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HBD3 alters matrix metalloproteinase production in human dendritic cells exposed to *Porphyromonas gingivalis* hemagglutinin B (HagB)

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Abstract

Background—Matrix metalloproteinases (MMP) are zinc- or calcium-dependent proteinases involved in the normal maintenance of the extracellular matrix. When elevated, they contribute to the tissue destruction seen in periodontal disease. Recently, we found that human beta defensin 3 (HBD3), a cationic antimicrobial peptide, alters chemokine and pro-inflammatory cytokine responses in human myeloid dendritic cells exposed to *Porphyromonas gingivalis* hemagglutinin B (HagB). In this study, we tested the hypothesis that HagB induces MMP production in dendritic cells and that HBD3 mixed with HagB prior to treatment alters HagB-induced MMP profiles.

Methods—Dendritic cells were exposed to 0.2 μ M HagB alone and HagB+HBD3 (0.2 or 2.0 μ M) mixtures. After 16 hours, concentrations of MMPs in cell culture media were determined with commercial multiplex fluorescent bead-based immunoassays. An integrated cell network was used to identify the potential HagB-induced signaling pathways in dendritic cells leading to the production of MMPs.

Results—0.2 μ M HagB induced MMP1, 2, 7, 9, and 12 responses in dendritic cells. 0.2 μ M HBD3 enhanced the HagB-induced MMP7 response ($p < 0.05$) and 2.0 μ M HBD3 attenuated HagB-induced MMP1, 7, and 9 responses ($p < 0.05$). The MMP12 response was not affected. In the

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CONFLICT OF INTEREST

Kim A. Brogden has had a Cooperative Research and Development Agreement with Cellworks Group Inc., San Jose, CA. Monica Raina, Amber Bates, Carol Fischer, Ann Progulske-Fox, and Kim A. Brogden declare no competing financial interests in the findings of this study or with Cellworks Group Inc., San Jose, CA USA or Cellworks Research India Ltd, Whitefield, Bangalore, India. Shireen Vali is employed by Cellworks Group Inc., San Jose, CA USA.

predicted network, MMPs would be produced via activation of MYD88/TRAF6 and SHC1/ERK pathways. Signals converge at the activation of MAPKs, AKT, and PKC to activate the transcription factors NFkB, AP1, SP1, NFAT, CTNNB1, and HIF1A, which transcribe different MMPs.

Conclusion—HagB was a MMP stimulus and HBD3 was found to decrease the HagB-induced MMP-1, 7, and 9 responses in dendritic cells at 16 hours, an observation that suggests HBD3 can alter microbial antigen-induced production of MMPs.

Keywords

Matrix Metalloproteinases; *Porphyromonas gingivalis*; Hemagglutinins; Defensins; HagB protein

Matrix-metalloproteinases (MMPs) are a family of 24 homologous human, zinc- or calcium-dependent proteinases important in angiogenesis, bone remodeling, morphogenesis, tooth eruption, cell communication, wound healing, tissue repair, and remodeling after injury.^{1–3} They also play critical roles in immune responses by either modifying proteins post-translationally to promote rapid delivery to other cells or by inactivating these proteins to initiate or terminate the immune process.⁴ These functions allow MMPs to direct systematic inflammation and regulate cytokine biosynthesis through activation of signal transduction pathways. When their balance is disrupted, MMPs become initiators of extracellular matrix destruction and exacerbate local and systemic diseases such as rheumatoid arthritis, atherosclerosis, and periodontal disease.^{1, 2}

Multiple cell types in both healthy and periodontally diseased tissues are capable of expressing MMPs.^{5, 6} These cell types include gingival sulcular epithelial cells, gingival fibroblasts, endothelial cells, monocytes, macrophages, plasma cells, neoplastic cells, and polymorphonuclear (PMN) leukocytes. MMPs also play a major role in the development and progression of chronic periodontitis by breaking down and destroying collagen, the major substrate of gingival and periodontal connective tissues and bone matrix.⁷ Elevated concentrations of various MMPs have been detected in gingival crevicular fluid around teeth displaying periodontitis.^{7–9}

Recently, we found that human beta defensin 3 (HBD3), a cationic antimicrobial peptide, and histatin 5 regulate chemokine and pro-inflammatory cytokine responses in dendritic cells exposed to *Porphyromonas gingivalis* hemagglutinin B (HagB).^{10, 11} HagB is a major virulence factor of *P. gingivalis* involved in non-fimbrial adhesion of the microorganism to host cells. It is a 49.0 kDa protein composed of 350 amino acid residues with a pI of 8.42. Recombinant HagB induces a robust chemokine and cytokine response in dendritic cells.^{10, 11}

HBD3 is a dynamic host defense peptide produced by a variety of cells and tissues of the oral cavity.^{12, 13} It is a small, 45 amino acid residue molecule with a strong cationic charge (+11) and monoisotopic mass of 5,157.7 Da.¹⁴ It has a strong role in innate and adaptive immunity.¹⁵ HBD3 kills microorganisms;^{13, 14, 16, 17} enhances the barrier function of mucosa by regulating permeability and membrane tight junctions in keratinocytes;¹⁸ chemoattracts monocytes, macrophages, T cells, neutrophils, immature dendritic cells, and

mast cells; promotes differentiation of human mesenchymal stem cells and osteoblasts;¹⁹ and regulates complement activation.^{20, 21} Important to this application, HBD3 can also modulate biomarker responses. We have demonstrated that HBD3 (at low concentrations) attenuates pro-inflammatory agonist-induced chemokine and pro-inflammatory cytokine responses of dendritic cells. HBD3 (at high concentrations) enhances agonist-induced chemokine and pro-inflammatory cytokine responses of dendritic cells.^{10, 11}

We used a computational and experimental modeling approach to show that the biomarker responses were strongly influenced by the concentration and timing of HBD3 or histatin 5 treatment. We also found that dendritic cells exposed to HagB produce MMPs.¹⁰ Here, we confirm that HagB induces the production of MMPs in dendritic cells and show that HagB-induced MMP responses were lower when HagB was mixed with HBD3 in dendritic cell cultures.

MATERIAL AND METHODS

Solutions

HagB was prepared as previously described in a number of studies.^{10, 11, 22, 23} HBD3 was purchased commercially. * 0.01 M sodium phosphate buffer with 0.14 M NaCl, pH 7.2 (PBS) was prepared and used as a control solution and diluent to prepare 0.2 μ M HagB, 0.2 μ M HBD3, and 2.0 μ M HBD3 solutions. All solutions were closely monitored for endotoxin content[†]. PBS contained 4.0 pg endotoxin/ml (n=3), reconstituted HBD3 solution contained 0.07 pg endotoxin/ μ g peptide (n=3), and HagB contained 14.0 pg endotoxin/ μ g protein (n=3).

Dendritic cells

Dendritic cells were obtained from multiple sources. In replication 1, dendritic cells were prepared from primary peripheral human blood monocytes.[‡] In replication 2 and 3, dendritic cells were obtained from different individuals. § Cells in the latter preparations were of myeloid origin and stained 31.5% CD1c⁺/79.1% CD11c⁺ and 54.4% CD141⁺/79.1% CD11c⁺, ** Dendritic cells were cultivated in LGM-3.^{††}

Experimental design

In each of 3 replications, 0.8 ml of 1.0×10^5 viable dendritic cells in LGM-3 were added to 24-well plates^{‡‡} at a density of 4.1×10^4 cells/cm². After 2 hours, LGM-3 media containing non-adherent cells and debris was removed and 0.9 ml of LGM-3 was added back. 0.1 ml test and control solutions were added to adhered cells. Thus, dendritic cells were exposed to (final concentrations): PBS (Grp 1), 0.2 μ M HagB (Grp 2), 0.2 μ M HBD3 (Grp 3), 0.2 μ M

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**Multi-color flow cytometry kit FMC016, R&D Systems, Inc., Minneapolis, MN.

††Lymphocyte Growth Media-3, Lonza Walkersville, Inc., Walkersville, MD.

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HagB+0.2 μ M HBD3 (Grp 4), 2.0 μ M HBD3 (Grp 5), or 0.2 μ M HagB+2.0 μ M HBD3 (Grp 6).

In our prior work¹⁰, we observed that *P. gingivalis* HagB induced robust chemokine and cytokine responses in dendritic cells in 16 hours and these HagB-induced responses could be altered by adding HBD3 to the dendritic cell cultures. That time period was used in this study. At 16 hours, cell culture supernatants were removed and centrifuged at $13,200 \times g$ (16,100 rpm) at 25°C for 5 minutes to pellet non-adherent cells and cell debris. Supernatants were removed and frozen at -80°C.

Viability

We have recently found that high concentrations of HBD3 can be cytotoxic for dendritic cells.²⁴ To ensure that changes in MMP concentrations were not due to cytotoxic effects of treatment solutions on dendritic cells, their viability was monitored in each replication. The cytotoxic effects of PBS, HagB, HBD3, and HagB+HBD3 mixtures on dendritic cells were determined by measuring the median fluorescence intensity (MFI) of the metabolic conversion of resazurin^{§§} to resorufin as previously described.²⁴ The percent viability was determined as the (MFI of the test solution/MFI of the PBS (Grp 1) solution) $\times$ 100.

Determination of MMPs

Concentrations (pg/ml) of MMP1, MMP2, MMP7, MMP9, and MMP12 were determined in dendritic cell culture media supernatants in triplicate from each of the three replications with commercial multiplexed fluorescent bead-based immunoassays^{***} in the particle counter^{†††} as previously described in detail.^{10, 11, 23} Standard curves ranged from 5.0–20,000.0 pg/ml (MMP1), 12.0–50,000.0 pg/ml (MMP2), 98.0–400,000.0 pg/ml (MMP7), 2.4–10,000.0 pg/ml (MMP9), and 24.0–100,000.0 pg/ml (MMP12).

Modeled HBD3 effects on HagB-induced MMP responses

An integrated cell network was used to create a predictive computational simulation model of human dendritic cells as previously described.^{10, 11} This model was used to predict the effect of HBD3 on the HagB-induced MMP responses of dendritic cells. This model provides a dynamic and transparent view of dendritic cell physiology at the functional proteomics abstraction level and predicts outcomes for different experimental scenarios in an automated high-throughput system. The model was manually aggregated, and is currently updated, from published research and collaborative data on signaling pathways, intermediates, transcription factors, enzyme kinetics and gene regulations comprising 3,860 biological species with over 15,433 cross-talk interactions. The technology has been recently used to predict a) elastin triggered transient pro-inflammatory responses by human dermal fibroblasts;²⁵ b) anti-aggregation of alpha-synuclein in M17 dopaminergic cells;²⁶ ATPase activity of heat shock protein 90 in tumor cells, endothelial cells, and stromal cells;²⁷ c) effects of progesterone and Cox2 inhibitors on progesterone receptor isoforms A and B in

§§Alamar Blue, Invitrogen Corp., Carlsbad, CA.

***EMD Millipore, Billerica, MA.

†††Luminex 100 IS Instrument, Luminex, Austin, TX.

myometrial cells;²⁸ d) butein inhibition of STAT3 expression in HepG2, SNU-387, and PLC/PRF5 human hepatocellular carcinoma cells;²⁹ and e) effects of isorhamnetin on the peroxisome proliferator-activated receptor gamma signaling cascade.³⁰ It has also been used to predict the PD-L1 expression on oral squamous cell carcinoma cells.^{31, 32}

The predictive computational simulation model of the effects of HBD3 and histatin 5 on HagB-induced chemokines and cytokines in dendritic cells was extensively validated in the prior studies by Harvey et al.¹⁰ and Borgwardt et al.¹¹, respectively. The dendritic cell network was used to predict the HagB-induced signaling pathways in dendritic cells leading to the production of MMPs and influencing effects of HBD3 on HagB-induced signal transduction.

Statistical analysis

Concentrations of MMPs in dendritic cell culture media supernatants were interpolated from curves constructed from the MMP standards and their respective MFI readings directly on the instrument^{†††} and readout files.^{§§§}

Dendritic cells in each of the 3 replications produced low concentrations of MMPs (Grp 1). The responses were unique to the 3 dendritic cell sources in each replication. HagB (Grp 2) induced the production of MMP1, MMP2, MMP7, MMP9, and MMP12 above baseline levels (Grp 1). The responses and magnitude of these responses varied among replications for each MMP and the differences of MMP production across replications were normalized. The percent increase was determined as the ((MMP concentration of test groups) – (MMP concentration of control Grp 1)/MMP concentration of Grp 1) × 100.

An ANOVA was then fit to the HagB-induced percent increase responses and the effect of HBD3 on HagB-induced MMP responses was determined versus that induced by HagB alone using Dunnett's test.³³ A 0.05 level was used to determine statistically significant differences. All analyses were conducted using a commercial software package.^{****}

RESULTS

Viability

Solutions containing PBS (Grp 1), 0.2 µM HagB (Grp 2), 0.2 µM HBD3 (Grp 3), 2.0 µM HBD3 (Grp 5), 0.2 µM HagB+0.2 µM HBD3 (Grp 4), and 0.2 µM HagB+2.0 µM HBD3 (Grp 6) were not cytotoxic for dendritic cells in any of the three replications. Percent viabilities ranged from 95.0–106.4% (Fig. 1).

HagB-induced MMP responses in dendritic cells

Dendritic cells in each replication produced low concentrations of MMP1, 2, 7, 9, and 12 in control cultures exposed to PBS. The responses were unique to dendritic cell sources in each replication. HagB (Grp 2) induced the production of MMP1, 2, 7, 9, and 12 above these

^{†††}xPonent v3.1 software, Luminex, Austin, TX.

^{§§§}Milliplex Analyst v5.1 software, EMD Millipore, Billerica, MA.

^{****}JMP, Version 10.0, SAS, Cary, NC.

baseline levels and responses varied among replications. Therefore, the differences of MMP concentrations across replications were normalized to a percent increase of MMP and the results for 3 samplings per each of 3 replications (n=9) were combined. HagB-induced MMP1 (172.0 ± 51.7 SEM % increase), MMP2 (118.9 ± 106.8 SEM % increase), MMP7 (24.6 ± 14.0 SEM % increase), MMP9 (92.7 ± 43.0 SEM % increase), and MMP12 (210.6 ± 51.6 SEM % increase) responses (Fig. 2).

Influence of HBD3 on HagB-induced MMP response

The effects of HBD3 on HagB-induced MMP responses were then determined and compared to that induced by HagB alone using the method of Dunnett. Three patterns were observed (Figs. 3a–3e). First, 0.2 μ M HBD3 (Grp 4) significantly enhanced the 0.2 μ M HagB-induced MMP7 response and 2.0 μ M HBD3 (Grp 6) significantly attenuated 0.2 μ M HagB-induced MMP7 response (Fig 3c). Second, 2.0 μ M HBD3 (Grp 6), but not 0.2 μ M HBD3 (Grp 4), significantly attenuated the 0.2 μ M HagB-induced MMP1 and MMP9 responses (Fig 3a and 3d). Finally, neither 0.2 μ M HBD3 (Grp 4) or 2.0 μ M HBD3 (Grp 6) attenuated or enhanced the 0.2 μ M HagB-induced MMP2 and MMP12 responses (Figs. 3b and 3e).

Predicted influence of HBD3 on a HagB-induced MMP response

In model simulations (Fig. 4), HagB was predicted to bind to CD14 and activin receptors.¹⁰ In the predicted network schematic MMPs would be produced via activation of MYD88/TRAF6 and SHC1/ERK pathways, respectively. Both activin and CD14 signals converge at the activation of MAPKs, AKT, and PKC to activate the transcription factors NFkB, AP1, SP1, NFAT, CTNNB1, and HIF1A, which transcribe different MMPs.

Previously we found that HBD3 had an inhibitory effect on dendritic cells.¹⁰ It binds to G-protein coupled receptors CCR2B and CCR6, stimulates Gq and Gi respectively, and activates the LYN/SYK/PLC/PKC-DAG pathway converging at the activation of AKT, NFkB, and NFAT. HBD3 negatively regulates MMP production by inhibiting CD14, CD40, and IFNG receptors.

DISCUSSION

Previously, *P. gingivalis* HagB was found to be a potent chemokine and cytokine stimulus.¹⁰ HagB-treated dendritic cells produced ~ 37 chemokines and cytokines in 16 hours. In that study, HBD3 was found to alter the HagB-induced chemokine and cytokine response in dendritic cells.¹⁰ In this present study, 2.0 μ M HagB was found to be a MMP stimulus and HBD3 was found to alter the HagB-induced MMP1, 7, and 9 responses in dendritic cells at 16 hours. The role of HBD3 in regulating MMP responses to specific pro-inflammatory antigenic exposure is largely unknown, particularly the effects of HBD3 concentration and antigen exposure to dendritic cells.

Dendritic cells in this study were from different sources and produced different background levels of MMPs. In replication 1, dendritic cells were prepared from primary peripheral human blood monocytes. In replications 2 and 3, dendritic cells were obtained as commercial preparation. Because of the different background levels of MMPs, we

normalized the responses and determined the HagB-induced percent change. This allowed the different replications to be pooled. An ANOVA was then fitted to the HagB-induced percent increase responses and the effect of HBD3 on HagB-induced MMP responses was determined versus that induced by HagB alone using Dunnett's test.³³ Similar to our previous work,¹⁰ HagB did induce MMP1, 2, 7, 9, and 12 responses (Fig. 2).

Higher concentrations of HBD3 are known to be cytotoxic for dendritic cells.²⁴ In the present study, inocula containing 0.2 and 2.0 μM HBD3 were not cytotoxic for dendritic cell cultures alone or in combination with HagB (Fig. 1).

HBD3 was found to alter HagB-induced MMP1, 7, and 9 responses in human dendritic cells. A lower 0.2 μM HBD3 concentration significantly enhanced the 0.2 μM HagB-induced MMP7 response and a higher 2.0 μM HBD3 concentration significantly attenuated 0.2 μM HagB-induced MMP1, 7, and 9 responses. The MMP12 response was not affected. The exact reason for this is not yet known. The concentration effect of HBD3 on HagB-induced MMP responses in human myeloid dendritic cells was similar to the concentration effect of HBD3 on HagB-induced chemokine and pro-inflammatory cytokine responses stimulus.¹⁰

HagB and HBD3 influence similar signaling pathways. HagB stimulates dendritic cells in a TLR4-dependent manner, and MyD88 and TRIF are required for their optimal activation.³⁴ HBD3 affects the activity of pro-inflammatory pathways associated with MyD88 and TRIF.^{35, 36} Therefore, to determine the parameters of HBD3 influencing the HagB-induced responses seen at 16 hours, we utilized a computational simulation dendritic cell model to predict MMP signaling pathways.

There are a number of influencing factors on HagB-induced MMP production. For example, Activating Transcription Factor-3 (ATF3) is a negative regulator of TLR signaling in dendritic cells,³⁷ which can suppress the transcription of MMP2^{38, 39} and MMP1, MMP3, and MMP10 expressions.⁴⁰ MMP2 did not have much of a response in our study. ATF3 is transcribed by ATF2, which comes downstream to the MAPK pathways. With HagB stimulation, there could be an increase in MAPK activation. Increase in MAPK activation positively influences MMP2 expression in dendritic cells through AP-1, SP-1 and HIF1A but at the same time, it negatively regulates MMP2 expression via ATF3. With HagB stimulation and increased MAPKs, MMP2 is activated as well as inhibited via MAPKs, hence levels of MMP2 may not vary.

There are additional influencing factors on MMP production. p38 negatively regulates MMP1 and MMP13 expression.^{41–43} MMP13 was activated by AP-1 and NF κ B in our model. All these transcription factors can be activated by MAPKs (p38, ERK etc.), which gets activated with HagB stimulation. If we consider the link in dendritic cells where p38 negatively regulates MMP13 expression, then like in the case of MMP2, maybe we can show MMP13 expression not getting changed with HagB stimulation since downstream of p38, we have activation of MMP13 expression via AP-1 and NF κ B as well as inhibition of MMP13 expression. To what extent these factors influence the effect of HBD3 on HagB-induced MMP production by dendritic cells will have to be determined using pathway inhibitors.

In prior studies,^{10, 11} we used HagB as a pro-inflammatory stimulus to induce chemokine and cytokine responses in human dendritic cells. We demonstrated that HBD3 binds to select sites on HagB, and the interaction among these molecules attenuated or enhanced pro-inflammatory HagB-induced chemokine and cytokine responses.^{11, 10, 11} In this study, we used HagB in similar concentrations to induce MMP1, 2, 7, 9, and 12 responses in human dendritic cells. We observed differing patterns of responses (Figs. 3a–3e) similar to HagB-induced chemokine and cytokine responses.¹⁰ Lower, 0.2 μ M concentrations of HBD3 (Grp 4) significantly enhanced the HagB-induced MMP7 response and higher, 2.0 μ M concentrations of HBD3 (Grp 6) significantly attenuated the HagB-induced MMP7 response (Fig 3c). Higher, 2.0 μ M concentrations of HBD3 (Grp 6), but not lower, 0.2 μ M concentrations of HBD3 (Grp 4), significantly attenuated the HagB-induced MMP1 and MMP9 responses (Fig 3a and 3d). Finally, neither concentrations of HBD3 (Grp 4, Grp 6) attenuated or enhanced the HagB-induced MMP2 and MMP12 responses (Figs. 3b and 3e). HBD3 treatment alone (Grp 3, Grp5) induced some MMP production, but this response was less than the MMP production induced by HagB treatment. These results suggest that HBD3 has the potential to regulate the production of MMPs from dendritic cells exposed to a pro-inflammatory stimulus. To what extent this mechanism occurs with other cells that are capable of expressing MMPs^{5, 6}, like gingival sulcular epithelial cells, gingival fibroblasts, endothelial cells, monocytes, macrophages, plasma cells, neoplastic cells, and PMN leukocytes, is not yet known. Because MMPs also play critical roles in immune responses by either modifying proteins post-translationally to promote rapid delivery to other cells or by inactivating these proteins to initiate or terminate the immune process,⁴ inhibition of their production by a pro-inflammatory stimulus would dampen the roles of MMPs in local and systematic inflammation and regulate cytokine biosynthesis through activation of signal transduction pathways. Again, a better understanding of the biochemical and regulatory pathways of these proteins will assist in identifying ways to target MMPs in hopes to arrest or attenuate the progression of inflammation and tissue damage in periodontal disease.

Both HBD3 and HagB are associated with the MyD88 and TRIF pathways, which when activated can lead to downstream MMP production. With the predicted pathways proposed in this study, we suspect the inhibition of CD14 by HBD3, decreasing the amount of HagB able to bind to CD14, resulted in decreased MMP production in the groups treated with HBD3 and HagB. This prediction needs to be further tested using pathway inhibitors. Another possibility, which has been explored in some of our previous work,¹⁰ is that multiple pathways are involved simultaneously. We have seen that in the presence of HagB, low amounts of HBD3 can cause a slightly enhanced MMP7 production (Grp 4) while higher amounts of HBD3 can attenuate MMP7 production (Grp 6). In reference to Fig. 4, with HagB and lower amounts of HBD3 present multiple pathways (both the right and left sides of Fig. 4) might be activated, while with HagB and higher amounts of HBD3 present the HBD3 might inhibit certain pathways (left side of Fig. 4) downstream of HagB causing less MMP production. Again, this prediction needs to be further tested using pathway inhibitors.

CONCLUSIONS

In this study, 2.0 μ M HagB was found to be an MMP stimulus and HBD3 was found to decrease the HagB-induced MMP-1, 7, and 9 responses in dendritic cells at 16 hours, an observation that suggests HBD3 can alter microbial antigen-induced production of MMPs.

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SUMMARY SENTENCE

In this study, *Porphyromonas gingivalis* hemagglutinin B (HagB) was found to be a MMP stimulus and HBD3 was found to alter the HagB-induced MMP1, 7, and 9 responses in dendritic cells, an observation that suggests HBD3 can alter microbial antigen-induced production of MMPs.

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Figure 1.

The percent viability of solutions containing PBS (Grp 1), 0.2 μ M HagB (Grp 2), 0.2 μ M HBD3 (Grp 3), 0.2 μ M HagB+0.2 μ M HBD3 (Grp 4), 2.0 μ M HBD3 (Grp 5), and 0.2 μ M HagB+2.0 μ M HBD3 (Grp 6) for dendritic cells is shown for 3 samplings in each of 3 replications (n=9). The percent viability was determined from the median fluorescent intensity (MFI) of resorufin (MFI of the test solution/MFI of the PBS solution in Grp 1) $\times$ 100. 0.2 μ M HagB, 0.2 μ M HBD3, and 2.0 μ M HBD3 were not cytotoxic alone or in combination and there were no significant differences in the viability among groups ($p > 0.05$).

Figure 2.

0.2 μ M *P. gingivalis* hemagglutinin B (HagB)-induced MMP responses in dendritic cells. Dendritic cells in each of the 3 replications produced low concentrations of MMPs that were unique to the 3 dendritic cell sources in each replication. HagB induced the production of MMP1, MMP2, MMP7, MMP9, and MMP12 above baseline levels and the percent increase in MMP response was determined to normalize the data. The percent increase was determined as the ((MMP concentration of test groups) – (MMP concentration of control Grp 1))/MMP concentration of Grp 1) $\times$ 100.

Figure 3. MMP responses of dendritic cells exposed to 0.2 μ M HagB (Grp 2), 0.2 μ M HBD3 (Grp 3), 0.2 μ M HagB+0.2 μ M HBD3 (Grp 4), 2.0 μ M HBD3 (Grp 5), and 0.2 μ M HagB+2.0 μ M HBD3 (Grp 6). * indicated statistically significant differences ($p < 0.05$).

Figure 4.

A schematic of the predicted signalling pathways for HagB-induced MMP production in dendritic cells. HagB provides activating signals to CD14 and activin receptors and stimulates the production of MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, MMP12, and MMP13 via activation of MYD88/TRAF6 and SHC1/ERK pathways, respectively. Both activin and CD14 signals converge at the activation of MAPKs, AKT and PKC to activate the transcription factors NFKB, AP1, SP1, NFAT, CTNNB1 and HIF1A which transcribes different MMPs. HBD3 exerts an inhibitory effect on dendritic cells. It binds to G-protein coupled receptors CCR2B and CCR6, which stimulate Gi and Gq respectively and activate LYN/SYK/PLC/PKC-DAG pathway converging at the activation of AKT, NFKB and NFAT. HBD3 also negatively regulates the matrix metalloproteinases by inhibiting CD14, CD40, and IFNG receptors. The dotted red lines in the figure indicate inhibitory links and dotted

fluorescent green lines indicate activation links. Downstream effects (activation links) of each transcription factor are shown by unique colored solid lines.