**VIROLOGY**

**O-GlcNAc transferase promotes influenza A virus–induced cytokine storm by targeting interferon regulatory factor–5**

Qiming Wang†, Peining Fang†, Rui He†, Mengqi Li†, Haisheng Yu†, Li Zhou†, Yu Yi†, Fubing Wang†, Yuan Rong‡, Yi Zhang‡, Aihong Chen‡, Nanfang Peng‡, Yong Lin‡, Mengji Lu§, Ying Zhu‡, Guoping Peng†, Liqun Rao†, Shi Liu†,*

In this study, we demonstrated an essential function of the hexosamine biosynthesis pathway (HBP)–associated O-linked β-N-acetylglucosamine (O-GlcNAc) signaling in influenza A virus (IAV)–induced cytokine storm. O-GlcNAc transferase (OGT), a key enzyme for protein O-GlcNAcylation, mediated IAV-induced cytokine production. Upon investigating the mechanisms driving this event, we determined that IAV induced OGT to bind to interferon regulatory factor–5 (IRF5), leading to O-GlcNAcylation of IRF5 on serine-430. O-GlcNAcylation of IRF5 is required for K63-linked ubiquitination of IRF5 and subsequent cytokine production. Analysis of clinical samples revealed that IRF5 is O-GlcNAcylated, and higher levels of proinflammatory cytokines correlated with higher levels of blood glucose in IAV-infected patients. We identified a molecular mechanism by which HBP-mediated O-GlcNAcylates IRF5 function during IAV infection, highlighting the importance of glucose metabolism in IAV-induced cytokine storm.

**INTRODUCTION**

Glucose serves as a major nutrient that fuels cellular metabolic activities (1). After uptake through the glucose transporter, glucose flows through three major glucose metabolic pathways: glycolysis, the pentose phosphate pathway (PPP), and the hexosamine biosynthesis pathway (HBP) (2). These metabolic pathways have distinct destinations and functions. Compared with glycolysis and PPP, a small portion of glucose is metabolized through the HBP, generating its end product, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) (3). O-linked β-N-acetylglucosamine (O-GlcNAc) transferase (OGT) mediates the transfer of UDP-GlcNAc to serine (S) or threonine (T) residues of target proteins, referred to as protein O-GlcNAcylation (4). Conversely, O-GlcNAcase (OGA) removes the modification (5). Because of OGT and OGA, O-GlcNAcylation is a highly dynamic process (6). O-GlcNAcylation plays an important role in many key cellular processes, including transcription, translation, nuclear transport, protein stability, and protein–protein interactions (7). O-GlcNAcylation regulates transcription through OGT-associated chromatin-modifying complexes and direct modification of transcription factors such as nuclear factor κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) (8, 9).

Influenza A virus (IAV) is a highly contagious single-stranded RNA virus that has a serious impact worldwide (10). IAV infects both the upper and lower respiratory tracts via endocytosis (10). After infection, some viral components are recognized by several pathogen recognition receptors that, in turn, promote downstream cellular and humoral responses, including the “cytokine storm” (11). This term was first used in 2003 to describe an immune response to influenza infection in reference to influenza-associated encephalopathy (12). Influenza-induced cytokine storm has been linked to aggressive proinflammatory responses and insufficient control of anti-inflammatory responses (13). Several experimental studies and clinical trials suggested that cytokine storm correlates directly with widespread tissue damage and unfavorable prognosis of severe influenza (14). Nevertheless, we have little understanding of the mechanisms that promote cytokine storm or why some individuals exhibit an excessive response to the virus to an extent that leads to hospitalization or death.

Reprogramming cellular metabolic activities has recently been demonstrated to play a critical role in the activation of the immune system and hyperinflammation (15). A widely accepted concept is that embedded in immune cell physiology are metabolic pathways and metabolites that not only provide energy and substrates for growth and survival but also direct effector functions, differentiation, and gene expression (16, 17). Previous studies reported increased glucose uptake and utilization in immune cells, serving as a hallmark feature of acute inflammation (18). Nevertheless, the overall effect of OGT in the IAV–induced cytokine storm remains unknown. We recently reported that IAV induces cytokine storm via interferon (IFN) regulatory factor–5 (IRF5) (19). In this study, we found that genetic deletion of OGT markedly impaired IAV–induced cytokine storm in vivo and in vitro. Furthermore, we characterized OGT-mediated IRF5 O-GlcNAcylation on S430, induced by IAV, as a key mechanism for IRF5 ubiquitination and activation of inflammatory cytokine expression. Together, our findings demonstrate a mechanism whereby IAV infection leads to glucose metabolism reprogramming toward
the HBP pathway, boosting O-GlcNAc signaling and promoting inflammatory cytokine expression by targeting IRF5.

**RESULTS**

**IAV induced inflammatory cytokine expression by affecting glucose metabolism**

To achieve a comprehensive understanding of metabolic changes in response to IAV infection, we challenged freshly isolated peripheral blood mononuclear cells (PBMCs) from healthy donors with IAV and performed a metabolomics assay. Principal components analysis revealed a markedly altered metabolic profile upon IAV infection (fig. S1A). Pathway enrichment analysis identified several crucial metabolic pathways with substantial changes, including amino and nucleotide sugar metabolism, glutathione metabolism, and glycolysis (fig. S1B). Many intermediate metabolites involved in glycolysis and the HBP showed increased abundance upon IAV challenge (Fig. 1, A and B). Metabolic tracer analysis using $^{13}$C-glucose tracing also revealed increased incorporation of glucose-derived carbon in glycolysis, PPP, and HBP metabolites (fig. S1, C and D). The

![Fig. 1. GlcN enhances IAV-induced inflammatory cytokine expression.](image)

(A and B) PBMCs were infected with the WSN virus (MOI (multiplicity of infection) = 1) or mock-infected for 12 hours and harvested for metabolomics analysis. Quantification analysis of some intermediates in glycolysis (A) or HBP (B). G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F16BP, fructose 1,6-bisphosphate; 3PG, 3-phosphoglyceric acid; PEP, phosphoenolpyruvate. (C) PBMCs were infected with the WSN virus (MOI = 1) or mock-infected for the indicated times before Western blot analyses. MW, molecular weight. (D) ATII cells were infected with the WSN virus (MOI = 1) or mock-infected and treated with or without the indicated concentration of GlcN for 24 hours. The viral titers in the culture supernatants were measured using a plaque assay (left) and relative RNA levels of NP-specific mRNA, cRNA, and vRNA were measured using quantitative polymerase chain reaction (qPCR) (right). (E) Experiments were performed as described in (D), except that ATII cells were treated with or without GlcN (10 mM) for the indicated times. (F) PBMCs were infected with the WSN virus (MOI = 1) or mock-infected and treated with or without GlcN (10 mM) for 12 hours and subjected to plaque analyses (left) or Western blot analyses (right). (G) Experiments were performed as described in (F), except that enzyme-linked immunosorbent assay (ELISA) analyses were performed. (H and I) C57BL/6 mice were treated with phosphate-buffered saline (PBS) or GlcN (50 mg/kg per day) by intraperitoneal injection for 1 week and intranasally with $10^4$ plaque-forming units (pfu) of WSN IAV. Body weights were recorded daily (H). Survival curves show data collected until day 14 after infection (I). Statistical analysis was performed using the log-rank test ($n = 5$ for each group). (J) Experiments were performed as described in (I), except that levels of proinflammatory cytokines and chemokines in the bronchoalveolar lavage fluid (BALF) were measured 48 hours after infection. (K) Experiments were performed as described in (I), except that lung viral titers were measured by plaque assay (left) or IAV M1 were measured using Western blot analyses (right) on day 5 after infection. In the qPCR experiments, the control was designated as 1. All experiments were repeated at least three times. Bar graphs present means ± SD or means ± SEM ($n = 3$; **$P < 0.01$).
availability of UDP-GlcNAc is an important determinant of OGT enzymatic activity (4). Consistent with increased HBP end product UDP-GlcNAc abundance, total protein O-GlcNAcylation in PBMCs showed substantial increases after the challenge with IAV (Fig. 1C).

In addition, the HBP rate-limiting enzyme glucamine fructose-6-phosphate transaminase 1, OGT, and OGA moderately increased during IAV infection (fig. S1E).

Glucosamine (GlcN) activates the HBP pathway to generate the end product UDP-GlcNAc, used in glycan synthesis, and induces O-GlcNAc protein modifications (20). We examined the effects of GlcN on IAV transcription and replication by measuring the production of three forms of the IAV RNA [mRNA, complementary RNA (cRNA), and viral RNA (vRNA)] using an approach described previously (21). As shown in Fig. 1 (D and E), GlcN induced virus titers as well as the expression of three forms of IAV RNA in a time- and dose-dependent manner in primary human alveolar type II (ATII) cells, the major target cells during IAV infection (22). Treatment of PBMCs with GlcN led to a substantial increase in the production of those proinflammatory cytokines and chemokines and induced IAV replication (Fig. 1, F and G, and fig. S1F). Similar results were obtained in primary ATII cells (fig. S1, G and H). The effect of GlcN on IAV-induced proinflammatory cytokine and chemokine expression was further investigated in vivo. As shown in Fig. 1H, GlcN treatment plus IAV infection led to lower body weights than IAV infection. Moreover, in response to the IAV infection alone, GlcN treatment and IAV-infected mice displayed shortened survival times and lower survival rates (Fig. 1I). We next investigated whether production of proinflammatory cytokines and chemokines was altered in GlcN-treated mice during IAV infection. As expected, levels of the IFN-γ, tumor necrosis factor–α (TNF-α), interleukin-6 (IL-6), IL-8, chemokine (C-C motif) ligand 2 (CCL2), and CCL5 mRNAs and proteins were notably greater in GlcN-treated mice than in wild-type (WT) mice during IAV infection (Fig. 1J and fig. S1I). Consistent with this result, GlcN also induced IAV replication in mice (Fig. 1K). These findings suggest that IAV infection promotes HBP activity and downstream OGT-mediated protein O-GlcNAcylation and enhances inflammatory cytokine expression.

**IAV induces cytokine expression through OGT**

In light of the increased HBP activity and protein O-GlcNAcylation upon IAV infection, we hypothesized that OGT- or OGA-mediated O-GlcNAc signaling would play a role in IAV-induced cytokine expression. Overexpression of OGT increased IAV titers and the expression of three forms of IAV RNA, whereas overexpression of OGA decreased IAV titers and the expression of three forms of IAV RNA (fig. S2, A and B). Quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assays (ELISAs) revealed that OGT overexpression promoted IAV-induced production of IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 mRNAs and proteins (Fig. 2A). As previously described, the K908A mutation totally abolished the enzymatic function of OGT without damaging its protein abundance (23). As expected, OGT K908A mutant failed to affect IAV-induced cytokine expression (Fig. 2A). Similarly, OGT overexpression, but not the OGT K908A mutant, promoted the expression of the IAV viral protein (M1) and virus titers (Fig. 2B). We designed two specific small interfering RNAs (siRNAs) for OGT (siRNA-OGT #1 and #2) and tested their efficiency to confirm the effects of OGT on an IAV-induced cytokine storm (fig. S2C). In qPCR and ELISA experiments, OGT knockdown inhibited IAV-induced production of IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 mRNAs and proteins (Fig. 2C). Consistent with this result, OGT knockdown inhibited expression of IAV viral protein (M1) and virus titers (Fig. 2D). The effect of OGT expression on IAV-induced cytokine expression was not cell type specific because similar results were observed in ATII cells (fig. S2, D to G). OGA overexpression also decreased IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 mRNAs and protein levels (fig. S2, H and I).

PUGNAc is a widely used inhibitor of O-GlcNAcylase (OGA) that potently enhances protein O-GlcNAcylation, whereas OSMI-1 is a recently discovered OGT inhibitor that decreases protein O-GlcNAcylation (24). In line with previous results, treatment of PBMCs with PUGNAc, which markedly promoted protein O-GlcNAcylation, resulted in elevated cytokine production in response to IAV infection (Fig. 2, E and F). By contrast, levels of IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 mRNAs and proteins, as well as expression of IAV viral protein (M1) and virus titers, were lower in PBMCs treated with OSMI-1 than in vehicle-treated control (Fig. 2, G and H). Similar results were observed in ATII cells (fig. S2, J to M). Together, the findings with both genetic and pharmacological approaches suggest that OGT regulates IAV-induced cytokine storm through its enzymatic activity.

**IRF5 is O-GlcNAcylated at S430 during IAV infection**

We sought to determine the level at which OGT exerts its promoting effect on IAV-induced cytokine expression. In a qPCR assay, OGT knockdown inhibited myeloid differentiation primary response gene 88 (MyD88), TANK-binding kinase 1, IkB kinase (IKKβ), transforming growth factor β–activated kinase 1, and IRF5-induced IL-6 expression, suggesting that OGT functions at the transcription factor level (fig. S3A). In our previous studies, IRF5 was shown to be critical for IAV-induced cytokine storm (19). We hypothesized that OGT regulates IAV-induced cytokine storm via interacting with IRF5. Co-immunoprecipitation (Co-IP) and reverse Co-IP results suggested that IRF5 interacts with OGT and OGA (Fig. 3, A and B). By contrast, another adaptor protein involved in IFN signaling, melanoma differentiation-associated gene 5 (MDA5), did not interact with IRF5 (Fig. 3C). O-GlcNAcylation of exogenously expressed IRF5 could be detected in A549 cells, and this signal was much enhanced when OGT was coexpressed (Fig. 3D). The O-GlcNAc signal on IRF5 was abolished when anti–O-GlcNAc antibody was preincubated with 500 mM GlcNAc, suggesting the specificity of O-GlcNAc signal (Fig. 3D). Conversely, OGA overexpression inhibited the O-GlcNAc signal on IRF5 (Fig. 3E). By contrast, neither retinoic acid inducible gene I (RIG-I) nor MDA5 was O-GlcNAcylated, even when OGT was coexpressed (fig. S3, S and B and C). Further endogenous Co-IP experiment results suggested that OGT was weakly associated with IRF5 in unstimulated cells, and this association, as well as O-GlcNAcylated IRF5, increased after stimulation with IAV (Fig. 3F).

OGA/IRF5 interaction was abolished during IAV infection (Fig. 3F).

To further investigate the role of OGT in IRF5 O-GlcNAcylation, we generated mice with a myeloid-specific Ogt deletion (Ogt<sup>Δmye</sup>) by crossing the Ogt<sup>Δmye</sup> mice with lysosome M-Cre mice (6). Ogt<sup>Δmye</sup> mice were used as WT controls. IAV infection induced a marked elevation of endogenous IRF5 O-GlcNAcylation in WT mouse bone marrow–derived macrophages (BMMs) but not Ogt<sup>Δmye</sup> BMMs (Fig. 3G). OGA-mediated IRF5 O-GlcNAcylation required its enzymatic activity because the OGT K908A mutant did not catalyze IRF5 O-GlcNAcylase (Fig. 3H). We further generated N-terminal (1 to 220 amino acids) and C-terminal (221 to 498 amino acids)
Fig. 2. OGT is critical for IAV-induced inflammatory cytokine expression. (A) PBMCs were transfected with the indicated plasmids or vector controls for 24 hours, infected with the WSN virus (MOI = 1) or mock-infected for 24 hours, and subjected to qPCR (top) and ELISA (bottom) analyses. (B) Experiments were performed as described in (A), except that viral titers were measured by plaque assay (top) or IAV M1 protein in cells were measured using Western blot analyses (bottom). (C and D) Experiments were performed as described in (A) and (B), except that the indicated si-OGTs were used. (E) PBMCs were infected with the WSN virus (MOI = 1) or mock-infected for 12 hours, treated with or without PUGNAc (50 μM) for 12 hours, and subjected to qPCR (top) and ELISA (bottom) analyses. (F) Experiments were performed as described in (E), except that viral titers were measured by plaque assay (top) or IAV M1 protein in cells were measured using Western blot analyses (bottom). (G and H) Experiments were performed as described in (E) and (F), except that OSMI-1 (20 μM) were used. In the qPCR experiments, the control was designated as 1. All experiments were repeated at least three times. Bar graphs present means ± SD (n = 3; **P < 0.01; n.s., not significant).
Fig. 3. O-GlcNAcylation of IRF5 on S430 is critical for IAV-induced inflammatory cytokine expression. (A) A549 cells were transfected with Flag-tagged IRF5 (Flag-IRF5) and Myc-tagged OGT (Myc-OGT) for 48 hours. Co-immunoprecipitation (Co-IP) and immunoblot (IB) analyses were performed with the indicated antibodies. IgG, immunoglobulin G. (B and C) Experiments were performed as described in (A), except that Myc-OGA (B) or Myc-MDA5 (C) was used. (D and E) A549 cells were transfected with the indicated plasmid for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WCL, whole cell lysates. (F) A549 cells were infected with the WSN WT virus for the indicated times. Immunoprecipitation and immunoblot analyses were performed with the indicated antibodies. (G) Experiments were performed as described in (F), except that Ogtfl/fl and OgtΔmye BMMs were infected with the WSN virus for 24 hours. (H) A549 cells were transfected with the indicated plasmid for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. (I) Domain mapping of the IRF5 and OGT interaction. A549 cells were transfected with the indicated plasmid for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. The schematic representations of IRF5 truncations are shown at the top. (J) LC-MS/MS analysis was performed to identify S430 as an IRF5 O-GlcNAcylation site after Tm challenge. MS/MS spectrum of the 2+ ion at a mass/charge ratio (m/z) of 1115.06575 corresponding to IRF5 peptide ARLLLEMFSGELSWSA. (K) A549 cells were transfected with empty vector, Myc-OGT, Flag-IRF5 WT, or mutant constructs of IRF5 for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. All experiments were repeated at least three times.
truncated fragments of IRF5 and detected O-GlcNAcylation on its C terminus (Fig. 3I). To identify the O-GlcNAcylation site(s) on IRF5, mass spectrometry (MS) analysis was performed. Several detectable O-GlcNAcylation sites were shared between the full-length and C-terminal fragment of IRF5 revealed by liquid chromatography–tandem MS (LC-MS/MS) (Fig. 3I). Further analysis with single mutations revealed that a mutation (S430A) caused the loss of O-GlcNac signal without affecting IRF5 protein abundance, suggesting that IRF5 was O-GlcNAcylated on S430 (Fig. 3K). IRF5 S430 is well conserved among metazoan species (fig. S3D). Furthermore, IRF5 S430 failed to induce IAV-mediated regulation of levels of IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 mRNAs and proteins, confirming that S430 plays an important role in IRF5 function (fig. S3, E and F). These findings support the notion that IRF5 O-GlcNAcylation on S430 is critical for IAV-induced cytokine storm.

**IRF5 O-GlcNAcylation promotes its K63-linked ubiquitination**

Because previous studies identified TNF receptor–associated factor 6 (TRAF6)–mediated K63-linked ubiquitination of IRF5 as a critical protein modification for IRF5 function (25), we next sought to determine whether O-GlcNAcylation of IRF5 was vital for its ubiquitination using both overexpression and endogenous systems. IAV infection induced further enhancement in total and K63-linked ubiquitination of IRF5 but showed no effect on K48-linked ubiquitination (Fig. 4A). Furthermore, K63-linked ubiquitination of IRF5 was greater in IAV-infected cells than in controls (Fig. 4B). In an overexpression system, Myc-OGT enhanced K63-linked, but not K48-linked, polyubiquitination of IRF5 (Fig. 4C). Another experiment with an OGT enzymatic-dead K908A mutant revealed that OGT-mediated enhancement in IRF5 ubiquitination requires the enzymatic function of OGT (Fig. 4D). IRF5 with loss-of–O-GlcNAcylation S430A mutation showed less K63-linked ubiquitination than did WT IRF5 (Fig. 4E). We further investigated whether IAV regulated IRF5 ubiquitination via OGT. As shown in Fig. 4 (F and G), IRF5 ubiquitination was markedly attenuated in OgtΔmye BMMs or OGT–knocked down A549 cells. When cells were transfected with plasmids expressing V5-tagged TRAF6 and Flag-tagged IRF5, coexpression of OGT induced further enhancement in IRF5 K63 ubiquitination that was dependent on OGT enzymatic activity (Fig. 4H). A Co-IP assay showed that OGT promoted the association between IRF5 and TRAF6 via OGT enzymatic activity (Fig. 4H). Compared with WT IRF5, the S430A mutant lost its association with TRAF6 (Fig. 4I). To dissect the relationship between OGT, IRF5, and TRAF6, we used a CRISPR-Cas9–based gene targeting strategy to generate TRAF6-KO (knockout) cells. While IRF5 ubiquitination was markedly attenuated by TRAF6 deletion, IRF5 O-GlcNAcylation remained largely intact in TRAF6-KO cells (Fig. 4, J and K), suggesting that induced IRF5 O-GlcNAcylation on S430 mediated by OGT is an upstream step for IRF5 K63 ubiquitination.

We next examined the effect of OGT on the translocation of IRF5 from the cytosol to the nucleus, a hallmark of cytokine production. Western blot analyses revealed that OGT promoted IAV-induced IRF5 nucleocytoplasmic transport, and this was dependent on OGT enzymatic activity (fig. S4A). By contrast, OGA prevented IRF5 translocation from the cytosol to the nucleus during IAV infection (fig. S4B). As expected, IAV-induced IRF5 WT, but not IRF5 S430A mutation, translocated from the cytosol to the nucleus (fig. S4C). To test whether the O-GlcNAcylation on IRF5 mediated GlcN-regulated cytokine expression, we generated mice deficient in the Ifr-5 gene (Ifr-5−/− mice) using standard CRISPR-Cas9 technology. Ifr-5−/− BMMs in medium with GlcN showed markedly increased cytokine generation, including IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 (fig. S4, D and E). Nevertheless, GlcN failed to cause any increase in production of these cytokines in Ifr-5−/− BMMs (fig. S4, D and E). GlcN also induced IRF5 O-GlcNAcylation, as well as IRF5 ubiquitination in a time-dependent manner (fig. S4F). Consistently, treatment with GlcN led to interaction between IRF5 and TRAF6 (fig. S4G). Together, these data suggest that IAV promotes K63-linked ubiquitination of IRF5 via O-GlcNAcylation of IRF5.

**IAV induced inflammatory cytokine expression through OGT and IRF5 in mice**

To examine the function of OGT/IRF5 axis in IAV-induced cytokine storm in vivo, we crossed OgtΔmye mice with IRF5-deficient mice to generate OgtΔmye mice carrying the IRF5 null allele (OgtΔmye;Ifr-5−/−). We next infected WT, OgtΔmye, Ifr-5−/−, and OgtΔmye;Ifr-5−/− mice with the WSN strain of the influenza virus and monitored body weights. Compared with OgtΔmye and Ifr-5−/− mice, OgtΔmye;Ifr-5−/− mice showed complete abolition of the effect of IAV on body weights (Fig. 5A). Moreover, OgtΔmye;Ifr-5−/− mice were completely rescued from IAV-induced lethality (Fig. 5B). Consistent with this result, OgtΔmye and Ifr-5−/− mice exhibited lower IAV titers and nucleoprotein (NP)–specific mRNA, cRNA, and vRNA, and OgtΔmye;Ifr-5−/− mice exhibited the lowest IAV titers and NP-specific mRNA, cRNA, and vRNA than did WT mice during IAV infection (Fig. 5C). Similar results were also obtained using the lethal mouse-adapted influenza virus A/FM/1/47 (H1N1) (fig. S5, A and B). We next investigated whether the production of proinflammatory cytokines and chemokines was altered in OgtΔmye;Ifr-5−/−, and OgtΔmye;Ifr-5−/− mice during IAV infection. As expected, levels of IFN-β, TNF-α, IL-6, IL-8, CCL2, and CCL5 mRNAs and proteins were significantly lower in PBMCs, bronchoalveolar lavage fluid (BALF), and BMMs of OgtΔmye and Ifr-5−/− mice than in WT mice during IAV infection (Fig. 5D and fig. S5C). Increased production of inflammatory cytokines was abolished in OgtΔmye;Ifr-5−/− mice (Fig. 5D and fig. S5C). The blood glucose level was also lower in OgtΔmye;Ifr-5−/−, and OgtΔmye;Ifr-5−/− mice than in WT mice during IAV infection (Fig. 5E). Similar results were obtained in A/FM/1/47 (H1N1)–infected mice (fig. S5D). Furthermore, activation of O-GlcNac signaling by thiamet G (TMG) pretreatment failed to cause increased inflammatory cytokine levels in Ifr-5−/− BMMs reconstituted with IRF5 S430A mutant (Fig. 5G). We next asked whether there was a promoting effect of the OGT/IRF5 axis on inflammatory cytokine expression in physiological conditions by treatment with GlcN. As shown in fig. S5E, OgtΔmye and Ifr-5−/− mice exhibited significantly reduced inflammatory cytokine production in PBMCs, BALF, and BMMs in response to GlcN treatment, and GlcN failed to induce inflammatory cytokine production in OgtΔmye;Ifr-5−/− mice. Together, these findings suggest that OGT/IRF5 positively regulates IAV-induced cytokine storm in vivo.

**OGT-regulated cytokine expression and IAV replication through IRF5**

Since OGT associates with IRF5, we next examined the role of IRF5 in OGT-regulated cytokine expression and IAV replication. In ELISAs, overexpression of IRF5 promoted OGT-regulated inflammatory cytokine expression, whereas knockdown of IRF5 altered the induction effects of OGT (fig. S6, A and B). The role of IRF5 on OGT-regulated IAV replication was also investigated. As shown in fig. S6C, the
knockdown of IRF5 inhibited OGT-induced IAV replication. Conversely, high virus titers and the expression of three forms of IAV RNA were present in cells when OGT and IRF5 were overexpressed (fig. S6D). We also investigated whether OGT/IRF5 is important in poly(I:C)-induced lung injury. Endogenous Co-IP experiment results suggested that OGT was weakly associated with IRF5 in unstimulated cells, and this association increased after stimulation with poly(I:C) (fig. S6E). Lung sections from Ogtfl/fl and OgtΔmye and Irf-5 −/− mice showed reductions in inflammation and lung injury compared with those from control mice in response to poly(I:C) treatment (fig. S6F). Poly(I:C)-induced lung injury was almost abolished in Ogtfl/fl;Irf-5 −/− mice (fig. S6F). As expected, levels of inflammatory cytokine expression were significantly lower in BALF of Ogtfl/fl and Irf-5 −/− mice than in WT mice (fig. S6G). These results suggest that IRF5 is associated with OGT, and this association positively regulates OGT-mediated signaling pathway.

Fig. 4. O-GlcNAcylation of IRF5 on S430 promotes its K63-linked ubiquitination. (A) A549 cells were transfected with Flag-IRF5 and the indicated ubiquitin (Ub) plasmids. Twenty-four hours after transfection, cells were infected with the WSN virus (MOI = 1) for 24 hours before Co-IP and immunoblot analyses were performed with the indicated antibodies. (B) A549 cells were transfected with the WSN virus (MOI = 1) for the indicated times. Co-IP and immunoblot analyses were performed with the indicated antibodies. (C) A549 cells were transfected with Flag-IRF5, Myc-OGT, and the indicated ubiquitin plasmids for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. (D and E) Experiments were performed as described in (C), except that Myc-OGT (K908A) (D) or Flag-IRF5 (S430A) (E) was used. (F) Ogtfl/fl and OgtΔmye BMMs were infected with the WSN virus (MOI = 1) for 24 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. (G) A549 cells were transfected with si-ctrl or si-OGT for 24 hours and infected with the WSN virus (MOI = 1) for 24 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. (H and I) A549 cells were transfected with the indicated plasmids for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. (J) TRAF6+/+ or TRAF6−/− A549 cells were infected with the WSN virus (MOI = 1) for 24 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. (K) TRAF6+/+ or TRAF6−/− A549 cells were transfected with the vector control or Myc-OGT for 48 hours. Co-IP and immunoblot analyses were performed with the indicated antibodies. All experiments were repeated at least three times.
Blood glucose correlates with inflammatory cytokine expression, and IRF5 is O-GlcNAcylated in patients infected with IAV

To further elucidate the roles of the glucose metabolism in IAV infection, we obtained fresh blood from healthy individuals and from patients diagnosed with IAV infection between 2018 and 2019. There were two independent cohorts of IAV-infected patients. Cohort #1 and #2 included 107 and 12 IAV-infected patients, respectively. To investigate whether glucose metabolism predicted clinical outcome in IAV-infected patients, we analyzed blood glucose levels in cohort #1. As shown in Fig. 5A, blood glucose levels were greater in IAV-infected patients than in healthy individuals. Furthermore, total protein O-GlcNAcylation was greater in PBMCs from four randomly selected IAV-infected patients in cohort #1 (#6, #8, #46, and #82) than in three randomly selected healthy individuals (#14, #33, and #90) (Fig. 5B). Accordingly, IL-6 and IL-8 were expressed at significantly higher levels in IAV-infected patients than in the healthy individuals (Fig. 5, C and D). Elevated IL-6 and IL-8 expression in IAV-infected patients was tightly correlated with high levels of blood glucose (Fig. 5, E and F). Subsequently, we analyzed the IRF5 O-GlcNAcylation levels in cohort #2. Co-IP results suggested that IRF5 is O-GlcNAcylated and interacts with OGT in the PBMCs of IAV-infected patients (Fig. 5G). These findings provide further evidence that glucose metabolism and IRF5 O-GlcNAcylation are potentially involved in IAV-regulated cytokine storm.

DISCUSSION

Glucose metabolism and the inflammatory cytokine signal network evolved together. Nevertheless, it remains unclear whether the two systems interact with each other during IAV infection. In this study, we identified a previously undescribed mechanism of IAV-regulated cytokine storm, in which IAV induces cytokine storm by increasing glucose metabolism. Mechanistically, IAV promotes OGT-mediated O-GlcNAcylation of transcription factor IRF5 on serine-430 that is required for K63-linked ubiquitination of IRF5 and subsequent downstream inflammatory cytokine production (Fig. 5H).

It has been increasingly recognized that inflammatory responses must be coupled to specific metabolic programs to support their energetic demands (26, 27). Several reports have suggested the...
potential role of glucose metabolism in IAV infection. One recent study found that glucose availability and utilization are critical to surviving influenza infection. Further research showed that mice challenged with 2-deoxyglucose, which blocks glucose metabolism by inhibiting hexokinase activity, exhibited defects in control of body temperature and respiratory and heart rates but not antiviral inflammatory mediators (28). Two other studies showed that IAV infection–driven macrophage and dendritic cell glycolytic metabolism

Fig. 6. Analysis of glucose levels, inflammatory cytokine expression, and IRF5 O-GlcNAcylation levels in IAV-infected patients. (A) Serum glucose levels in healthy individuals (n = 107) and IAV-infected patients (n = 107). Data represent means ± SEM. (B) Healthy individuals (#14, #33, and #90) and IAV-infected patients (#6, #8, #46, and #82) were randomly selected to isolate PBMCs. The relative expression of protein O-GlcNAcylation, OGT, and IRF5 was measured using Western blot analyses. (C and D) Serum IL-6 (C) and IL-8 (D) protein levels in healthy individuals (n = 107) and IAV-infected patients (n = 107). Data represent means ± SEM. (E and F) Glucose levels and IL-6 (E) or IL-8 (F) protein levels in IAV-infected patients subjected to Pearson's correlation analysis. (G) PBMCs were isolated from healthy individuals (n = 12) and IAV-infected patients (n = 12). Co-IP and immunoblot analyses were performed with the indicated antibodies (***P < 0.01). (H) A hypothetical model for how IAV uses OGT promoting inflammatory cytokine expression by O-GlcNAcylation of IRF5.
are crucial components of innate antiviral defenses (29, 30). Here, we found that IAV infection led to rapid increases in activities of glucose metabolic pathways, and we identified an essential role for HBP-associated O-GlcNac signaling in IAV-induced inflammatory cytokine expression. Treatment with GlcN, which is used in glycan synthesis, induces HBP and O-GlcNac protein modifications, effectively activating inflammatory responses. This finding is consistent with those of two recent studies showing that elevated glucose metabolism upon viral infection was important for antiviral immune responses. To the best of our knowledge, this phenomenon suggests that IAV may have developed independent strategies to activate the HBP and glycolytic metabolism.

We further determined that OGT-mediated O-GlcNAcylation plays a pivotal role in IAV-regulated cytokine storm. Mechanistically, we identified IRF5 as a target of OGT-mediated O-GlcNAcylation in IAV-induced cytokine storm, and IRF5 O-GlcNAcylation on S430 is critical for the induction of cytokine storm. Previous studies have shed light on several key molecules in the innate immune system as targets for O-GlcNAcylation, including IKKβ, NF-κB, TGF-β activated kinase 1 binding protein 1, and STAT3 (8, 9, 31, 32). We suspected that OGT targets other than IRF5 that contribute to IAV-induced cytokine storm may exist. If other targets existed, activation of the O-GlcNAc signaling should enhance IAV-regulated cytokine storm in the presence of loss-of-O-GlcNAc mutant of IRF5. However, Irf-5−/− BMMS treated with TMG and reconstituted with IRF5 S430 mutant failed to cause any increase in cytokine expression (Fig. 5G). These results suggest that IRF5 is at least the major, if not the only, functional OGT target that promotes IAV-regulated cytokine storm. Moreover, we recently found that microRNA-302a suppresses IAV-stimulated IRF5 expression and cytokine storm induction (19).

In light of our previous and current results, we propose that IRF5 is a key transcription factor in IAV-induced cytokine storm, and IAV has developed at least two strategies to control the function of IRF5.

IRF5, a member of the IRF family, is critical for regulating immune and inflammatory responses in health and disease (19). IRF5 contains a DNA binding domain, an IRF-association domain, and a serine-rich region (SRR) at the C terminus (33). Compared with IRF3 and IRF7, much less is known about how IRF5 is activated. It was previously identified that serine-445 is an important phosphorylation site of IRF5 (34). Here, we demonstrated that IAV infection caused a robust increase in IRF5 O-GlcNAcylation. O-GlcNAcylation of IRF5 on S430 in SRR domain is an essential step for its ubiquitination and, subsequently, activation of inflammatory responses. However, note that other O-GlcNAcylation site(s) may exist on IRF5 that can be indirectly affected by IRF5 S430 O-GlcNAcylation. Previous studies have reported that the overall effect of protein O-GlcNAcylation on inflammation enhanced activation of inflammatory immune signaling. Nevertheless, currently, little is known about additional mechanisms, other than the regulation of protein phosphorylation, by which O-GlcNAc signaling modulates protein functionality. Given the essential roles of several forms of posttranslational modification other than phosphorylation, including ubiquitination and acetylation, this is of particular importance to inflammatory and immune signal networks. Our study demonstrates a working model in which OGT-mediated O-GlcNAcylation regulates protein function via modification of ubiquitination.

Pathogenic influenza virus infection causes robust cytokine production, including proinflammatory and anti-inflammatory cytokines. The role of robust cytokine production on influenza replication is not well understood. One study has shown that in Socs4-deficient animals, there is an increased cytokine production and IAV replication in the lungs (35). Another study found that down-regulation of c-Jun significantly suppressed not only viral replication but also the expression of inflammatory cytokines both in vitro and in vivo (36).

In this work, we showed that OGT/IRF5 increases virus replication as well as cytokine production. In light of previous and our current results, we propose that IAV replication is induced or viral clearance is delayed by this excessive release of chemokines and proinflammatory cytokines. IRF5 knockdown inhibited OGT-induced cytokine production. However, IRF5 knockdown cannot entirely abolish OGT-induced cytokine production and IAV replication (fig. S6, A and B).

These data suggest that OGT may regulate IAV replication via other mechanisms. We would like to highlight the fact that the herein suggested axis comprises several very potent regulators of cytokine storm (OGT and IRF5). Each of these proteins might also affect cytokine and chemokine production by other means and/or in conjunction with additional interaction partners.

A few studies have shown clinical efficacy of influenza vaccination with reduction in health complications, hospitalization, and deaths, causing loss of metabolic control, leading to increased levels of glycosylated serum proteins (37, 38). Nevertheless, little is known about the molecular mechanism underlying this phenomenon. In the present study, we found that elevated inflammatory cytokine expression levels in IAV-infected patients were tightly correlated with high levels of blood glucose. Furthermore, IRF5 O-GlcNAcylation was greater in IAV-infected patients than in healthy individuals. Although more research is needed to understand the delicate regulatory mechanisms between IAV-induced cytokine storm and glucose metabolism, our current findings may provide a potential target for the treatment of IAV infection in the future.

METHODS
Ethics statement
The collection of clinical samples was conducted according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of Wuhan University in accordance with guidelines for the protection of human subjects. All study participants provided written informed consent for the collection of samples and subsequent analyses.

All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the institutional animal care and use committee of Wuhan University (project license WDSKY0201302).

Clinical samples
Clinical samples of patients were collected from volunteers during physical examinations between April 2017 and April 2019 at the Renmin Hospital of Wuhan University and Zhongnan Hospital of Wuhan University. Blood samples were obtained from patients (cohort #1: 50 males and 57 females, aged 39.25 ± 18.2 years; cohort #2: 6 males and 6 females, aged 30.11 ± 11.48 years) confirmed to be infected with H1N1 IAV. Blood samples from healthy individuals (cohort #1: 53 males and 54 females, aged 33.25 ± 9.67 years; cohort #2: 6 males and 6 females, aged 32.71 ± 12.84 years) were randomly selected as controls from the local blood donation center. Patients with influenza recruited in the present study were confirmed as being infected with H1N1 virus by qPCR. PBMCs were isolated using standard Ficoll gradient centrifugation from the peripheral blood of either infected
subjects or uninfected controls. PBMCs were transfected with plasmid DNA by electroporation with an Amazia Nucleofector II device according to the manufacturer’s protocol and then resuspended in RPMI 1640 supplemented with penicillin (100 U/ml) and streptomycin (100 mg/ml).

Reagents, constructs, and mice
The details of critical reagents are listed in table S1. Unless specified otherwise, all biochemical reagents were purchased from Sigma-Aldrich. The details of antibodies are listed in table S2. The coding regions of IFRF5 and mutants, OGT, OGA, MDA5, and TRAF6 were created in our laboratory. The IFN-β promoter was provided by H. Shu of Wuhan University, China. To verify constructs and the specificity of antibodies, all constructs were transfected into 293T cells, and expression was analyzed using Western blot. All constructs were confirmed by DNA sequencing (Sangon Biotech, Shanghai, China).

Cytokine and chemokine analyses
The tracheas of euthanized mice were exposed, transected, and intubated with a blunt 18-gauge needle. One milliliter of phosphate-buffered saline (PBS) supplemented with cOmplete, Mini, EDTA-free protease inhibitor mixture (Roche Applied Science) was infused and collected four times. The recovered BALF was centrifuged at 3000g for 3 min at 4°C and stored at −80°C until use. A multiplex ELISA was performed on supernatants using ELISA kits (R&D Systems).

Cell culture and virus
Human lung epithelial cells (A549) were cultured in F-12K medium ( Gibco-BRL, Gaithersburg, MD, USA). Human embryonic kidney cells (293T) were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco-BRL). A549 cells and 293T cells were purchased from China Center for Type Culture Collection and supplemented with 10% heat-inactivated fetal bovine serum (FBS). Primary human ATII cells were purchased from Wuhan Pricells Company ( Wuhan, China). ATII cells were grown in DMEM supplemented with 1% charcoal stripped-FBS, KGF (K; 10 ng/ml); R&D Systems Inc., Minneapolis, MN, USA), 0.1 mM IBMX (I), 0.1 mM 8-Br-cAMP (A), 10 mM dexamethasone (D) (Sigma), 2 mM glutamine, amphotericin B (2.5 µg/ml), streptomycin (100 µg/ml), penicillin G (100 U/ml), and gentamicin (10 µg/ml; Gibco-BRL, Rockville, MD, USA). PBMCs were isolated from blood of healthy donors by standard Ficoll gradient centrifugation and cultured in RPMI 1640 without FBS and antibiotics. All cell cultures were maintained at 37°C in a 5% CO₂ incubator.

The recombinant human IAV A/WSN/33 (H1N1) was generated by transfecting Madin-Darby canine kidney cells with the eight-plasmid transfection system to generate IAV, as previously described (19). The eight-plasmid transfection system was a gift from R. G. Webster (Department of Infectious Diseases, St. Jude’s Children’s Research Hospital, Memphis, TN, USA). The IAV A/FM/1/47 (H1N1) was a gift from W. Hou (School of Basic Medical Sciences, Wuhan University). The stock virus was propagated in 10-day-old embryonated chicken eggs (Shijun Li laboratory at Central China Agricultural University) for 36 to 48 hours at 37°C. Allantoic fluid was then harvested, and aliquots were stored at −80°C before use.

Mice
WT C57BL/6 mice were purchased from the Animal Facility at Wuhan University Zhongnan Hospital. Irf-5−/− mice were created in our laboratory as previously described (19). Ogt−/− mice were generated by crossing the Ogt−/− mice with lysosome M-Cre mice. C57BL/6 mice and lysosome M-Cre mice were purchased from the Jackson Laboratory. All animals were housed under specific pathogen–free conditions at 21°C and 31% humidity.

Quantitative real-time PCR
Total RNA was extracted using TRizol reagent according to the manufacturer’s instructions. qPCR assays were performed using the ABI StepOne real-time PCR (RT-PCR) system (Applied Biosystems, Waltham, MA) and SYBR RT-PCR kits (Applied Biosystems). Primers specific to either human or murine genes are listed in table S3. The relative expression of each gene was calculated and normalized using the $2^{-\Delta\Delta C_{\text{t}}}$ method.

Western blot analysis
Western blot analyses were performed as previously described (19). Briefly, cells were harvested by low-speed centrifugation and washed with PBS. Cells were lysed in radioimmunoprecipitation assay buffer (Cell Signaling Technology, Boston, MA, USA), and protein concentrations were determined using bichinchoninic acid (BCA) assays (Cell Signaling Technology). Forty micrograms of each protein sample was separated using 12% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked with 1× tris-buffered saline with Tween 20 and 5% (w/v) nonfat milk for 1 hour at room temperature. Then, membranes were incubated with primary antibodies overnight at 4°C. Subsequently, blots were incubated with horseradish peroxidase–linked secondary antibodies (Jackson ImmunoResearch) for an additional 1 hour. Immunoreactive bands were visualized using an enhanced chemiluminescence system (GE Healthcare).

Transfection and luciferase reporter gene assays
Cells were seeded on 24-well dishes and transfected using Lipofectamine 3000 (Invitrogen) for 24 hours, after which they were serum-starved for an additional 24 hours before harvest. A Renilla luciferase reporter vector pRL-TK was used as the internal control. Luciferase assays were performed using a dual-specific luciferase assay kit (Promega, Madison, WI, USA). Firefly luciferase activities were normalized on the basis of Renilla luciferase activities.

Metabolomics analysis
PBMCs (5 × 10⁶) from male C57BL/6 mice (8 weeks of age) left untreated or challenged with IAV for 12 hours were harvested. The metabolite extraction was performed as previously described (16, 17). Briefly, the media were aspirated, and the cells were washed twice before lysing the cells. The metabolites were extracted using cold 80% methanol/water mixture and resuspended in 50% methanol/water mixture for further analysis using LC-MS/MS. A selected reaction monitoring LC-MS/MS method with positive and negative ion polarity switching on a Xevo TQ-S mass spectrometer was used for analysis. Peak areas integrated using MassLynx 4.1 were normalized to the respective protein concentrations. The data acquisition was carried out using Analyst 1.6 software, and peaks were integrated with MultiQuant (AB SCIEX, Framingham, MA, USA). MetaboAnalyst 3.0 software was used for heat map, principal components analysis, and pathway impact analysis.

LC-MS/MS analysis for 13C-UDP-GlcNAc
13C labeling of UDP-GlcNAc from U-13C6-glucose was detected from cell lysates using nuclear magnetic resonance (NMR) spectroscopy–based metabolomics analysis (16, 17). Briefly, samples were dried and
then reconstituted using phosphate buffer in 100% D\textsubscript{2}O with 500 mM TMSP as a chemical shift reference. The supernatant (500 ml) was transferred to a 5-mm NMR tube for data acquisition. The NMR data were collected at 298 K on an AVANCE III HD 700-MHz spectrometer (Bruker) equipped with a 5-mm quadruple-resonance QCI-P CryoProbe (\textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N, and \textsuperscript{31}P) using IconNMR software (Bruker). A two-dimensional (2D) \textsuperscript{1}H-\textsuperscript{13}C heteronuclear single-quantum coherence (HSQC) spectrum was collected for each of the samples. The 2D \textsuperscript{1}H-\textsuperscript{13}C HSQC spectra were collected with 1 K data points and a spectrum width of 11,160 Hz in the direct dimension and 128 data points and a spectrum width of 29,052 Hz in the indirect dimension. The 2D \textsuperscript{1}H-\textsuperscript{13}C HSQC spectra were processed with NMRPipe. The spectra were Fourier-transformed, manually phased, zero-filled, apodized with a sine–bell window function, and baseline-corrected following solvent subtraction. All spectra were referenced to TMSP at 0 parts per million (ppm). The processed spectra were then analyzed using NMRView version 8.0 (Johnson, 2018).

**IRF5 O-GlcNAcylation site mapping**

IRF5 O-GlcNAcylation sites were identified using MS strategy as previously described (8, 9). Briefly, immunoprecipitated IRF5 from IAV-infected A549 cells were subjected to SDS-PAGE. The corresponding bands were excised and the proteins were reduced with dithiothreitol, alkylated with iodoacetamide, digested with trypsin overnight, and trypsin digests were transferred to a 5-mm NMR tube for data acquisition. The NMR data were collected at 298 K on an AVANCE III HD 700-MHz spectrometer (Bruker) equipped with a 5-mm quadruple-resonance QCI-P CryoProbe (\textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N, and \textsuperscript{31}P) using IconNMR software (Bruker). A two-dimensional (2D) \textsuperscript{1}H-\textsuperscript{13}C heteronuclear single-quantum coherence (HSQC) spectrum was collected for each of the samples. The 2D \textsuperscript{1}H-\textsuperscript{13}C HSQC spectra were collected with 1 K data points and a spectrum width of 11,160 Hz in the direct dimension and 128 data points and a spectrum width of 29,052 Hz in the indirect dimension. The 2D \textsuperscript{1}H-\textsuperscript{13}C HSQC spectra were processed with NMRPipe. The spectra were Fourier-transformed, manually phased, zero-filled, apodized with a sine–bell window function, and baseline-corrected following solvent subtraction. All spectra were referenced to TMSP at 0 parts per million (ppm). The processed spectra were then analyzed using NMRView version 8.0 (Johnson, 2018).

**Statistical analysis**

Data were obtained from three independent reproducible experiments. Data were expressed as means ± SD or means ± SEM. Student’s t-test was used for statistical comparisons between two groups. One-way analysis of variance (ANOVA) was used to compare three or more groups. P < 0.05 was considered significant and was indicated with an asterisk (*).

**SUPPLEMENTARY MATERIALS**

Supplemental material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/16/eaaz7086/DC1

**REFERENCES AND NOTES**

1. R. J. DeBerardinis, C. B. Thompson, Cellular metabolism and disease: What do metabolic outliers teach us? Cell 148, 1132–1144 (2012).
2. X. Liu, D. E. Cooper, A. A. Cluntun, M. O. Warmoes, S. Zhao, M. A. Reid, J. Liu, P. J. Lund, M. Lopes, B. A. Garcia, K. E. Welling, D. G. Kirsch, J. W. Locasale, Acetate production from glucose and coupling to mitochondrial metabolism in mammalians. Cell 175, 502–513.e13 (2018).
3. S. Hardivillé, G. W. Hart, Nutrient regulation of signaling, transcription, and cell physiology by O-GlcNAcylation. Cell Metab. 20, 208–213 (2014).
4. X. Yang, K. Qian, Protein O-GlcNAcylation: Emerging mechanisms and functions. Nat. Rev. Mol. Cell Biol. 18, 452–465 (2017).
5. Y. R. Yang, M. Song, H. Lee, Y. Yeon, J.-E. Choi, H.-J. Jung, H. Y. Moon, H.-Y. Byun, E.-K. Kim, D. H. Kim, M. N. Lee, A. Köh, J. Ghim, J. H. Choi, W. Lee-Kwon, K. T. Kim, S. H. Ryu, P.-G. Suh, O-GlcNAcase is essential for embryonic development and maintenance of genomic stability. Aging Cell 11, 439–448 (2012).
6. R. Shafi, S. P. N. Iyer, L. G. Ellies, O. D’Orenatten, K. W. Marek, D. Chui, G. W. Hart, J. D. Marth, The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc. Natl. Acad. Sci. U.S.A. 97, 5735–5739 (2000).
7. G. W. Hart, M. P. Housley, C. Slawson, Cycling of O-linked β-N-acetylgalactosamine on nucleocyttoplasmic proteins. Nature 446, 1017–1022 (2007).
8. D. F. Allison, J. J. Wamsley, M. Kumar, D. Li, L. G. Gray, G. W. Hart, D. R. Jones, M. W. Mayo, Genetically targeted receptor-interacting serine/threonine-protein kinase 3. Proc. Natl. Acad. Sci. U.S.A. 109, 16888–16893 (2012).
9. X. Li, Z. Zhang, L. Li, W. Gong, A. J. Lazebny, B. J. Swanson, L. E. Herring, J. M. Asara, J. D. Singer, H. Wen, Myeloid-derived cullin 3 promotes STAT3 phosphorylation by inhibiting OGT expression and protects against intestinal inflammation. J. Exp. Med. 214, 1093–1109 (2017).
10. L. Si, H. Xu, X. Zhou, Z. Zhang, Z. Tian, Y. Wang, Y. Wu, B. Zhang, Z. Niu, C. Zhang, G. Fu, S. Xiao, Q. Xia, L. Zhang, D. Zhou, Generation of influenza A viruses as live but replication-incompetent virus vaccines. Science 354, 1170–1173 (2016).
11. A. Iwasaki, R. Medzhitov, A new shield for a cytokine storm. Cell 141, 861–862 (2011).
12. X.-z. J. Guo, P. G. Thomas, New fronts emerge in the influenza cytokine storm. Cell Host Microbe 24, 791–803.e6 (2018).
13. T. Liu, X. Li, K. S. Attri, C. Liu, L. Li, L. E. Herring, J. M. Asara, Y. L. Lei, P. K. Singh, C. Gao, H. Wen, O-GlcNAc transferase links glucose metabolism to MAVS-mediated antiviral innate immunity. Cell Host Microbe 24, 791–803.e6 (2018).
14. X. Li, W. Gong, H. Wang, T. Li, K. S. Attri, R. E. Lewis, A. C. Kalil, F. Bhinderwala, R. Powers, G. Yin, L. E. Herring, J. M. Asara, Y. L. Lei, X. Yang, D. A. Rodriguez, M. Yang, D. R. Green, P. K. Singh, H. Wen, O-GlcNAc transferase suppresses inflammation and necroptosis by targeting receptor-interacting serine/threonine-protein kinase 3. Immunity 50, 1115 (2019).
17. W. Zhang, G. Wang, Z.-G. Xu, H. Tu, F. Hu, J. Dai, Y. Chang, Y. Chen, Y. Lu, H. Zeng, Z. Cai, F. Han, C. Xu, G. Jin, L. Sun, B.-S. Pan, S.-W. Lai, C.-C. Hsu, J. Xu, Z.-Z. Chen, H.-Y. Li, P. Seth, J. Hu, X. Zhang, H. Li, H.-K. Lin. Lactate is a natural suppressor of RLR signaling by targeting MAVS. Cell 178, 176–189.e15 (2019).

18. B. Everts, E. Amiel, S.-C.-C. Huang, A. M. Smith, W. Y. Lam, V. Redmann, T. C. Freitas, J. Blagih, G. J. W. van der Windt, M. N. Artymov, R. G. Jones, L. E. Pearce, E. J. Pearce, TLR-driven early glycolytic reprogramming via the kinases TBK1-IRK supports the anabolic demands of dendritic cell activation. Nat. Immunol. 15, 323–332 (2014).

19. X. Chen, L. Zhou, N. Peng, H. Yu, M. Li, Z. Cao, Y. Lin, X. Wang, Q. Li, J. Wang, Y. She, C. Zhu, M. Lu, Y. Zhu, S. Liu. MicroRNA-302a suppresses influenza A virus-stimulated interferon regulatory factor 5 expression and cytokine storm induction. J. Biol. Chem. 292, 21291–21303 (2017).

20. M. S. Denzel, N. J. Storm, A. Geschwind, B. Raddi, Y. Hinze, E. Jarosch, T. Sommer, T. Hoppe, A. Antebi, Hexoseamine pathway metabolites enhance protein quality control and prolong life. Cell 156, 1167–1178 (2014).

21. E. Kawakami, T. Watanabe, K. Fujii, H. Goto, S. Watanabe, T. Noda, Y. Kawaoka. Strand-specific real-time RT-PCR for distinguishing influenza vRNA, cRNA, and mRNA. J. Virol. Methods 173, 1–6 (2011).

22. R. J. Mason, Biology of alveolar type II cells. Annu. Rev. Biochem. 825–858 (2011).

23. C. Martinez-Fleites, M. S. Macauley, Y. He, D. L. Shen, D. J. Vaccolio, G. J. Davie. Structure of an O-GlcNAc transferase homolog provides insight into intracellular glycosylation. Nat. Struct. Mol. Biol. 15, 764–765 (2008).

24. G. W. Hart, C. Clawson, G. Ramirez-Corra, O. Lagerof, Cross talk between O-GlcNAcylation and phosphorylation: Roles in signaling, transcription, and chronic disease. Annu. Rev. Biochem. 80, 825–858 (2011).

25. M. Y. Bahlk, K. A. Fitzgerald, P. M. Pitha, Functional regulation of MyD88-activated interferon regulatory factor 5 by N6-arginine polyubiquitination. Mol. Cell. Biol. 28, 7296–7308 (2008).

26. A. Brand, K. Singer, G. E. Koehl, M. Kolitzus, G. Schoenhammer, A. Thiel, C. Matos, C. Bruss, S. Klobuch, K. Peter, M. Kastenberger, C. Bogdan, U. Schleicher, A. Mackensen, E. Ullrich, S. Fichtner-Feigl, R. Kesselring, M. Mack, U. Ritter, M. Schmid, C. Blank, K. Dettmer, P. J. Oeffner, P. Hoffmann, S. Wollner, K. E. Geissler, J. Pouysseguer, A. Villunger, A. Steven, B. Seliger, S. Schreml, S. Haferkamp, E. Kohl, S. Karrer, M. Berneburg, W. Herr, S. Helfrich-Weber, M. G. C. Schmidt, M. Schuster, L. Desmedt, O-GlcNAc-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. Cell Metab. 24, 657–671 (2016).

27. E. J. Pearce, A. I. M. Hoepelman, G. E. H. M. Rutten, Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin. Infect. Dis. 41, 281–288 (2005).

28. W. H. Yang, S. Y. Park, H. W. Nam, D. H. Kim, J. G. Kang, E. S. Kang, Y. S. Kim, H. C. Lee, K. S. Kim, J. W. Cho. NFκB activation is associated with its O-GlcNAcylation state under hyperglycemic conditions. Proc. Natl. Acad. Sci. U.S.A. 105, 17345–17350 (2008).

29. D. Kim, H. Lee, J. Koh, J. S. Ko, B. R. Yoon, Y. K. Jeon, Y. M. Cho, T. H. Kim, Y. S. Suh, H. J. Lee, H. K. Yang, K. S. Park, H. Y. Kim, C. W. Lee, W. W. Lee, D. H. Chung. Cytosolic Pellino-1-mediated K63-linked ubiquitination of IRF5 in M1 macrophages regulates glucose intolerance in obesity. Cell Rep. 20, 832–845 (2017).

30. J. Ren, X. Chen, Z. J. Chen, RKKI is an IRF5 kinase that instigates inflammation. Proc. Natl. Acad. Sci. U.S.A. 111, 17438–17443 (2014).

31. E. L. Pearce, TLR-driven early glycolytic reprogramming via the kinases TBK1-IRK supports the anabolic demands of dendritic cell activation. Nat. Immunol. 15, 323–332 (2014).

32. A. Brand, K. Singer, G. E. Koehl, M. Kolitzus, G. Schoenhammer, A. Thiel, C. Matos, C. Bruss, S. Klobuch, K. Peter, M. Kastenberger, C. Bogdan, U. Schleicher, A. Mackensen, E. Ullrich, S. Fichtner-Feigl, R. Kesselring, M. Mack, U. Ritter, M. Schmid, C. Blank, K. Dettmer, P. J. Oeffner, P. Hoffmann, S. Wollner, K. E. Geissler, J. Pouysseguer, A. Villunger, A. Steven, B. Seliger, S. Schreml, S. Haferkamp, E. Kohl, S. Karrer, M. Berneburg, W. Herr, S. Helfrich-Weber, M. G. C. Schmidt, M. Schuster, L. Desmedt, O-GlcNAc-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. Cell Metab. 24, 657–671 (2016).

33. E. J. Pearce, A. I. M. Hoepelman, G. E. H. M. Rutten, Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin. Infect. Dis. 41, 281–288 (2005).

Acknowledgments: Funding: This work was supported by the National Natural Science Foundation of China (81872262 to S.L., 31570870 to L.Z., 81301428 to Y.Y., and 81802627 to Y.J.Y.), the Fundamental Research Funds for the Central Universities (20142017kf0221 to S.L. and 2042015kf0188 to L.Z.), the Science and Technology Innovation Fostering Foundation of Zhongnan Hospital of Wuhan University (cxpy20160025 to F.W.), and the Deutsche Forschungsgemeinschaft (TRR60 and RTG1949 to M. Lu and M. Trilling). Author contributions: Q.W. participated in carrying out all the experiments. P.F., R.H., M. Li, and H.Y. participated in gene cloning. L.Z., Y.Y., Y. Zhang, and A.C. participated in mouse experiments. F.W., Y.R., N.P., and T.L. collected and analyzed clinical samples for the study. M. Lu and Y. Zhu participated in the design of the study. G.P. and L.R. discussed the results and participated in the critical review of the manuscript. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in this paper are present in the paper and/or the Supporting Materials. Additional data related to this paper may be requested from the authors.

Submitted 2 October 2019
Accepted 21 February 2020
Published 15 April 2020
10.1126/sciadv.aaz7086

Citation: Q. Wang, P. Fang, R. He, M. Li, H. Yu, L. Zhou, Y. Yi, F. Wang, Y. Rong, Y. Zhang, A. Chen, N. Peng, Y. Lin, M. Lu, Y. Zhu, G. Peng, L. Rao, S. Liu. O-GlcNAc transferase promotes influenza A virus–induced cytokine storm by targeting interferon regulatory factor–5. Sci. Adv. 6, eaaz7086 (2020).