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OASL – a new player in controlling antiviral innate immunity

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Abstract

The cellular innate immune system plays a critical role in mounting the initial resistance to virus infection. It is comprised of various pattern-recognition receptors that induce type I interferon production, which further shapes the adaptive immunity. However, to overcome this resistance and promote replication, viruses have evolved mechanisms to evade this host innate immune response. Here we discuss a recently described mechanism of boosting the innate immunity by oligoadenylate synthetase-like (OASL) protein, which can potentially be used to overcome viral evasion and enhance innate immunity.

Keywords

Influenza; OASL; interferon; RIG-I

Introduction

Despite remarkable advances in vaccination and treatment, diseases caused by viral infections remain one of the leading causes of death worldwide; as we have seen in recent years, a number of viruses also threaten the global health with pandemic potential. Among the different types of viruses that are human pathogens, RNA viruses pose unique challenges due to their rapid replication kinetics, high mutation rates, and complex evolutionary dynamics. The co-evolution of the virus and the host has resulted in competing strategies to protect and propagate over a long time. As a result, often times the host antiviral immunity that prevents viral infection is targeted by viruses [1]. Therefore, understanding the inner workings of the host innate immune response – the first line of defense against viral infection, and how it is subverted by viruses gives us one of the most promising opportunities to combat diseases caused by RNA viruses.

Host defense against RNA or DNA virus infection is initiated by the innate immune receptors such as RIG-I (Retinoic acid-inducible gene I)-like receptors (RLR), Toll-like

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receptors (TLR) and specific DNA-sensors through the detection of non-self nucleic acids. This initiates the cellular innate immune response, primarily mediated by type I interferons (IFN), and shapes subsequent adaptive immunity [2–4]. IFN induced by RLR or TLR signaling acts in both autocrine and paracrine manner to induce many IFN-stimulated genes (ISGs), which mediate most of the pleiotropic effects of IFN. Due to common transcriptional elements in their promoters, several ISGs are also directly induced by virus infection (via IRF3/IRF7) without requiring IFN signaling [5,6]. Recently, a large number of ISGs were tested for their antiviral activities against multiple viruses [7–9]. However, among ~ 400 ISGs, the biochemical functions of only a handful have been delineated [10,11]. The best understood mechanism for the antiviral activity of ISG is the generalized inhibition of protein synthesis by dsRNA-activated enzymes (e.g., RNA activated Protein Kinase (PKR), Oligoadenylate Synthetase (OAS) etc.). Recent studies have shed light on the mechanism of antiviral activities of some ISGs, such as Viperin [12], IFIT family proteins [13], IFI16 [14], and cGAS [15]. We have recently described the mechanism of antiviral activity of one such ISG, Oligoadenylate Synthetase-Like (OASL) [16]. Here we discuss some of the unique features of OASL, and the potential for developing broad acting antiviral therapy exploiting this pathway.

OAS family of proteins - Structural features and conservation

Oligoadenylate synthetases (OAS) belong to a family of ISGs characterized by their ability to synthesize 2'–5' oligoadenylates, which induce RNA degradation by activating a latent RNase, RNase L [17]. However, the recent identification of the cytoplasmic DNA sensor cyclic GMP-AMP synthetase (cGAS), which is another member of the OAS family, shows potentially diverse functions of this family of proteins [18]. Human oligoadenylate synthetase-like (*OASL*), is related to the OAS proteins by its N-terminal OAS-like domain, but harbors characteristic changes in the active site, and is thus devoid of 2'–5' oligoadenylate synthetase activity (Fig. 1). 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 [17]. *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 [8,19]. A number of epidemiological studies have also linked various SNPs in human OASL gene to altered susceptibility to hepatitis C and West Nile virus infections [20–22]. However, in the absence of the catalytic activity to synthesize 2'–5' oligoadenylates, the mechanism of human OASL antiviral activity remained elusive until recently.

The presence of enzymatically active OAS-like proteins was reported in marine sponges [23]. However, as the IFN system is restricted to the jawed vertebrates, the significance of this finding and its contribution in the innate immunity remained largely unappreciated. The discovery of cGAS and its obvious structural similarity with OAS proteins has generated new attention to this family of proteins. It is now clear that these proteins, including the bacterial dinucleotide cyclase (DncV), belong to an ancient nucleotidyltransferase superfamily (NTase domain in Fig. 1) [24,25], and are widely found throughout various forms of life [26]. Interestingly, not all the homologs in this family are predicted to be enzymatically active [26], which indicates divergent functions of these proteins arising

during evolution. However, as demonstrated by human OASL, which is devoid of enzyme activity, the absence of enzymatic activity is seldom the defining characteristic of OAS proteins' involvement in innate immunity. As discussed below, OASL, which seems to have evolved from OAS1 and is confined in vertebrates [27,28], has orthologs in various other vertebrate species with enzyme activity.

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* [17]. While mouse *Oasl1* is enzymatically inactive, mouse *Oasl2* contains two crucial Asp residues in its active site and exhibits OAS enzyme activity [29] (Fig. 1). Similar enzymatically active OASL othologs have also been reported in chickens [30]. 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* [31]. Furthermore, *Oasl1*$^{-/-}$ mice showed better control of viremia and a better virus-specific CD8^{+} T-cell differentiation upon chronic LCMV infection [32]. 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 viral replication suggesting that Oasl2 acts as the functional equivalent of human OASL [16].

Mechanism of action of OASL proteins - Enhancement of RIG-I activity by OASL

Human OASL promotes antiviral activity by enhancing the sensitivity of RIG-I activation. From a number of biochemical and structural studies [33], 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 RIG-I 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 that in the case of longer RNA, RIG-I oligomerization occurs without pUb [34]. 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 allows the RIG-I sensor to avoid aberrant activation of antiviral innate immunity and IFN induction. It has been shown that the synthesis of short K63-linked polyubiquitin chains, or the K63-linked polyubiquitination of RIGI is carried out by the ubiquitin ligase TRIM25 [35,36]. However, we have shown that in presence of OASL, RIG-I can be activated by viral RNA in the absence of TRIM25 [16]. This and additional OASL-RIG-I interaction studies allowed us to propose the following model for the enhancement of RIG-I activity by OASL (Fig. 2). 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. However, it will be premature to conclude that this is the

sole mechanism of OASL function, and further investigations are necessary to delineate other possible mechanisms of its activity. For example, OASL expression inhibited HSV-1 replication in HEK293 cells [16]. As HEK293 cells are known to be defective in cGAS-STING-mediated DNA sensor signaling, this antiviral activity was attributed to RIG-I activated through RNA pol III [37]. However, it is not yet clear how OASL affects HSV-1 replication in cells where it is sensed through cGAS pathway.

Viral evasion of innate immunity - Targeting of OASL by viral proteins

As the primary mediators of antiviral innate immunity, the RLR and the IFN pathways are targeted by multiple RNA viruses. Respiratory syncytial virus (RSV), the causative agent for severe bronchiolitis and pneumonia in children and the elderly, accomplishes this with the nonstructural proteins, NS1 and NS2. These proteins specifically promote the degradation of various proteins involved in IFN pathways [38]. Our recent finding that OASL is targeted for degradation by RSV NS1, again supports its physiological importance in providing cellular innate immunity. Interestingly, for the mouse *Oasl* isoforms, it is only the Oasl2 that inhibits RSV replication, and is degraded in the presence of RSV NS1, while Oasl1 is not (unpublished observation). Similarly, for the influenza virus multiple mechanisms are known to subvert antiviral innate immunity [39]. The influenza non-structural protein, NS1 can directly bind RIG-I and/or limit ligand availability by binding RNA [40,41]. Additionally, NS1 can attenuate activation of RIG-I via inhibition of ubiquitination by the ubiquitin ligase, TRIM25 [35]. As it has been shown that in the presence of OASL, RIG-I activation can be carried out without TRIM25, it is expected that boosting OASL expression may provide strong antiviral activity against all strains of influenza viruses. However, the picorna viruses that are primarily sensed through MDA5 (Melanoma differentiation associated gene 5, another member of the RLR family) are not inhibited by OASL showing specificity. Interestingly, OASL is not targeted by picorna viruses to subvert innate immunity (unpublished observation). These findings together argue in favor of using the OASL-pathway to provide broad antiviral activity against viruses that are primarily sensed through RIG-I, which might help overcome viral subversion of innate immunity.

Conclusions – Unmet needs and how OASL may be useful

Two aspects about OASL-mediated enhancement of RIG-I signaling make it unique for combating viral infection. First, OASL has the potential to overcome the innate immune evasion. According to our results with influenza virus, despite targeting of TRIM25 by NS1, RIG-I can be activated in presence of OASL (unpublished observations). 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. It makes the RIG-I-based RNA detection system much more sensitive to viral RNA, where it can be activated with comparatively sub-threshold levels of virus infection [16]. 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 virus infections. However, the successful delivery of a cytoplasmic protein to obtain therapeutic efficacy is a daunting challenge for the current drug delivery technologies. Thus, we are focusing on the respiratory viruses, which affect the respiratory system that is much more accessible to non-invasive manipulations. In summary, 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.

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Highlights

- Human OASL provides antiviral activity by enhancing RIG-I signaling.
- The presence of OASL allows cells to overcome viral evasion for some viruses.
- Targeting the OASL-pathway may be an effective way to combat viral infection.

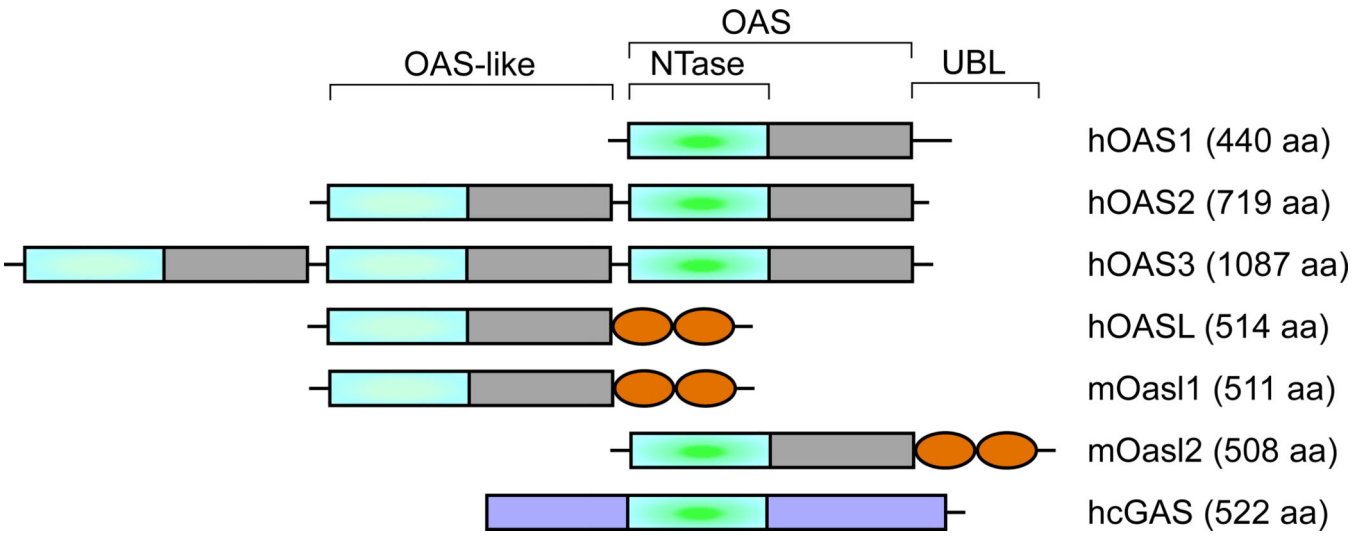

Fig. 1.

Domain organizations of human OAS family and mouse Oasl proteins. Human OAS1, OAS2 and OAS3 (hOAS) contain 1, 2 and 3 OAS-like domains respectively. Among them only one from each has active nucleotidyltransferase (NTase) activity (aligned at the center). Human and mouse OASL proteins have the OAS domain, but the NTase activity is lost in hOASL and mOasl1, whereas mOasl2 has NTase activity. All the OASL proteins contain two repeats of ubiquitin-like domains (UBL) at the C-terminal. Another member of the family, cGAS harbors structurally similar NTase domain, but no other similarity.

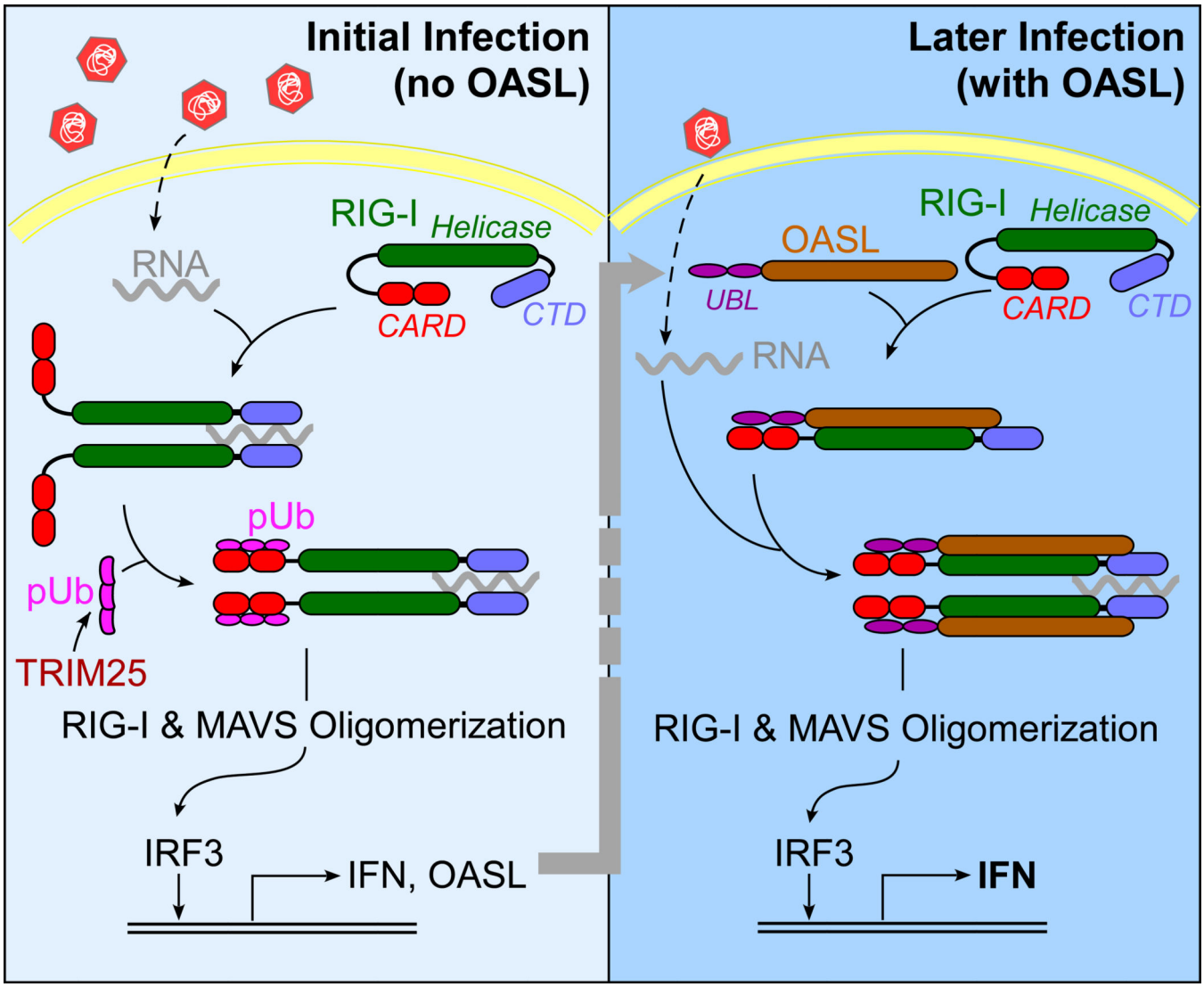

Fig. 2.
A schematic model of OASL-mediated enhancement of RIG-I signaling.