Role of the chicken oligoadenylate synthase-like gene during *in vitro* Newcastle disease virus infection

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ABSTRACT The enzyme 2′-5′ oligoadenylate synthase (**OAS**) is one of the key interferon-induced antiviral factors that act through inhibition of viral replication. In chickens, there is a single well-characterized *OAS* gene, oligoadenylate synthase-like (***OASL***) that has been shown to be upregulated after infection with various viruses. However, a deeper understanding of how chicken OASL acts against viral infection is still necessary. In this study, we tested the hypothesis that *OASL* short interfering RNA (**siRNA**)–mediated knockdown would decrease the host gene expression response to the Newcastle disease virus (**NDV**) by impacting antiviral pathways. To assess our hypothesis, a chicken fibroblast cell line (**DF-1**) was infected with the NDV (LaSota strain) and *OASL* expression was knocked down using a specific siRNA. The level of NDV viral RNA in the cells and the expression of interferon response- and apoptosis-related genes were evaluated by quantitative PCR at 4, 8, and 24 h postinfection (**hpi**). Knockdown of *OASL* increased the level of NDV viral RNA at 4, 8, and 24 hpi ($P < 0.05$) and eliminated the difference between NDV-infected and noninfected cells for expression of interferon response- and apoptosis-related genes ($P > 0.05$). The lack of differential expression suggests that knockdown of *OASL* resulted in a decreased response to NDV infection. Within NDV-infected cells, *OASL* knockdown reduced expression of signal transducer and activator of transcription 1, interferon alfa receptor subunit 1, eukaryotic translation initiation factor 2 alpha kinase 2, ribonuclease L, caspase 8 (***CASP8***) and caspase 9 (***CASP9***) at 4 hpi, *CASP9* at 8 hpi, and caspase 3, *CASP8*, and *CASP9* at 24 hpi ($P < 0.05$). We suggest that the increased NDV viral load in DF-1 cells after *OASL* knockdown was the result of a complex interaction between *OASL* and interferon response- and apoptosis-related genes that decreased host response to the NDV. Our results provide comprehensive information on the role played by *OASL* during NDV infection in vitro. Targeting this mechanism could aid in future prophylactic and therapeutic treatments for Newcastle disease in poultry.

Key words: chicken fibroblast cell line, DF-1 cells, interferon, small interfering RNA, viral chicken disease

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INTRODUCTION

Newcastle disease, caused by a single-stranded RNA virus belonging to the Paramyxoviridae family, is one of the main viral diseases that reduce efficiency in poultry production causing significant economic losses (Saba Shirvan and Mardani, 2014). Different outcomes of Newcastle disease, ranging from lower egg production to neurologic problems and widespread mortality are dependent on Newcastle disease virus (**NDV**) strain pathogenicity (Cattoli et al., 2011), and the severity of NDV infection is also determined by other factors including host resistance to the virus (Dortmans et al., 2011).

Antiviral host defense initiates with the prompt recognition of possible pathogens, which elicits responses from the innate immune system (Goraya et al., 2017). Newcastle disease virus recognition leads to the activation of signaling pathways and transcription factors that result in the production of cytokines, chemokines, and interferons to contain viral replication (Kapczynski et al., 2013). The importance of type I (Rue et al., 2011) and type II (Susta et al., 2013) interferons and interferon effectors (Wilden et al., 2009; Pagala et al., 2013) has been shown during NDV infection. The

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transcriptional profile obtained from chick embryonic fibroblasts showed that among the 8,433 differentially expressed transcripts associated with NDV infection, 3,616 were in pathways associated with interferon-stimulated genes (**ISG**) (Liu et al., 2018). The ISG have different functions to create a strong and fast response to viruses, but despite their importance, only a few ISG have been well characterized in chickens. Characterized ISG include the M × 1 protein, which can block early stages of virus replication, protein kinase R (**PKR**), which can contain virus replication by repressing viral RNA translation, and the 2′-5′ oligoadenylate synthase (**OAS**)/ribonuclease L (**RNase L**) pathway, which can cleave viral RNA transcripts and restrain viral replication (Santhakumar et al., 2017).

One of the key antiviral factors induced by interferons, 2′-5′ OAS acts through inhibition of viral replication (Choi et al., 2015). In humans, the OAS family consists of 4 proteins, OAS1, OAS2, OAS3, and OASL (Zhu et al., 2014; Leisching et al., 2017). The biological functions of the OAS family in humans and other mammals have been extensively explored (Justesen et al., 2000). In chickens, there is only a single OAS gene, oligoadenylate synthase like (***OASL***). The induction of *OASL* expression by type I, II, and III interferons has been shown in a chicken fibroblast cell line (Masuda et al., 2012). In goose, overexpression of OASL protein reduced the replication of NDV (Yang et al., 2016). Oligoadenylate synthase like has also been shown to have anti-*Flavivirus* activity in ducks and geese (Tag-EL-Din-Hassan et al., 2018) and in chickens (Tag-El-Din-Hassan et al., 2017). Avian OASL can produce 2′-5′-oligoadenylates from ATP and activate the OAS/RNase L pathway to inhibit the replication of positive single-stranded RNA viruses, double-stranded RNA viruses, and negative single-stranded RNA viruses in the duck and ostrich (Rong et al., 2018). In our previous studies, the expression of *OASL* was upregulated by thr NDV in the trachea epithelium tissue (Deist et al., 2017) and in the spleen (Zhang et al., 2018; Del Vesco et al., 2020) of inbred chickens.

Despite its documented importance, studies focused on OASL in chickens are scarce, and to our knowledge, there is no information elucidating OASL antiviral mechanisms in chickens. In this study, we test the hypothesis that *OASL* siRNA-mediated knockdown would decrease the host response to the NDV by impacting gene expression in different antiviral pathways. Therefore, the objectives of this study were to determine the level of NDV viral RNA and the expression of interferon response- and apoptosis-related genes in a chicken fibroblast cell line (**DF-1**) infected with the NDV (LaSota strain) in which *OASL* expression was knocked down using a specific siRNA.

MATERIAL AND METHODS

DF-1 Cell Culture

DF-1 cells (chicken fibroblast cell line; ATCCCRL-12203, Manassas, VA) were cultured with 10% fetal bovine serum and 1% antibiotics (Gibco Antibiotic-Antimycotic 100X, Thermo Fisher scientific, Waltham, Massachusetts) in Dulbecco's Modified Eagle's Medium 1X (Gibco, Thermo Fisher scientific, Waltham, Massachusetts, US) at 39°C and 5% CO2. DF-1 cells from passage 6 were used throughout the entire experiment.

Experimental Design

To test the role of the *OASL* gene in inhibiting the efficiency of cellular responses to NDV infection in vitro, we evaluated 4 experimental groups: 1) NDV-infected cells transfected with an *OASL*-targeted siRNA (**siOASL**); 2) NDV-infected cells transfected with a scrambled nontargeting siRNA (**siNT**) as a negative control; 3) Noninfected cells transfected with siOASL; and 4) Noninfected cells transfected with siNT. Cells from each group were harvested at 4, 8, and 24 h postinfection (**hpi**) with the NDV. The experiment was replicated 4 times (different ds) with 3 technical replicates at each time.

Oligoadenylate Synthase-Like Gene Knockdown

Three specific siRNA (siOASL#1, siOASL#2, and siOASL#3) targeting different regions of the *OASL* gene (Table 1) and an siNT (catalog number 4390843) were designed by Thermo Fisher Scientific (Waltham, Massachusetts). To test the designed siRNA and perform the knockdown assays, DF-1 cells were prepared in 6-well plates, seeded at 2 × 105 cells/well and then grown to 80% cell confluency. In each well, 25 pmol of the appropriate siRNA (siOASL#1, siOASL#2, siOASL#3, or siNT) was transfected using Lipofectamine (Lipofectamine 3000 Transfection Reagent; Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts) as per the manufacturer's instructions.

Knockdown efficiency rate was calculated by the comparative cycle threshold (**CT**) method (**ΔΔCT**) using the ribosomal 28S gene as the housekeeping gene. Oligoadenylate synthase-like gene expression in cells transfected with siNT was compared with gene expression in cells transfected with each siOASL#1–3 to calculate the knockdown rate. After testing the *OASL* gene–targeting siRNA, siOASL#2 was used in the downstream knockdown assays owing to its higher knockdown efficiency rate (Table 1). Because chicken OASL has 2 alternatively spliced isoforms, ChOAS-A and ChOAS-B (Tag-El-Din-Hassan et al., 2012), siOASL#2 was designed to target both of the isoforms. Subsequently, siOASL#2 is designated as siOASL in the following descriptions.

To test the effect of transfection regents, we had a control group for the transfection reagent (Lipofectamine) together with the treatment groups every time the experiment was replicated (4 different days). These samples were analyzed and there was no effect of transfection regents on OASL mRNA expression or on the expression of the other genes.

Table 1. Sequences and knockdown rate of oligoadenylate synthetase-like (OASL) siRNA.

	Sequence		
siRNA	*Sense, 5′-3′*	*Antisense, 5′-3′*	*KD rate*[1]
siOASL#1	GGACAGUAACAAGACCACATT	UGUGGUCUUGUUACUGUCCTT	58.53
siOASL#2	GUAUUUACUGGGAGAAGUATT	UACUUCUCCCAGUAAAUACAG	65.36
siOASL#3	CUGUGAAGGUGCAAGUGAATT	UUCACUUGCACCUUCACAGGT	59.75

Abbreviation: siRNA, short interfering RNA.
[1]KD rate = knockdown rate presented as percentage of gene expression.

Newcastle Disease Virus Infection

Newcastle disease LaSota strain vaccine (Newcastle disease vaccine, B1 type, LaSota strain, Live virus; Zoetis Inc., Charles City, Iowa) was used to infect DF-1 cells. At 24 h after transfection with siOASL or siNT RNA, the culture medium was replaced with fresh culture medium without antibiotics, and cells were infected with the NDV at a multiplicity of infection of 1 (NDV-infected groups) or PBS (NDV noninfected groups). After 1 h of exposure to the NDV or PBS, the medium was replaced with fresh medium and incubated. At 4, 8, and 24 hpi (time of incubation excluding the initial hour of exposure), cells were washed with PBS and harvested with 0.25% trypsin EDTA (Gibco, Thermo Fisher scientific, Waltham, Massachusetts) for 5 min at 39°C. Cell viability was assessed by trypan blue exclusion using the formula: % viable cells = [1.00 – (Number of dead cells/Number of total cells)] × 100). Samples were stored at -80°C until RNA isolation.

Newcastle Disease Virus Viral Load

Total RNA was isolated from cells of all the 4 groups (3 independent samples per group, each with 3 technical replicates) for a total of 48 samples using the Ambion RNAqueous Total RNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA) as per the manufacturer's protocol. Viral RNA within each sample was quantified by quantitative PCR using the VetMAX NDV real-time PCR kit (Life Technologies, Carlsbad, CA). Specific probes to the matrix protein (**M**) gene were used, and dilutions of inoculum virus were used as standards as described in the study by Deist et al. (2017). The log copy number of isolated viral RNA was calculated as the mean for each sample (run in triplicate) and was normalized by the average number of cells in the well at each time. At 4 hpi, viral load is presented as log copy number/2 × 106 cells; at 8 hpi, viral load is presented as log copy number/2.5 × 106 cells; and at 24 hpi, as log copy number/3.5 × 106 cells.

Host Gene Expression

After total RNA isolation, all 48 samples were DNase-treated using the DNA-free kit (Thermo Fisher Scientific, Waltham, MA) as per the manufacturer's recommendations. Proper quantity and high purity of the RNA samples before and after DNase treatment were ensured by NanoDrop ND-1000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA) evaluation.

Host gene expression was evaluated through One-Step quantitative PCR using the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Germantown, MD). The amplification reaction for all the target genes and for the housekeeping gene consisted of 1 µL of RNA at 50 ng/µL, 1 µL of each primer (forward and reverse) at 15 pM, 12.5 µL of QuantiTect SYBR Green RT-PCR master mix, 0.5 µL of QuantiTect RT mix, and water to a total volume of 25 µL per well of a 96-well plate. The thermal cycling parameters for all genes were as follows: incubation at 50°C for 30 min, hot start at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, and ending with a melt curve from 65°C to 95°C. All of the reactions were performed in triplicate. Chicken-specific primers (Table 2) used for the amplification reactions were designed based on the gene sequences deposited in the RefSeq database at www.ncbi.nlm.nih.gov (*Gallus gallus* Annotation Release 104) using the Primer-BLAST Tool. Primer sequences for the housekeeping gene (*28S* gene) were previously reported (Kogut et al., 2003). In the present study, the 28S gene expression levels for all samples were evaluated by ANOVA, and no significant effects of any treatment were found. This shows its stability of expression in the samples studied and among the treatments applied in our study, thus validating the appropriateness of 28S as a reference in this study.

Statistical Analysis

The effect of *OASL* knockdown on NDV viral load was analyzed by pairwise comparisons between treatments using an F-test in JMP 14.1.0 software (SAS Institute, Cary, North Carolina, 2018). The statistical model included the main effect of siRNA treatment and quantitative PCR plate.

Host gene expression data were evaluated as the adjusted CT value using the following formula: 40 − ([mean test gene CT] + [median *28S* CT − mean *28S* CT] × [test gene slope/*28S* slope]) (Coble et al., 2011; Kaiser et al., 2012; Lian et al., 2012). Slopes were determined with a series of 10-fold dilutions using pooled RNA for the housekeeping gene and target-specific amplicons as the template to determine PCR efficiency of each primer/gene set. The median *28S* CT represents the median CT value of all individual samples for the housekeeping gene, while mean CT values for the test

Table 2. Primer sequences used for quantitative real-time PCR.

	Sequence (5′-3′)		
Gene	*Forward primer*	*Reverse primer*	*Accession number/Reference*
*OASL*1	GTCGACATCCTGCCTGCTTAC	GAAGCTGGGGGAGAAATCGC	XM_015293006.2
JAK1	AGAGGCTGAGGGGTACGG	ATCTTCACGCTCTCCAAGGG	XM_015290965.2
STAT1	CGTCCGTGCGGGTATTTCTG	AGCTGGTGAACTTGCTCCAA	XM_025152161.1
IFNA	GACAGCCAACGCCAAAGC	GTCGCTGCTGTCCAAGCATT	(Lian et al., 2012)
IFNB	CTGGATTGACCGCACACGCCA	GGGAGCGCGTGCCTTGGTTTA	(Lian et al., 2012)
IFNAR1	TCAGGTTCGAAAAATGTGGCT	GGTAGTCTCTGGAGCAAGATCA	XM_015299270.2
EIF2AK2	CGTCGACGTGGACATGAGAG	GCTGCAGCTTTTGCTTCCTT	XM_015283611.2
RNASEL	AGATCACAGACCTACTGAGGCT	GATGCTGGCGTAAACGGTTG	NM_001031267.1
CASP3	CGCTCAGGGGAAGATGTATCA	CCAGAGTCCACAGACTTGCTA	NM_204725.1
CASP8	GCTTGTAACAGAGGGGCAAA	CACAGATGATGCCAGCCAAA	NM_204592.3
CASP9	TTTCAGGTCCCTGTGCTTCC	TTCCGCAGCTCCACATCAA	XM_424580.6
28S	GGCGAAGCCAGAGGAAACT	GACGACCGATFGCACGTC	(Kogut et al., 2003)

Abbreviations: *CASP3*, caspase 3; *CASP8*, caspase 8; *CASP9*, caspase 9; *EIF2AK2*, eukaryotic translation initiation factor 2 alpha kinase 2; *IFNA*, interferon *alfa; IFNB,* interferon beta*; IFNAR1,* interferon alfa receptor subunit 1; *JAK1*, janus kinase 1; *RNASEL*, Ribonuclease L; *STAT1*, signal transducer and activator of transcription 1; 1*OASL*, 2′-5′-oligoadenylate synthetase-like; *28S*, ribosomal 28S gene.

genes and *28S* were calculated individually from the triplicates of each sample.

For each time point, the expression of each gene (mean adjusted CT values) was analyzed by pairwise comparisons using an F-test in JMP 14.1.0 software (SAS Institute, Cary, North Carolina, 2018). Pairwise comparisons were performed between siRNA groups (siOASL and siNT) within the same infection status, or between noninfected and NDV-infected samples within the same siRNA group. Differential gene expression was considered significant at $P < 0.05$ and is graphically displayed as log2 fold change.

RESULTS

Antiviral Response Against NDV Infection in DF-1 Cells

In this study, the role of the *OASL* gene in inhibiting the efficiency of NDV replication was assessed by siRNA-mediated knockdown in DF-1 cells. Cell viability was more than 95% at all time points, and there was no significant effect of treatments on cell viability. The average knockdown rate of *OASL* gene expression in noninfected and NDV-infected groups was approximately 61% at 4 hpi, 64% at 8 hpi, and 60% at 24 hpi.

The effect of infection of DF-1 cells with NDV LaSota vaccine strain within siNT and siOASL groups was evaluated by assaying the expression of interferon response- and apoptosis-related genes at 4, 8, and 24 hpi (Figure 1). Within the siNT group, NDV-infected cells had higher expression of *OASL*, *JAK1*, *EIF2AK2* (gene coding for PKR), *CASP8,* and *CASP9* genes than the noninfected cells at 4 hpi ($P < 0.05$). The NDV-infected group also had higher expression of *STAT1*, *IFNB, IFNAR1, EIF2AK2, RNASEL, CASP3, CASP8,* and *CASP9* at 8 hpi and higher expression of *OASL, JAK1, EIF2AK2, CASP3, CASP8,* and *CASP9* than the noninfected group at 24 hpi ($P < 0.05$). However, *OASL* was the only gene to be significantly upregulated by NDV infection within the siOASL group at 4 and 24 hpi. No significant effect of NDV infection on the expression of the other evaluated genes was observed within the siOASL group at any time point.

OASL Role in the Antiviral Response Against NDV Infection

Because *OASL* knockdown decreased the host gene expression responses to the NDV, we further investigated its antiviral activity by assessing the effect of the *OASL* knockdown on NDV viral load (Figure 2). Knockdown of *OASL* significantly increased the level of NDV viral RNA by 12.65% at 4 hpi, 6.78% at 8 hpi, and 7.20% at 24 hpi ($P < 0.05$).

We compared the expression of interferon response- and apoptosis-related genes between the siOASL and siNT groups at 4, 8, and 24 hpi to determine if the knockdown of *OASL* would increase NDV replication in a chicken line (Figure 3). Within NDV-infected cells, the siOASL group had lower expression of *OASL, STAT1, IFNAR1, EIF2AK2, RNASEL, CASP8,* and *CASP9* than the siNT group at 4 hpi ($P < 0.05$). The *OASL* knockdown group also had lower expression of *OASL* and *CASP9* than the siNT group at 8 hpi and lower expression of *OASL, CASP3, CASP8*, and *CASP9* than the siNT group at 24 hpi (Figure 3; $P < 0.05$).

DISCUSSION

In chickens, the importance of the interferon-coordinated responses against different viruses has recently been described (Goossens et al., 2013; Qu et al., 2013). This includes virulent NDV which is known to induce expression of not only type I and II interferons but also multiple interferon effectors (Rue et al., 2011). The NDV can induce strong immune responses in vivo by activating several ISG (Liu et al., 2018); however, there is a lack of knowledge on how any individual

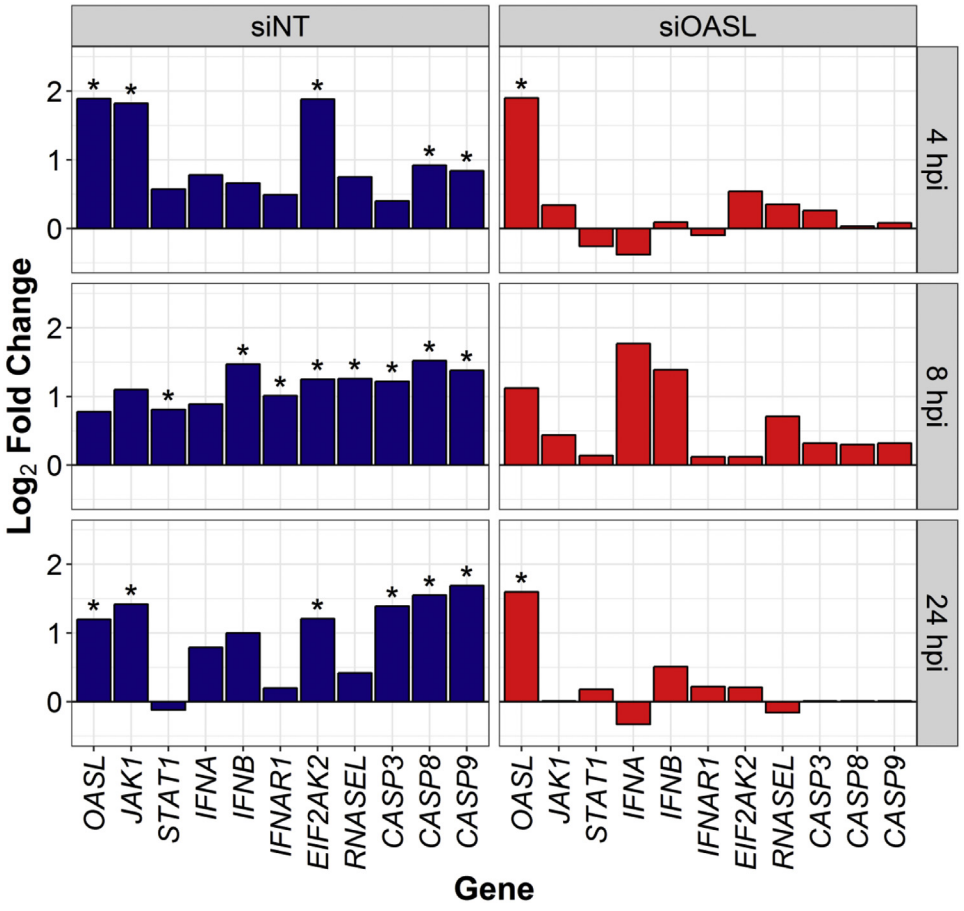

Figure 1. Effect of Newcastle disease virus (NDV) infection on gene expression within a chicken fibroblast cell line (DF-1) transfected with a nontargeting siRNA (siNT) or transfected with a siRNA targeting the 2′-5′-oligoadenylate synthetase-like gene (*OASL*) (siOASL) at 4, 8, and 24 h postinfection (hpi). The siOASL used in the analyses was chosen from a group of 3 specific siRNA targeting different regions of the OASL gene owing to its higher knockdown efficiency rate (65.36%). The bars represent the log2 fold change between the mean adjusted CT of the NDV-infected and noninfected cells. $^*P < 0.05$. Abbreviations: *CASP3*, caspase 3; *CASP8*, caspase 8; *CASP9*, caspase 9; CT, cycle threshold; *EIF2AK2*, eukaryotic translation initiation factor 2 alpha kinase 2; *IFNA*, interferon *alfa; IFNB,* interferon beta*; IFNAR1,* interferon alfa receptor subunit 1; *JAK1*, janus kinase 1; *OASL* = 2′-5′-oligoadenylate synthetase-like; *RNASEL,* Ribonuclease L; *STAT1*, signal transducer and activator of transcription 1.

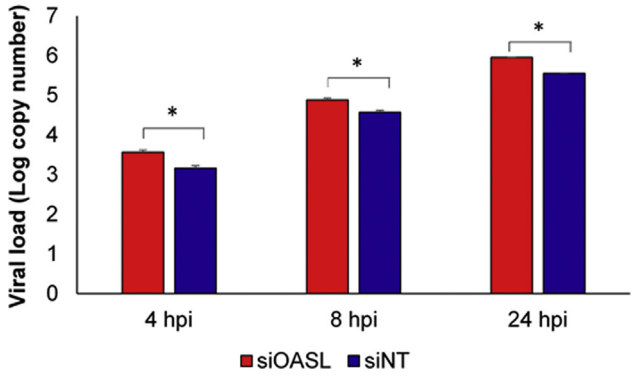

Figure 2. Effect of 2′-5′-oligoadenylate synthetase-like (*OASL)* knockdown on Newcastle disease virus (NDV) viral load at 4, 8, and 24 h postinfection (hpi). The siOASL used in the analyses was chosen from a group of 3 specific siRNA targeting different regions of the OASL gene owing to its higher knockdown efficiency rate (65.36%). The bars show the level of viral RNA given as log copy number normalized by the number of viable cells at each time point. Log copy number is given per 2 × 106 cells at 4 hpi, per 3.5 × 106 cells at 8 hpi, and per 3.5 × 106 cells at 24 hpi. $^*P < 0.05$. Abbreviations: siNT, cells transfected with a scrambled nontargeting siRNA; siOASL, cells transfected with an *OASL*-targeted siRNA; siRNA, short interfering RNA.

gene helps to contain NDV infection. We previously showed that a high-titer infection with even a vaccine strain of NDV induced the expression of multiple ISG in various chicken tissues (Deist et al., 2017, 2018; Zhang et al., 2018). In addition, an important result in our studies was the consistent effect of infection with NDV on *OASL* gene expression. Using 2 inbred chicken lines, of 21 genes related to the interferon signaling pathway and the *eIF2* family, *OASL* was the only one to be upregulated by NDV in the spleen of both resistant and susceptible inbred lines, suggesting an important role of *OASL* in the host response to the NDV (Del Vesco et al., 2020). The OAS family has been shown as one of the main interferon effectors that induce the degradation of viral RNA resulting in the inhibition of viral replication (Choi et al., 2015).

In this study, we tested the effect of *OASL* siRNA–mediated knockdown on the host gene expression response to the NDV. Comparing the expression of interferon response- and apoptosis-related genes between the siOASL and siNT groups, there was no differential

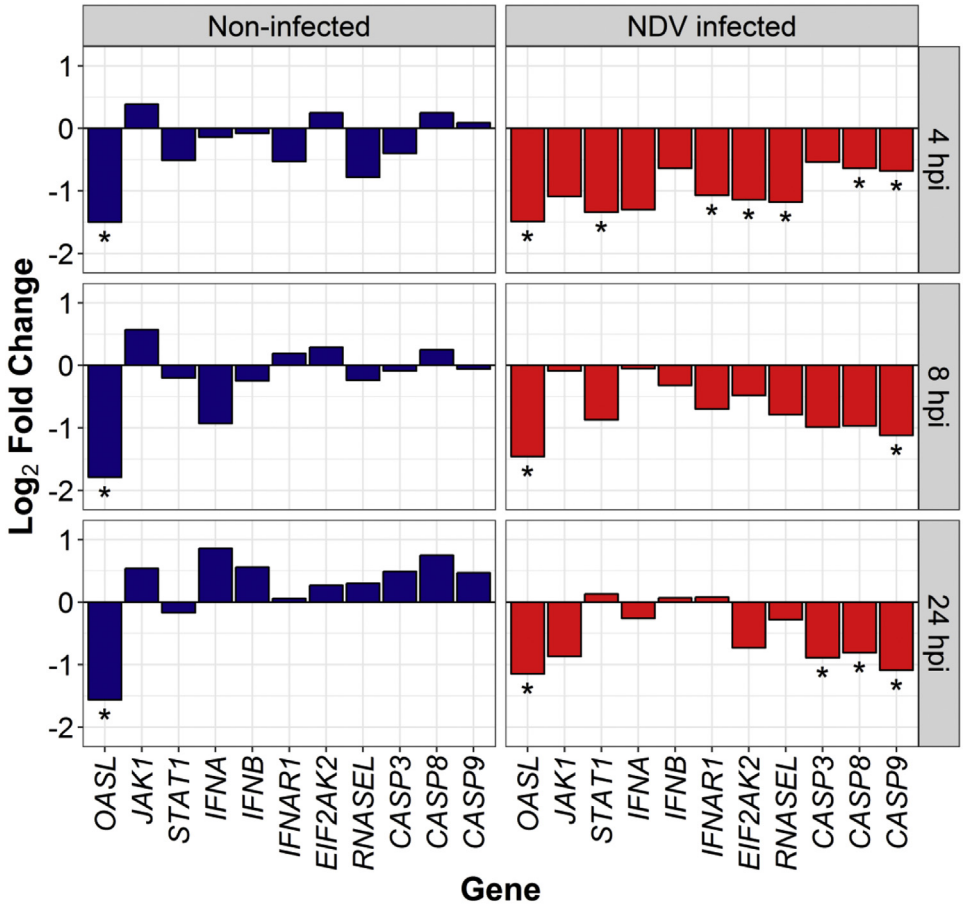

Figure 3. Effect of 2′-5′-oligoadenylate synthetase-like *(OASL)* knockdown on gene expression within a chicken fibroblast cell line (DF-1) infected with theNewcastle disease virus (NDV) or noninfected at 4, 8 and 24 h postinfection (hpi). The siOASL used in the analyses was chosen from a group of 3 specific siRNA targeting different regions of the OASL gene owing to its higher knockdown efficiency rate (65.36%). The bars represent the log2 fold change between the mean adjusted CT of the siOASL and siNT groups. $^*P < 0.05$. Abbreviations: *CASP3*, caspase 3; *CASP8*, caspase 8; *CASP9*, caspase 9; CT, cycle threshold; *EIF2AK2*, eukaryotic translation initiation factor 2 alpha kinase 2; *IFNA*, interferon *alfa; IFNB,* interferon beta*; IFNAR1,* interferon alfa receptor subunit 1; *JAK1*, janus kinase 1; *OASL* = 2′-5′-oligoadenylate synthetase-like; *RNASEL,* Ribonuclease L; siOASL = cells transfected with an *OASL*-targeted siRNA; siNT = cells transfected with a scrambled non-targeting siRNA *STAT1*, signal transducer and activator of transcription 1

expression for any of the tested genes within the noninfected group. This shows that the treatment with siRNA did not induce an antiviral response. This is important because specific small RNA can impact the activation of the type I interferon response and antiviral activity (Watson et al., 2019).

To determine whether infection with the LaSota strain of the NDV could induce an antiviral response in DF-1 cells, the expression of 11 genes related to the interferon response and apoptosis was characterized. Our results showed that NDV infection could induce an immune response within the siNT group by upregulating the expression of signaling molecules, ISG, and apoptosis-related genes. Importantly, our results also showed that the knockdown of *OASL* reduced the host response because there was no significant difference in the expression of the evaluated genes between the infected and noninfected cells within the siOASL group. This lack of response can be due in part to the complex role that OASL plays in the interferon pathway as well as its relationship with other ISG. In the cytoplasm, OAS is activated by virus double-stranded RNA, a replicative intermediate of single-strand RNA viruses, and promotes the synthesis of 2-5 oligoadenylate, which activates RNase L and results in the cleavage of host and viral RNA (Chakrabarti et al., 2011). The outcome of antiviral activity of the OAS/RNase L pathway in mammals occurs through the elimination of the viral genome, state inhibition of viral protein synthesis, damage to the host cell machinery required for viral replication, induction of interferon beta by the produced small duplex of RNA and, ultimately, through the elimination of the virus-infected cells by apoptosis (Silverman, 2007).

We further investigated the antiviral activity of *OASL* by assessing the effect of the *OASL* knockdown on the NDV viral load. Similar to the findings previously observed in the duck and ostrich (Rong et al., 2018), knockdown of *OASL* significantly increased the level of NDV viral RNA in DF-1 cells at all 3 time points investigated. Previous studies have shown that the OAS family can regulate viral replication through different mechanisms (Kristiansen et al., 2010; Chakrabarti

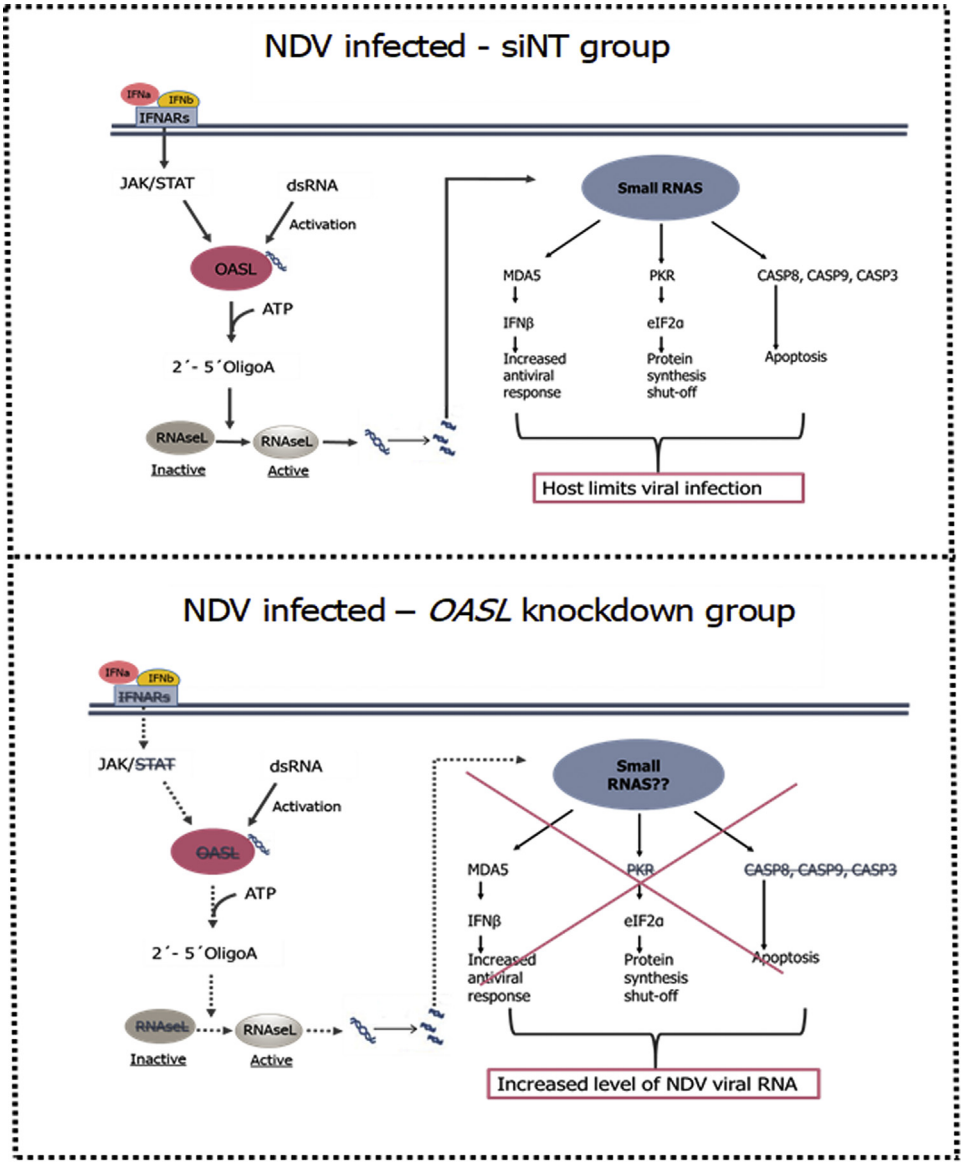

Figure 4. Proposed mechanism for the role of 2′-5′-oligoadenylate synthetase-like (OASL) during infection with the Newcastle disease virus (NDV) in a chicken fibroblast cell line (DF-1). Crossed out genes were downregulated by *OASL* knockdown. Partial *OASL* silencing was associated with the lower expression of IFN-α receptor subunit 1 (*IFNAR1*), signal transducer and activator of transcription 1 (*STAT1*), ribonuclease L (*RNASEL*), eukaryotic translation initiation factor 2 alpha kinase 2 (*EIF2AK2*; PKR), caspase 3 (*CASP3*), caspase 8 (*CASP8*) and caspase 9 (*CASP9*) genes, resulting in higher level of NDV viral RNA.

et al., 2011), and despite the characterized activity of *OASL* against some viruses in chickens (Lee et al., 2014; Ranaware et al., 2016), the pathways regulated by OASL to contain viral replication are not well characterized. To better understand how OASL could repress NDV infection, we evaluated the effect of *OASL* knockdown on the level of gene expression within NDV-infected cells.

At 4 hpi, we observed that decreased expression of *OASL* also caused lower expression of *RNASEL*, showing the OASL-RNase L interaction in a chicken cell line. The knockdown of *OASL* also resulted in lower expression of signaling molecules (*STAT1* and *IFNAR1*), genes related to the apoptosis regulation (*CASP8* and *CASP9*), and lower expression of PKR, another ISG that represses NDV replication (Zhang et al., 2014). Protein kinase R recognizes double-stranded RNA and acts by phosphorylating eukaryotic initiation factor 2 subunit alpha to prevent virus RNA translation (Daher et al., 2017). A possible relationship between RNase L and PKR after virus infection or poly I:C stimulation has been recently proposed (Zheng, 2019). However, this interaction is still largely unknown in chickens infected with the NDV. Because high expression of all these genes at this early stage would be required to induce a strong immune response, the downregulation caused by the knockdown of *OASL* may result in the increased viral load also observed in the siOASL group. Thess results suggest that OASL can act together with other effectors to increase interferon signaling and thereby prevent NDV replication (Figure 4).

Besides the well-characterized RNase L–dependent pathway, OAS can limit viral replication through different mechanisms. Chicken OASL exerts anti-*Flavivirus* activity in a manner independent of its enzymatic activity (Tag-El-Din-Hassan et al., 2017). Porcine OASL interacts with melanoma differentiation–associated gene 5 to enhance the melanoma differentiation–associated gene 5–mediated type I interferon–signaling pathway and inhibit the replication of the classical swine fever virus (Li et al., 2017), and OAS protein can also directly inhibit viral proliferation without the activation of known antiviral signaling pathways in mice and in mouse and human cells (Kristiansen et al., 2010). The apoptosis-mediated role of OAS has also been extensively studied (Castelli et al., 1997; Díaz-Guerra et al., 1997; Yamane et al., 2006). Our results demonstrate that knockdown of *OASL* in NDV-infected cells caused decreased expression of *CASP3, CASP8,* and *CASP9* genes at 24 h. Domingo-Gil and Esteban (2006) presented a detailed mechanism on how the OAS-RNase L system can induce apoptosis in a caspase-dependent manner in HeLa cells. Caspases are cysteine proteases that have the capacity to activate each other in an intracellular cascade that results in the activation of the effector caspases (caspase 3, caspase 6, and caspase 7) leading to the cell death (Thomson, 2001). Apoptosis acts as an important mechanism of host defense against virus. Although apoptosis can prevent the spread of many viruses by blocking viral replication through the death of infected cells, in some situations, apoptosis might also facilitate viral egress (Kaminskyy and Zhivotovsky, 2010). Chu et al. (2018) showed that NDV V protein can inhibit cell apoptosis to complete NDV replication inside DF-1 cells. More examples of apoptosis inhibition mechanisms induced by NDV to promote virus replication have been reported in recent years (Kang et al., 2017; Wang et al., 2018). In a similar way, our results of lower expression of *CASP3, CASP8,* and *CASP9* in NDV-infected cells treated with siOASL may help explain the higher viral load observed in that group. All these results also suggest that apoptosis can be used by host cells as an important mechanism to prevent NDV replication.

CONCLUSION

In summary, we show that *OASL* knockdown reduced the antiviral host gene expression response and increased the level of NDV RNA at 4, 8, and 24 hpi. Our results suggest that OASL may act in different pathways at different time points to increase the host antiviral response and limit NDV replication because the decrease in *OASL* expression by gene knockdown resulted in lower expression of interferon response-related genes at 4 hpi and lower expression of apoptosis-related genes at 24 hpi. The difference in the patterns of gene expression caused by the knockdown of *OASL* at different time points can aid understanding of how OASL can reduce the replication of NDV. We suggest that the increased NDV viral load in DF-1 cells with partial silencing of *OASL* expression was a result of a complex interaction between OASL and interferon response- and apoptosis-related genes, which down regulated the genes in these pathways and, thus, decreased the host response to the NDV. Our study points to the importance of future research to determine if overexpression of OASL has a beneficial effect on NDV control and if the changes at the mRNA level also change protein levels. Our results are consistent with previous studies on the role played by *OASL* during NDV infection in vitro, expand knowledge of potential mechanisms, and could aid in future prophylactic and therapeutic treatments for Newcastle disease in poultry.

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DISCLOSURES

The authors declare no conflicts of interest.

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