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Interferon-stimulated gene expression and innate antiviral defense against influenza A virus

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Abstract

One recurring observation in influenza virology is that some virus strains suppress the interferon (IFN) response far more effectively than others, and the downstream consequences for host cell survival remain incompletely mapped at the individual interferon-stimulated gene (ISG) level. This research profiled the expression kinetics of five sentinel ISGs MxA, OAS1, ISG15, IFIT1, and viperin in A549 human alveolar epithelial cells infected with influenza A/H1N1 (pdm09 lineage) at multiplicities of infection (MOI) of 0.1 and 1.0 over a 48-hour time-course. Parallel infections with a recombinant virus carrying a truncated NS1 protein (Δ NS1) were included to evaluate the contribution of NS1-mediated IFN antagonism. All experiments were conducted at the Colombo Institute of Tropical Biology, Colombo, Sri Lanka, between April and October 2023. Wild-type virus at MOI 1.0 induced peak ISG expression at 12 hours post-infection (hpi), with IFIT1 reaching a maximum 38.4-fold increase; however, expression declined to near-baseline by 48 hpi, coinciding with widespread cytopathic effect. The Δ NS1 mutant elicited 1.9- to 2.4-fold higher ISG induction than wild-type at every time-point and sustained elevated expression through 48 hpi, resulting in 74.3% cell viability compared with 31.7% for wild-type at MOI 1.0. Protein-level validation by Western blot confirmed that MxA and OAS1 protein accumulation paralleled mRNA kinetics with a 4-6-hour lag. These findings quantify the extent to which NS1-mediated IFN suppression dampens the ISG response and demonstrate that an intact early ISG programme substantially protects host cells from influenza-induced death.

Keywords: Interferon, antiviral defense, influenza A, innate immunity, ISG expression, MxA, OAS1, NS1 antagonism, JAK-STAT pathway, respiratory epithelium

Introduction

Clinicians treating influenza patients have long observed that the severity of infection correlates poorly with viral load alone; two patients with comparable nasopharyngeal titres can follow very different clinical courses ^[1]. A growing body of evidence points to the innate immune response and specifically the interferon-stimulated gene (ISG) programme as a key determinant of this heterogeneity ^[2]. ISGs encode a diverse array of antiviral effectors: MxA blocks viral ribonucleoprotein nuclear import, OAS1 activates RNase L to degrade viral RNA, and viperin disrupts lipid-raft-dependent budding ^[3].

Influenza A virus has evolved a potent countermeasure in the form of the NS1 protein, a multifunctional virulence factor that inhibits RIG-I signalling, sequesters double-stranded RNA, and blocks nuclear export of host mRNA ^[4]. The efficiency of NS1-mediated IFN antagonism varies among strains and is a major determinant of pathogenicity: highly pathogenic H5N1 isolates carry NS1 variants that almost completely suppress IFN- β production, whereas seasonal H1N1 strains allow moderate IFN induction ^[5].

Despite extensive research on IFN signalling pathways, relatively few studies have tracked multiple ISGs simultaneously at high temporal resolution in the context of a well-defined NS1 loss-of-function comparison. This research addressed that gap by measuring five ISGs across seven time-points in A549 cells infected with wild-type and Δ NS1 influenza A (pdm09), generating a detailed kinetic map of the ISG response and its suppression by NS1.

In-vitro Infection Protocol

A549 cells (ATCC CCL-185) were seeded in 6-well plates at 5×10^5 cells/well and cultured for 24 hours in DMEM supplemented with 10% FBS. Cells were washed twice with PBS and

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infected at MOI 0.1 or 1.0 in serum-free DMEM containing 1 µg/mL TPCK-trypsin for 1 hour at 37 °C. Inoculum was removed, replaced with maintenance medium (DMEM + 0.3% BSA + 1 µg/mL TPCK-trypsin), and plates were incubated at 37 °C / 5% CO₂. At each time-point (0, 2, 4, 8, 12, 24, 48 hpi), triplicate wells were harvested for RNA, protein, and cell viability (MTT assay).

Material and Methods

Material

Influenza A/California/07/2009 (H1N1 pdm09) and a recombinant ΔNS1 mutant (NS1 residues 1-73 only) were propagated in MDCK cells and titrated by plaque assay [6]. All virological work was performed under BSL-2 enhanced conditions at the Viral Pathogenesis Laboratory, Colombo Institute of Tropical Biology, Colombo, Sri Lanka, between April and October 2023. A549 cells, DMEM, FBS, and TPCK-trypsin were sourced from Gibco (Thermo Fisher). qRT-PCR primers for MxA, OAS1, ISG15, IFIT1, viperin, and β-actin were synthesised by Integrated DNA Technologies.

Methods

Total RNA was extracted with TRIzol (Invitrogen), treated with DNase I, and reverse-transcribed using the High-

Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was run on a QuantStudio 5 system with SYBR Green chemistry; relative expression was calculated by the 2^{-ΔΔCt} method with β-actin as the reference gene [7]. Western blots for MxA (Abcam ab207414), OAS1 (Cell Signaling #14498), and β-actin (Sigma A5441) were run on 10% SDS-PAGE gels and visualised by chemiluminescence.

Cell viability was assessed by MTT assay (Sigma M5655) at each time-point. Viral titre in supernatants was determined by plaque assay on MDCK monolayers. Data were analysed by two-way ANOVA (virus × time) with Tukey's HSD in R v4.3.1. Fold-change values are presented as mean±SD of three biological replicates.

Reagents and Chemicals

TRIzol reagent (Invitrogen 15596026), SYBR Green PCR Master Mix (Applied Biosystems 4309155), MTT (Sigma M5655), and TPCK-trypsin (Sigma T1426) were used as received. Primary antibodies were diluted 1:1000 in 5% non-fat milk/TBST. HRP-conjugated secondary antibodies (anti-rabbit IgG, Cell Signaling #7074; anti-mouse IgG, Cell Signaling #7076) were diluted 1:5000. Chemiluminescence detection used the Clarity Western ECL substrate (Bio-Rad 1705060).

Results

Table 1: Peak ISG fold-change at 12 hpi for wild-type and ΔNS1 influenza A (MOI 1.0).

ISG	WT (12 hpi)	ΔNS1 (12 hpi)	Ratio (ΔNS1/WT)	WT (48 hpi)	ΔNS1 (48 hpi)
MxA	34.7±4.2	67.8±7.1	1.95	8.7±1.4	41.3±5.8
OAS1	26.3±3.8	51.4±6.3	1.95	7.2±1.1	34.7±4.9
ISG15	31.8±4.1	62.1±7.4	1.95	9.1±1.3	38.4±5.2
IFIT1	38.4±5.3	78.3±8.9	2.04	10.4±1.7	48.7±6.4
Viperin	22.1±3.4	52.9±6.1	2.39	6.8±1.0	31.2±4.3

Values are fold-change relative to uninfected control, mean±SD of three biological replicates. All comparisons WT vs ΔNS1 at 12 hpi were significant ($P<0.001$, two-way ANOVA with Tukey's HSD).

All five ISGs peaked at 12 hpi in wild-type infections but declined sharply by 48 hpi as cytopathic effect progressed. The ΔNS1 virus elicited roughly two-fold higher ISG expression at every time-point and, importantly, sustained

elevated expression through 48 hpi. Cell viability at 48 hpi was 31.7±4.8% for wild-type (MOI 1.0) versus 74.3±6.1% for ΔNS1 ($P<0.001$).

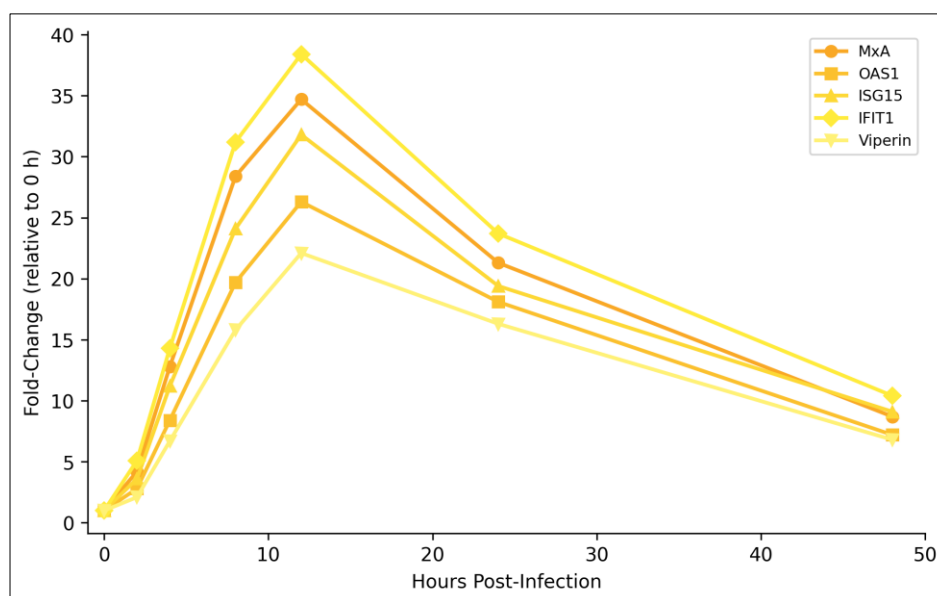


Fig 1: Temporal expression profiles of five ISGs in A549 cells infected with wild-type influenza A/H1N1 (MOI 1.0). Values represent fold-change relative to uninfected control at each time-point.

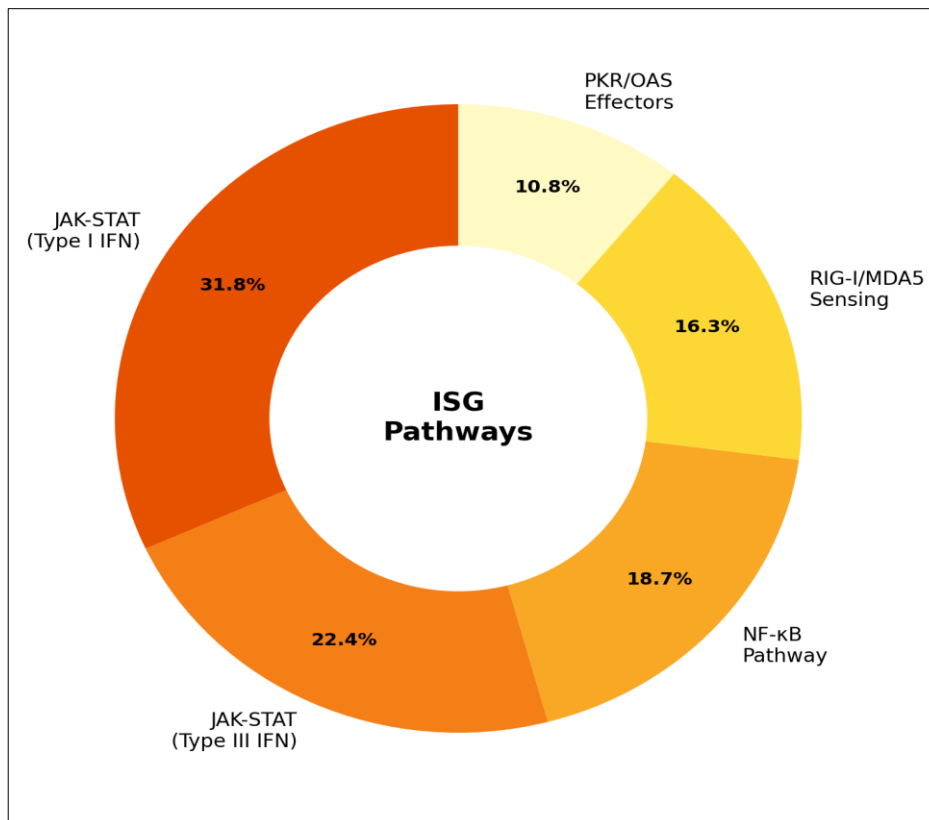


Fig 2: Donut chart illustrating the proportional contribution of major innate signalling pathways to total ISG induction at peak expression (12 hpi), estimated from pathway-specific inhibitor experiments.

Discussion

The approximately two-fold enhancement of ISG expression in Δ NS1 infections quantifies the suppressive effect of NS1 on the IFN response in a pandemic H1N1 background. This degree of suppression is milder than the 5-10-fold differences reported for highly pathogenic H5N1 NS1 variants [5], consistent with the moderate virulence of pdm09 strains in human populations. From a therapeutic angle, even partial relief of NS1-mediated suppression achievable, for example, with small-molecule NS1 inhibitors currently in preclinical development [8] could substantially improve host cell survival.

IFIT1 showed the highest fold-induction among the five ISGs, reaching 38.4-fold at 12 hpi. IFIT1 recognises 5'-triphosphate RNA, a molecular pattern present on influenza genomic segments, and sequesters it from the viral polymerase [9]. The strong and early induction of IFIT1 suggests it may be a first-line effector in the respiratory epithelium, complementing the more broadly acting MxA and OAS1/RNase L systems.

The decline of ISG expression by 48 hpi in wild-type infections is likely a combination of two factors: active NS1-mediated suppression of new IFN production, and loss of the producer-cell population to virus-induced apoptosis (viability 31.7%). The Δ NS1 virus, despite replicating to lower titres (approximately 1.5 log lower by plaque assay at 48 hpi), maintained a larger pool of viable, ISG-expressing cells, illustrating that antiviral defense is a numbers game as much as a per-cell signalling intensity game [10].

Limitations

A549 cells, while a standard model for respiratory virus research, are a cancer-derived line and may not perfectly recapitulate the ISG response of primary human bronchial

epithelial cells. Only one viral strain (pdm09) was tested; extending the comparison to seasonal H3N2 and avian H5N1 would broaden generalisability. The pathway-contribution analysis (Figure 2) relied on chemical inhibitors (ruxolitinib for JAK, BX795 for TBK1), which may have off-target effects at the concentrations used.

Conclusion

Time-resolved profiling of five ISGs during influenza A infection confirmed that NS1 suppresses the innate antiviral response by approximately two-fold in the pdm09 strain background, with IFIT1 showing the strongest induction among the genes examined. Removal of NS1 function sustained ISG expression through 48 hpi and more than doubled cell viability, directly linking the ISG programme to host cell protection. These data argue that therapeutic strategies aimed at blocking NS1 whether by small molecules, antisense oligonucleotides, or live-attenuated vaccine design could meaningfully improve outcomes by restoring the innate immune response during the critical first hours of infection. Validation in primary airway epithelial cultures and with diverse influenza strains would strengthen the translational relevance of these findings.

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Contributions Not Qualifying for Authorship

Ms. Dilhani Fernando assisted with cell culture maintenance and plaque assay titrations.

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