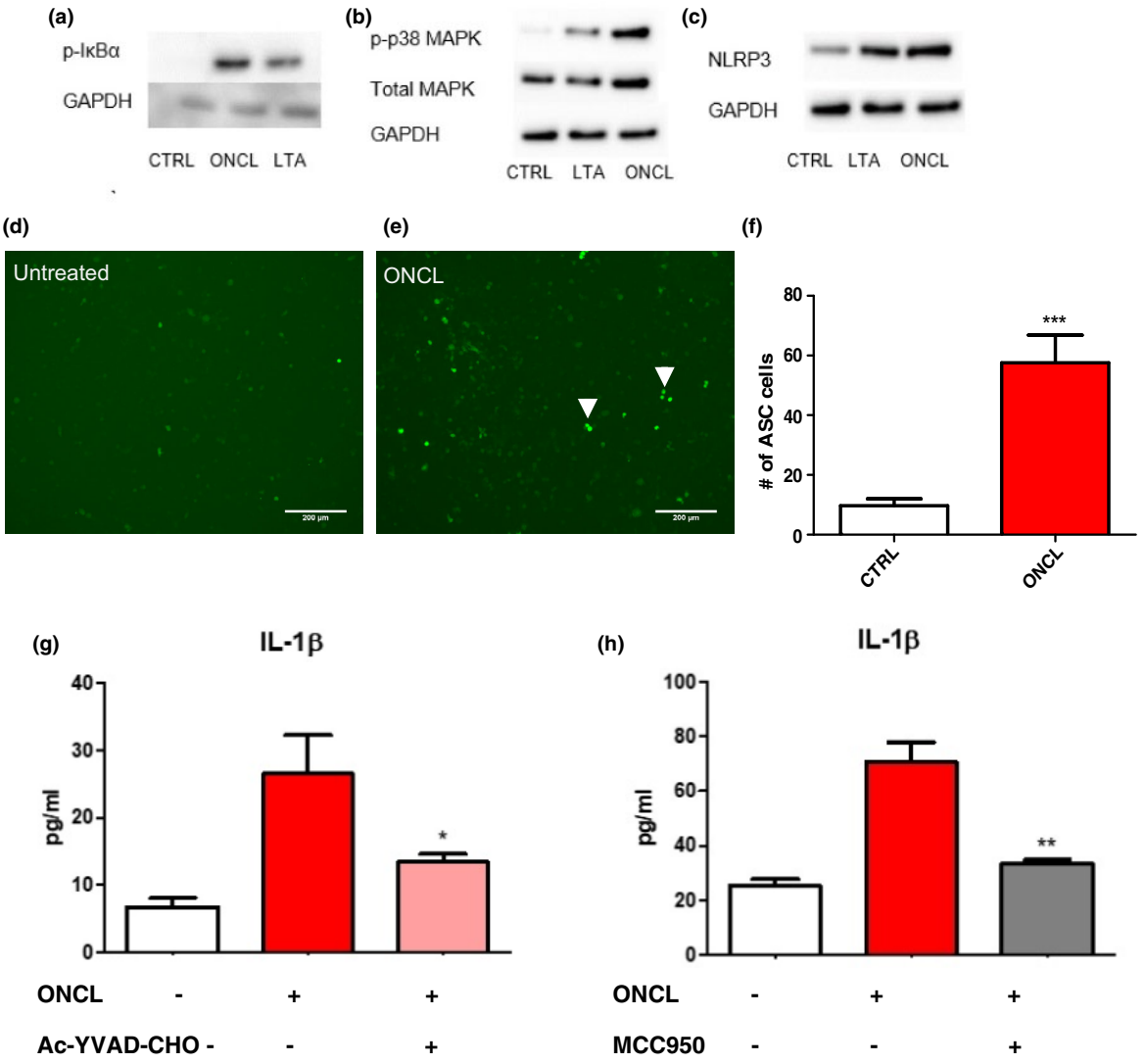

Figure 3 ONCL induced inflammation-signalling pathway. Representative images of western blots for (a) Phospho-IκBα (b) phosphorylated p38 MAPK, total MAPK, and (c) NLRP3 at the predicted molecular size on THP-1 macrophages treated with ONCL. LTA was used as positive control. (d, e) representative images from live cells imaging of THP-1 ASC-FGP macrophages showing ASC formation following ONCL treatment. The number of ASC positive cells is higher in ONCL treated compared to untreated cells (f). To confirm inflammasome activation with ONCL, pretreatment with both caspase-1 inhibiter, Ac-YVAD-CHO and NLRP3 inhibitor MCC950 significantly reduced the levels of IL-1 β release (g, h). Data represent avenge of three independent experiments and presented as mean ± SEM.*$P < 0.01$, **$P < 0.001$, ANOVA with Bonferoni correction.

Discussion

The mechanisms underlying cell death induced sterile inflammation and repair in the dental pulp are not fully understood. This study demonstrated for the first time that the death of odontoblasts could lead to a sterile inflammatory response, mediated by the NLRP3 inflammasome. The findings that dead odontoblasts could orchestrate an inflammatory response that facilitates repair are intriguing. Pioneer studies on pulpal wound healing suggested that the presence of necrosis in the direct pulp capping experiments is crucial for the healing process and reparative dentine formation (Schröder & Sundström 1974, Mejare & Cvek 1993). Although necrosis is usually accompanied by inflammation, the mechanism by which dead cells induce

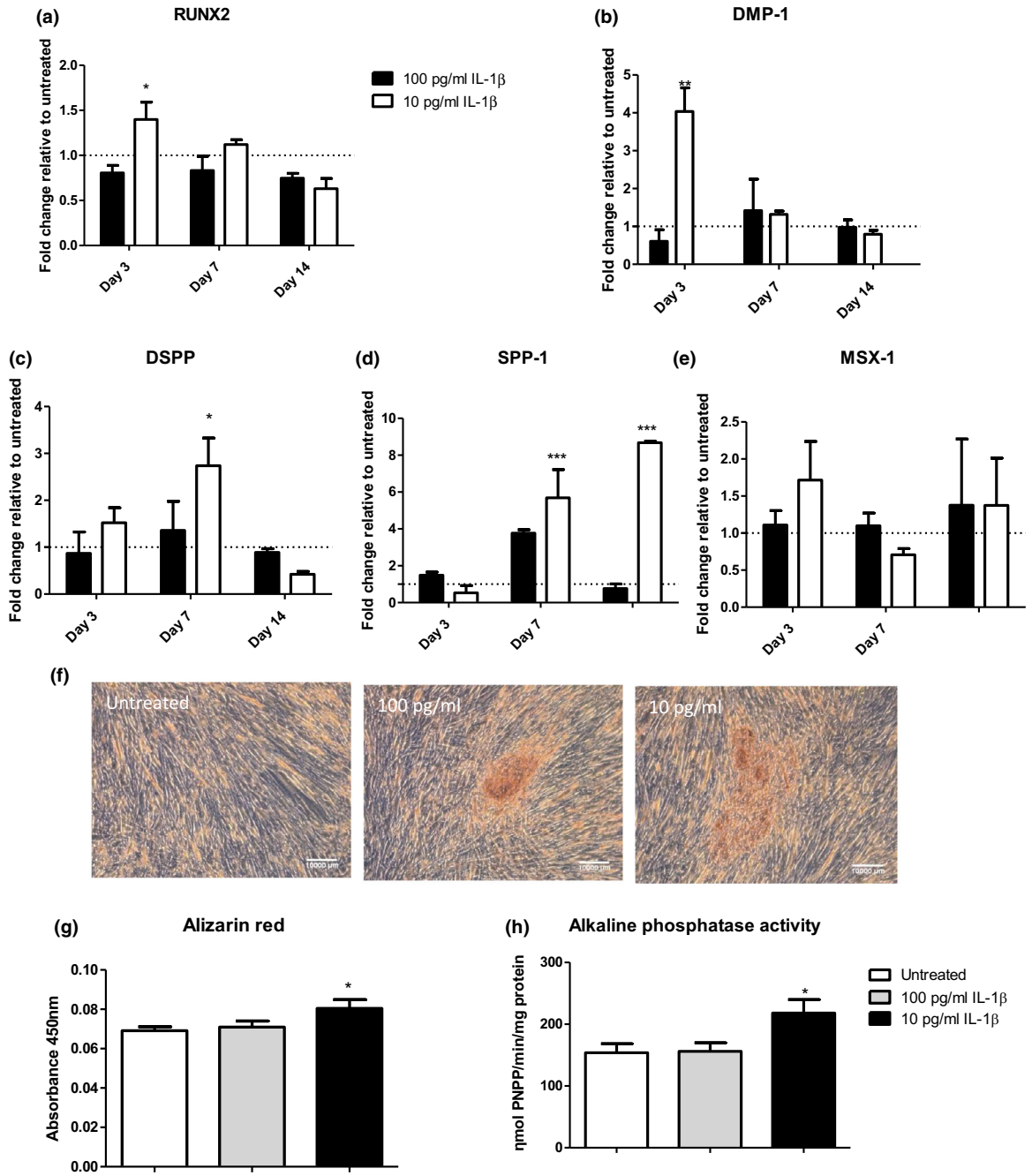

Figure 4 IL-β enhances osteo/odontogenic differentiation of DPCs. Expression of the osteo/odontogenic gene RUNX2, DMP-1 was significantly increased at day 3 with 10 pg/ IL-1β (a, b). DSPP and SPP-1 were significantly increased at day 7 (c, d) with SPP-1 expression continued to increase at day 14 (d). There was no significant change in the expression of MSX-1 (e). Treatment with IL-1β 10 pg mL^{-1} induced mineralization in DPCs (f, g) and alkaline phosphatase activity (h). Data represent average of three independent experiments and presented as mean ± SEM $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$; ANOVA and Kruskal–Wallis test. Scale bar = 10 000 μm.

inflammation is in its infancy with the discovery that DAMPs released from dead cells can activate the immune response (Chen *et al.* 2007). To date, many DAMPs including high-mobility group box 1 (HMGB1), extracellular adenosine triphosphate (eATP), heat shock proteins (HSPs), S100A8/A9 proteins, nucleic acids and proteoglycans amongst others have been identified (Sarhan *et al.* 2018). DAMPs-induced inflammation is considered essential to restoring tissue homoeostasis by activating tissue repair mechanisms. DAMPs activate macrophages, dendritic cells, and endogenous precursor stem cells that are required for initiating tissue repair (Limana *et al.* 2005, Vezzoli *et al.* 2011). DAMPs have been shown to induce the proliferation of smooth muscle cells (Kuraitis *et al.* 2012), fibroblasts and to stimulate angiogenesis (De Mori *et al.* 2007). They also promote regeneration of renal tubule cells and liver cells (Yang *et al.* 2009, 2012, Romagnani & Anders 2013). It is likely therefore that induction of DPCs proliferation and migration observed with ONCL is related to DAMPs released from dead odontoblasts that facilitate these initial steps in the process of reparative dentine formation (Tziafas 1994, Smith 2002). The nature of odontoblast released DAMPs and their specific function therefore merits further investigation.

In addition to a possible direct role on the activation of DPCs as outlined above, DAMPs could indirectly influence healing and repair by the activation of inflammasomes. Many studies reported that activation of inflammasome with DAMPS contributes to tissue regeneration through the inflammasome-dependent cytokines, promoting effective clearance of damaged cells and tissue repair (Artlett 2013, Santana *et al.* 2016). Indeed the findings of this study demonstrated that IL-1-β, the product of inflammasome activation, enhanced odontogenic differentiation of DPCs as evident by induction of odontogenic gene SPP1, DSP, DMP-1, Runx2 and increased alkaline phosphate activity and mineralization. IL-1β is a known pro-inflammatory cytokine that has been reported to increase collagen synthesis and the levels of vascular cell adhesion molecule1 in dental pulp cells (Barkhordar *et al.* 2002, Chang *et al.* 2012), however, its role in repair is not fully understood. Previous studies have shown that bone marrow-derived mesenchymal stem cells cultured under inflammatory conditions with IL-1β, developed a mineralization phenotype (Ferreira *et al.* 2013). In periodontal ligament stem cells, IL-1β had a dual effect where it enhanced mineralization at low doses 10 pg mL^{-1} and inhibited mineralization at higher concentrations (Mao *et al.* 2016). The level of IL-1β in the inflamed dental pulp tissue was reported to be in the pictogram range (Abd-Elmeguid *et al.* 2013) comparable to that which induced the odontogenic effect reported in this study and suggesting a role for IL-1β in the dental pulp repair mechanisms.

Conclusion

DAMPs released by dead odontoblast activated DPC migration and proliferation and induced NLRP3 inflammasome-dependent sterile inflammatory response that enhanced odontogenic differentiation of DPCs.

Acknowledgements

The authors would like to thank Ms Catherine Fulton and Dr Xenia Khodji for their technical assistance and advice. The work was funded by Jordan University of Science and Technology (PhD Scholarship to Banan Al Natour).

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

References

Abd-Elmeguid A, Abdeldayem M, Kline LW, Moqbel R, Vliagoftis H, Yu DC (2013) Osteocalcin expression in pulp inflammation. *Journal of Endodontics* **39**, 865–72.

Anders HJ, Schaefer L (2014) Beyond tissue injury - damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis. *American Society of Nephrology* **25**, 1387–400.

Artlett CM (2013) Inflammasomes in wound healing and fibrosis. *The Journal of Pathology* **229**, 157–67.

Barkhordar R, Ghani Q, Russell T, Hussain M (2002) Interleukin-1β activity and collagen synthesis in human dental pulp fibroblasts. *Journal of Endodontics* **28**, 157–9.

Bianchi ME (2006) DAMPs, PAMPs and alarmins: all we need to know about danger. *Journal of Leukocyte Biology* **81**, 1–5.

Chang M-C, Lin L-D, Zwei J *et al.* (2012) Regulation of vascular cell adhesion molecule-1 in dental pulp cells by interleukin-1β: the role of prostanoids. *Journal of Endodontics* **38**, 774–9.

Chen CJ, Kono H, Golenbock D, Reed G, Akira S, Rock KL (2007) Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nature Medicine* **13**, 851–6.

De Mori R, Straino S, Carlo AD *et al.* (2007) Multiple effects of high mobility group box protein 1 in skeletal muscle regeneration. *Arteriosclerosis Thrombosis Vascular Biology.* **27**, 2377–83.

El Karim IA, Linden GJ, Curtis TM *et al.* (2011) Human odontoblasts express functional thermo-sensitive TRP channels: Implications for dentin sensitivity. *Pain* **152**, 2211–23.

Farges JC, Alliot-Licht B, Renard E *et al.* (2015) Dental pulp defence and repair mechanisms in dental caries. *Mediators of Inflammation* **2015**, 230251.

Ferreira E, Porter RM, Wehling N *et al.* (2013) Inflammatory cytokines induce a unique mineralizing phenotype in mesenchymal stem cells derived from human bone marrow. *Journal of Biological Chemistry* **288**, 29494–505.

Goldberg M, Njeh A, Uzunoglu E (2015) Is pulp inflammation a prerequisite for pulp healing and regeneration? *Mediators of Inflammation* **2015**, 347649.

Gong T, Liu L, Jiang W, Zhou R (2020) DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nature Reviews Immunology* **20**, 95–112.

Gregory CA, Grady Gunn W, Peister A, Prockop DJ (2004) An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Analytical Biochemistry* **329**, 77–84.

Kuraitis D, Ebadi D, Zhang P *et al.* (2012) Injected matrix stimulates myogenesis and regeneration of mouse skeletal muscle after ischaemic injury. *European Cells and Materials* **24**, 175–95.

Lamkanfi M (2011) Emerging inflammasome effector mechanisms. *Nature Reviews Immunology* **11**, 213–20.

Limana F, Germani A, Zacheo A *et al.* (2005) Exogenous high-mobility group box 1 protein induces myocardial regeneration after infarction via enhanced cardiac C-kit+ cell proliferation and differentiation. *Circulation Research* **97**, e73–83.

Liu Y, Kimura K, Orita T, Sonoda KH (2015) Necrosis-induced sterile inflammation mediated by interleukin-1α in retinal pigment epithelial cells. *PLoS One* **10**, 1–17.

Mao CY, Wang YG, Zhang X, Zheng XY, Tang TT, Lu EY (2016) Double-edged-sword effect of IL-1β on the osteogenesis of periodontal ligament stem cells via crosstalk between the NF-κB, MAPK and BMP/Smad signaling pathways. *Cell Death & Disease* **7**, e2296.

Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Molecular Cell* **10**, 417–26.

Mejare I, Cvek M (1993) Partial pulpotomy in young permanent teeth with deep carious lesions. *Dental Traumatology* **9**, 238–42.

Romagnani P, Anders H-J (2013) What can tubular progenitor cultures teach us about kidney regeneration? *Kidney International* **83**, 351–3.

Santana PT, Martel J, Lai HC *et al.* (2016) Is the inflammasome relevant for epithelial cell function? *Microbes and Infection* **18**, 93–101.

Sarhan M, Land WG, Tonnus W, Hugo CP, Linkermann A (2018) Origin and consequences of necroinflammation. *Physiological Reviews* **98**, 727–80.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671–5.

Schröder U, Sundström B (1974) Transmission electron microscopy of tissue changes following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odontologisk Revy* **25**, 57–68.

Smith AJ (2002) Pulpal responses to caries and dental repair. *Caries Research* **36**, 223–32.

Song Z, Lin Z, He F *et al.* (2012) NLRP3 is expressed in human dental pulp cells and tissues. *Journal of Endodontics* **38**, 1592–7.

Tziafas D (1994) Mechanisms controlling secondary initiation of dentin. *International Endodontic Journal* **27**, 61–74.

Vezzoli M, Castellani P, Corna G *et al.* (2011) High-mobility group box 1 release and redox regulation accompany regeneration and remodeling of skeletal muscle. *Antioxidants and Redox Signaling* **15**, 2161–74.

Wang L, Jin H, Ye D *et al.* (2016) *Enterococcus faecalis* lipoteichoic acid–induced NLRP3 inflammasome via the activation of the nuclear factor kappa B pathway. *Journal of Endodontics* **42**, 1093–100.

Wang L, Manji GA, Grenier JM *et al.* (2002) PYPAF7, a novel PYRIN-containing Apaf1-like protein that regulates activation of NF-kappa B and caspase-1-dependent cytokine processing. *The Journal of Biological Chemistry* **277**, 29874–80.

Wang W, Wang X, Chun J *et al.* (2013) Inflammasome-independent NLRP3 augments TGF-β signaling in kidney epithelium. *The Journal of Immunology* **190**, 1239–49.

Winning L, El Karim IA, Lundy FT (2019) A comparative analysis of the osteogenic potential of dental mesenchymal stem cells. *Stem Cells and Development* **28**, 1050–8.

Yang D, de la Rosa G, Tewary P, Oppenheim JJ (2009) Alarmins link neutrophils and dendritic cells. *Trends in Immunology* **30**, 531–7.

Yang R, Zhang S, Cotoia A, Oksala N, Zhu S, Tenhunen J (2012) High mobility group B1 impairs hepatocyte regeneration in acetaminophen hepatotoxicity. *BMC Gastroenterology* **12**, 1–8.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Characterization of odontoblast-like cells.

