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IL-29 Exhibits Antiviral Activity by Inducing RIG-I and IFI-16 Expression in Oral Epithelial Cells

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Abstract

Interleukin-29 (IL-29) is a cytokine belonging to the type III interferon family, which regulates a similar set of genes as type I interferons. Although type I interferons act globally, type III interferons primarily target epithelial cells and protect them against the frequent viral attacks that are common for barrier tissues. The antiviral effects of IL-29 have been demonstrated on barrier surfaces in the respiratory and gastrointestinal tracts, liver, blood-brain barrier, and skin, but it remains unknown whether IL-29 exhibits these effects in oral epithelial cells. In this study, we found that the functional IL-29 receptor, interferon-lambda receptor 1, is expressed in epithelial cells from both human oral mucosa and gingiva, but not inhuman gingival fibroblasts. Although IL-29 stimulation did not induce pro-inflammatory cytokine mRNA expressions, such as IL-6 and IL-8, it did induce retinoic acid-inducible gene-I (RIG-I) and interferon-gamma-inducible protein 16 (IFI-16) production via a signal transducer and activator of transcription 1 (STAT1)-dependent pathway in gingival epithelial cells. RIG-I and IFI-16 sense viral nucleic acids, and the stimulation of these receptors induces interferon-beta production. Moreover, we confirmed that the augmenting effects of IL-29 on 5' triphosphate double-stranded RNA (a synthetic ligand for RIG-I)-induced interferon-beta production in gingival epithelial cells. These data suggest the therapeutic potential of IL-29 for preventing viral infections in the oral mucosa.

Keywords

Type III interferon
Type I interferon
Virus infection
Innate immunity
Oral mucosa

1. Introduction

Oral lesions are predominantly mucosal lesions, which can be caused by a variety of factors but are often caused by infectious diseases, particularly viral infections. Mucosal lesions of the oropharynx caused by viral infection present as blisters, aphthae, erosions, and ulcers, and leukemia and squamous cell carcinoma of the oral cavity have also been shown to be caused

by viral infection. Type I interferon (IFN) has been used as antiviral therapy, but various adverse effects have been reported because type I IFN receptors are expressed in all cells except target organs and target cells. In contrast, interleukin (IL)-29 (IFN-λ1), a type III IFN, has an antiviral activity similar to type I IFNs, but its receptor expression is localized and is expected to be an IFN with fewer side effects. Type III IFNs have been studied in the liver, intestinal epithelium, airway mucosal epithelium, skin, blood, and blood-brain barrier [1], but there are few reports on IL-29 receptor expression and its function in the oral mucosa. We are conducting daily research aiming at the clinical application of IL-29 as an antiviral agent in oral mucosa. In this article, we give an overview of viruses causing oral mucosal lesions and type III IFN and then discuss the possibility of its clinical application, including the results of our research.

2. Viral oral mucosal diseases

2.1. Aimple viruses and varicelloviruses [2]

The genus Herpes simplex viruses (HSV) and varicella-zoster viruses (VZV) are DNA viruses with double-stranded DNA genomes. Herpesviruses are characterized by latent infection in tissues. HSV and VZV in particular are latently infectious in the ganglia and are known to cause recurrent infections. They are reactivated by a reduction in the host's immunity and present with symptoms.

2.2. Enteroviruses [3]

Enteroviruses are single-stranded RNA viruses without an envelope and include enterovirus 71 and coxsackievirus A16, which are the main causative viruses of hand-foot-and-mouth disease. The route of infection is still unknown, but infection causes painful blisters and ulcers on the oral mucosa. The causative virus of herpangina, which is also known as a typical summer cold, is often coxsackievirus A4, which causes blisters and ulcers.

2.3. Morbillivirus genus [4]

Morbillivirus is a single-stranded RNA virus with an envelope, to which the measles virus belongs. Various routes of infection, such as droplet and contact transmission, are used, and the viruses are extremely contagious. After an incubation period of 10–12 days following viral infection, fever and upper respiratory tract inflammation symptoms are followed by the appearance of a rash, which is preceded by the appearance of small white spots (Koplik spots) on the buccal mucosa of the molars around 1–2 days before the rash.

2.4. Papillomaviruses [5]

Human papillomavirus (HPV) is thought to infect epithelial cells, especially proliferating basal cells. In the oral mucosa, HPV has been reported to be involved in the development of benign lesions such as leukemia, erythroplakia, and papillomas, as well as in the development of squamous cell carcinoma, a malignant lesion.

2.5. Lentivirus genus [6]

Lentiviruses are single-stranded RNA viruses with an envelope, to which the human immunodeficiency virus (HIV) belongs. Oral lesions associated with HIV infection are known to include recurrent oral candida, angulus stomatitis, and stomatitis. Oral lesions often trigger the diagnosis of acquired immunodeficiency syndrome (AIDS), and it is considered important to lower the threshold for HIV testing in clinical practice.

3. Type III interferons

Type III IFNs, also known as the IFN-λ family, are relatively new IFNs, ranging from IFN-λ1 to λ4. λ1, λ2, and λ3 are encoded by IFNL1 (IL-29), IFNL 2 (IL-28A), and IFNL3 (IL-28B), respectively, and their antiviral effects have been reported to be stronger in the order IFN-λ1 > IFN-λ3 > IFN-λ2 [7]. IFN-λ4 has low homology with other IFN-λs and has different functions [8]. Unlike

type I IFNs (IFN-α and IFN-β), whose receptors are expressed on all cell types, the IFN-λ receptors, especially IFN-λR1 which has a high affinity for its ligand, are restricted to hepatocytes, epithelial cells, and dendritic cells. When IFN-λ binds to the receptor, it phosphorylates signal transducers and activators of transcription 1 (STAT1) and STAT2 proteins, which in turn induces interferon stimulating gene (ISG) and exerts antiviral effects [1]. In other words, type III IFNs share the same downstream signaling molecules as type I IFNs after binding to the receptor; although the ISG-inducing capacity of type III IFNs is known to be weaker than that of type I IFNs [7], type III IFN signaling is believed to induce ISGs in a sustained manner.

4. IL-29 receptor (IFN-λR1) expression in the oral mucosa and its functional analysis.

Based on the aforementioned background, we analyzed the gene expression levels in cells constituting the oral mucosa and epidermal-derived keratinocytes, which are known to express the IL-29 receptor, using the polymerase chain reaction (PCR) method. As a result, we confirmed that IFN-λR1 was expressed at the gene level in keratinocytes derived from the oral mucosa and gingiva as well as epidermal-derived keratinocytes. Furthermore, we found that IFN-λR1 was expressed at the protein level using flow cytometry. On the other hand, it was not expressed in gingiva-derived fibroblasts, suggesting that the receptor may be expressed only in epithelial cells in the oral mucosa. Next, we confirmed by Western blotting that STAT1 was phosphorylated in a concentration-dependent manner when oral mucosa-derived and gingiva-derived keratinocytes were stimulated with IL-29. As a recognition mechanism by which host cells sense pathogens such as bacteria and viruses, they express pattern recognition receptors that recognize characteristic structures conserved in pathogens. As described in section 2, both RNA viruses and DNA viruses are responsible for oral mucosal diseases, hence in this study, we focused on retinoic acid-inducible gene-I (RIG-I), the pattern recognition receptor responsible for nucleic acid recognition in RNA viruses, and interferon gamma-inducible protein 16 (IFI-16), the pattern-recognition receptor responsible for recognizing double-stranded DNA viruses [9]. Western blotting analysis confirmed that RIG-I and IFI-16 expressions were induced in human gingiva-derived keratinocytes in an IL-29 concentration-dependent manner. The expression of these two receptors induced by IL-29 was significantly suppressed by STAT1 inhibitors, suggesting a STAT1-dependent response. Since IL-29 induces the expression of receptors that recognize viral nucleic acids without inducing inflammatory cytokines, we examined whether IL-29 could enhance the antiviral activity of IL-29 in the oral mucosa. We investigated whether IL-29 pre-treatment enhanced IFN-β production in human gingival-derived keratinocytes by stimulation with 5' triphosphate double-stranded RNA, a synthetic ligand for RIG-I. The results using real-time PCR and ELISA confirmed the enhancement effect (**Figure 1**). Further experiments using live viruses are under consideration, and further evidence for clinical application is considered necessary.

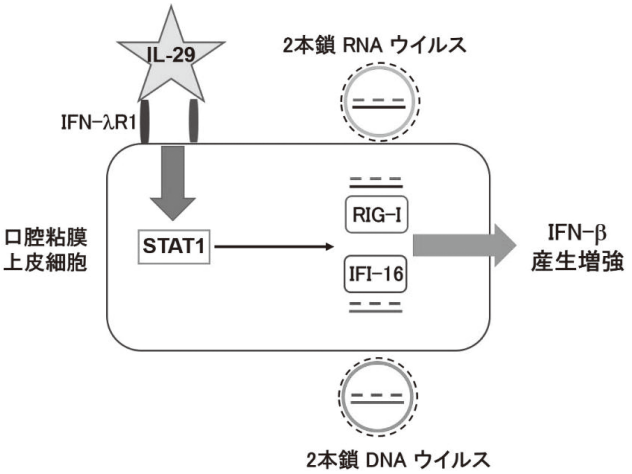

Figure 1. Mechanism of enhanced antiviral activity of IL-29 in the oral mucosa.

5. Conclusion

Although research on viruses has been conducted in a very wide range of fields, few studies have focused on the enhancement and activation of antiviral activity in the oral mucosa. Among dental diseases, the development of preventive and therapeutic methods for diseases caused by viral infections is of great clinical importance. We are currently aiming to develop novel therapeutic strategies based on our findings.

Disclosure statement

The authors declare no conflict of interest.

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