Aryl Hydrocarbon Receptor Interacting Protein Targets IRF7 to Suppress Antiviral Signaling and the Induction of Type I Interferon*

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Background: IRF7 is known as the master regulator of type I IFN production, yet little is known about its negative regulation.

Results: AIP interacts with IRF7 and inhibits IRF7 nuclear localization.

Conclusion: AIP suppresses virus-induced type I IFN production by targeting IRF7 for inactivation.

Significance: Understanding IRF7 regulation is important to elucidate the host innate immune response to virus infection.

The transcription factor IRF7 (interferon regulatory factor 7) is a key regulator of type I interferon and plays essential roles in restricting virus infection and spread. IRF7 activation is tightly regulated to prevent excessive inflammation and autoimmunity; however, how IRF7 is suppressed by negative regulators remains poorly understood. Here, we have identified AIP (aryl hydrocarbon receptor interacting protein) as a new binding partner of IRF7. The interaction between AIP and IRF7 is enhanced upon virus infection, and AIP potently inhibits IRF7-induced type I IFN (IFNα/β) production. Overexpression of AIP blocks virus-induced activation of IFN, whereas knockdown of AIP by siRNA potentiates virally activated IFN production. Consistently, AIP-deficient murine embryonic fibroblasts are highly resistant to virus infection because of increased production of IFNα/β. AIP inhibits IRF7 function by antagonizing the nuclear localization of IRF7. Together, our study identifies AIP as a novel inhibitor of IRF7 and a negative regulator of innate antiviral signaling.

There are two classes of pattern recognition receptors that recognize and respond to viral RNAs: cytoplasmic RIG-I (retinoic acid-inducible gene I)-like helicase receptors and membrane-bound Toll-like receptors. The cytoplasmic helicases RIG-I and MDA-5 (melanoma differentiation associated gene 5) are key RIG-I-like helicase receptors that detect 5’-triphosphate RNA and dsRNA (or the dsRNA mimetic poly(I:C)) respectively (2). RIG-I contains tandem N-terminal CARD domains, a central DECH-box RNA helicase, and a C-terminal regulatory domain. RIG-I is autoinhibited because of intramolecular interactions of the CARD and helicase domains. RIG-I binding to viral RNA triggers a conformational change that exposes the CARDs and promotes lysine 63-linked polyubiquitination of RIG-I by the E3 ligase TRIM25 (tripartite motif-containing 25) (5). RIG-I lysine 63-linked polyubiquitination facilitates multimerization and CARD–CARD interactions with the outer mitochondrial membrane adaptor MAVS (also known as IPS-1, CARDIF, or VISA) (6). MAVS forms prion-like aggregates and nucleates a signaling complex containing TRAF E3 ligases (TRAF2, TRAF3, TRAF5, and TRAF6), the adaptor molecule NEMO, and the noncanonical IκB kinases TBK1 and IκKε (7–9). TBK1 and IκKε phosphorylate IRF transcription factors (IRF3 and IRF7) that trigger their dimerization, nuclear translocation, and activation of type I IFN gene expression (10). In addition to IRF activation, the NF-κB transcription factor is also activated downstream of RIG-I-like helicase receptors and contributes to the induction of type I IFN (11).

IRF3 and IRF7 are key transcription factors that together orchestrate the production of type I IFN. IRF3 is expressed constitutively in a variety of tissues and is responsible for the early phase of virus-mediated type I IFN induction (12). Conversely, IRF7 is expressed at very low levels in most cell types, except for lymphoid cells and plasmacytoid dendritic cells (pDCs), and is inducible by interferon (13). Although IRF3 plays important roles in the initial phase of type I IFN induction, IRF7 is the major player in establishing a secondary and larger wave of IFN...
production (14). Indeed, knock-out studies in mice have revealed that IRF7 is essential for virus-mediated MyD88-independent production of type-I IFN (15), and hence IRF7 is known as the master regulator of type I IFN.

Antiviral signaling requires a precise balance between activation and termination to restore homeostasis after viral clearance because deregulated and overproduction of type I IFN may promote inflammatory or autoimmune diseases. As such, a growing number of inhibitors of RIG-I, MAVS, TRAF3, and TBK1 in antiviral signaling have been described, including RNF125, USP21, Itch, Triad3A, NLRP4, A20, TAX1BP1, and ABIN (16–23). In addition, several inhibitors have been found to target either IRF3 (24, 25) and/or IRF7 (26–29) by distinct mechanisms to restrict antiviral signaling.

AIP (aryl hydrocarbon receptor interacting protein; also known as XAP2, ARA9, and FKBP37) was first identified as an interacting protein of the aryl hydrocarbon receptor (AhR)3 in a yeast two-hybrid screen using AhR as bait (30–32). The human AIP gene encodes a 37-kDa protein of 330 amino acids. AIP contains an N-terminal immunophilin-like domain, three C-terminal tetratricopeptide repeat (TPR) domains, and an alpha-7 helix (32, 33). Previous studies have established that AIP forms a tetrameric complex together with AhR and a dimer of Hsp90 in the cytoplasm (34, 35). Hsp90 and AIP retain AhR in the cytoplasm, and AIP prevents the ubiquitination and degradation of AhR (36). Upon AhR ligand binding, AIP is dissociated from the complex, allowing AhR to enter the nucleus, dimerize with AhR nuclear translocator, and activate genes involved in the xenobiotic response (37, 38). AIP was also identified as a protein associated with several viral proteins, such as the X protein of the hepatitis B virus (31) and EBNA-3 of the Epstein-Barr virus (39). Emerging studies indicate that AIP has the X protein of the hepatitis B virus (31) and EBNA-3 of the Epstein-Barr virus (39). Emerging studies indicate that AIP has roles outside of its well known regulation of AhR and the xenobiotic response. AIP can interact with the CARMA1 adaptor molecule in T lymphocytes and enhances CARMA1 binding to MALT1 and BCL-10 to promote T cell receptor-mediated NF-κB activation and IL-2 production (40). In this study, we describe a novel function of AIP as a negative regulator of IRF7 and antiviral signaling.

**Experimental Procedures**

**Cells, Plasmids, and Reagents—** 293T cells were obtained from ATCC. Aip−/− MEFs were kindly provided by Dr. Auli Karhu (University of Helsinki) (41). Human AIP cDNA was PCR-amplified and cloned in pCDNA-Myc. Myc-AIP was provided by Dr. Marta Karbonits (William Harvey Research Institute) (33). AIP deletion mutants were amplified by PCR and cloned in pCDNA-Myc. Flag-AIP and deletion mutants (1–503, 1–475, 1–416, 1–340, 1–246, and 1–298) were previously described (42). AIP siRNAs were purchased from Dharmacon/GE Healthcare. AIP siRNAs were performed using Lipofectamine 2000 (Life Technologies). Control scrambled and siGENOME SMARTpool AIP siRNAs were purchased from Dharmacon/GE Healthcare. Control and AIP siRNA were also purchased from Sigma-Aldrich. For luciferase assays, cells were lysed in 1× passive lysis buffer (Promega) 1 day after transfections, and luciferase activity was measured with the Dual-Luciferase assay system (Promega) according to the manufacturer’s instructions. Firefly luciferase values were normalized to Renilla luciferase values (internal control), and luciferase activities were presented as “relative activity” compared with nontreated controls: either empty vector, control siRNA, or mock infection.

**Co-immunoprecipitations and Western Blotting—** The following antibodies were used in this study: AIP (ARA9) ab88470 (Abcam), AIP (ARA9) ab181230 (Abcam), Flag M2 (Sigma-Aldrich), Flag (2368; Cell Signaling Technology), LDH1 (Santa Cruz Biotechnology), and poly-ADP-ribose polymerase (Cell Signaling Technology). Cells were lysed in radioimmunoprecipitation assay buffer, and co-immunoprecipitations (co-IPs) and Western blotting were performed as described before (47).

**Subcellular Fractionation—** Cells were lysed in hypotonic buffer (20 mm HEPES, pH 8.0, 10 mm KCl, 1 mm MgCl2, 0.1% (v/v) Triton X-100, 400 mm NaCl). After 15 min of incubation on ice and centrifugation, supernatants representing cytoplasmic fractions were harvested. The pellets were washed with hypotonic buffer three times and further lysed using nuclear extraction buffer (20 mm Tris, pH 8.0, 150 mm NaCl, 1% (v/v) Nonidet P-40, 10 mm iodoacetamide) in a water bath sonication system, after which supernatants were collected representing nuclear fractions. All lysis buffers were supplemented with 1 mm PMSF and a protease inhibitor mixture tablet (Roche Applied Science) before use.

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3 The abbreviations used are: AhR, aryl hydrocarbon receptor; TPR, tetratricopeptide repeat; MEF, mouse embryonic fibroblast; co-IP, co-immunoprecipitation; SeV, Sendai virus; VAD, virus-activated domain; ID, inhibitory domain; SRD, signal response domain; VSV, vesicular stomatitis virus.
AIP Suppresses IFN-α and Antiviral Signaling

**Immunofluorescence Microscopy**—HEK293T and MEFs were cultured overnight on glass coverslips in 12-well plates. After transfection/infection, cells were washed with PBS and fixed with 1% paraformaldehyde for 15 min followed by two washes with 1× PBS. The cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min and incubated with Super-Block buffer (Thermo Scientific) for 45 min followed by staining with rabbit anti-Flag and mouse anti-Myc antibodies for 1 h and five washes with 0.1% Triton X-100 in PBS. Next, coverslips were incubated with Alexa Fluor 555-conjugated donkey anti-mouse and Alexa Fluor 488-conjugated donkey anti-rabbit (Life Technologies) and DAPI (1:10,000; Sigma-Aldrich). Images were obtained using a Nikon Eclipse E800 fluorescence microscope with CCD camera using Elements Software for imaging of fluorescent slides.

**ELISA**—The mouse IFN-α4 and IFN-β kits were purchased from PBL Assay Science. ELISA was performed using cell supernatants according to the manufacturer’s instructions. A standard curve was made using the recombinant IFNs provided with the kit and used to calculate the values (pg/ml) for each sample.

**RT-PCR and Quantitative Real Time PCR**—RNA was harvested from cells using the RNeasy mini kit (Qiagen). RNA was converted to cDNA using avian myeloblastosis virus reverse transcriptase (Promega). RT-PCR was performed using platinum PCR SuperMix (Life Technologies). The following primers were used for RT-PCR: AIP, cloning primers from above were used for PCR; IRF7 forward, 5′-TAC CAT CTA CCT GGG CTT CG-3′; IRF7 reverse, 5′-AAG GAA GCA CTC GAT GTC GT-3′; 18S forward, 5′-GTAACCCGTTGAACCCCATT-3′; and 18S reverse, 5′-CATCCAATCGGTAGTAGCG-3′. Real time PCR was performed using SYBR Green quantitative PCR (Sigma-Aldrich). Gene expression was normalized to the internal control 18S rRNA. The following primers were used for quantitative real time PCR: mouse IFN-β forward, 5′-ATG AAC AAG AGG TTC ATC TTC C-3′; mouse IFN-β reverse, 5′-AGG AGC TTC TGA CAT TTC CGA A-3′, mouse IFN-α4 forward, 5′-AAGCTGTGTGATGCAGGAA-3′; mouse IFN-α4 reverse, 5′-TGGTTGAGGAAGAGAGGGCT-3′; mouse IL-6 forward, 5′-TAGTCCTTCCTACCCCAATTTCC-3′; and 18S reverse, 5′-TTGGTCCCTTAGCACTCCTTCTC-3′; and 18S, same primers as above.

**Virus Infections**—Sendai virus (Cantell strain) was purchased from Charles River. VSV-GFP was provided by Dr. Siddharth Balachandran (Fox Chase Cancer Center) (48). Cells were infected with Sendai virus (20 HA/ml) or VSV-GFP at multiplicity of infections of 0.001 (293T) or 1.0 (MEFs) in serum-free DMEM for 1 h. After infections, virus-containing supernatant was removed and replaced with complete medium.

**Statistical Analysis**—Error bars represent the standard deviation of triplicate samples. Two-tailed unpaired $t$ test was performed, and the level of significance is indicated $p < 0.001 (***)$, $p < 0.01 (**)$, and $p < 0.05 (*)$.

**Results**

**AIP Interacts with IRF7**—A previous study using mass spectrometry to map the innate immune interactome in response to virus infection found that IRF7 may serve as a potential binding partner of AIP (49). To investigate this possibility, we conducted co-IP assays in 293T cells transfected with Myc-AIP and Flag-IRF7 plasmids. IRF7 was detected upon immunoprecipitation of AIP, and AIP was found in IRF7 immunoprecipitates (Fig. 1A), indicating that ectopic AIP can interact with IRF7. The binding between AIP and IRF7 was specific and was not observed with overexpressed MDA5 (Fig. 1B). We next examined the interaction of endogenous AIP with Flag-IRF7, in the absence or presence of Sendai virus (SeV) infection. Interestingly, endogenous AIP interacted with IRF7, and the interaction was progressively enhanced over time by virus infection (Fig. 1C). Increased virus-induced AIP-IRF7 interaction was observed despite similar amounts of AIP immunoprecipitated and comparable expression levels of AIP and IRF7 in the lysates (Fig. 1C). Therefore, virus infection appears to enhance the interaction between endogenous AIP and IRF7.

AIP contains an N-terminal peptidyl-prolyl cis-trans-isomerase-like domain, three C-terminal TPR domains (TPR1, TPR2, and TPR3), and an alpha-7 helix (32). To identify the domain(s) within AIP required for its interaction with IRF7, we generated a series of N- or C-terminal deletion mutants of AIP. AIPΔN lacked the peptidyl-prolyl cis-trans-isomerase domain; AIPΔC1 lacked the alpha-7 helix; AIPΔC2 lacked the alpha-7 helix and TPR3; AIPΔC3 lacked the alpha-7 helix, TPR2 and TPR3; and AIPΔC4 lacked the alpha-7 helix, TPR1, TPR2, and TPR3 (Fig. 1D). As expected, full-length AIP interacted with IRF7 (Fig. 1F). The basal interactions between AIPΔC1 and ΔC2 and IRF7 appeared to be diminished; however, SeV-induced binding between these mutants and IRF7 was observed (Fig. 1F). AIPΔC3 and ΔC4 mutants were completely impaired in binding to AIP (Fig. 1F), indicating that AIP TPR2 is important for the observed binding with IRF7. AIPΔN was still able to interact with IRF7, indicating that the peptidyl-prolyl cis-trans-isomerase-like domain was dispensable for the binding (Fig. 1F). Therefore, it appears that the TPR2 domain of AIP mediates interactions with IRF7.

IRF7 contains an N-terminal DNA-binding domain, a constitutive activation domain between amino acids 151 and 246, a virus-activated domain (VAD) between amino acids 278 and 305, an inhibitory domain (ID) between amino acids 372 and 467, and a signal response domain (SRD) containing key virus-induced phosphorylation sites between amino acids 468 and 491 (42). Concurrently, we utilized a series of IRF7 C-terminal deletion mutants to identify the essential domain(s) involved in its interaction with AIP (Fig. 1E). IRF7Δ1 lacks the SRD; IRF7Δ2 lacks part of the ID and SRD; IRF7Δ3 lacks the entire ID and SRD; IRF7Δ4 lacks the VAD, ID, and SRD; IRF7Δ5 lacks part of the constitutive activation domain, VAD, ID, and SRD. Full-length IRF7 and IRF7Δ1, Δ2, and Δ3 mutants interacted with AIP (Fig. 1G). However, IRF7Δ4 and Δ5 mutants did not interact with AIP (Fig. 1G). Thus, it appears that the VAD comprises the AIP interaction motif within IRF7.

**AIP Inhibits Antiviral Signaling**—IRF7 is known as the master regulator of type I IFN and is critical for virus-induced type I IFN in a variety of cell types (15). Therefore, we next investigated whether AIP played a role in virus-induced type I IFN production. First, we conducted a luciferase reporter assay using an IFN-β luciferase reporter plasmid. Interestingly, AIP
inhibited SeV-induced IFN-β promoter activation in a dose-dependent manner (Fig. 2A). AIP inhibition of the IFN-β promoter appeared to be specific because AIP did not inhibit TNF-induced activation of an NF-κB luciferase reporter (Fig. 2B). Because IRF7 is essential for virus-mediated induction of IFN-α production (15), we next examined whether AIP could modulate IRF7 activation of an IFN-α4 luciferase reporter. We transfected small amounts of IRF7 to activate the reporter because 293T cells express miniscule amounts of IRF7. Indeed, robust activation of the IFN-α4 promoter was observed with the combination of SeV infection and ectopic IRF7 (Fig. 2C). However, overexpression of AIP significantly diminished IFN-α4 activation by SeV and IRF7 (Fig. 2C). Together, these results indicate that AIP functions as a negative regulator of antiviral signaling pathways that control virus-induced production of type I IFN.

We next performed loss of function studies to determine whether loss of AIP enhanced virus-mediated IFN-α4 promoter activation. Expression of endogenous AIP was suppressed by transfection of a pool of four distinct siRNAs into 293T cells and confirmed by Western blotting (Fig. 2D, bottom panel). Knockdown of endogenous AIP did not affect basal levels or the induction of IFN-α4 promoter activation by IRF7 alone (Fig. 2D). However, siRNA-mediated knockdown of AIP significantly enhanced IFN-α4 promoter activation by SeV infection together with IRF7 transfection (Fig. 2D).
To determine whether AIP regulation of type I IFN production played any role in modulating virus replication, we next investigated whether either overexpression or knockdown of AIP influenced the replication of vesicular stomatitis virus expressing GFP (VSV-GFP). Cells were transfected with Myc-AIP, infected with VSV-GFP, and then subjected to immunofluorescence microscopy and Western blotting to examine GFP expression as a surrogate for virus infection. Overexpression of AIP strongly enhanced VSV-GFP replication as observed by microscopy and Western blotting for GFP (Fig. 2E). Conversely, knockdown of AIP using a pool of four siRNAs suppressed replication of VSV-GFP (Fig. 2F). Transfection of cells with a single independent AIP siRNA (siAIP-2) similarly reduced VSV-GFP replication, indicating that the siRNA inhibition of VSV-GFP replication was likely not due to off target effects (Fig. 2G). Taken together, these data provide strong evidence that AIP inhibits antiviral signaling pathways and type I IFN activation that leads to increased virus replication.

**AIP-deficient Cells Overproduce Type I IFN and Are Resistant to Virus Infection**—Genetic ablation of AIP in mice leads to embryonic lethality caused by cardiac malformation (41, 50). To provide genetic evidence for a role of AIP in the regulation of antiviral signaling, we used Aip−/− MEFs. Whereas WT MEFs were readily infected by VSV-GFP, Aip−/− MEFs were profoundly resistant to virus infection at an identical multiplicity of infection (Fig. 3A). To demonstrate that these effects were due
solely to loss of AIP, we reconstituted Aip<sup>−/−</sup> MEFs with an AIP plasmid. Reconstitution of the AIP-deficient MEFs with AIP restored virus replication as determined by immunofluorescence microscopy and Western blotting for GFP (Fig. 3B).

Based on our earlier results, we hypothesized that the resistance of Aip<sup>−/−</sup> MEFs to virus infection was imparted by deregulated antiviral signaling and increased levels of virus-mediated type I IFN production. To examine this notion, we conducted ELISAs using Aip<sup>−/−</sup> MEFs to quantify the magnitude of virus-triggered type I IFN production. Initially, Aip<sup>−/−</sup> MEFs were transfected with poly(I:C), a synthetic analog of double-stranded RNA, to activate antiviral signaling. IFN-β in the cell supernatants was quantified by ELISA. In wild-type MEFs, transfection with poly(I:C) induced ~50 pg/ml of IFN-β (Fig. 3C). However, poly(I:C) induced over 400 pg/ml of IFN-β in supernatants from Aip<sup>−/−</sup> MEFs (Fig. 3C). Wild-type and Aip<sup>−/−</sup> MEFs were also infected with SeV, and IFN-α4 in cell supernatants was quantified by ELISA. SeV infection induced ~10 pg/ml of IFN-α4 from wild-type MEFs, whereas Aip<sup>−/−</sup> MEFs produced over 200 pg/ml after SeV infection (Fig. 3D). Therefore, antiviral signaling is clearly deregulated in Aip<sup>−/−</sup> MEFs, and both IFN-β and IFN-α4 are overproduced upon virus infection or...
poly(I:C) transfection. These data further confirm that AIP is an essential negative regulator of antiviral signaling.

We next conducted quantitative real time PCR assays to examine the effect of either AIP overexpression or deficiency on type I IFN mRNAs. In wild-type MEFs, transfection of IRF7 inhibited the expression of IFN-α mRNA, which was further potentiated by SeV infection (Fig. 3E). However, AIP overexpression significantly diminished SeV/IRF7-induced expression of IFN-α mRNA (Fig. 3E). We next reconstituted Aip−/− MEFs by transient transfection with an AIP plasmid infected with SeV and examined the expression of IFN-β, IFN-α4, and IL-6 mRNAs by quantitative real time PCR. As expected, SeV infection up-regulated the expression of IFN-β, IFN-α4, and IL-6 mRNAs in Aip−/− MEFs; however, reconstitution with AIP suppressed virus-mediated induction of type I IFN (IFN-β and IFN-α4), but not IL-6 (Fig. 3F). Given that IL-6 is predominantly regulated by the NF-κB pathway downstream of RIG-I, these data indicate that AIP specifically suppresses the IRF pathway that controls type I IFN.

**AIP Specifically Targets IRF7 for Inhibition by Interfering with Its Nuclear Localization**—Thus far, we have demonstrated that AIP interacts with IRF7 in a virus-inducible manner and that AIP suppresses virus-induced type I IFN production. IRF3 is a key transcription factor activated by TBK1 downstream of RIG-I and MAVS that induces the expression of type I IFN (51). The next series of studies were undertaken to determine whether AIP inhibited IRF7 and/or IRF3 function. First, we conducted an IFN-β luciferase reporter assay to determine whether siRNA-mediated knockdown of AIP enhanced activation by a super-active form of IRF3 (IRF3SA), a phospho-mimetic containing five C-terminal Ser/Thr to Asp mutations (52). Although knockdown of AIP augmented SeV-mediated IFN-β activation, it had no effect on IRF3SA-induced activation of IFN-β (Fig. 4A).

IRF3 and IRF7 are activated upon phosphorylation by the IKK-related kinases TBK1 and IKKe (10). Western blotting was performed to determine whether AIP blocked the phosphorylation of IRF3 and/or IRF7. IRF3 phosphorylation was detected by a phosphorylation specific antibody that recognizes phosphorylated Ser-396. As expected, overexpression of a constitutively active form of RIG-I (ΔRIG-I) comprising the N-terminal CARD domains elicited phosphorylation of endogenous IRF3 (Fig. 4B). Overexpression of AIP had no effect on IRF3 phosphorylation; however, the ubiquitin-editing enzyme A20 blocked IRF3 phosphorylation as previously reported (Fig. 4B) (21). To investigate IRF7 phosphorylation, we transfected IRF7 in 293T cells together with a TBK1 expression plasmid. Because of a lack of functional phospho-IRF7 antibodies, we monitored IRF7 phosphorylation by a band shift caused by a slower migration pattern on SDS-PAGE gels. Indeed, TBK1 triggered the phosphorylation of IRF7, which was not inhibited by AIP overexpression (Fig. 4C). Therefore, AIP does not block either IRF3 or IRF7 phosphorylation.

Upon TBK1-induced phosphorylation, IRF3 and IRF7 form homodimers and translocate to the nucleus to activate type I IFN genes (52, 53). Therefore, we next examined whether AIP inhibited virus-triggered nuclear localization of IRF7 and IRF3. First, we conducted biochemical fractionation experiments to examine virus-induced IRF7 nuclear translocation in the absence or presence of AIP. As expected, SeV infection promoted the nuclear translocation of IRF7 (Fig. 5A). However, exogenous AIP blocked SeV-induced IRF7 nuclear localization (Fig. 5A). AIP expression was mainly detected in the cytoplasmic compartment (Fig. 5A). Therefore, AIP may inhibit IRF7 by antagonizing its nuclear localization. To further examine this notion, we conducted immunofluorescence microscopy experiments in Aip−/− MEFs reconstituted with an AIP plasmid and subsequently infected with SeV. We also transfected an IRF7 plasmid because MEFs expressed extremely low levels of endogenous IRF7 (data not shown). In uninfected Aip−/− MEFs (in the absence or presence of AIP transfection), IRF7 was mostly found in the cytoplasm (Fig. 5B). After SeV infection, IRF7 was mostly found in the nucleus; however, reconstitution of Aip−/− MEFs with AIP blocked SeV-mediated IRF7 nuclear localization (Fig. 5B). This effect was specific to IRF7 because AIP did not inhibit SeV-induced nuclear translocation of IRF3 (Fig. 5C). Therefore, AIP specifically blocks virus-induced IRF7, but not IRF3, nuclear localization.

Finally, we examined the effect of AIP on IRF7 transactivation using a Gal4-IRF7 fusion comprising the DNA-binding domain of Gal4 fused to the coding sequence of IRF7 (46). Luciferase assays were conducted using a reporter plasmid containing the luciferase coding gene driven by Gal4 binding sites.
Expression of Gal4-IRF7 strongly activated the Gal4-Luc reporter; however, AIP suppressed Gal4-IRF7 activation in a dose-dependent manner (Fig. 5D), indicating that AIP blocks IRF7 transactivation.

**Discussion**

Antiviral signaling and the production of type I IFN are tightly regulated to ensure effective viral clearance with minimal collateral tissue damage and without excessive inflammation. However, chronic virus infection or genetic factors may perturb the delicate balance between activation and termination of antiviral signaling pathways. In this regard, uncontrolled production of type I IFNs has been implicated in a number of cancers and immune disorders such as psoriasis, systemic lupus erythematosus, and multiple sclerosis (54–57). In this study, we have identified AIP as a novel inhibitor of the RIG-I antiviral pathway and virus-induced type I IFN production. In addition to its well studied role as a chaperone and regulator of AhR subcellular localization, AIP also interacts with and limits the activation of IRF7 during virus infection.

Our results indicate that AIP interacts with IRF7 and inhibits virus-induced nuclear localization of IRF7. We have mapped the AIP binding domain in IRF7 to the VAD. The IRF7 VAD domain between amino acids 278 and 305 is required for optimal activation of IRF7 and is necessary for virus-induced IRF7 nuclear translocation (42). Therefore, it is tempting to speculate that AIP blocks IRF7 nuclear translocation by interacting with and masking the VAD domain. AIP may also maintain IRF7 in an inactive “closed” form similar to what has been proposed for Kaposi’s sarcoma herpesvirus ORF45 inhibition of IRF7 (58). The following mechanisms may also contribute to the inactivation of IRF7: 1) AIP may induce a conformational
change in IRF7 that impairs NLS recognition or binding to import machinery; 2) AIP may enhance nuclear export of IRF7; and 3) AIP may promote the posttranslational turnover of nuclear IRF7. Interestingly, the inhibition of IRF7 nuclear localization by AIP is strikingly similar to the regulation of AhR by AIP. AIP binds to AhR and inhibits AhR nuclear translocation by triggering a conformational change that prevents binding of its bipartite NLS to importin-β (38). Therefore, AIP may antagonize the nuclear localization of AhR and IRF7 transcription factors by similar mechanisms, although further studies are needed to address this possibility.

AIP is ubiquitously expressed in human tissues and cell lines, and it appears that AIP expression is not induced by virus infection (data not shown). However, our results indicate that the interaction between endogenous AIP and IRF7 is enhanced by virus infection and thus raises the possibility that AIP functions as a negative feedback inhibitor of antiviral signaling. It is likely that AIP undergoes specific post-translational modifications in response to virus infection that facilitates enhanced interactions with IRF7. Indeed, our preliminary studies suggest that overexpression of TBK1 alters the mobility of AIP on SDS-PAGE gels and promotes a band shift that could represent phosphorylation (Fig. 4C). Future studies will identify potential virus-triggered post-translational modifications in AIP and how these regulate AIP inhibition of IRF7 and antiviral signaling.

Because IRF7 functions as the master regulator of type I IFN production, not surprisingly it is targeted for inactivation by a number of viruses. For example, Ebola Zaire virus VP35 (59), Epstein-Barr virus BZLF1/LF2 (60, 61), and Kaposi's sarcoma herpesvirus RTA/ORF45/viIRF3 (62–64) all directly target IRF7 for inhibition. Another potential mechanism by which viruses may inactivate IRF7 is by hijacking host-encoded inhibitors of antiviral signaling. In this regard, we have reported that human T cell leukemia virus type 1 Tax hijacks a host factor, SOCS1 (suppressor of cytokine signaling 1), to inhibit antiviral signaling and dampen type I IFN production (65). In light of our findings that AIP functions as a negative regulator of IRF7 and antiviral signaling, it is interesting that AIP has been found to interact with several viral proteins such as hepatitis B virus X protein (31) and EBNA-3 (39). It is plausible that certain viruses may hijack AIP to restrict antiviral signaling and promote virus replication.

AhR has been long known as a regulator of the host xenobiotic response to aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzop-dioxin (66). More recently, AhR has been implicated as an important regulator of adaptive immunity and the development of regulatory T cells and Th17 T cells (67, 68). AhR also regulates the host response to virus infection. Treatment of mice with a low dose of the AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin increased morbidity and mortality in mice infected with influenza A virus (69), by impairing the priming of CD8+ T cells in the lung (70). Given the broad roles of AhR in the regulation of multiple facets of immune cell development, activation, and differentiation, it will be interesting to determine whether AhR inhibits innate antiviral signaling and type I IFN together with AIP.

Gene targeting studies in mice have revealed that AIP plays essential roles in cardiac development and maintaining productive erythropoiesis (50). In humans, germ line mutations in AIP have been identified and linked to the development of pituitary adenomas (71). AIP mutations have been found in 30% of familial isolated pituitary adenomas, and ~80% of patients with AIP-related familial isolated pituitary adenomas exhibit acromegaly or gigantism caused by excessive growth hormone secretion (72). Many of the reported AIP mutations lead to loss of function and result in truncated or no protein (73), suggesting that AIP functions as a tumor suppressor gene in the pituitary gland. Whether familial isolated pituitary adenomas patients with loss of function mutations in AIP are more resistant to virus infection and/or exhibit heightened inflammatory responses because of increased IRF7 activation remains to be seen. In summary, we have identified AIP as an inhibitor of IRF7 and virus-induced type I IFN. AIP expression and/or function may potentially be modulated to control IRF7 activation and the production of type I IFN during virus infections or in the setting of autoimmunity.

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