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Anti-viral Activity of Human β -Defensin 3 Against Vaccinia Virus

Michael D. Howell, Ph.D.¹, Joanne E. Streib, B.A.¹, and Donald YM Leung, M.D., Ph.D.^{1,2,3}

¹Division of Allergy and Immunology, Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO 80206, USA

²Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO 80262 USA

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TO THE EDITOR

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that is associated with recurrent bacterial and viral skin infections.¹ This has prompted the Center for Disease Control and Prevention (CDC) to exclude individuals with AD from smallpox vaccination with vaccinia virus (VV) due to the increased risk of developing eczema vaccinatum.

Anti-microbial peptides (AMPs) are an integral component of the innate immune response due to their activity against invading pathogens.^{2,3} Recent studies by our laboratory have demonstrated that two main families of AMPs – β -defensins and cathelicidins – are deficient in the skin of AD patients.^{4,5} This deficiency may explain, in part, the increased propensity of AD patients to recurrent skin infections.

Human β -defensin (HBD)-3 is a 5-kDa peptide produced primarily by keratinocytes following skin injury or inflammation. Additionally it has been shown to exhibit anti-bacterial activity against a variety of bacteria,² however its activity against VV is unknown. In the current study, we hypothesized that HBD-3 kills VV and thereby contributes to the innate immune response against VV.

To test this hypothesis, we obtained the Wyeth strain of VV from the CDC and maintained the virus in HeLa S3 (ATCC#CCK-2.2) human adenocarcinoma cells as previously described.⁶ The anti-viral activity of HBD-3 was evaluated by incubating VV with 0 – 100 μ M of HBD-3 (Peptides International, Louisville, KY) or the control peptide 8044 (GLNGPDIYKGUYQFKSVEFD) for 24 hours. The virus:peptide solution was then added to BS-C-1 African green monkey kidney cells and evaluated using a standard viral plaque assay or real-time RT-PCR as previously described.⁶ As shown in Figure 1, HBD-3 significantly ($p < 0.001$) reduced the expression of the DNA-dependent RNA polymerase, which is expressed within two hours of viral entry, and the number of viral plaques in a concentration-dependent manner. The control peptide 8044 did not exhibit any anti-viral activity against VV.

³Address correspondence and reprint requests to: Donald YM Leung, MD, PhD, National Jewish Medical and Research Center, Department of Pediatrics, Room K926, 1400 Jackson Street, Denver, CO 80206, Phone: 303-398-1186; Fax: 303-270-2182; Email: leungd@njc.org.

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HBD-3 has been shown to be induced in keratinocytes by tissue injury and pro-inflammatory cytokines.^{2, 5} Therefore we investigated whether infection with VV would induce the expression of HBD-3 in keratinocytes. The HaCaT human keratinocyte cell line was grown in Dulbecco's Modified Eagle's Medium (Cellgro, Herndon, VA), supplemented with 10% fetal calf serum (Gemini Bio Products, Calabasas, CA) and 1% of the following: penicillin/streptomycin, L-glutamine, minimal essential medium (MEM) with non-essential amino acids (GIBCO, Grand Island, NY), MEM vitamins solution (GIBCO) until confluent. Once confluent, cells were infected with 0.1 or 0.05 plaque forming units (pfu) of VV per cell. At designated time points, RNA was isolated from the infected keratinocytes and levels of HBD-3 evaluated by real-time RT-PCR as previously described.⁵ VV infection induced HBD-3 gene expression in a time dependent manner, with significantly ($p < 0.05$) higher levels observed as early as 18 hours following infection (Figure 2A).

The skin of AD patients is characterized by the over-expression of IL-4 and IL-13;^{7, 8} therefore we investigated the effect of these cytokines on VV induced expression of HBD-3. Primary keratinocytes (Cascade Biologics, Portland, OR) were routinely grown in serum-free keratinocyte growth medium (EpiLife®, Cascade Biologics), supplemented with 1% human keratinocyte growth supplement V2 (Cascade Biologics), 0.06 mM calcium chloride and 1% of antibiotics (penicillin/streptomycin). Prior to infection, primary keratinocytes were differentiated in the presence of 1.3 mM CaCl_2 for five days. Keratinocytes were then incubated with 50 ng/ml of IL-4 (R&D Systems; Minneapolis, MN) and 50 ng/ml of IL-13 (R&D Systems) for 24 hours. After 24 hours, fresh cytokines were added to the culture and cells were then infected with VV (0.1 or 0.05 pfu/cell) for an additional 24 hours. Following the final 24 hour incubation, media was removed and RNA isolated from keratinocytes using RNeasy kits according to the manufacturer's guidelines (Qiagen, Valencia, CA) for real-time RT-PCR. HBD-3 gene expression was significantly elevated in keratinocytes infected with 0.1 pfu/cell (0.41 ± 0.04 ng; $p < 0.001$) or 0.05 pfu/cell (0.39 ± 0.07 ; $p < 0.001$) as compared to media alone (0.05 ± 0.01). However, pre-incubation of keratinocytes with IL-4 and IL-13 prior to VV infection in these studies significantly ($p < 0.001$) reduced the expression of HBD-3 (0.1 pfu/cell: 0.08 ± 0.01 ; 0.05 pfu/cell: 0.09 ± 0.01) as compared to infection with VV alone. Previously we have shown that the addition of IL-4 and IL-13 augments VV replication in keratinocytes,⁹ suggesting a relationship between HBD-3 expression and VV replication.

In summary, we have shown that HBD-3 exhibits potent anti-viral activity against VV, making it unique from HBD's 1 and 2 which have previously been shown not to exhibit activity against VV.⁶ Additionally, we demonstrate that IL-4 and IL-13 inhibit VV induced HBD-3 expression in keratinocyte cultures. It is not known why AD patients develop eczema vaccinatum, however the combination of our current data and previous data demonstrating deficient HBD-3 production in AD skin,⁵ suggests that a deficiency in HBD-3 increases the susceptibility of AD patients to systemic VV infection following smallpox vaccination.

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Abbreviations

AD	atopic dermatitis
AMP	anti-microbial peptide
CDC	Center for Disease Control and Prevention
HBD	human beta defensin
MEM	minimal essential medium
VV	vaccinia virus

Figure 1A

Figure 1B

Figure 1.

HBD-3 exhibits anti-viral activity against VV. VV was incubated with HBD-3 or the control peptide 8044 for 24 hours and then evaluated using real-time RT-PCR to measure viral replication (A) and a standard viral plaque assay to measure functionally active VV (B). Data are expressed as the mean $\pm$ SEM with an $n=6$. ** and *** indicate significant differences of $p<0.01$ and $p<0.001$, respectively.

Figure 2A

Figure 2B

Figure 2.

HBD-3 is induced by VV and down-regulated by Th2 cytokines. **A.** HaCaT cells were infected with 0.1 or 0.05 pfu of VV per cell for 6 to 48 hours. **B.** Primary keratinocytes were infected with VV in the presence and absence of IL-4 (50 ng/ml) and IL-13 (50 ng/ml) for 24 hours. For both A and B, HBD-3 gene expression was evaluated by real-time RT-PCR. Data are expressed as the mean $\pm$ SEM with an n=6. * and *** indicate significant differences of $p < 0.05$ and $p < 0.001$, respectively.