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## Original Article

# Evaluation of the Role of Herpes Simplex Virus Type 1 in the Secretion of Interleukin-1β (IL-1β) by Cultured Gingival Epithelial Cells

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## Abstract

**Background and Aims:** Several studies have shown that there is greater number of viruses in deep periodontal pockets than in normal gingival sulcus. We also know that virus may have an important role in induction of Il-1β secretion by different human cells. In this study we intended to determine the capacity of Herpes simplex virus type 1 to stimulate Il-1β secretion in human gingival keratinocytes.

**Materials and Methods:** primary gingival keratinocytes were obtained from samples extracted during crown lengthening surgical procedure. The cells were cultured in 24 well flasks and stimulated by one of these stimulants: LPS, HSV1, LPS+HSV1 and LPS+ATP. After incubation and collecting the supernatants, the amount of secreted Il-1β was measured by ELISA technique.

**Results:** none of the stimulants were able to cause significant amount of Il-1β secretion in any of the incubation times.

**Conclusion:** It seems that HSV1 does not have the capacity to induct IL-1β secretion as a single factor alone or in combination with bacterial LPS.

**Keywords:** Inflammasome, HSV1, IL-1b, Lipopolysaccharide, gingival keratinocytes

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## Introduction

Various herpesviruses are associated with severe types of periodontal disease (1). Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are the most commonly researched viruses in periodontology (2). Herpes virus pathogenicity is complex and is executed through direct virus infection and replication, or via a virus induced alteration of the host immune defense. The herpesvirus infection triggers a release of proinflammatory cytokines that have the potential to activate osteoclasts and matrix metalloproteinase and to impair antibacterial immune mechanisms, causing an up-growth of periodontopathic bacteria (3). EBV and CMV infection up-regulate the expression of interleukine-1 beta (IL-1β) and tumor necrosis factor-alpha (TNFα) of monocytes, and macrophages (4).

One of the other mechanisms that virus may be involved is their role in the production and maturation of IL-1β (5). The role of IL-1β in periodontitis has been highlighted (6). Indeed, like many other pathogens, viruses can up regulate IL-1β gene expression in gingival cells (7). IL-1βneeds a double stimulatory process for activation and secretion (8). In

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order to be fully functional, IL-1β needs to be cleaved by caspase 1 (8) that is activated via intracellular macromolecules called inflammasomes (8). It has been shown that DNA viruses can activate at least two types of inflammasomes named NALP3 and AIM2 (9). It is known that gingival epithelial cell harbor NALP3 that can be activated by HSV-1 in lymphocytes (10). Yilmaz et al have shown that gingival epithelial cells can secret IL-1βthrough activation of NALP3 after initial stimulation by LPS (10). In this study we aim to investigate the influence of HSV1 and bacterial LPS (as a co-factor) in aggregation of IL-1β production and secretion in keratinocytes. We assume that bacterial LPS despite being capable to induct other cytokines needs a co-factor (such a virus) to fully complete the IL-1β production cycle .

## Methods

**Cells and viruses**

Primary gingival keratinocytes were obtained from surgical crown lengthening, as described previously (11). Cells were grown in monolayers in Dulbecco's modified eagle's medium (DMEM) supplemented with 5% fetal bovine serum (Gibco). All cells were grown at 37oC in an atmosphere containing 5% CO2. HSV-1 was achieved from clinical lesions and typed using HSV-1 monoclonal antibody. Confluent cell monolayers grown in 24-well dishes were infected with HSV-1 at MOI1 and incubated at 37oC for 24h.

**Treatment of cells**

Monolayer of cells were divided in to four groups and each group was treated with one of the following stimulants: 4 µg/ml E-coli LPS for 12hr, HSV-1 as described previously for24hr, LPS for 12hr followed by HSV-1 for 24hr and LPS for 12hr followed by ATP 5mM 24). After the incubations, supernatants were harvested, and after a low-speed centrifugation they were used for cytokine assay.

**Measurement of cytokine secretion by ELISA**

Cell culture supernatants were collected and assayed for IL-1β activity by ELISA using a commercial cytokine ELISA kit for IL-1β(human platinium- bioscience).

## Results

Our results indicate that primary gingival keratinocytes do not support the secretion of IL-1β (above basal levels) following treatment with either LPS or HSV-1. As shown in figure 1, no increase in IL-1βsecretion with HSV-1 after incubation with LPS was observed .

There was higher level of IL-1βsecretion when primary gingival keratinocytes were treated with LPS, followed by ATP .

Primary gingival keratinocytes were found to be sensitive to infection with HSV1 and supported replication of this virus producing

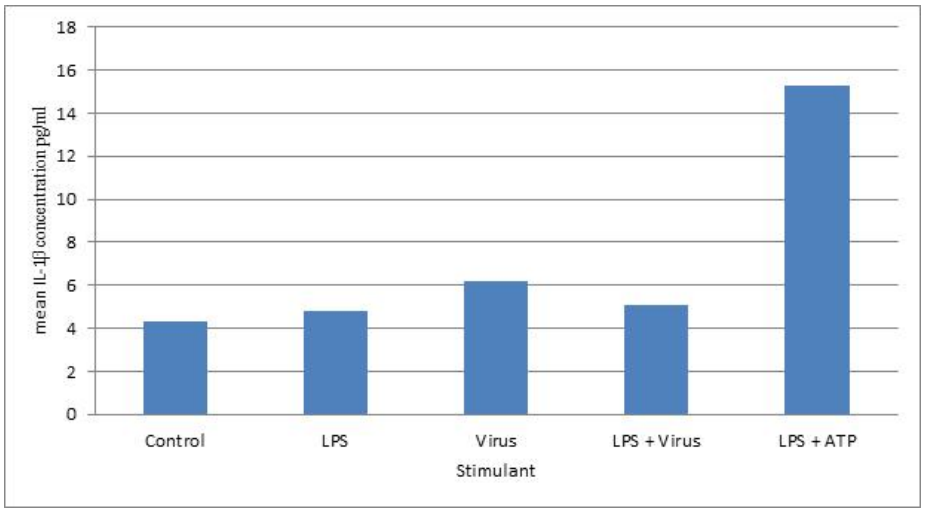



**Fig. 1.** IL-1β secretion was measured in suppernatants from primary gingival keratinocytes incubated with control buffer, incubated with 4µgml$^{-1}$ E-coli LPS for 12 hours, Infected with HSV-1 for 24 hours, incubated with 4µgml$^{-1}$ E-coli LPS for 12 h and then infected with HSV-1 for 24 hours, incubated with 4µgml$^{-1}$ E-coli LPS for 12 hours and then incubated with 5mM ATP for 24 hours.



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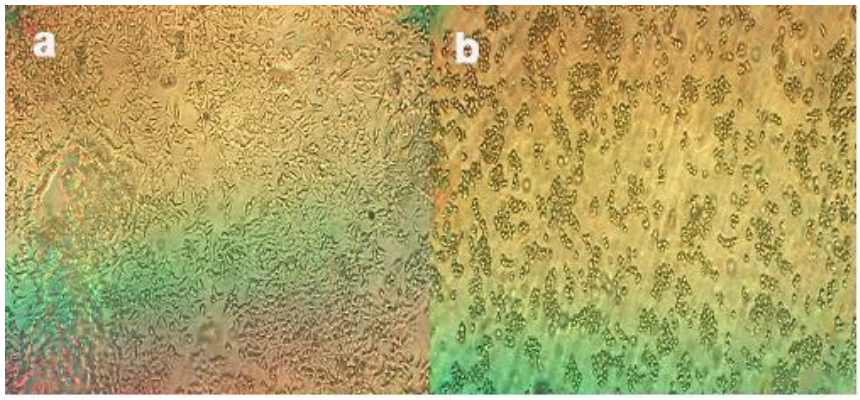



**Fig. 2.** a) Monolayer of primary gingival keratinocytes. b) a complete alteration in infected cells at 24hr post infection with HSV-1.

morphological changes in these cell monolayers. As it is shown in Fig 2, there was a complete alteration in infected cells at 24hr post infection as compared with uninfected control cells (Fig 2. a, b).

## Discussion

The association of herpes simplex family viruses and periodontitis has been shown by several studies(12-15) although these findings were not supported by others(16, 17). Different methodological methods and target populations appear to be behind these controversies .

As known, IL-1 βis one of the few cytokines that need a double stimulatory process for production and secretion (12). It has been well documented that TLRs and other cellular receptors have the ability to induce the transcription of the IL-1βgene (13). However, following translation only a non-functional pro-IL-1β is produced that needs to be cleaved in order to get matur and secreted. In 1990s it was discovered that caspase1 has a critical role in the cleavage of pro-IL-1β (19, 20). Later the inflammasomes were discovered as intra-cellular sensors that activate caspase1 after being stimulated by a variety of factors. Up till now, four types of Inflammasome has been discovered: IPAF, NALP3, AIM2 and NALP1 each having a specific stimulatory factor (14, 15). Viruses can incite different cellular receptors. In addition, they can trigger TLRs, RLRs and the inflammasomes (mainly NALP3 and AIM2) (9, 16). AIM2 is a general intra cellular DNA censor that can also be triggered by the DNA of the DNA-virus (17). It has been shown that several DNA viruses can cause IL-1βsecretion in an AIM2 dependent manner (18, 19). NALP3 is mostly activated by ROS which is mainly formed in phagosomes (20). The content of the phagosome can be different ranging from a non-organic crystal to a pathogen such as a virus .

Many studies have investigated the role of virus in the secretion of IL-1β in different cell types. Pirhonen,et al for the first time observed that RNA virus (Sendai and Influenza virus) can induce macrophages to secret a significant amount of IL-1β in a caspase1 dependent manner (After 24 hour of Sendai virus infection, macrophages released up to 1400 pg/ml of IL-1β) (21). Furthermore, Kanneganti et al. observed that the NALP3 is an important factor in activating caspase1 after stimulation with RNA virus (Murine Sendai virus, Rotavirus and influenza virus (22).

Nalp3 not only being essential in response to RNA virus, it has also an important role in case of DNA virus infection (beside AIM2). In this regard, Nour et al. discovered that the confection of HELF (human embryonic lung fibroblasts) cells with Varicella zoster virus, a DNA virus, and LPS, significantly increased the IL-1βsecretion (up to 200pg/ml) compared to mock infected cells. in presence or absence of LPS (1µg/ml) it was also more than cells infected with virus alone (up to 60pg/ml) (23). Delaloyeet al. saw that NALP3 is essential for IL-1βsecretion in Modified vaccinia virus Ankara (MVA) infection of T-helper1 (up to 200pg/ml after 24h MOI:5) (19). But this only

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happened if the virus where entered the cells by endocytosis .

It has also been shown that viral DNA can induce AIM2. Rathinam et al. found that several DNA viruses such as mCMV (mouse cytomegalic virus) and vaccinia virus can cause AIM2 dependent secretion of IL-1β although HSV-1could cause secretion ofIL-1β in AIM2- cells (18). The authors also found that HSV-1 stimulated secretion of IL-1β in macrophages was only dependent on NALP3 (in contrast to other DNA virus it had examined)(18). Although not mentioning the reason, it seemed that AIM2 senses mCMV and Vaccinia virus differently but not HSV-1. The second reason is that the mechanism of viral entry appears to be an important factor in the activation of NALP3. Activation of NALP3 had a limitation in Delaloye's study; it was only activated if the DNA virus was entered in the THP-1 cell by endocytosis (19). Accumulation of viral particles, leads to phagosomal destabilization and finally activation of NALP3 (9, 24).

Viruses have the capacity to both activate the TLRs and inflammasomes. LPS solely cannot activate the inflammasomes so we didn't expect to observe a significant amount of IL-1β in the samples that were stimulated only by IL-1β .

The way HSV-1 enters the cells may play a critical role in the activation of NALP3. Since HSV-1 is an enveloped virus it was generally supposed it can only enter the cell by direct membrane fusion without endocytosis (25). But now we know that endocytosis may occur in some cells in certain conditions. In HeLa cells and human keratinocytes, endocytosis may occur in a low pH environment (26, 27), which is present in deep periodontal pockets. In addition, it should be taken into account that in some settings HSV1 had the potential to evade the host immunity by inhibiting IL-1β secretion (28, 29).

## Conclusion

HSV-1 was not able to accelerate IL-1β production in gingival keratinocyte cells either alone or in connection with LPS. This may be due to the importance of NALP3 in sensing the virus. It seems that endocytosis is critical for the virus to be detected by NALP3. For future research we suggest that this experiment can be done in a low pH environment.

## Acknowledgement

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