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Type I Interferon and Interferon-stimulated Gene Expression in Oral Epithelial Cells

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Abstract

Oral epithelial cells (OEC) represent the first site of host interaction with viruses that infect the body through the oral route, however their innate antiviral defense mechanisms have yet to be defined. Previous studies have determined that OEC express pathogen-, damage- or danger-associated molecular patterns (PAMPs, or DAMPs), but their expression of key antiviral innate immune mediators, including type I interferons (type I IFN) and interferon-stimulated genes (ISGs) has not been studied extensively. We used the oral keratinocyte cell line, OKF6/TERT1 in the presence and absence of the viral mimics poly(I:C) and unmethylated CpG DNA, to define the expression of type I IFN and ISGs. We identified the basal expression of novel type I IFN genes IFNE and IFNK, while IFNB1 was induced by viral mimics, through the nuclear translocation of IRF3. Numerous ISGs were expressed at basal levels in OEC, with an apparent correlation between high expression and antiviral activity at the earlier stages of viral infection. Stimulation of OECs with poly(I:C) led to selective induction of ISGs, including *MX1*, *BST2*, *PML*, *RSAD2*, *ISG15* and *ZC3HAV1*. Together, our results demonstrate that OECs exhibit a robust innate antiviral immune defense profile, which is primed to address the wide variety of pathogenic viruses that are transmitted orally.

Keywords

virus; innate immunity; oral; epithelial; interferon; interferon-stimulated gene

Introduction

Many viruses are spread via saliva and/or begin infection through the mouth, including herpesviruses (Fülöp, Larbi, & Pawelec, 2013; Looker et al., 2015; Toussiro, Roudier, Roudier, & al., 2008), coxsackievirus (Corsino & Linklater, 2018), hepatitis B virus (Scott, Snitbhan, Bancroft, Alter, & Tingpalapong, 1980), cytomegalovirus (Plosa, Esbenshade, Fuller, & Weitkamp, 2012), Epstein-Barr virus (Niedobitek, Meru, & Delecluse, 2001) and Kaposi's sarcoma-associated herpesvirus (Cesarman, Chang, Moore, Said, & Knowles, 1995; Chang et al., 1994; Soulier et al., 1995). The site of first contact, the oral epithelial

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cells (OECs), act as the first line of defense against these pathogens. While specific mechanisms of how other tissues in the body respond to viruses, not much is known about the innate antiviral defenses of OECs, especially how OECs respond to actively replicating infections.

In general, host cells are able to detect the presence of certain molecular patterns, such as nucleic acid, extracellular ATP, and bacterial components, collectively termed pathogen-, damage-, or danger-associated molecular patterns (PAMPs or DAMPs) (Vénéreau, Ceriotti, & Bianchi, 2015). Originally only thought to detect evolutionarily distinct objects (Akira, Uematsu, & Takeuchi, 2006; Medzhitov & Janeway, 2000), the receptors for these molecular patterns, pattern recognition receptors (PRRs), are now hypothesized to have evolved by way of binding to objects in the environment and cell that are signals of cellular danger (Matzinger, 1994).

Regardless of the origins of the PRRs, these proteins, located either on the cell surface, endosomes, or in the cytoplasm, act as the first step toward clearing infections inside host cells. In humans, these PRRs fall under a number of categories: membrane-bound toll-like receptors (TLRs) and C-type lectin receptors (CLR) and cytoplasmic RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), and dsDNA sensors (e.g. cyclic GMP-AMP synthase, cGAS) (reviewed in Brubaker, Bonham, Zanoni, & Kagan, 2015)). While these PRRs bind to a wide variety of molecular patterns, each with their own degree of promiscuity for ligands (Seong & Matzinger, 2004), their activation pathway includes a number of similar transcription factors: nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), activator protein 1 (AP-1), and interferon regulatory factors (IRFs) (Brubaker et al., 2015). These transcription factors translocate to the nucleus upon activation and lead to the expression of genes encoding for pro-inflammatory cytokines and interferons (Parkin & Cohen, 2001) as well as antimicrobial peptides, which exhibit antiviral activity (Brice & Diamond, 2019).

Type I interferons (type I IFN) are the most well studied interferons in the context of direct inhibitions of viral infections (reviewed previously (McNab, Mayer-Barber, Sher, Wack, & O'Garra, 2015)). These proteins, through either autocrine or paracrine signaling, lead to the expression of interferon-stimulated genes (ISGs). The protein products of these genes act directly to inhibit almost every aspect of the viral lifecycle and even increase the ability of NK cells to kill infected cells (Schneider, Chevillotte, & Rice, 2014; Schoggins & Rice, 2011). The goal of this study was to further define the innate antiviral defense mechanisms of OECs, specifically which type I IFN and ISGs are being expressed in these cells, and how their expression responds to DAMP stimulation that can mimic viral infection.

Materials and Methods

Cells

The oral mucosal keratinocyte cell line OKF6/Tert-1 (Dickson et al., 2000) was cultured in keratinocyte serum-free media (K-SFM) (Gibco) supplemented with 2 mM L-glutamine (Corning), 1 IU/ml penicillin (Corning), 100 μ g/ml streptomycin (Corning), 75 μ g/ml bovine

pituitary extract (Gibco), 0.3 mM CaCl₂ (Sigma-Aldrich), and 0.6 ng/ml epidermal growth factor (EGF) (Gibco).

RNA Library Construction for Sequencing

RNA of cells from three biological replicates per condition was isolated using the RNEasy Plus Mini kit (Qiagen, 74136). TruSeq RNA library construction was performed at the Interdisciplinary Center for Biotechnology Research (ICBR) Gene Expression Core, University of Florida (UF). RNA quantitation was done by the QUBIT (ThermoFisher), purity was evaluated on a NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.), and sample quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc). Protein-free, intact total RNA (RIN>7) was used for library construction using the reagents provided in the Illumina TruSeq RNA sample preparation kit and following the manufacturer's low-sample number protocol (Part # 15026495 Rev. F). Briefly, 0.1–1.0 µg of RNA was fragmented using a divalent cations solution and incubation at 94°C. This step was followed by first strand cDNA synthesis using reverse transcriptase and random primers. Synthesis of ds cDNA was done using the 2nd strand master mix provided in the kit, followed by end-repair and dA-tailing. At this point, Illumina adaptors were ligated to the sample. Finally, library was enriched by 10–12 cycles of amplification using barcodes-containing primers (6 bp). The final library product was purified by Agencourt AMPure beads (Beckman Coulter, catalog # A63881). The library size and mass were assessed by analysis in the Bioanalyzer. Typically, a 200–1000 broad library peak was observed with the highest peak at ~500 bp. Quantitative PCR was used to validate the library's functionality, using the KAPA library quantification kit (Kapa Biosystems, catalog number: KK4824) and monitoring on the ABI7900HT real-time PCR system.

RNA Sequencing (RNASeq)

Sequencing was performed using the reagents provided in the Illumina 150-cycles (2×75 paired-end format), high output NextSeq500 sequencing kit (Cat# FC-404–1005). Ten µl of library (4 nM) is mixed with 10 µl 0.1 N NaOH for 5 minutes, then the library is diluted to 20 pM in HT1 buffer. A final dilution to 1.3–1.5 pM is made with HT1 buffer for a final volume of 1 ml. A volume of 600 µl is loaded onto the reagent cartridge provided in the kit for sequencing. Runs are set by choosing the 'Generate FASTQ only' workflow in Illumina Experiment Manager (Illumina, USA). Under these run conditions, cluster density typically falls in the 200–220 K/mm² range. Each NextSeq500 generated approximately 400 million single-end reads with ~80 % of reads having a greater than Q30 quality score. Up to 12 barcoded samples were pooled for sequencing on a single run, with an expected output of ~33 million pair-reads per sample. The reads that passed Illumina quality control filtering were used as raw data for further bioinformatics analysis. Transcripts designated as interferon-stimulated genes and type I interferon were selected for further examination.

RT-qPCR

OKF6/Tert-1 cells were treated with either 1 µg/ml double-stranded RNA mimetic poly(I:C) (Invivogen, tlr1-picw) in cell culture media for up to six hours. RNA of cells was isolated using the RNEasy Plus Mini kit (Qiagen, 74136) and subsequently converted to cDNA via reverse transcription using the iScript Supermix (Bio-Rad, 1708840). qPCR was performed

on the cDNA using SsoAdvanced SYBR Green (Bio-Rad, 172–5274) in the CFX96-C1000 Touch thermocycler (Bio-Rad). Relative expression of type I interferon or interferon-stimulated genes were measured using the 2^{-Cq} calculations (Winer, Kwang, Jung, Shackel, & Williams, 1999) in the CFX Manager software (Bio-Rad) with *ACTB* serving as the reference gene. Primers sequences were generated using the PrimerQuest tool (IDT). The list of primers used in these experiments can be found in Table 1.

Immunofluorescence Microscopy

OKF6/Tert-1 cells were plated at 30,000 cells/well of an 8-well chamber slide. Cells were treated with either or 5 μ M unmethylated CpG DNA (Invivogen, tlr1-2216) or dH₂O vehicle control for two hours. After treatment, cells were washed with PBS and fixed with ice-cold acetone for ten minutes at -20°C . After blocking cells overnight with 2 % v/v BSA/PBS, rabbit anti-human IRF3 IgG antibody (Abcam, 25950) at 1 μ g/ml in 2 % v/v BSA/PBS was added to cells for two hours at room temperature. Secondary antibody goat anti-rabbit IgG-Alexa Fluor 488 (Thermo Fisher, A-11008) at 4 μ g/ml in 2 % v/v BSA/PBS was then added to cells for two hours at room temperature. Cells were then mounted with Fluoroshield mounting medium with DAPI (Abcam, 104139) for ten minutes before the cover glass was sealed. The Zeiss Axiovert 200 M microscope with a Zeiss AxioCam MRm camera using a 20x objective was used to capture fluorescent images. Cells were washed three times with PBS between each step. Nuclear localization of IRF3 for fifty cells per condition was analyzed using ImageJ (Eliceiri, Schneider, Rasband, & Eliceiri, 2012).

Results

Basal Expression of Oral Epithelial Cell Interferon-stimulated Genes and Type I Interferon

To determine which, if any, ISGs were expressed in oral epithelial cells, RNA sequencing (RNASeq) was performed using RNA from untreated oral keratinocyte cell line OKF6/Tert-1. Many of the canonical ISGs were found to be expressed at baseline conditions (Table 2). Interestingly, the degree of expression of ISGs seemed to be highest with those having functions in earlier aspects of viral infection (entry, transcription, and translation) and lowest with those who act at later points in the defense against viruses (e.g. pro-NK cell functions). Surprisingly, this high level of basal expression of ISGs is observed in the absence of prior stimulation by IFN.

The same RNASeq data set was then analyzed to determine the expression of type I IFN at baseline conditions. As expected, neither *IFNB* nor most subtypes of *IFNA*, the major type I IFN, appear to be expressed at baseline conditions. However, other type I IFN, including *IFNE* and *IFNK* are present without stimulation (Table 3). Suggesting these type I IFN are responsible for the presence of downstream ISG expression.

Type I Interferon Expression in Oral Epithelial Cells Upon Pattern Recognition Receptor Stimulation

We next determined whether other type I IFN beyond those found to be expressed at baseline levels in OECs can be upregulated upon PRR stimulation. OKF6/Tert-1 treated with 1 μ g/ml dsRNA mimetic poly(I:C), which signals through TLR3, for up to six hours. *IFNB1*

expression, but not *IFNA4*, was upregulated (Fig. 1). A similar spike of *IFNB1* expression at two hours post-poly(I:C) treatment was observed in human corneal epithelial cells (Kumar, Zhang, & Yu, 2006). This temporal regulation of *IFNB1* expression is connected to the inherent negative feedback by downstream ISGs (Imaizumi et al., 2018). The treatment, however, did not increase the expression of the type I IFN, *IFNE* (Fig. 1), that was already expressed at high levels at baseline (Table 3), suggesting a different mechanism leads to a sustained expression of this type I IFN that could potentially act as a way to keep certain ISGs constitutively expressed.

Oral Epithelial Cell IRF3 Localization Upon Pattern Recognition Receptor Stimulation

Since viral mimetics and TLR agonists poly(I:C) (Fig. 1) and unmethylated CpG DNA (data not shown) increased the expression of *IFNB1*, we next assessed the effect of the TLR9 ligand on the movement to the nucleus of IRF3, a transcription factor associated with both *IFNB1* and *IFNA* regulation. After two hours, IRF3 appeared to localize to the nucleus upon unmethylated CpG DNA treatment compared to dH₂O vehicle control, as measured by immunofluorescence microscopy (Fig. 2). This movement of the IRF3 transcription factor is most likely connected to the increase in type I IFN expression we observed when treating cells with TLR agonists.

Interferon-stimulated Gene Expression in Oral Epithelial Cells Upon Pattern Recognition Receptor Stimulation

To measure downstream effects of IRF3 movement to the nucleus and type I IFN expression, we next determined the effect of the TLR3 agonist poly(I:C) on ISG expression. A number of ISGs were found to be upregulated upon poly(I:C) treatment of OKF6/Tert-1 cells (Fig. 3). Interestingly, each of the tested ISGs originally categorized in either the “Low” or “Medium” expression level groups at basal conditions were dramatically increased over time when treated with poly(I:C) compared to those ISGs in the “High” basal expression category (Fig. 3A and B compared to Fig. 3C). This difference suggests that OECs have evolved to express at high levels certain ISGs that defend against early aspects of viral infection and, upon stimulation by DAMPs associated with active viral replication, can upregulate other ISGs that dampen later aspects of the infection.

Discussion

The cells in the oral cavity are one of the first to be infected by pathogens that are spread orally, including a number of viruses (Corsino & Linklater, 2018; Fülöp et al., 2013; Looker et al., 2015; Scott et al., 1980; Toussiroit et al., 2008). In addition to the constitutive and induced production of host defense peptides (HDPs) with antiviral activities (Brice, Toth, & Diamond, 2018), cells can also express ISGs upon recognition of signals associated with dangerous levels of viral replication and subsequent production of type I IFN (Brubaker et al., 2015; McNab et al., 2015; Schneider et al., 2014; Schoggins & Rice, 2011). This intracellular innate pathway is so crucial for the early antiviral immune response that viruses have evolved ways to counteract this specific process (Schulz & Mossman, 2016), including KSHV, which dedicates about one quarter of its genome to subverting this pathway and its downstream effects (Lee, Lee, Chaudhary, Gill, & Jung, 2010). Similarly, deficiencies in

type I IFN have also been utilized to study viral infections including the common use of Vero cells, which do not successfully secrete IFN- α or IFN- β (Desmyter, Melnick, & Rawls, 1968), and *IFNAR* knockout mice (Müller et al., 1994). Despite this pathway garnering lots of attention in other tissues of the body, role of the type I IFN/ISG pathway in OECs is not as well studied. The results described here represent the first analysis of overall OEC type I IFN and ISG expression.

Much of the previous work of oral epithelial cell innate immunity has revolved around antibacterial aspects (McClure & Massari, 2014; Shnawa, 2016; Sugawara, Uehara, Tamai, & Takada, 2002), with the majority of IFN research involving the ability of OEC-produced IFN- γ , a type II IFN, to prime other OECs to secrete cytokines necessary for T cell and macrophage activation in the clearance of pathogenic, dysbiotic bacterial infections in the oral cavity (Mutlu, Scully, & Prime, 1991; Suga et al., 2013; Sugawara, Uehara, & Takada, 2002; Yumoto et al., 2001). While IFN- α and IFN- β have been shown to be secreted by OECs previously (Cervantes et al., 2016; Teixeira, Zhao, Kinane, & Benakanakere, 2019), our results demonstrate the ability of OECs to express not only *IFNB* and subtypes of *IFNA*, but also (for the first time) other lesser-studied type I IFN genes: *IFNK* and *IFNE* (Table 3). *IFNA* and *IFNB*, but not *IFNK*, induction by virus-related DAMPs, suggests a different role for *IFNK* and *IFNE* as potential constitutive activators of antiviral immunity in preparation for viral infections before the cell recognizes dangerous levels of viral replication. This early antiviral response that precedes, and independent of, canonical IFN signaling has been observed previously (Paladino, Cummings, Noyce, & Mossman, 2006) and may involve *IFNK* and *IFNE* signaling.

Similar to type I IFN research in OECs, not much is known about the expression of ISGs. This study represents the first transcriptome-wide analysis of OEC ISGs. The protein products of these ISGs have direct antiviral effects, with an overarching trend toward cell apoptosis to prevent productive viral infection (Schneider et al., 2014; Schoggins & Rice, 2011). A surprisingly high amount of ISGs are expressed in OECs without stimulation (Table 2). Interestingly, the expression of these ISGs is arranged in such a way that earlier aspects of viral replication are primed to be inhibited, while ISGs that address later steps of infection are not as highly expressed. This skewing towards preventing the first steps of viral infection make sense evolutionarily in that it is more energetically favorable to only produce ISGs and downstream proteins that affect the first steps of infection as opposed to all ISGs at the same time. Selective ISG expression at basal conditions also does not put unnecessary apoptotic stress on uninfected cells. As for how these ISGs are expressed without PAMP stimulation, the *IFNK* and *IFNE* expression observed at basal conditions may be responsible for the presence of a number of ISGs found without DAMP stimulation (Table 2). However, more testing is needed to determine not only if IFN- κ and IFN- ϵ are produced and lead to ISG expression, but also if these proteins have functional roles in the context of an actual viral infection.

The upregulation, in addition to expression, of these ISGs has similarly not been well described in OECs. Representative genes for each group of basally expressed ISGs, according to Table 2, also exhibited similar upregulation patterns after stimulation with poly(I:C). *MX1* and *RSAD2*, members of the “Low” basal expression group of ISGs, lead to

inhibition of post-entry and exit stages of viral infection (Haller, Staeheli, & Kochs, 2007; Seo, Yaneva, & Cresswell, 2011). These genes were the most robustly induced group after DAMP stimulation (Fig. 3A). Members of the “Medium” basal expression group, *BST2* and *ISG15*, are part of earlier aspects of viral infection, including prevention of viral protein functioning (le Tortorec, Willey, & Neil, 2011; Morales & Lenschow, 2013). These genes were the next highly expressed after poly(I:C) stimulation (Fig. 3B). The “High” basal expression group ISGs tested, *PML* and *ZC3HAV1*, are part of the antiviral pathway targeting viral replication and transcription (Everett & Chelbi-Alix, 2007.; Gao, Guo, & Goff, 2002), upstream of the other ISGs tested. In line with the trend established with the other two groups, these genes were least upregulated after poly(I:C) stimulation (Fig. 3C). The overall upregulation upon viral DAMP stimulation of the originally lower expressed ISGs, and a less robust increase of those ISGs of higher expression at basal conditions, underscores the hypothesis that OECs can exist as either “ready” to be infected or “active” in their antiviral activities, each with a different group of type I IFN and ISGs—in addition to pro-inflammatory cytokines and host defense peptides—orchestrating those actions (Fig. 4). More transcriptomic studies are necessary to determine exactly if, when, and how these states are defined and fluctuate.

Taken together, our results suggest that OECs express a certain subset of type I IFN and ISGs, while others can be induced upon different stresses associated with productive viral infections. The expressions of these antiviral genes point toward a natural defense mechanism evolved to protect against viral infections that begin their pathogenesis in the mouth. While these genes, and their protein products, have been previously characterized in other tissues (Schneider et al., 2014; Schoggins & Rice, 2011) and even used as therapies for viral infections in general (Galvani, Griffiths, & Cawley, 1988; Hsu, Chao, Lin, Chen, & Kao, 2015; Rivero-Juárez, Frias, & Rivero, 2016; Smith et al., 1999), more remains to be studied in OECs, the first cells viruses encounter when orally infecting individuals. Understanding the intricacies of our endogenous type I IFN and ISGs in OECs will eventually lead to targets for either preventative measures or therapies for diseases associated with oral viral infections.

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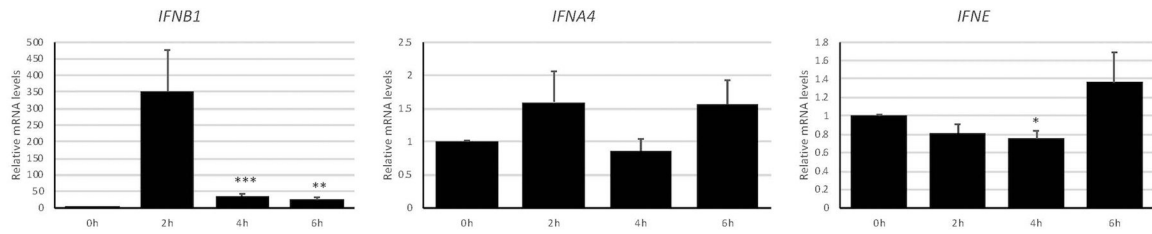


Figure 1. Type I interferon expression in oral epithelial cells upon pattern recognition receptor stimulation.

OKF6/Tert-1 cells were treated with 1 $\mu\text{g/ml}$ poly(I:C) for up to six hours and measured for changes in type I interferon expression by RT-qPCR. Bar graphs and error bars represent the means and standard errors of measurement (SEM) of three independent experiments, each with three biological replicates per condition. * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.0005$ compared to 0h, using a two-tailed, unpaired Student's t -test. All data analysis was performed using GraphPad Prism version 8.2.0 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

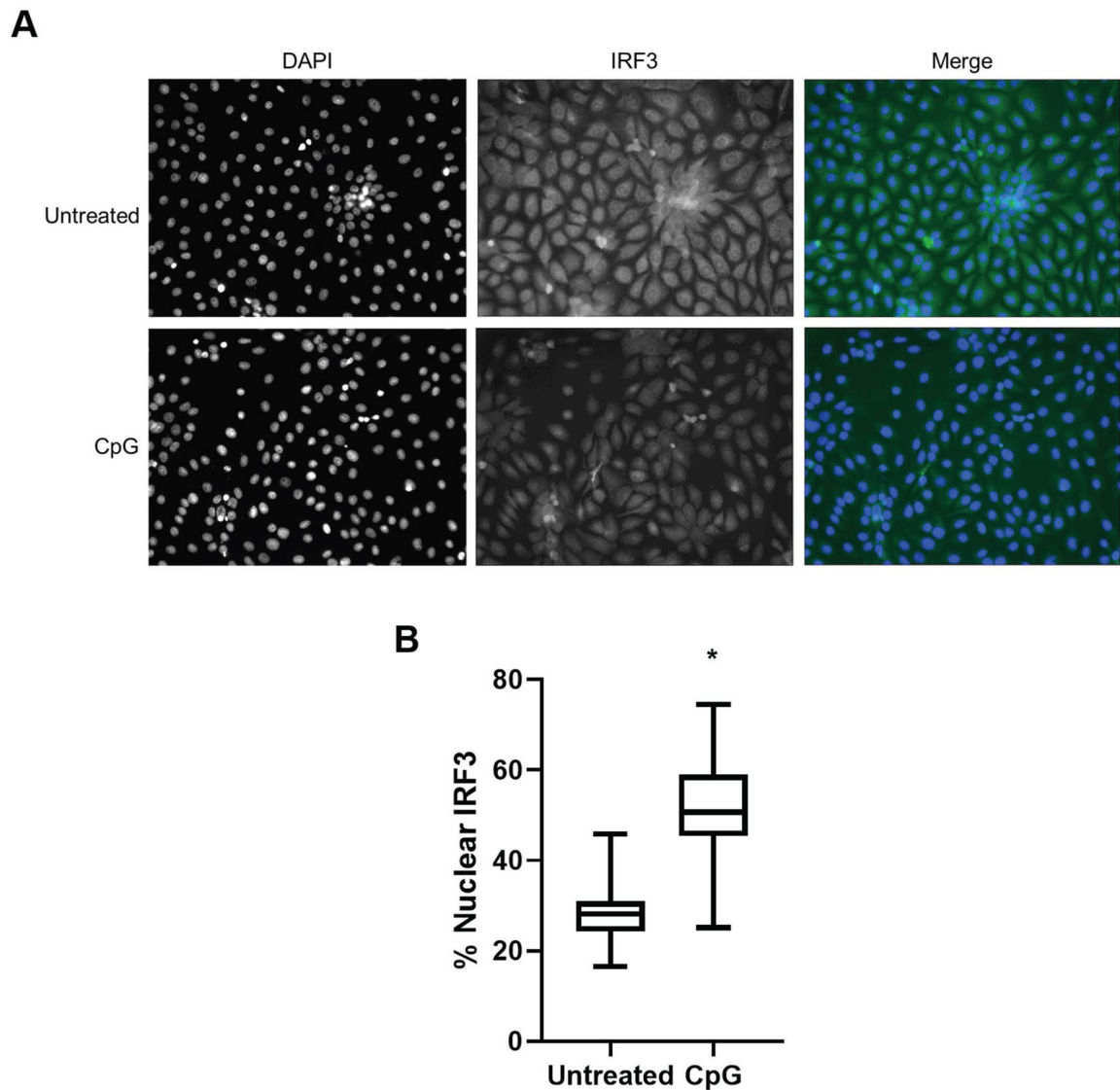


Figure 2. Oral epithelial cell IRF3 localization upon pattern recognition receptor stimulation. OKF6/Tert-1 cells were treated with with 5 μ M unmethylated CpG DNA for two hours. Localization of IRF3, either within or outside the nucleus as determined by co-localization with DAPI staining, was determined by immunofluorescence microscopy (A). Percentage of IRF3 localized to the nuclei of cells was measured for fifty cells per condition (B). * $p < 0.0001$ compared to the untreated condition, a two-tailed, unpaired Student's *t*-test after determining data from both conditions were normally distributed using the Shapiro-Wilk test.

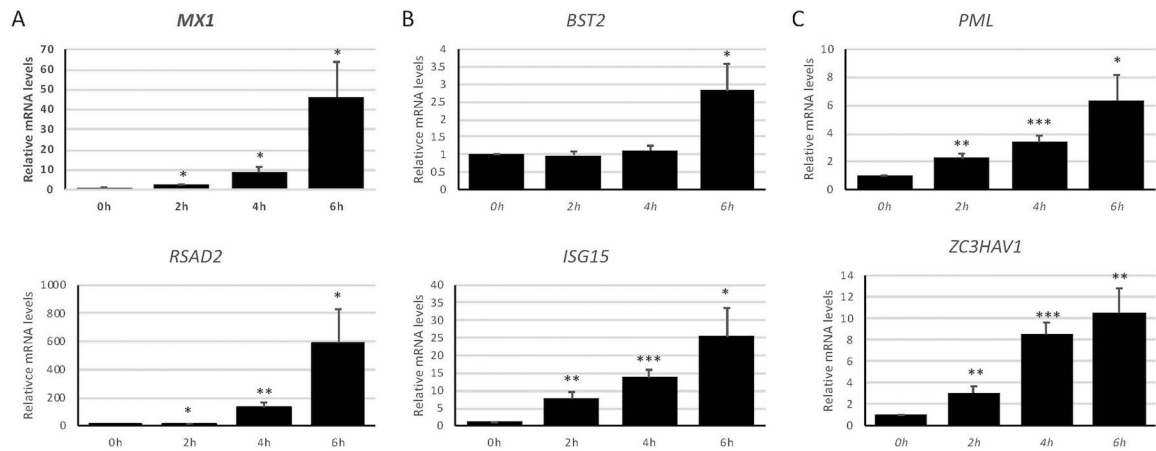


Figure 3. Interferon-stimulated gene expression in oral epithelial cells upon pattern recognition receptor stimulation.

OKF6/Tert-1 cells were treated with 1 $\mu\text{g}/\text{ml}$ poly(I:C) for up to six hours and measured for changes in interferon-stimulated gene (ISG) expression by RT-qPCR. Measured genes were divided into each ISG basal expression group, according to Table 2: Low (A), Medium (B), and High (C). Bar graphs and error bars represent the means and standard errors of measurement (SEM) of three independent experiments, each with three biological replicates per condition. * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.0005$ compared to 0h, using a two-tailed, unpaired Student's *t*-test.

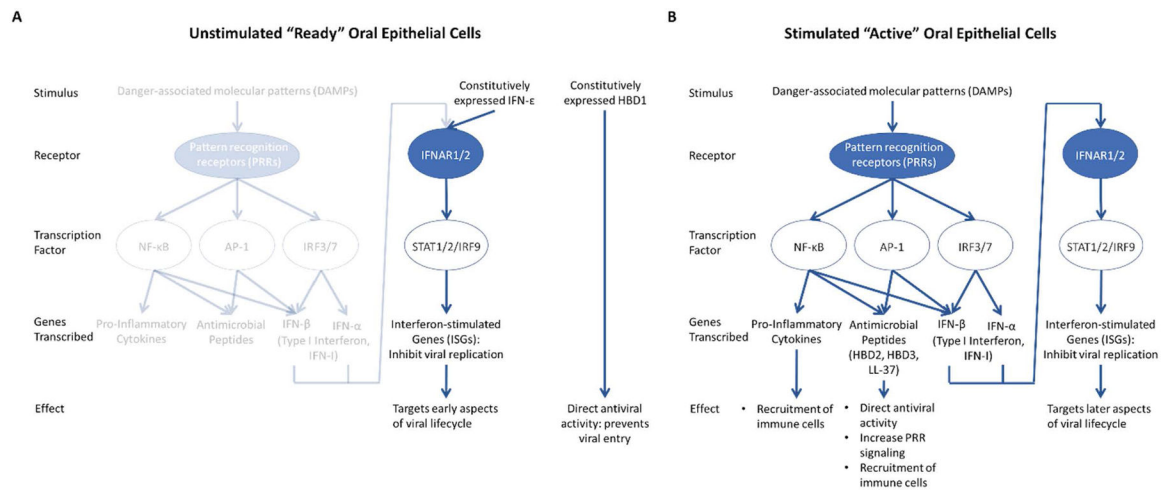


Figure 4. Proposed model of oral epithelial cell innate antiviral defenses.

Oral epithelial cells (OECs) either exist as unstimulated and “ready” for a potential viral infection (A) or stimulated and “active” in the defense against a replicating virus (B). (A) “Ready” OECs are not stimulated by danger-associated molecular patterns (DAMPs) and, therefore, do not express pro-inflammatory cytokines. However, despite not expressing the full repertoire of either type I interferon (type I IFN) or antimicrobial peptides (also known as host defense peptides, HDPs), OECs do express some of these proteins constitutively, leading to direct antiviral activity mediated by human beta defensin 1 (HBD1) (Brice & Diamond, 2019; Zhao, Wang, & Lehrer, 1996) and certain interferon-stimulated genes (ISGs) that target early aspects of the viral lifecycle. (B) OECs become “active” when stimulated with DAMPs, leading to transcription of a larger number of pro-inflammatory cytokines, HDPs, and type I IFN, each with their own antiviral effects.

Table 1.

Primers used for RT-qPCR

Gene	Primer Sequence 5'–3'
<i>ACTB</i>	F: GGATCAGCAAGCAGGAGTATG
	R: AGAAAGGGTGTAACGCAACTAA
<i>BST2</i>	F: CTGGATGGCATCTACTTCGTATG
	R: CAGGAGCACCAGAATTCCTATC
<i>IFNA4</i>	F: GAGGCCGAAGTTCAAGTTAT
	R: AGCACGGCCATCAGTAAAG
<i>IFNB1</i>	F: GCCGATTGACCATCTATGA
	R: GCCAGGAGGTTCTCAACAATAG
<i>IFNK</i>	F: CTGCAATACACCCAACCTATGA
	R: GAAGGTGTGTTGGCTGAAGA
<i>ISG15</i>	F: GAGGCAGCGAACTCATCTTT
	R: CCAGCATCTTCACCGTCAG
<i>MX1</i>	F: CTGGTGCTGAAACTGAAGAAAC
	R: TACCTCTGAAGCATCCGAAATC
<i>PML</i>	F: GCTAAGGCATGGCTGAGATT
	R: AGGGA CT CAGAATACAGGAGAG
<i>RSAD2</i>	F: TGGTGAGGTTCTGCAAAGTAG
	R: GTCACAGGAGATAGCGAGAATG
<i>ZAP</i>	F: GCAGTCCGAGCGGAATTTA
	R: CAGTCCAGAGAGTTCGTGATTT

Table 2.

Basal expression of Oral Epithelial Cell Interferon-Stimulated Genes

ISG	High Base Mean	Rank of all transcripts (out of 23,557)	Inhibition	Expression Level	Viral Inhibitions
ZC3HAV1 (ZAP)	5326.347284	1279	Transcription		
OAS3	3785.979053	1765	Replication/ Transcription		
SP100	2147.193058	2681	Replication/ Transcription	High	Entry, Transcription, Replication
IFITM2	1694.801492	3095	Entry		
PML	1622.004495	3185	Replication/ Transcription		
IFIT2	1009.976154	3972	Transcription		
IFITM3	650.3736959	4725	Entry		
ISG15	548.096075	4998	Viral Protein Function		
BST2 (Tetherin)	465.888201	5239	Exit		
ISG20	397.6777008	5514	Transcription	Medium	Viral Protein Function, Exit
OAS2	305.0472818	5964	Replication/ Transcription		
APOBEC3G	248.470772	6332	Replication/ Transcription		
IFITM1	245.6929232	6352	Entry		
OAS1	74.7362237	8733	Replication/ Transcription		
TRIM5	54.19553982	9471	Post-entry		
MX2	52.96851709	9524	Post-entry		
ADAR	43.92095899	9970	Replication/ Transcription		
IFIT1	41.49804198	10135	Transcription		
OASL	39.05579773	10295	Replication/ Transcription	Low	Post-entry, Pro-NK cell
RSAD2 (Viperin)	27.16107836	11268	Entry/Exit		
MICB	25.12520191	11495	Pro-NK cell		
MICA	20.39141631	12106	Pro-NK cell		
EIF2AK2 (PKR)	19.8998665	12169	Translation		
IFIT3	14.84120697	13109	Transcription		
MX1	13.41645478	13433	Post-entry		
CH25H	6.15382301	16147	Entry		

Basal expression levels of ISGs from OKF6/Tert-1 cells were measured by RNASeq analysis, ranked by high base mean (base mean of most highly expressed transcript variant of particular gene from three biological replicates), and grouped based on overall expression level.

Table 3.

Basal expression of Oral Epithelial Cell Type I Interferon

Base Mean	Gene
113.6056	Homo sapiens interferon, epsilon (IFNE), mRNA
7.783875	Homo sapiens interferon, kappa (IFNK), mRNA
0.793833	Homo sapiens interferon, alpha 1 (IFNA1), mRNA
0.282971	Homo sapiens interferon, omega 1 (IFNW1), mRNA

Basal expression levels of type I IFN from OKF6/Tert-1 cells were measured by RNASeq analysis and ranked based base mean from three biological replicates.