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ORIGINAL ARTICLE

Immunostimulatory activity of low-molecular-weight hyaluronan on dendritic cells stimulated with *Aggregatibacter actinomycetemcomitans* or *Porphyromonas gingivalis*

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Abstract

Objectives Periodontitis is a chronic inflammatory disease characterized by tooth-supporting tissue destruction, which is elicited by the host's immune response triggered against periodonto-pathogen bacteria. During periodontal tissue destruction, extracellular matrix components are metabolized and fragmented. Some extracellular matrix component-derived fragments, such as low-molecular-weight hyaluronan (LMW-HA), have potent immunogenic potential, playing a role as damage-associated molecular patterns (DAMPs) during activation of immune cells. Dendritic cells (DCs) play a central role in the host's immune response displayed during periodontitis; thus, this study aimed to analyze whether LMW-HA has an immunostimulatory activity on DCs when stimulated with periodonto-pathogen bacteria.

Materials and methods LMW-HA-treated and non-treated DCs were stimulated with *Aggregatibacter actinomycetemcomitans* or *Porphyromonas gingivalis* and the mRNA expression for cytokines tumor necrosis factor-α (TNF-alpha), interleukin-1β (IL-1B), interleukin-6 (IL-6), and interleukin-23 (IL-23A) was quantified by RT-qPCR. In addition, transcription factors interferon regulatory factor 4 (IRF4), interferon regulatory factor 8 (IRF8), neurogenic locus notch homolog protein 2 (NOTCH2), and basic leucine zipper ATF-like transcription factor 3 (BATF3), involved in DC activation, were analyzed.

Results Higher expression levels of TNF-alpha, IL-1B, IL-6, and IL-23A were detected in LMW-HA-treated DCs after bacterial infection, as compared with non-treated DCs. When LMW-HA-treated DCs were infected with *A. actinomycetemcomitans*, higher levels of IRF4, NOTCH2, and BATF3 were detected compared with non-treated cells; whereas against *P. gingivalis* infection, increased levels of IRF4 and NOTCH2 were detected.

Conclusion LMW-HA plays an immunostimulatory role on the immune response triggered by DCs during infection with *A. actinomycetemcomitans* or *P. gingivalis*.

Clinical relevance Detection of extracellular matrix component-derived fragments produced during periodontal tissue destruction, such as LMW-HA, could explain at least partly unsuccessful periodontal treatment and the chronicity of the disease.

Keywords Hyaluronan · LMW-HA · Dendritic cells · Cytokines · Transcription factors · *Aggregatibacter actinomycetemcomitans* · *Porphyromonas gingivalis*

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Introduction

Hyaluronic acid (HA), also known as hyaluronan, is a non-sulphated glycosaminoglycan ubiquitously distributed in the extracellular matrix of several tissues, including periodontal tissues [1–3]. In physiological conditions, HA possesses a high-molecular-weight structure (HMW-HA) with over 1000 kDa [2, 4]; nevertheless, in inflamed tissues, HA can be degraded to low-molecular-weight fragments (LMW-HA) of < 500 kDa [2, 5]. LMW-HA can act as an immunogen on

immune cells, promoting the expression of transcription factors and pro-inflammatory cytokines via expression and activity of nuclear factor-kappa B (NF-κB) [5–8].

During periodontitis, dendritic cells (DCs) fulfill a central role as antigen-presenting cells, along the beginning of the host's immune response and regulating it against periodontal pathogenic bacteria [9]. DCs have the ability to recognize not only pathogen-associated molecular patterns (PAMPs) but also damage-associated molecular patterns (DAMPs) [9], such as the LMW-HA released during periodontal tissue destruction. Thus, activation of DCs triggered by bacterial antigens could be modified in the presence of LMW-HA. In this context, considering the distinct patterns of transcription factor expression and cytokine production, three subsets of DCs have been described: conventional type 1 DCs (cDC1s) and type 2 DCs (cDC2s), which are mainly involved in bacterial antigen recognition and presentation, and plasmacytoid DCs (pDCs) [10, 11]. Thereby, during antigen presentation to T lymphocytes, each of these DC subsets could induce a distinct pattern of immune response depending on the DAMPs and/or PAMPs that they recognize. In fact, cDC1 and pDC subsets produce interleukin-1β and interleukin-12 and are associated with priming of Th1 lymphocytes [12–14]. Otherwise, cDC2 subset produces high amounts of interleukin-1β, interleukin-6, and interleukin-23, leading to the priming of Th1 and/or Th17 lymphocytes [13–17].

Higher levels of LMW-HA are detected in the periodontal tissues of patients affected with periodontitis as compared with healthy individuals [18, 19]. Therefore, the immune response triggered by DCs against periodontal pathogenic bacterial challenge could vary in the presence of increased levels of LMW-HA. In this study, we analyzed the immunogenic potential of LMW-HA in DCs infected with *P. gingivalis* or *A. actinomycetemcomitans*, quantifying the production of cytokines and the expression of transcription factors that determine the activation of the distinct DCs subtypes.

Materials and methods

A. actinomycetemcomitans and *P. gingivalis* strains and culture conditions

The strain Y4 (ATCC® 43718™) of *A. actinomycetemcomitans* was cultured on tryptone soy agar (TSA; Oxoid Ltd., Hampshire, England), supplemented with 0.6% yeast extract (Oxoid Ltd.) and 10% heat inactivated horse serum (Biological Industries USA Inc., Cromwell, CT, USA), at 37 °C and under capnophilic conditions generated with a microaerobic environment conditioner (CampyGen™; Oxoid Ltd). The strain W83 (ATCC® BAA-308™) of *P. gingivalis* was cultured on blood agar base no. 2 (Oxoid Ltd.), supplemented with 5% defibrinated horse blood (Blood and Hemoderivatives Laboratory, Public Health Institute, Santiago, Chile), 5 mg/l hemin (Calbiochem®, EMD Chemicals Inc., San Diego, CA, USA), and 1 mg/l menadione (Sigma-Aldrich Co., St. Louis, MO, USA), at 37 °C and under anaerobic conditions in an anaerobic chamber (Bactronez-2; Sheldon Manufacturing Inc., Cornelius, OR, USA). In order to obtain a reliable number of live bacteria having their whole antigenic potentiality for DC stimulation, bacterial growth curves were made in liquid brain-heart infusion medium (Oxoid Ltd.), supplemented with 10% heat inactivated horse serum or 5 mg/l hemin and 1 mg/l menadione for *A. actinomycetemcomitans* or *P. gingivalis*, respectively, and bacteria were taken at the exponential growth phase at 48 h.

Blood donors

Blood cells were obtained during platelet-apheresis procedures from healthy donors consecutively enrolled at the Blood Bank of the Clinic Hospital Dr. José Joaquín Aguirre, Universidad de Chile. The study group consisted of 10 adults who did not have periodontal disease as determined by the absence of gingival inflammation, clinical attachment loss, or increased probing depths. Further exclusion criteria were being positive for human immunodeficiency virus, being positive for hepatitis B or C virus, history of manifest infections during the last month, fever, symptomatic allergies, abnormal blood cell counts, increased liver enzymes, or medication of any kind except vitamins and oral contraceptives. The study design (Protocol 2010/14) was approved by the Ethics Committee for Human Research of the Faculty of Dentistry, Universidad de Chile, and conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. The investigation protocol was clearly explained to all the individuals, who agreed to participate in the study by signing an IRB-approved informed consent.

Dendritic cell generation

DCs were generated using a three-step protocol as previously described [20]. First, peripheral blood mononuclear cells were isolated using a density gradient centrifugation (Ficoll-Paque Plus; GE Healthcare, Uppsala, Sweden). From them, monocytes were purified by magnetic cell sorting using an anti-CD14 monoclonal antibody conjugated to magnetic beads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. Finally, DCs were generated by culturing the $CD14^+$ monocytes at 10^6 cells/ml in RPMI-1640 supplemented with 10% fetal bovine serum (Gibco Invitrogen Corp., Grand Island, NY, USA), 20 ng/ml granulocyte-monocyte colony stimulating factor (GM-CSF), and 20 ng/ml interleukin-4 (IL-4) (R&D Systems Inc., Minneapolis, MN, USA) for 6 days.

Hyaluronan treatment of dendritic cells

In order to analyze the dose-dependent response of DCs against hyaluronan, cells were treated with 25, 50, or 100 μg/ml of LMW-HA (hyaluronan 20 kDa; R&D Systems Inc.) applied from day 3 every 24 h, as previously described [21, 22]. On day 7, DCs were harvested for RNA extraction and subsequent RT-qPCR analysis. Non-treated DCs were used as negative control.

Bacterial stimulation of dendritic cells

In bacteria stimulation experiments, DCs were treated with 100 μg/ml of LMW-HA from day 3 every 24 h, and on day 7, cells were infected at a multiplicity of infection (MOI) = 100 with the *A. actinomycetemcomitans* or *P. gingivalis* strains. Two days post-infection, DCs were recovered to perform the RT-qPCR experiments. Non-treated DCs were used as control. Non-treated and non-infected DCs were used for comparisons. In each experimental step, cell counting was performed using a Neubauer® chamber and a phase contrast microscope (AxioVert 100; Carl Zeiss Co., Göttingen, Germany) and cell viability was assessed by Trypan blue dye exclusion. For each individual, the experiments were performed separately.

Expression of cytokines and transcription factors

For RT-qPCR analysis, total RNA was isolated from DCs using 400 μl ice-cold cell lysis buffer containing 0.5% Igepal® CA-630 (Sigma-Aldrich Co.) as previously described [20]. The first cDNA strands were synthesized using a reverse transcription kit (SuperScript III®; Invitrogen, Grand Island, NY, USA) following the manufacturer's protocol. The mRNA expression for the pro-inflammatory cytokines tumor necrosis factor-α (TNF-alpha), interleukin-1β (IL-1B), interleukin-6 (IL-6), and intereukin-23 (IL-23A), as well as for the transcription factors interferon regulatory factor 4 (IRF4), interferon regulatory factor 8 (IRF8), neurogenic locus notch homolog protein 2 (NOTCH2), and basic leucine zipper ATF-like transcription factor 3 (BATF3) involved in DC activation and polarization, was analyzed using the appropriate primers (Table 1) and a qPCR reagent (KAPA™ SYBR® Fast; KAPA Biosystems, Woburn, MA, USA). For this, 50 ng of cDNA was analyzed in a qPCR equipment (StepOnePlus®; Applied Biosystems, Singapore) with the following amplification protocol: 95 °C for 3 min and 40 cycles of 95 °C for 3 s and 60 °C for 30 s. For detection of non-specific product formation and false-positive amplification, a melt curve of 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s was performed. As an endogenous control, 18S rRNA expression levels were quantified.

Table 1 Forward and reverse primers used for cytokine and transcription factor mRNAs and 18S rRNA amplifications by RT-qPCR

Target	Forward primer	Reverse primer
TNF-alpha	cagcctcttctccttcctgat	gccagagggctgattagaga
IL-1B	ctgtcctgcgtgttgaaaga	ttgggtaatttttgggatctaca
IL-6	gcccagctatgaactccttct	gaaggcagcaggcaacac
IL-23A	agcttcatgcctccctactg	ctgctgagtctcccagtggt
IRF4	gacaacgccttacccttcg	aggggtggcatcatgtagtt
IRF8	tggggatgatcaaaaggagcc	aactggctggtgtcgaagac
NOTCH2	cagttacccacccacaggtc	ccatacaggcagtcaatggaa
BATF3	agacccagaaggctgacaag	ctccgcagcatggtgttt
18S rRNA	ctcaacacgggaaacctcac	cgctccaccaactaagaacg

BATF3, basic leucine zipper ATF-like transcription factor 3; *IL-1B*, interleukin-1β; *IL-6*, interleukin-6; *IL-23A*, interleukin-23; *IRF4*, interferon regulatory factor 4; *IRF8*, interferon regulatory factor 8; *NOTCH2*, neurogenic locus notch homolog protein 2; *TNF-alpha*, tumor necrosis factor-α

Statistical analysis

Data were presented as mean ± standard deviation of relative fold changes by normalizing the cytokine or transcription factor mRNA expression to the 18S rRNA expression using the $2^{-\Delta\Delta Ct}$ method (StepOne 2.2.2; Applied Biosystems). Statistical analysis was performed using the SPSS 22.0 software (IBM Corp., Armonk, NY, USA). The normality of data distribution was determined using the Kolmogorov-Smirnov test. Differences were analyzed using the analysis of variance (ANOVA) together with the Bonferroni post hoc tests. Probabilities of 0.05 or less were accepted as significant.

Results

Expression of cytokines in LMW-HA-treated dendritic cells

The levels of TNF-alpha, IL-1B, IL-6, and IL-23A expressed in DCs treated with 25, 50, or 100 μg/ml of LMW-HA are shown as fold change (Fig. 1). A dose-dependent increase in the expression levels for each cytokine was detected, with significantly higher expression levels of TNF-alpha ($p < 0.001$), IL-1B ($p = 0.031$), IL-6 ($p < 0.001$), and IL-23A ($p < 0.001$) when DCs were treated with 100 μg/ml of LMW-HA compared with non-treated cells.

Expression of cytokines in LMW-HA-treated and bacteria-stimulated dendritic cells

An increment in the expression levels for TNF-alpha, IL-1B, IL-6, and IL-23A mRNAs was detected in DCs stimulated

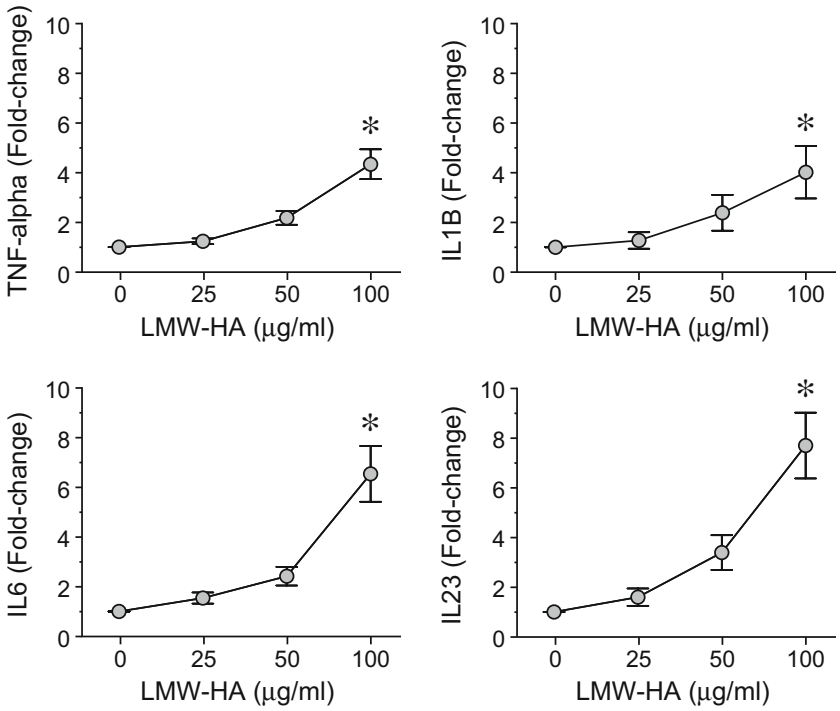

Fig. 1 Dose-dependent immunostimulatory potential of hyaluronan. Quantification of the mRNA levels for the cytokines tumor necrosis factor-α (TNF-alpha), interleukin-1β (IL-1B), interleukin-6 (IL-6), and interleukin-23 (IL-23A) is represented as fold change in dendritic cells treated with low-molecular-weight hyaluronan (LMW-HA = 25, 50, or 100 μg/ml). The cytokine mRNA expression in LMW-HA non-treated dendritic cells was considered as 1, as a reference for fold change in expression. Data from 8 independent experiments are shown as mean ± SD. Each experiment was performed in duplicate. *$p < 0.05$

with *A. actinomycetemcomitans* or *P. gingivalis* strains compared with non-infected cells (Fig. 2). When *A. actinomycetemcomitans*- or *P. gingivalis*-infected DCs were treated with LMW-HA, higher levels for TNF-alpha ($p < 0.001$), IL-1B ($p = 0.002$ and $p = 0.030$), IL-6 ($p = 0.038$ and $p = 0.002$), and IL-23A ($p = 0.031$ and $p = 0.011$) mRNAs were detected compared with non-treated cells.

Expression of transcription factors in LMW-HA-treated and bacteria-stimulated dendritic cells

Figure 3 depicts the expression levels for IRF4, IRF8, NOTCH2, and BATF3 mRNAs in infected DCs treated or non-treated with LMW-HA. When *A. actinomycetemcomitans*-infected DCs were treated with LMW-HA, higher levels for IRF4 ($p = 0.005$), NOTCH2 ($p = 0.032$), and BATF3 ($p = 0.040$) mRNAs were detected compared with non-treated cells. No differences were detected in the mRNA expression for IRF8. When *P. gingivalis*-infected DCs were treated with LMW-HA, higher levels for IRF4 ($p = 0.003$) and NOTCH2 ($p = 0.033$) mRNAs were detected compared with non-treated cells. No differences were detected in the mRNA expression for IRF8 and BATF3.

Discussion

At sites of inflammation, LMW-HA is generated due to fragmentation of HMW-HA via reactive oxygen species and enzymatic degradation by hyaluronidase, β-glucuronidase, and hexosaminidase [5]. From an immune point of view, LMW-HA acts as a DAMP, since it promotes the production of pro-inflammatory cytokines on immune cells, such as DCs [5, 6, 22–24]. During periodontitis, LMW-HA has been associated with the inhibition of the differentiation of osteoblasts responsible for alveolar bone formation [25] and the induction of apoptosis of gingival fibroblasts [26], which favors the loss of tissue homeostasis and destruction of periodontal tissues. In this study, the effect of LMW-HA on DCs when they are stimulated with *A. actinomycetemcomitans* or *P. gingivalis* was analyzed and it was demonstrated that LMW-HA induces an increment in the expression of immune-regulatory transcription factors and pro-inflammatory cytokines.

In DCs, LMW-HA induced cytokine expression in a dose-dependent manner, where the stimulation with 100 μg/ml triggered the highest expression levels of pro-inflammatory cytokines, which is consistent with previous results utilizing different concentrations of LMW-HA and determining that the stimuli with 100 μg/ml induced the highest proliferation of T and B lymphocytes [21]. In addition, it has been previously reported that this LMW-HA concentration falls in the pathophysiological range detected in inflamed tissues [27, 28]. In this context, the effect of the stimulation with *A. actinomycetemcomitans* or *P. gingivalis* on DCs after their exposure to 100 μg/ml of LMW-HA was analyzed. The results suggest that LMW-HA could be involved in the generation of a local cellular microenvironment that favors and eventually enhances the immunogenic effect of periodontal pathogenic bacteria on DCs.

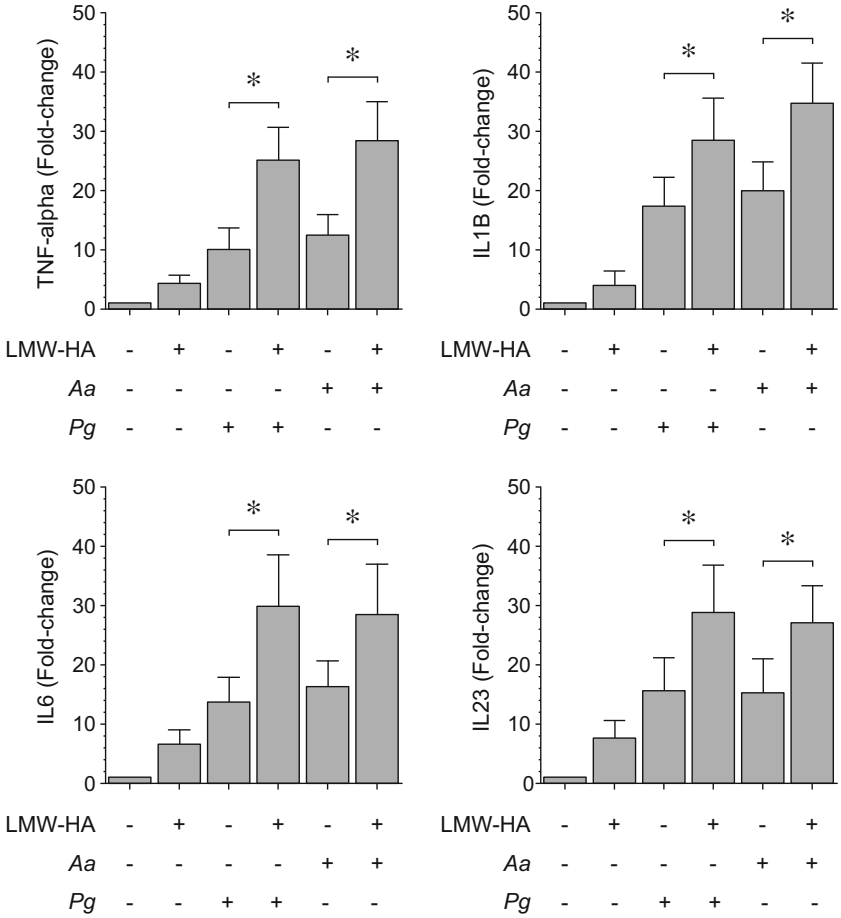

Fig. 2 Cytokine expression in infected dendritic cells. Quantification of the mRNA levels for the cytokines tumor necrosis factor-α (TNF-alpha), interleukin-1β (IL-1B), interleukin-6 (IL-6), and interleukin-23 (IL-23A) is represented as fold change in dendritic cells non-treated or treated with low-molecular-weight hyaluronan (LMW-HA = 100 μg/ml) and stimulated with *A. actinomycetemcomitans* (*Aa*) or *P. gingivalis* (*Pg*) (MOI = 100). The cytokine mRNA expression in LMW-HA non-treated and non-infected dendritic cells was considered as 1, as a reference for fold change in expression. Data from 10 independent experiments are shown as mean ± SD. Each experiment was performed in duplicate. $*p < 0.05$

The cytokines expressed by DCs depend on the cellular subset that has been stimulated [29], which consequently defines the pattern of adaptive immune response induced in T lymphocytes [30]. From this basis, LMW-HA alone was not able to induce an overexpression of the analyzed transcription factors. Conversely, DCs exposed to LMW-HA and bacteria elicited an overexpression of IRF4, NOTCH2, and BATF3. The transcription factor BATF3 is key to the activation of the cDC1 subtype, which is associated with the induction of a Th1 pattern of immune response [12]. In addition, IRF4 is the transcription factor that determines the activation of the cDC2 phenotype, also associated with Th1-type immunity [13]. Nevertheless, the overexpression of both IRF4 and NOTCH2 in cDC2s leads them to produce interleukin-6 and interleukin-23, which in turn induce a Th17 pattern of immune response [14–17, 31].

In the present investigation, *A. actinomycetemcomitans* in the presence of LMW-HA induced the overexpression of the transcription factors IRF4, NOTCH2, and BATF3, associated with the cDC1 and cDC2 lineages [13, 32]. On the other hand, *P. gingivalis* in the presence of LMW-HA induced the overexpression of the transcription factors IRF4 and NOTCH2, which only promote the activation of cDC2s. Thus, it could be established that *A. actinomycetemcomitans* is implied in the activation and polarization of more DC subsets involved in host's immuno-inflammatory response as compared with *P. gingivalis*, at least in the context of our experiments, which is consistent with the higher immunogenicity of *A. actinomycetemcomitans* on DCs, as compared with *P. gingivalis* [20]. In particular, the Th1 and Th17 patterns of immune response against *A. actinomycetemcomitans* would be associated with the activity of cDC1s and cDC2s, and the Th1 pattern of immune response against *P. gingivalis* would be associated with cDC2 activity. Even though different patterns of immune response could be detected between both bacteria, in both cases, overexpression of Th1 and Th17 types of cytokines was detected, with enhanced TNF-alpha, IL-1B, IL-6, and IL-23A expression when infected DCs were treated with LMW-HA.

The transcription factors implied in the activation and polarization of cDC1s and cDC2s have been described in studies using conventional myeloid DCs, which differ from the monocyte-derived DCs used in the present investigation [14, 32]. Monocyte-derived DCs show more functional and transcriptional similitudes with cDC2s than with cDC1s or pDCs [31]. Meanwhile, GM-CSF used during

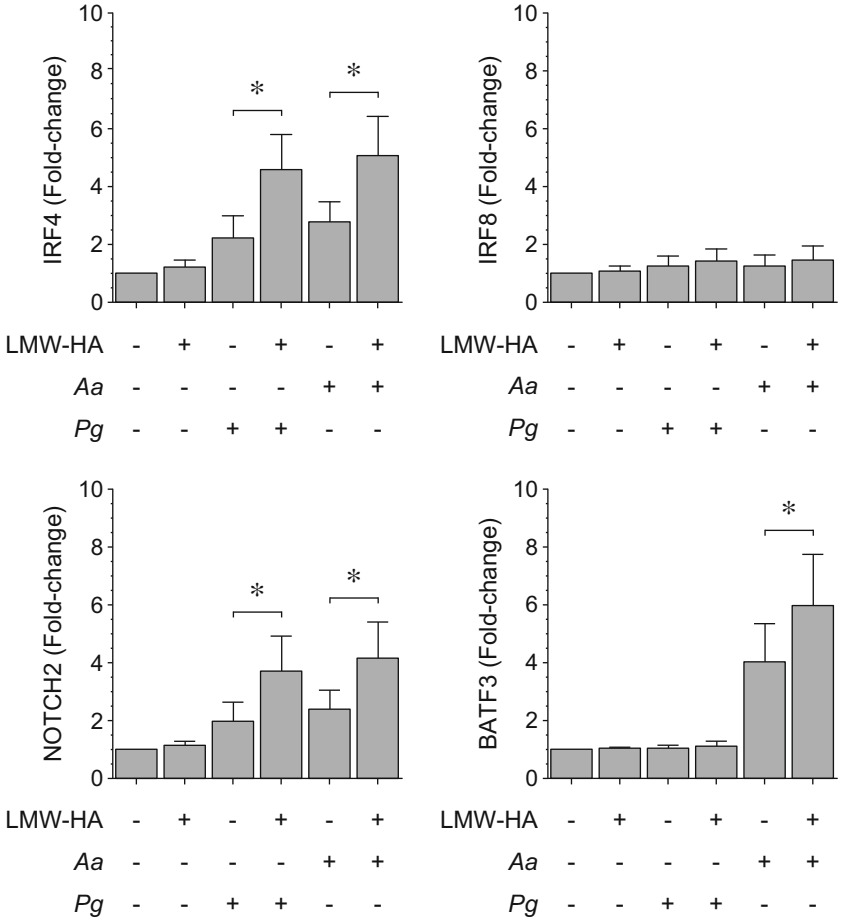

Fig. 3 Transcription factor expression in infected dendritic cells. Quantification of the mRNA levels for the transcription factors interferon regulatory factor 4 (IRF4), interferon regulatory factor 8 (IRF8), neurogenic locus notch homolog protein 2 (NOTCH2), and basic leucine zipper ATF-like transcription factor 3 (BATF3) is represented as fold change in dendritic cells non-treated or treated with low-molecular-weight hyaluronan (LMW-HA = 100 μg/ml) and stimulated with *A. actinomycetemcomitans* (*Aa*) or *P. gingivalis* (*Pg*) (MOI = 100). The cytokine mRNA expression in LMW-HA non-treated and non-infected dendritic cells was considered as 1, as a reference for fold change in expression. Data from 10 independent experiments are shown as mean ± SD. Each experiment was performed in duplicate. *$p < 0.05$

the differentiation protocol of DCs could induce the early inhibition of IRF8 and the modulation of transcription factors associated with the plasmacytoid subtype [31]. Furthermore, the obtainment of DCs using a protocol that stimulates monocytes with GM-CSF and IL-4 is dependent of IRF4 but does not require nor inhibits the expression of BATF3 [33]. IRF8 is expressed during early stages of hematopoiesis and by direct and indirect competition with other transcription factors promotes the polarization of DCs to the cDC1 and pDC lineages [31]. Moreover, its expression directly competes with IRF4 and consequently controls the cDC1:cDC2 balance, while the expression of BATF3 determines the final stage of maturation to a cDC1 lineage [31]. Accordingly, the early inhibition of IRF8 and the unaffected expression of IRF4 and BATF3 in the DCs analyzed in the present investigation suggests that the results regarding the expression levels of IRF8 could be, at least in part, due to the use of a linage of DCs that could present phenotypical variations in the transcriptional programs of human cDC1 and pDC, but that presents similarities with the cDC2 subtype. This could represent a limitation of this study that could be further improved by using cDC and pDC obtaining protocols [34].

Periodontitis is characterized by a deregulated immunoinflammatory response that leads to chronic inflammatory status and tooth-supporting tissue destruction. During extracellular matrix destruction, the generation of LMW-HA activates DCs and consequently promotes the adaptive immune response [5]. In fact, LMW-HA favors antigen presentation by inducing the expression of the co-stimulatory molecules CD80 and CD86 [35], stimulates DC migration towards the draining lymph nodes [36], and enhances the expression of cDC1/cDC2-related cytokines and transcription factors, as demonstrated in the present investigation. Therefore, during periodontitis, LMW-HA might act as a strong adjuvant of adaptive immune response against periodonto-pathogens, promoting breakdown of periodontal tissues, additional formation of LMW-HA, and, thus, the chronicity of the disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol (Protocol 2010/14) was approved by the Ethics Committee for Human Research of Faculty of Dentistry, Universidad de Chile.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Bansal J, Kedige SD, Anand S (2010) Hyaluronic acid: a promising mediator for periodontal regeneration. Indian J Dent Res 21(4):575
2. Jiang D, Liang J, Noble PW (2011) Hyaluronan as an immune regulator in human diseases. Physiol Rev 91(1):221–264
3. Necas J, Bartosikova L, Brauner P, Kolar J (2008) Hyaluronic acid (hyaluronan): a review. Vet Med 53(8):397–411
4. Bowden DJ, Byrne CA, Alkhayat A, Eustace SJ, Kavanagh EC (2017) Injectable viscoelastic supplements: a review for radiologists. AJR Am J Roentgenol 209(4):883–888
5. Powell JD, Horton MR (2005) Threat matrix. Low-molecular-weight hyaluronan (HA) as a danger signal. Immunol Res 31(3): 207–218
6. Petrey AC, de la Motte CA (2014) Hyaluronan, a crucial regulator of inflammation. Front Immunol 5:101
7. Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR (2006) Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. J Immunol 177(2):1272–1281
8. Termeer C, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, Miyake K, Freudenberg M, Galanos C, Simon JC (2002) Oligosaccharides of hyaluronan activate dendritic cells via toll-like receptor 4. J Exp Med 195(1):99–111
9. Song L, Dong G, Guo L, Graves DT (2018) The function of dendritic cells in modulating the host response. Mol Oral Microbiol 33(1):13–21
10. Mildner A, Jung S (2014) Development and function of dendritic cell subsets. Immunity 40(5):642–656
11. Satpathy AT, Wu X, Albring JC, Murphy KM (2012) Re(de)fining the dendritic cell lineage. Nat Immunol 13(12):1145–1154
12. Chandra J, Kuo PT, Hahn AM, Belz GT, Frazer IH (2017) Batf3 selectively determines acquisition of $CD8^+$ dendritic cell phenotype and function. Immunol Cell Biol 95(2):215–223
13. Nizzoli G, Krietsch J, Weick A, Steinfelder S, Facciotti F, Gruarin P, Bianco A, Steckel B, Moro M, Crosti M, Romagnani C, Stölzel K, Torretta S, Pignataro L, Scheibenbogen C, Neddermann P, De Francesco R, Abrignani S, Geginat J (2013) Human $CD1c^+$ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. Blood 122(6):932–942
14. Tussiwand R, Gautier EL (2015) Transcriptional regulation of mononuclear phagocyte development. Front Immunol 6:533
15. Bedoui S, Heath WR (2015) Krüppel-ling of IRF4-dependent DCs into two functionally distinct DC subsets. Immunity 42(5):785–787
16. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hägerbrand K, Marsal J, Gudjonsson S, Håkansson U, Reizis B, Kotarsky K, Agace WW (2013) IRF4 transcription-factor-dependent $CD103^+CD11b^+$ dendritic cells drive mucosal T helper 17 cell differentiation. Immunity 38(5):958–969
17. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, Ho AW, See P, Shin A, Wasan PS, Hoeffel G, Malleret B, Heiseke A, Chew S, Jardine L, Purvis HA, Hilkens CM, Tam J, Poidinger M, Stanley ER, Krug AB, Renia L, Sivasankar B, Ng LG, Collin M, Ricciardi-Castagnoli P, Honda K, Haniffa M, Ginhoux F (2013) IRF4 transcription factor-dependent $CD11b^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity 38(5):970–983
18. Casale M, Moffa A, Vella P, Sabatino L, Capuano F, Salvinelli B, Lopez MA, Carinci F, Salvinelli F (2016) Hyaluronic acid: perspectives in dentistry. A systematic review. Int J Immunopathol Pharmacol 29(4):572–582
19. Yamalik N, Kilinç K, Çağlayan F, Eratalay K, Çağlayan G (1998) Molecular size distribution analysis of human gingival proteoglycans and glycosaminoglycans in specific periodontal diseases. J Clin Periodontol 25(2):145–152
20. Vernal R, Leon R, Herrera D, Garcia-Sanz JA, Silva A, Sanz M (2008) Variability in the response of human dendritic cells stimulated with *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans*. J Periodontal Res 43(6):689–697
21. Ke C, Wang D, Sun Y, Qiao D, Ye H, Zeng X (2013) Immunostimulatory and antiangiogenic activities of low molecular weight hyaluronic acid. Food Chem Toxicol 58:401–407
22. Rizzo M, Bayo J, Piccioni F, Malvicini M, Fiore E, Peixoto E, García MG, Aquino JB, Gonzalez Campaña A, Podestá G, Terres M, Andriani O, Alaniz L, Mazzolini G (2014) Low molecular weight hyaluronan-pulsed human dendritic cells showed increased migration capacity and induced resistance to tumor chemoattraction. PLoS One 9(9):e107944
23. Braza F, Brouard S, Chadban S, Goldstein DR (2016) Role of TLRs and DAMPs in allograft inflammation and transplant outcomes. Nat Rev Nephrol 12(5):281–290
24. Hull RL, Bogdani M, Nagy N, Johnson PY, Wight TN (2015) Hyaluronan: a mediator of islet dysfunction and destruction in diabetes? J Histochem Cytochem 63(8):592–603
25. Fujioka-Kobayashi M, Müller HD, Mueller A, Lussi A, Sculean A, Schmidlin PR, Miron RJ (2017) In vitro effects of hyaluronic acid on human periodontal ligament cells. BMC Oral Health 17(1):44
26. Tanne Y, Tanimoto K, Okuma S, Kunimatsu R, Hirose N, Mitsuyoshi T, Tanne K (2013) Effects of hyaluronan oligosaccharides on apoptosis of human gingival fibroblasts. Open J Stomatol 3(01):19
27. Wang Y, Han G, Guo B, Huang J (2016) Hyaluronan oligosaccharides promote diabetic wound healing by increasing angiogenesis. Pharmacol Rep 68(6):1126–1132
28. Yamawaki H, Hirohata S, Miyoshi T, Takahashi K, Ogawa H, Shinohata R, Demircan K, Kusachi S, Yamamoto K, Ninomiya Y (2009) Hyaluronan receptors involved in cytokine induction in monocytes. Glycobiology 19(1):83–92
29. Murphy KM (2013) Transcriptional control of dendritic cell development. Adv Immunol 120:239–267
30. Bluestone JA, Mackay CR, O'Shea JJ, Stockinger B (2009) The functional plasticity of T cell subsets. Nat Rev Immunol 9(11):811–816
31. Collin M, Bigley V (2018) Human dendritic cell subsets: an update. Immunology 154(1):3–20
32. Geginat J, Nizzoli G, Paroni M, Maglie S, Larghi P, Pascolo S, Abrignani S (2015) Immunity to pathogens taught by specialized human dendritic cell subsets. Front Immunol 6:527
33. Briseño CG, Haldar M, Kretzer NM, Wu X, Theisen DJ, Kc W, Durai V, Grajales-Reyes GE, Iwata A, Bagadia P, Murphy TL, Murphy KM (2016) Distinct transcriptional programs control cross-priming in classical and monocyte-derived dendritic cells. Cell Rep 15(11):2462–2474

34. Balan S, Ollion V, Colletti N, Chelbi R, Montanana-Sanchis F, Liu H, Vu Manh TP, Sanchez C, Savoret J, Perrot I, Doffin AC, Fossum E, Bechlian D, Chabannon C, Bogen B, Asselin-Paturel C, Shaw M, Soos T, Caux C, Valladeau-Guilemond J, Dalod M (2014) Human $XCR1^+$ dendritic cells derived in vitro from $CD34^+$ progenitors closely resemble blood dendritic cells, including their adjuvant responsiveness, contrary to monocyte-derived dendritic cells. J Immunol 193(4):1622–1635

35. Termeer CC, Hennies J, Voith U, Ahrens T, Weiss JM, Prehm P, Simon JC (2000) Oligosaccharides of hyaluronan are potent activators of dendritic cells. J Immunol 165(4):1863–1870

36. Martín-Fontecha A, Sebastiani S, Höpken UE, Uguccioni M, Lipp M, Lanzavecchia A, Sallusto F (2003) Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med 198(4):615–621