The effects of Type-I & -II IFNs disclose a role for Dopamine in SARS-CoV-2 infection

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BACKGROUND

Recent studies highlight the dynamic nature of virus-host interaction during SARS-CoV-2 infection, raising intriguing questions about the role and timing of interferon (IFN) responses. In fact, SARS-CoV-2 delays/antagonizes Type-I, and to a definitely lesser extent, Type II-IFNs. While paving the way for potential antiviral therapies based on immune activation, the molecular mechanisms linking different IFN pathways to SARS-CoV-2 susceptibility remain to be elucidated. The present study investigates the role of Type-I & -II IFNs in SARS-CoV-2 infection, with a focus on molecular pathways related with innate and adaptive immunity in human lung cells. In the light of recent studies suggesting a role for dopamine (DA)-related pathways in SARS-CoV-2 infection, we also investigate potential mechanisms bridging DA pathways and IFN-induced responses in Sars-CoV-2 infection.

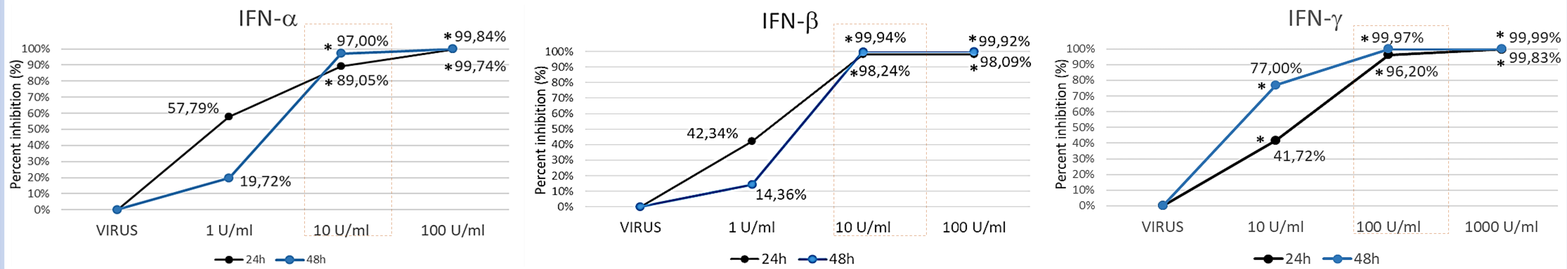
METHODS

Human lung adenocarcinoma cells (CaLu-3, ATCC, MALE) were treated with IFN-α, -β or -γ (from 1 to 1000 U/mL) 16h prior to infection with SARS-CoV-2 (MOI 0.05, 3h). In another set of experiments, IFNs were added only post-infection (p.i). Supernatants were harvested at 24 and 48h to assess viral replication by RT-qPCR, and to quantify the levels of cytokines/chemokines through ELISA Multiplex assay. At 48h p.i, cells were collected in RNAzol and RNA was extracted through the phenol/chloroform method and reverse-transcribed to investigate a variety of transcriptional targets. Results are presented as the average of the relative expression units to the GAPDH gene ±SEM, calculated by the 2−ΔΔCt equation. Cell viability was assessed by MTT. Statistical analyses were performed through the Students' t-test.

Downregulation of DA receptors bridges the anti-replicative and anti-inflammatory effects of Type-I & -II IFNs in SARS-CoV-2 infection

RESULTS

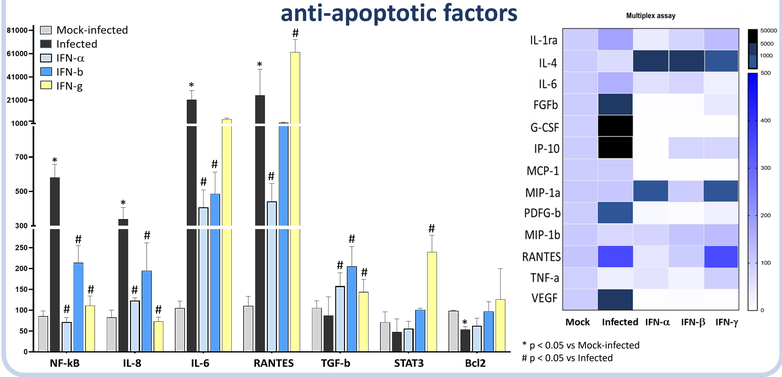
1. Type –I and –II IFNs dramatically reduce SARS-CoV-2 replication in CaLu-3 lung cells



Dose- and time-dependent anti-replicative effects of IFNs administered prior to and during SARS-CoV-2 infection. The antireplicative effects of Type-I and -II IFNs at 48h reach a plateau at the doses of 10 and 100 U/ml, respectively. Results are expressed as percentage with respect to controls (VIRUS, infected cells). *p < 0,05. IFNs, and mostly IFN-γ, retain antiviral activity even when added only p.i., albeit at a lower magnitude compared with pretreatment (**APPENDIX 1**). None of the IFN doses tested was toxic at 48h (**APPENDIX 2**).

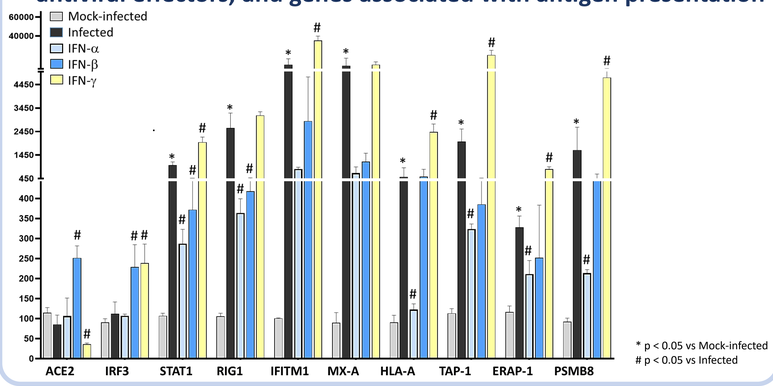
2. Type-I and –II IFNs inhibit SARS-CoV-2 replication through convergent and divergent mechanisms

A Both Types decrease the expression and/or protein levels of pro-inflammatory mediators while augmenting anti-inflammatory and anti-apoptotic factors



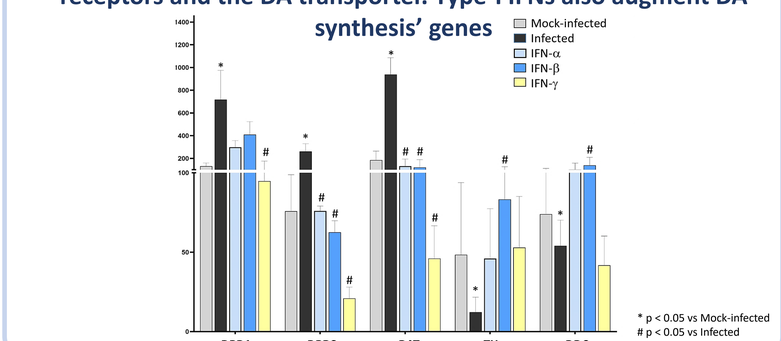
* p < 0.05 vs Mock-infected
\# p < 0.05 vs Infected

B Type-I and –II IFNs show divergent effects in the expression of ACE2, antiviral effectors, and genes associated with antigen presentation



* p < 0.05 vs Mock-infected
\# p < 0.05 vs Infected

C Type-I and –II IFNs converge in downregulating the expression of DA receptors and the DA transporter. Type-I IFNs also augment DA synthesis' genes



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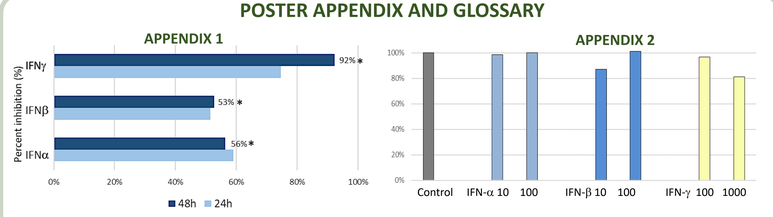
POSTER APPENDIX AND GLOSSARY



Anti-replicative effects of IFNs (IFN-α and -β 10 U/mL, and IFN-γ 100 U/mL) administered post SARS-CoV-2 infection. Values are expressed as percent inhibition of viral replication compared with infected cells (VIRUS).*p<0.05

Cell viabilty, assessed through the MTT method, showed that at 48h none of the IFNs is frankly toxic at the concentration employed (10-1000 U/mL). Values are expressed as percentage of Control.

GLOSSARY

Interferon(s) **IFN(s)**, Severe Acute Respiratory Coronavirus-2 **SARS-CoV-2,** Glyceraldehyde 3-phosphate dehydrogenase **GAPDH,** 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide **MTT,** Nuclear Factor kappa-light-chain-enhancer of activated B cells **NF-kB,** Interleukine **IL-6/8,** Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted **RANTES,** Transforming Growth Factor beta **TGF-b**, Signal Transducer And Activator Of Transcription **STAT1/3,** B-cell lymphoma 2 **Bcl-2,** Angiotensin-Converting Enzyme 2 **ACE2**, Interferon Regulatory Factor 3 **IRF3,** Retinoic acid-Inducible Gene I **RIG-I,** Interferon-inducible transmembrane **IFITM1,** Interferon-mediated host factors myxovirus **MX-A,** Human Leukocyte Antigen **HLA-A,** Transporter associated with antigen processing 1 **TAP-1,** Endoplasmic Reticulum Aminopeptidase **ERAP1/2,** Proteasome Subunit Beta **PSMB8/9/10,** Dopamine **DA**, Dopamine Receptor D1/D2 **DRD1,DRD2,** Dopamine Transporter **DAT,** Tyrosine Hydroxylase **TH,** L-Dopa Decarboxylase **DDC**

CONCLUSIONS & DISCUSSION

- Type-I &-II IFNs are key to halt SARS CoV-2 replication by acting through both convergent and divergent (likely complementary) mechanisms.

A Type-I and -II IFNs converge in reducing the expression of pro-inflammatory genes (NF-kβ, IL-6/8) while increasing the expression of anti-inflammatory and anti-apoptotic pathways (TGF-β, STAT3/Bcl-2). This is recapitulated at protein levels. In fact, except for a few chemokines (RANTES and MIP-1a), IFNs α, b, and γ converge in reducing pro-inflammatory mediators that are strongly induced by SARS-CoV-2, meanwhile increasing anti-inflammatory factors (i.e IL-4).

B Albeit an increase in IRF3 expression induced by IFN-β, Type-I IFNs, fail to induce STAT-1 and antiviral effectors (MX-A, IFITM1, RIG-1). This suggests that, despite their exogenous administration, SARS-CoV-2 either strongly suppresses Type-I IFNs or overwhelms their effectiveness at late time-points of viral replication (48h). This contrasts with IFN-γ, which instead potentiates the expression of STAT1, antiviral effectors and genes associated with adaptive immunity-antigen processing/presentation (ERAP-1, PSMB8, TAP-1, and HLA-A). The latter may explain its paradoxical induction of inflammatory genes that are instead reduced by Type-I IFNs (i.e. RANTES).

Opposite effects are also observed at the level of ACE2 expression, which is increased by IFN-β and decreased by IFN-γ.

C Both Types of IFNs converge in reducing the expression of DA receptors (mostly D2DRs and the DA transporter (DAT). Contrarily to IFN-γ, Type-I IFNs also upregulate genes associated with DA synthesis (TH, and DDC).

These preliminary data disclose a potential role for DA activity/metabolism in SARS-CoV-2 infection in lung cells, which deserves to be investigated in the frame of multiorgan COVID-19 pathogenesis besides mere neurological manifestations.