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What is the oligoadenylate synthetases-like protein and does it have therapeutic potential for influenza?

John F. Alcorn^{1,2} and Saumendra N. Sarkar^{*,2,3,4}

¹Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine, Pittsburgh, PA

²Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA

³Cancer Virology Program, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA

⁴Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

Besides its pandemic potential, seasonal influenza infection is associated with an estimated 250,000 to 500,000 deaths worldwide every year. Part of this virulence of influenza virus can be attributed to its ability to evade the host innate immune response. Here we discuss the possibility of using a recently described mechanism of boosting the innate immunity by oligoadenylate synthetase-like protein, to combat influenza infections.

Keywords

Influenza; OASL; interferon; RIG-I

Introduction

Influenza viruses present a threat to global health with significant pandemic potential. The coexistence and co-evolution of the host and the virus have resulted in competing strategies to protect and propagate, respectively, over a long time. As a result the host innate immune system, the first line of defense against viral infection, has been a major target for evasion by almost all human viruses [1]. For influenza virus this is accomplished primarily by the non-structural protein, NS1 through various mechanisms [2]. One such mechanism to successfully subvert innate immune induction is by inhibiting primary antiviral cytokine interferon (IFN) induction. The recent description of a unique mechanism of IFN induction

*Corresponding Author: Saumendra N. Sarkar, Ph.D., University of Pittsburgh Cancer Institute, Hillman Cancer Research Pavilion, Suite 1.8, 5117 Centre Avenue, Pittsburgh, PA 15213, Phone: (412) 623-7720, Fax: (412) 623-7715, saumen@pitt.edu.

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[3] may provide ways to counter this subversion, and help develop new therapeutic approaches against multiple strains of influenza virus.

Viral RNA sensing and IFN induction

RNA viruses pose a great threat to public health due to their rapid replication kinetics, high mutation rates, and complex evolutionary dynamics. Cellular innate immunity, triggered by sensing of viral RNA through specialized receptors, regulates not only the outcome of a viral infection, but also tissue tropism, predisposition to disease, inflammation, and tumorigenesis [4]. Following entry into target cells, viral RNA is detected by cytosolic receptors such as RIG-I (retinoic acid-inducible gene I)-like (RLR) and toll-like receptors (TLR) through the detection of non-self nucleic acids. RLR engagement with viral RNA leads to activation of signaling pathways resulting in transcriptional induction of IFN (IFN α and IFN β). IFN induced by RLR or TLR signaling acts in an autocrine or paracrine manner to induce many IFN-stimulated genes (ISG). Due to common transcriptional elements in their promoters, several ISGs are also targets for direct induction by viral infection (via IRF3/IRF7), without requiring IFN signaling. Most, if not all, of the pleiotropic effects of IFN are mediated by ISGs. The best understood mechanism for the antiviral activity of ISG is the generalized inhibition of protein synthesis by dsRNA-activated enzymes (e.g., PKR, OAS, ADAR, etc.). However, among ~ 400 ISGs, the biochemical function of only a handful has been delineated [5]. Recently, a number of ISGs were tested for their antiviral activities against a variety of viruses [6]. However, the mechanisms of antiviral activity for most of these genes remain unknown.

Subversion of innate host defense by influenza

A primary component of the early, innate immune response to influenza infection is IFN production. The subsequent ISG response limits viral replication by modifying cell viability, gene transcription, and protein synthesis. Viral pathogens have developed several strategies to subvert the IFN response. The influenza NS1 gene attenuates IFN induction through several interactions. NS1 can directly bind RIG-I and/or limit ligand availability by binding RNA [7, 8]. Further, NS1 can attenuate activation of RIG-I via inhibition of ubiquitination by the ubiquitin ligase, TRIM 25 [9]. TRIM25 requires oligomerization for its functional activity on RIG-I, a process that is opposed by NS1. NS1 also has been shown to inhibit IRF3 nuclear translocation [10]. In addition, to these effects upon IFN production, NS1 has additional roles in evasion of host immunity. A role for NS1 in regulating cell signaling processes involved in inflammation has been demonstrated. NS1 induces the PI3K pathway to limit host cell apoptosis and benefit viral replication [11]. While, influenza virus lacking NS1 induces increased caspase-1 activity (presumably via the inflammasome) resulting in enhanced IL-1 β and IL-18 production [12]. IL-1 β and IL-18 are important mediators of the macrophage and neutrophil response at mucosal surfaces. These data suggest an expanded role of influenza NS1 in modulating innate host defense.

IFN-inducible Oligoadenylate Synthetase-Like (OASL) protein

Oligoadenylate synthetases (OAS) are a family of ISGs characterized by their ability to synthesize 2'-5' oligoadenylates, which induce RNA degradation by activating RNaseL [13].

However, the recent identification of the cytoplasmic DNA sensor cGAS, which is another member of OAS family, shows potentially diverse functions of this family of proteins [14]. Human oligoadenylate synthetase-like (*OASL*), is related to the OAS family by its N-terminal OAS-like domain, which is derived from an ancestral OAS protein, but harbors characteristic changes in the active site, and is thus devoid of 2'-5' oligoadenylates synthetase activity. Furthermore, *OASL* contains two tandem ubiquitin-like domains (UBL) in the C-terminus, which are not present in any of the other members of the OAS family [13]. *OASL* is directly and rapidly induced by virus infection via interferon regulatory factor (IRF)-3 as well as by IFN signaling and has been shown to have antiviral activities, which requires the UBL domain [15]. We have recently demonstrated that *OASL* interacts with RIG-I and enhances RIG-I mediated IFN induction [3].

Unlike in humans, two *OASL* orthologs have been identified in mice: *Oasl1* and *Oasl2*, sharing respectively 70% and 48% amino acid sequence identity with human *OASL* [13]. Uniquely, the mouse *Oasl2* contains two crucial Asp residues in its active site and it exhibits OAS enzyme activity. The mouse *Oasl1*, has been recently shown to inhibit IFN induction by binding to the 5' UTR of IRF7 and inhibiting its translation. Consequently, targeted deletion of *Oasl1* led to enhanced IFN induction and diminished viral replication *in vivo* [16]. In contrast to *Oasl1*, human *OASL* and mouse *Oasl2* do not bind to the IRF7 5'UTR and are devoid of IRF7 suppression activity. Targeted deletion of *Oasl2* in mice showed enhanced virus replication; suggesting that *Oasl2* acts as the functional equivalent of human *OASL*.

Mechanism of *OASL* action

OASL promotes antiviral activity by enhancing the sensitivity of RIG-I activation. From a number of biochemical and structural studies [17], a model for RIG-I activation has been proposed where RIG-I adopts a stable auto-inhibited conformation in the absence of RNA. Upon binding to viral RNA through the C-terminal domain (CTD), the helicase domain changes conformation, thereby enabling *OASL* to hydrolyze ATP and further interact with RNA. The N-terminal CARDs (Caspase activation and recruitment domains) then bind to K63-linked polyubiquitin (pUb), converting RIG-I to an active competent state, which is followed by CARD-mediated MAVS aggregation and signaling. Recent observations also suggest RNA dependent RIG-I aggregation in the case of longer RNA resulting in promotion of MAVS activation [18]. Although, for larger dsRNA the strict requirement of pUb for RIG-I activation has been a topic of debate, in most cases RIG-I activation is strongly regulated by a two-step mechanism requiring simultaneous binding of two ligands – RNA and pUb. This mechanism makes the RIG-I sensor likely to avoid aberrant activation of antiviral innate immunity and IFN induction. It has been shown that the short K63-linked polyubiquitin chains, or the K63-linked polyubiquitination of RIG-I is carried out by the ubiquitin ligase TRIM25 [19, 20]. However, we have shown that in presence of *OASL* RIG-I can be activated by viral RNA in the absence of TRIM25 [3]. Thus, we believe that following the initial viral infection and *OASL* induction in the infected and the surrounding cells through IFN signaling, *OASL* binds to RIG-I and mimics pUb. This makes RIG-I activation more sensitive requiring just one ligand, viral RNA, and leads to enhanced IFN induction.

Unmet needs and how OASL may be useful

The primary focus of protection against influenza on a global scale is centered upon vaccine development. Vaccination efforts have resulted in a reduction of seasonal illness related to influenza, but are limited in efficacy overall. A primary complication to vaccine strategies is influenza virus antigenic drift and shift. Over time, the influenza strain's antigen repertoire shifts reducing protective immunity. Antigenic shift occurs when novel influenza strains emerge through gene reassortment. The 2009 pandemic influenza strain is an example of this process where virus was passaged through avian and swine vectors. This emergence of novel strains presents limits on vaccine generation resulting in a large rate of infection prior to the availability of vaccine. Antiviral therapies are also currently available, however oseltamivir (Tamiflu) is mainly effective when delivered early during the infection time course and patients often present at the hospital beyond this window. Influenza strains also have shown the ability to develop oseltamivir resistance. For these reasons, there are significant limitations to current approaches for treatment of influenza pneumonia. OASL presents a novel molecule that may be able to boost innate host defense, even in the presence of viral inhibition, resulting in improved immunity. Two aspects about OASL-mediated enhancement of RIG-I signaling make it unique for combating influenza infection. First, OASL has the potential to overcome the NS1-mediated innate immune evasion. According to our results, despite targeting of TRIM25 by NS1, RIG-I can be activated in presence of OASL. Second, unlike RIG-I expression, which results in IFN induction that can lead to toxicity, expression of OASL by itself does not activate IFN induction. Therefore, delivering OASL protein or ectopically expressing OASL is less likely to have major toxic side effects and may prove a new mode of combating influenza infection.

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