nations of vaccine + adjuvants were administered intranasally in a maximum volume of 50 ml. Tissue samples were harvested from euthanized mice and processed for analysis of viral titers, quantitative RT-PCR, or ELISA. In survival experiments mice were sacrificed if their weight dropped more than 25% of their initial body weight as per institutional guidelines. The animal experiments were performed according to the guidelines of the German animal protection law. All animal protocols were approved by the relevant German authorities.

Flow cytometry. Lung samples were enzymatically digested with 2 mg/ml of Collagenase D (Roche). Mediastinal lymph nodes were disrupted mechanically. Tissue samples were processed for flow cytometry and red cells were lysed using BD Red Blood Cell Lysing Buffer. Samples were acquired on a FACS canto II instrument and analyzed with FlowJO software (Treestar).

Results. Engagement of TLR3 and type I IFN signaling by candidate adjuvants of cold-adapted influenza vaccines in mice, increased vaccine-induced cytokine production early after mucosal administration, including enhanced production of MCP1, IP-10 and G-CSF. This increase in vaccine-induced early inflammatory responses resulted in robust cytotoxic T lymphocyte (CTL) activity, rapid viral clearance, and enhanced survival to influenza challenge. Conversely, vaccine adjuvants did not influence antibody titers in vaccinated individuals, suggesting that the early events that take place during the innate phase of the immune response play a chief role on the establishment and modulation of adaptive immunity.

Conclusion. Our results indicated that specific adjuvants with the ability to enhance TLR3 and type I IFN signaling enhance cytokine responses associated to vaccine administration, which resulted in improved $CD8^{+}$T cell-dependent recall responses. Of note, adjuvant-vaccine regimens reduced the vaccine dose necessary to provide protection to lethal challenge. These findings indicate that modulation of innate immunity via TLR ligands and type I IFN agonists may serve as a therapeutic strategy to enhance T cell memory maintenance after influenza vaccination, and may provide means to reduce the amount of vaccine stock necessary to protect individuals at risk in the face of an influenza pandemic.

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Pathogenic Influenza A viruses and SARS-Coronaviruses modulate global interferon stimulated gene induction through diverse mechanisms

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Introduction: Successful respiratory viruses have developed means to overcome specific IFN and ISG effector functions. Yet globally, ISGs as a class have broad functions and impede both pathogenesis and viral replication. Therefore, highly successful respiratory viruses including SARS-CoV and Influenza A virus (IAV), likely attempt to short-circuit global ISG induction, potentially through diverse mechanisms.

Methods. In this work, global RNA (microarray) and proteomics data (mass spectrometry) are utilized to compare and contrast ISG control strategies employed by IAV H5N1, H1N1 2009, and SARS-CoV. Calu-3 cells, a human lung epithelial cell line, are treated with type I IFN to define a consensus list of IFN responsive genes. These genes are then examined in the context of virus infections to evaluate the global ISG response.

Results. SARS-CoV and IAV H5N1 utilize contrasting approaches to interfere with the global host ISG response. Whereas IAV H5N1 actively manipulates the ISG response with up and down regulation of ISG transcripts, SARS-CoV successfully limits ISG induction until late times during infection. Global proteomic analysis confirms the absence of ISG proteins following H5N1 infection; in contrast, SARS-CoV induces increased abundance of several ISG proteins, but not until after peak viral titers have been achieved. Together, the data illustrate that these two virulent respiratory viruses control the global ISG response.

Further examination reveals ISG manipulation is absent in a less virulent IAV strain. H1N1 2009 infection results in robust ISG transcript expression with minimal down regulation. Similarly, proteomics analysis finds increased abundance of ISG proteins absent in H5N1 infection. Transcriptional factor analysis found no direct antagonism; however, truncation of the C-terminal portion of NS1 ablated ISG down regulation in H5N1 infections. Meta-analysis finds a similar ablation is observed in A549 cells infected H1N1 1918 when NS1 is manipulated. Together, the data strongly implicate a role for NS1 in mediating control of the entire global ISG response following virulent IAV infections.

For SARS-CoV, the delayed ISG induction is maintained in both mutant and precursor viruses. DORF6, a SARS-CoV mutant lacking a major IFN antagonist, fails to induce ISG induction any earlier. Similarly, Bat-SRBD, a synthetic precursor virus, maintains the ability control the global ISG response, suggesting this trait is maintained in pre-cursors of the epidemic SARS-CoV. Additional work examines the ability of the SARS-CoV to camouflage its viral intermediates as well as replication within double membrane vesicles as mechanisms mediating delayed ISG induction.

Conclusion. Together, the work highlights the contrasting approaches used by SARS-CoV and IAV strains to limit global ISG induction. In addition, the results imply an important role for controlling ISG response in the context of virulent respiratory virus infection. Further examination of these results will likely produce novel approaches to treat important respiratory virus infection.

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