NIH Public Access
Author Manuscript
J Immunol. Author manuscript; available in PMC 2011 December 15.

Published in final edited form as:
J Immunol. 2010 December 15; 185(12): 7385–7393. doi:10.4049/jimmunol.1002468.

Aim2-deficiency stimulates the expression of interferon-inducible *Ifi202*, a lupus susceptibility murine gene within the *Nba2* autoimmune susceptibility locus

Ravichandran Panchanathan[1,2], **Xin Duan**[1], **Hui Shen**[1,2], **Vijay A. K. Rathinam**[3], **Loren D. Erickson**[4], **Katherine A Fitzgerald**[3], and **Divaker Choubey**[1,2]

[1] Department of Environmental Health, University of Cincinnati, 3223 Eden Avenue, P. O. Box-670056, Cincinnati, OH 45267

[2] Cincinnati VA Medical Center, 3200 Vine Street, Cincinnati, OH 45220

[3] Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA

[4] Department of Microbiology, University of Virginia, Charlottesville, VA 22908, USA

Abstract

Murine Aim2 and p202 proteins (encoded by the *Aim2* and *Ifi202* genes) are members of the interferon (IFN)-inducible p200-protein family. Both proteins can sense double-stranded DNA (dsDNA) in the cytoplasm. However, upon sensing dsDNA, only the Aim2 protein through its pyrin domain (PYD) can form an inflammasome to activate caspase-1 and induce cell death. Given that the p202 protein has been predicted to inhibit the activation of caspase-1 by the Aim2 protein and that increased levels of the p202 protein in female mice of certain strains are associated with lupus susceptibility, we compared the expression of *Aim2* and *Ifi202* genes between *Aim2*-deficient and age-matched wild type mice. We found that the *Aim2*-deficiency in immune cells stimulated the expression of *Ifi202* gene. The increased levels of the p202 protein in cells were associated with increases in the expression of IFN-β, STAT1, and IFN-inducible genes. Moreover, after knockdown of *Aim2* expression in the murine macrophage cell line J774.A1, IFN-β treatment of cells robustly increased STAT1 protein levels (as compared to control cells), increased the activating phosphorylation of STAT1 on Tyr-701, and stimulated the activity of an IFN-responsive reporter. Notably, the expression of *Aim2* in non lupus-prone (C57BL/6 and B6.*Nba2*-C) and lupus-prone B6.*Nba2*-ABC splenic cells and in a murine macrophage cell line that overexpressed p202 protein was found to be inversely correlated with *Ifi202*. Collectively, our observations demonstrate an inverse correlation between Aim2 and p202 expressions. We predict that defects in Aim2 expression within immune cells contribute to increased susceptibility to lupus.

Address Correspondence to: Divaker Choubey, Department of Environmental Health, University of Cincinnati, 3223 Eden Avenue, P.O. Box 670056, Cincinnati, OH 45267; Ph.: 513-558-1014, Fax: 513-558-0925, Divaker.choubey@uc.edu.

Disclosures

The authors have no financial conflicts of interest.

Publisher's Disclaimer: "This is an author-produced version of a manuscript accepted for publication in *The Journal of Immunology* (*The JI*). The American Association of Immunologists, Inc. (AAI), publisher of *The JI*, holds the copyright to this manuscript. This version of the manuscript has not yet been copyedited or subjected to editorial proofreading by *The JI*; hence, it may differ from the final version published in *The JI* (online and in print). AAI (*The JI*) is not liable for errors or omissions in this author-produced version of the manuscript or in any version derived from it by the U.S. National Institutes of Health or any other third party. The final, citable version of record can be found at www.jimmunol.org."

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Keywords

Aim2 inflammasome; p202; interferon; inflammation; SLE

Introduction

The interferon (IFN)-inducible *Ifi200*-gene family includes several genes that encode structurally and functionally-related proteins (the p200-family proteins) (1–4). The gene family includes the murine (for example, *Ifi202a*, *Ifi202b*, *Ifi203*, *Ifi204*, *Ifi205*, and *Aim2*) and human (for example, *IFI16*, *MNDA*, *IFIX*, and *AIM2*) genes. The murine *Ifi200*-family genes cluster within the New Zealand Black (NZB)-derived *Nba2* lupus susceptibility interval, which is syntenic to the 1q21–23 region in humans (2,4,5). Notably, the identification of the murine *Aim2* gene and its sequence analysis revealed that the Aim2 protein sequence is conserved (55% amino acid identities) between mice and humans (6).

Constitutive expression of the human *AIM2* gene is detectable in the spleen, small intestine and peripheral leukocytes (6,7). Furthermore, IFN-γ treatment of the human HL-60 cell line (6) or IFN-β treatment of the human THP-1 cell line (8) increases the *AIM2* mRNA levels. The *AIM2* gene contains a microsatellite instability site that results in the inactivation of the gene in certain human cancers (9). Also, the *AIM2* gene is silenced by DNA-methylation (9) and reduced levels of the *AIM2* mRNA have been noted in peripheral blood mononuclear cells from SLE patients (10). Like the human AIM2 protein, the expression of the murine Aim2 protein is detectable in splenic cells, thioglycollate-elicited macrophages (TEMs), and bone marrow-derived macrophages (BMDMs) (11). Moreover, the IFN-β treatment of TEMs further increased the levels of Aim2 protein (11).

We reported earlier that steady-state levels of the *Ifi202* mRNA and protein (the p202 protein) are higher in splenic cells from 4-month-old (preautoimmune) B6.*Nba2* congenic (congenic for the *Nba2* interval on C57BL/6 genetic background) female mice than the age-matched C57BL/6 (B6) females (2,5). In contrast to *Ifi202*, levels of the *Ifi203* mRNA were lower in the congenic female mice than the B6 female mice and the levels of *Ifi204* did not differ measurably (5). Levels of the *Aim2* mRNA were not compared (the *Aim2* probe was not present on the microarray GeneChip). Given that the *Ifi202*, *Ifi203*, and *Ifi204* genes are IFN-inducible genes (1,2) and the activation of IFN-signaling is associated with the development of lupus diseases (12), the increased expression of *Ifi202* gene in the B6.*Nba2* congenic female mice (as compared to B6 female mice) prompted us and others to further investigate the potential role of p202 protein in lupus susceptibility (2,4).

The expression of the *Ifi202* gene (possibly both *Ifi202a* and *Ifi202b* genes)is up-regulated after treatment of cells with type I or type II interferon (2–4). The up-regulation is shown to be through the IFN-responsive *cis*-elements (termed interferon-stimulated response elements or ISRE) in the promoter region of the *Ifi202* gene (2). We have demonstrated that the promoter polymorphisms, which are predicted to affect the transcription of *Ifi202*, contribute to increased constitutive expression of the *Ifi202a* gene in certain lupus-prone strains of mice (4,5). We have noted that steady-state levels of the *Ifi202* mRNA in splenic cells from non lupus-prone B6 and NZW mice are at least ~10–100-fold lower than the lupus-prone NZB or B6.*Nba2* congenic mice (5). However, steady-state levels of p202 protein in splenic B cells ($B220^{+}$) from B6.*Nba2* female mice are only 2–3-fold higher than age and gender-matched B6 mice (13). Interestingly, the B6.*Nba2* congenic female mice develop detectable levels of autoantibodies beginning ~6-months of age against the nuclear antigens and exhibit increased serum levels of type I IFNs and the expression of *Ifi202* (5,14). However, the B6.*Nba2* female mice that are deficient in the IFN-α/β-receptor fail to develop

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

autoantibodies and express reduced (~ 2-fold less) levels of *Ifi202* mRNA (15). Furthermore, ~7-month-old B6.*Nba2-C* sub-congenic female mice do not develop antinuclear antibodies and do not exhibit increased serum levels of type I IFNs (14).

Basal and induced levels of p202 protein are regulated by transcriptional and post-transcriptional mechanisms (2). Agents besides the IFNs that are known to induce the expression of *Ifi202* gene include IL-6 (16) and the female hormone estrogen (17). Interestingly, basal levels of the p202 protein are detected primarily in the cytoplasm of splenic B cells (13) and mouse embryonic fibroblasts (MEFs) (18) from the B6.*Nba2* congenic mice. Moreover, IFN-α treatment of cells potentiates the nuclear accumulation of p202 protein (13,18). Consistent with the above observations, the generation of congenic NZB mice that were deficient in the α-chain of IFN-α/β receptor revealed that the receptor deficiency did not affect the basal levels of p202 protein (19). However, the IFN receptor deficiency ameliorated the symptoms of lupus diseases (19).

Both Aim2 and p202 proteins can form homo- and heterodimers (6,20) and recognize dsDNA in the cytoplasm through oligosaccharide/oligonucleotide-binding fold (OB-fold) located within the 200-amino acids repeat (also called the HIN-200 domain) (6). The Aim2 protein contains a homotypic protein-protein interaction pyrin domain (PYD) in the N-terminus (6). After sensing dsDNA in the cytoplasm, the PYD of the human AIM2 and murine Aim2 proteins interacts with an adaptor protein apoptosis-associated speck-like protein containing a caspase-activating recruitment domain (ASC) and forms an inflammasome (6,8,21–23). The AIM2/Aim2 inflammasome activates caspase-1 (resulting in the generation of p20 and p10 fragments), which processes the pro-IL-1β and pro-IL-18 for release, and induces cell death by pyroptosis (caspase-1-dependent cell death) (6,23).

In contrast to the Aim2 protein, the p202 protein lacks the pyrin domain (6,24). Therefore, p202 protein, upon sensing dsDNA in the cytoplasm, can not form an inflammasome (6). Notably, the knockdown approach has identified p202 protein as an inhibitor of cytosolic DNA-induced caspase-1 (and caspase-3) activation (24). Accordingly, the caspase-1 activation in macrophages in response to dsDNA correlated inversely with the levels of the p202 protein in three strains of mice (24). Thus, it has been predicted that the Aim2 protein promotes and the p202 protein represses the activation of caspase-1 in response to cytoplasmic DNA (24,25).

Recent studies (11,26,27) involving the generation of *Aim*2-deficient mice revealed that *Aim*2-deficient mice are more susceptible to certain bacterial (for example, *Francisella tularensis* and *Listeria monocytogenes*) and viral (for example, murine CMV) infections in spite of increased serum levels of IFN-β. Moreover, immune cells (splenic cells and BMDMs) from the *Aim2*-deficient mice were defective in the activation of caspase-1, secretion of IL-1β and IL-18, and induction of cell death upon infection by certain intracellular pathogens (11,26,27). Additionally, these studies indicated that Aim2 expression is not needed for type I interferon production after mice are infected with certain pathogens or when cells are transfected with dsDNA (11,25–27). Instead, the Aim2 protein appears to act negatively toward regulating the IFN responses (11,25–27).

Given that the p202 protein can not form an inflammasome upon sensing dsDNA in the cytoplasm and that increased levels of p202 in female mice of certain strains are associated with increased lupus susceptibility (4,6), we compared the expression of *Aim2* and *Ifi202* genes between *Aim2*-deficient and age-matched wild type mice. Here we report that *Aim2*-deficiency in immune cells stimulates the expression of IFN-inducible *Ifi202* gene.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Materials and Methods

Mice

Generation of *Aim2*-deficient mice on the mixed (129×B6) genetic background has been described (11). Splenic and bone marrow cells were isolated from wild-type or age-matched *Aim2*-deficient male and female mice(age ~4–6 weeks) that were housed in pathogen-free animal facilities at the University of Massachusetts, Worcester, MA. Similarly, total splenic cells were isolated from ~4-month-old B6, B6.*Nba2*-ABC (same as B6.*Nba2*), and B6.*Nba2*-C sub-congenic male and age-matched female mice(both B6-*Nba2*-ABC and B6.*Nba2*-C mice were preautoimmune; ref. 14) that were housed in pathogen-free animal facilities at the University of Virginia, Charlottesville, VA. Age-matched male and female non-autoimmune B6 and pre-autoimmune young (age ~6–8 weeks) B6.*Nba2*, NZB, and (NZB × NZW)F_1 mice were purchased from The Jackson Laboratory (Bar Harbor, Main) and housed in pathogen-free animal facilities at the University of Cincinnati. The Institutional Animal Care and Use Committee (IACUC) at the institution, where the mice were housed, approved the protocol to use mice for studies described here.

Splenocytes and bone marrow cells isolation, cell culture, and treatments

Total single cell splenocytes were prepared from male or age-matched female mice as described previously (17). After the lysis of red blood cells, splenocytes werere-suspended in RPMI 1640 medium supplemented with 10% fetal bovineserum. Bone marrow cells (BMCs) were isolated as described previously (28). Unless otherwise indicated, splenic or bone marrow cells from two or more age-matched male or female mice were pooled to prepare total RNA or protein extracts. Bone marrow-derived macrophages (BMDMs) were purified using magnetic beads (purification kit purchased from Miltenayi Biotech, Auburn, CA) allowing the positive selection of CD11b$^+$ cells. The purified (>90–95% pure) BMDMs were cultured in RPMI-1640 supplemented with 10% fetal bovine serum and GM-CSF (10 ng/ml) for treatments.

Murine macrophage J774.A1 and RAW264.7 cell lines were purchased from the American Type Culture Collection (ATCC) and maintained as suggested by ATCC. When indicated, sub-confluent cultures of cells were treated with the murine IFN-β (1,000 u/ml; from PBL Biomedical laboratories, Piscataway, NJ) for the indicated times. Mouse embryonic fibroblasts (MEFs) that were isolated from wild type or *Aim2*-deficient embryo were cultured in DMEM (high glucose) cell culture medium (supplemented with 10% fetal bovine serum and antibiotics).

To overexpress p202 protein in RAW264.7 cells, sub-confluent cultures of cells (in a six well plate) were transfected with an empty vector (pCMV) or p202 expression plasmid (pCMV-202) using FuGene 6 transfection reagent (Roche Applied, Indianapolis, IN) as suggested by the supplier. 24 hours after transfections, cells were split and the transfected cells were selected in G418 (400 μg/ml) for ~10-days. The G418-resistant colonies (>200 colonies) were pooled and cell cultures were maintained without G418 in the medium for several days before performing experiments that are described here.

To knockdown *Aim2* or *Ifi202* expression in J774.A1 cells, sub-confluent cultures of cells (in a six well plate) were infected with lentivirus (purchased from Santa Cruz Biotech, Santa Cruz, CA) encoding either shRNA to *Aim2* (sc-140968-V) or *Ifi202* (sc-40698-V) gene. As a control, cells were infected with the lentivirus encoding a control shRNA (sc-108080). 24 hours after infections, cells were selected in puromycin (1 μg/ml) for at least 5-days. Puromycin-resistant cells were pooled and cell cultures were maintained without puromycin in the medium for three days before experiments.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Reporter assays

For reporter assays, sub-confluent cultures of J774.A1 cells(in a 6-well plate) were transfected with the reporter plasmid ISRE-luc (purchased from Clontech, Mountain View, CA; 2.5 μg) and pRL-TK (purchase from Promega, Madison, WI; 0.5 μg), using FuGENE 6 (Roche Applied, Indianapolis, IN), as suggested by the supplier. When indicated, cells were either left untreated (control) or treated with the murine IFN-β (1,000 u/ml for 15 h). After transfections, cells were harvested between 40–45 h. Cells were lysed and the firefly and *Renilla* dual luciferase activities were determined as described previously (17).

Isolation of RNA from splenocytes, BMCs, and RT-PCR

Total splenocytes or BMCs (5–8 × 10^6 cells) were used to prepare RNA using TRIzol (Invitrogen, Carlsbad, CA) method (17). Total RNA preparation was digested with DNase I (to remove the contaminating genomic DNA) and 0.5–2 μg of RNA was used for RT-PCR reaction using the Superscript one-step RT-PCR system (from Invitrogen). PCR reactions were performed using a pair of the *Ifi*202 (primers: forward: 5′-ggtcatctaccaactcagaat-3′; reverse primer: 5′-ctctaggatg ccactgctgttg-3′), *Ifi203* (primers: forward: 5′-gatggctgaatacaagaatattg-3′; reverse: 5′-tcagaagtatggttccagaga-3′), or the *Aim2* (primers: forward: 5′-acagtggccacggaga-3′; reverse: 5′-aggtgacttcactccaca-3′) gene-specific primers. The conditions for the regular PCR have been described previously (17).

Quantitative real-time TaqMan PCRs were performed using the 7300 Real-Time PCR System (from Applied Biosystems, Foster City, CA, USA) and the commercially available real-time TaqMan gene expression assays. The PCR cycling program consisted of denaturation at 95°C for 10 min, 40 cycles at 95°C for 15 seconds, followed by annealing and elongation at 60°C for 1 min. The TaqMan assays for *Ifi202* (Assay Id# Mm0304 8198_m1; the assay allowing the detection of both *Ifi202a* and *Ifi202b* mRNA levels), *Aim2* (Assay Id# Mm01295719_m1), *Ifnb* (Assay Id# Mm00439552_s1), *Rsad2* (Assay Id# Mm00491265_m1), *Syn25* (Assay Id# Mm00836412_m1), and the endogenous *Actb* control (cat # 4352933E) were purchased from Applied Biosystems (Foster City, CA) and used as suggested by the supplier.

Immunoblotting and cell fractionation

As described previously (17), total cell lysates from splenocytes or J774.A1 cells were prepared in a modified radio-immune precipitation assay (RIPA) lysis buffer, and the lysates containing equal amounts of protein were processed for immunoblotting. The p202 antiserum, which detects both p202a and p202b proteins in immunoblotting, has been described previously as well (29). To detect p202 protein in extracts from J774.A1 cells, we also used monoclonal antibodies to p202 (sc-166253) from Santa Cruz Biotech. Antibodies to detect mouse Aim2 protein were raised in rabbits using a C-terminal peptide (KVIKAAKPKTDMKSVE). The specificity of the antibodies was confirmed through immunoblotting that used bacterially expressed murine recombinant Aim2 protein. Antibodies to STAT1 (# 9172), p-STAT1$^{Tyr\text{-}701}$ (# 9171), and β-actin (# 4967) were purchased from Cell Signaling Technology (Danvers, MA). Antibodies to Viperin (sc-102099) and ASC (sc-22514) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies (AHZ0082) to detect caspase-1 and cleaved caspase-1 were purchased from Invitrogen.

Wild type or *Aim2*-deficient MEFs were lysed using a detergent lysis procedure to prepare cytoplasmic and nuclear fractions as described previously (29). Fractions containing equal amounts of proteins were analyzed by immunoblotting.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Statistical analyses

The measurement values are presented as means ± SEM. The statistical significance of differences in the measured mean frequencies between the two groups was calculated using the Student's two-tailed *t* test. A p value <0.05 was considered significant.

Results

Aim2-deficiency stimulates the Ifi202 expression

The generation of *Aim2*-deficient mice indicated that the Aim2 protein negatively regulates type I IFN responses (11,25–27). Therefore, we compared steady-state levels of the IFN-inducible *Ifi202* mRNA and protein between wild-type and age-matched *Aim2*-null mice at an early age (4–6 weeks). Because the expression of the *Ifi202* gene is gender-dependent (17), consequently, we included both male and age-matched female mice in our studies. As shown in Fig. 1A, *Aim2*-null splenic cells had higher basal levels of STAT1, p-STAT1, viperin, an IFN-inducible protein (11), and p202, as compared to the wild-type cells. Consistent with our previous observations (17), the p202 protein levels were higher in females than the age-matched males. Correspondingly, we noted increased steady-sate levels of the *Ifi202* mRNA in *Aim2*-null splenic and bone marrow-derived cells in the female mice as compared to the male mice (Fig. 1B–D). Moreover, consistent with our above observations, *Aim2*-deficient MEFs had higher levels of *Ifi202* mRNA (Fig. 1E) and protein (Fig. 1F) as compared to the wild-type MEFs and the p202 protein was detected in cytoplasmic as well as nuclear fractions (Fig. 1F). Furthermore, consistent with our previous observations (5), levels of *Ifi203* mRNA were inversely correlated with the *Ifi202* mRNA (Fig. 1G). Notably, no appreciable difference in the levels of the *Ifi203* mRNA was evident between males and females. Collectively, our above observations indicated that *Aim2*-deficiency in splenic cells, bone marrow cells, and MEFs results in increased levels of *Ifi202* mRNA and protein.

Aim2 expression is inversely correlated with the expression of IFN-β and the IFN-inducible genes

As noted above (Fig. 1A), our observations revealed that the *Aim2*-deficient splenic cells showed constitutive activation of IFN-signaling, as determined by increased levels of STAT1, p-STAT1, and IFN-inducible proteins (such as viperin and p202). Therefore, we compared steady-state levels of IFN-β mRNA between the wild type and *Aim2*-null splenic cells. As shown in Fig. 2A, steady-state levels of IFN-β (but not IFN-γ; data not shown) mRNA were higher in splenic cells from both male and female *Aim2*-deficient mice (in females more than males) as compared to the wild-type mice. Similarly, levels of the IFN-inducible viperin protein encoding mRNA (encoded by the *Rsad2* gene) (Fig. 2B) were significantly higher in *Aim2*-null cells than the wild type cells. Interestingly, levels of 2′-5′ synthetase mRNA were higher in *Aim2*-null male mice as compared to the wild type mice (Fig. 2C). However, no increase in mRNA levels was evident in the *Aim2*-null female mice. Although, it is not known whether gender-dependent factors regulate the expression of 2′-5′ synthetase gene, our above observations warrant further investigation. Collectively, the above observations indicated that the *Aim2* expression in splenic cells is inversely correlated with the IFN-β expression, the activation of IFN-signaling, and increased expression of certain IFN-inducible genes, including the *Ifi202*.

Knockdown of Aim2 expression potentiates the activating phosphorylation of STAT1 and the expression of IFN-inducible proteins

Our above observations that *Aim2*-deficient cells exhibit increased levels of IFN-β mRNA and p202 protein prompted us to investigate the molecular mechanisms by which the Aim2

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

protein suppresses the expression of IFN-inducible genes. For this purpose, we knocked down the expression of the *Aim2* gene in the murine macrophage cell line J774.A1. We chose these cells because they express detectable basal levels of Aim2 protein and the levels increased further (~2-fold) by type I or type II IFN treatment of cells (Fig. 3A). As shown in Fig. 3B and C, stable infection of cells with lentivirus encoding shRNA to *Aim2* gene efficiently reduced basal levels of Aim2 protein and mRNA as compared to cells infected with control shRNA. Unexpectedly, treatment of cells with IFN-β induced levels of the Aim2 protein (Fig. 3B) as well as mRNA (Fig. 3C) in *Aim2* knocked down cells. These observations are consistent with the possibility that the type I IFN-treatment of J774.A1 cells, by inhibiting the expression of Dicer (RNase III enzyme) (30), interferes with the knockdown of the *Aim2* gene by siRNA approach. Interestingly, the IFN-treatment resulted in the robust activating phosphorylation of STAT1 (Tyr-701) and increases in STAT1 and viperin protein levels. Given that the STAT1 expression is auto-stimulatory (31), these observations revealed that Aim2 expression in J774.A1 cells inhibits a step upstream to the activating phosphorylation of STAT1. Accordingly, the activity of an IFN-responsive reporter (the ISRE-luc) was stimulated ~5-fold more by IFN-β (1,000 u/ml) treatment in the Aim2 knocked down cells as compared to control cells (Fig. 3D). Importantly, the knockdown of Aim2 expression in J774.A1 cells also moderately (2–2.5-fold) increased basal levels of the *Ifi202* mRNA (Fig. 3E) and protein (Fig. 3F). This moderate increase in the p202 mRNA and protein levels is consistent with several previous observations (2,4,15). Collectively, our above observations revealed that knockdown of *Aim2* expression in J774.A1 cells potentiate the activating phosphorylation of STAT1 and the expression of IFN-inducible proteins, including the p202.

Lupus susceptible mice express reduced levels of the Aim2 protein

Given that the increased expression of p202 in immune cells is associated with increased lupus susceptibility in certain strains of female mice (4), the above observations that *Aim2*-deficiency stimulates the expression of p202 protein prompted us to compare steady-state levels of *Aim2* and *Ifi202* mRNAs and proteins in immune cells from non lupus-prone and lupus-prone mice. As shown in Fig. 4A and B, basal steady-state levels of *Aim2* mRNA were relatively higher in splenic cells from non lupus-prone B6 female mice than the age-matched NZB, B6.*Nba2* (same as B6.*Nba2*-ABC), and (NZB × NZW)F_1 females. In contrast, steady-state levels of the *Ifi202* mRNA were significantly higher in preautoimmune female mice than the age and gender-matched B6 mice. Because generation of the B6.*Nba2*-C mouse line (14), a sub-congenic line of the *Nba2* congenic mouse line (5), indicated that these mice do not produce antinuclear autoantibodies and type I IFNs at the age of ~7-months (14), our above observations that the expression of *Aim2* is inversely correlated with the expression of *Ifnb* and *Ifi202* genes, made it conceivable that B6.*Nba2*-C mice express *Aim2,* but not *Ifi202* gene. Therefore, we compared basal levels of the *Aim2*, *Ifi202*, and *Ifnb* mRNA among the B6, B6.*Nba2*-C, and B6.*Nba2*-ABC splenic cells isolated from ~4-month-old (preautoimmune) females. As shown in Fig. 4C & D, steady-state levels of *Aim2* mRNA were inversely correlated with both *Ifi202* and *Ifnb* mRNA in three strains of mice. Accordingly, the expression of the Aim2 protein was detectable in splenic cells from the B6 or B6.*Nba2*-C, but not B6.*Nba2*-ABC, female mice. Consistent with the post-transcriptional mechanisms regulating the p202 protein levels in a variety of cells (2,4), we did not detect the p202 protein levels in the B6.*Nba2*-C mice (Fig. 4E). Because both B6.*Nba2*-C and B6.*Nba2*-ABC females were preautoimmune (not expected to produce detectable levels of autoantibodies; 5, 14), we decided to compare levels of pro-caspase-1 and pro-IL-1β among B6, B6.*Nba2*-C, and B6.*Nba2*-ABC splenic cells. As shown in Fig. 4E, levels of the pro-caspase-1 protein were comparable among the three strains of female mice. However, levels of pro-IL-1β protein were significantly higher in the B6 mice as compared to the B6.*Nba2*-C or B6.*Nba2*-ABC females (Fig. 4E). Collectively, these observations indicated that lupus

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

susceptible B6.*Nba2*-ABC female mice express reduced levels of Aim2 protein in splenic cells before detection of any autoantibodies and that the reduced levels of the Aim2 protein in splenic cells are associated with increased levels of IFN-β mRNA, potentiation of IFN-signaling (as measured by the activating phosphorylation of STAT1), and increased steady-state levels of the p202 protein.

Expression levels of p202 protein are inversely correlated with Aim2 protein and the activation of caspase-1

Caspase-1 activation in macrophages, in response to dsDNA, inversely correlated with the levels of the *Ifi202* mRNA in three strains (B6, BALB/c, and NZB) of female mice (24). Given that the expression of p202 depends on gender (17), we compared the activation of caspase-1 in bone marrow-derived macrophages (BMDMs) between NZB male and age-matched female mice. We chose the NZB mice because basal levels of the p202 protein are readily detectable (5) and these mice generate detectable levels of the anti-DNA antibodies at an early age (19). As shown in Fig. 5A, the basal levels of p202 protein were several folds higher in BMDMs isolated from NZB females (age ~12 weeks) than the age-matched males. Interestingly, basal levels of Aim2 protein were inversely correlated with the p202 protein: detectable in BMDMs from the male mice, but not in female mice. Furthermore, IFN-β treatment of cells, which increased levels of the p202 protein, did not increase Aim2 protein levels. Moreover, basal levels of the caspase-1 p20 were lower in females than males (Fig. 5A) and IFN-β treatment of BMDMs from females, which further increased (~6-fold) the p202 protein levels, reduced the levels of caspase-1 p20 subunit.

Our above observations that increased basal and IFN-β-induced levels of the p202 protein in the NZB BMDMs inversely correlated with Aim2 protein levels prompted us to test whether the increased levels of p202 protein could regulate the expression of the *Aim2* gene. For this purpose, we overexpressed p202 protein in RAW264.7 cells, a murine macrophage cell line. We chose these cells because they do not express the adaptor protein ASC (24), therefore, allow stable overexpression of a gene of interest from a transfected plasmid. As shown in Fig. 5B and C, increased expression of *Ifi202* mRNA and protein in RAW264.7 cells reduced basal levels of *Aim2* mRNA and protein. Accordingly, knockdown of p202 expression in murine macrophage cell line J774.A1 also resulted in moderate (~2-fold) increases in Aim2 protein levels (Fig. 5D) and activation of the caspase-1 (as measured by the detection of p20 and p10 proteins) in response to transfection of synthetic DNA (poly dA.dT) into cells (Fig. 5D). Interestingly, under our experimental conditions, transfection of the synthetic DNA into J774.A1 cells increased levels of p202 protein, which slightly reduced levels of the activated caspase-1. Again, it is conceivable that the production of type I IFN in response to transfection of the synthetic DNA (poly dA.dT) into J774.A1 cells inhibited the efficiency of knockdown by siRNA approach through decreasing the levels of Dicer (30). Collectively, these observations indicated that the expression levels of p202 protein in cells are inversely correlated with Aim2 protein levels and the activation of caspase-1.

Discussion

Recent studies (11,26,27) demonstrated that *Aim2*-deficient mice are more susceptible to certain infections (than the wild type mice), which result in the release of pathogen-derived DNA into the cytoplasm of bone marrow-derived macrophages. These studies also indicated that *Aim2*-deficient mice are not defective in producing IFN-β after infections. Therefore, it has been proposed that the Aim2 inflammasome function is needed for the elimination of infected cells (through caspae-1-dependent cell death) to clear certain infections in mice and increase chances for survival.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Earlier, knockdown approach identified the p202 protein as a potential inhibitor of cytosolic DNA-induced caspase-1 (and caspase-3) activation by Aim2 inflammasome (24,25). Therefore, we compared the expression of *Aim2* and *Ifi202* genes between *Aim2*-deficient and age-matched wild type mice. Our experiments reveal that (i) *Aim2*-deficiency in mice increased levels of the IFN-β mRNA, levels of STAT1, p-STAT1 proteins, and the expression of IFN-inducible genes, including the *Ifi202*, as compared to the wild type mice (Figs. 1 and 2); (ii) IFN-β treatment of J774.A1 macrophage cells after knockdown of *Aim2* expression robustly increased levels of STAT1, its activating phosphorylation on Tyr-701, and stimulated the activity of an IFN-responsive reporter than the control cells (Fig. 3); (iii) the expression of *Aim2* in immune cells from certain strains of female mice (B6, B6.*Nba2*-C, and B6.*Nba2*-ABC) inversely correlated with the expression of *Ifnb* and *Ifi202* genes (Fig. 4); and (iv) expression levels of p202 protein in BMDMs from the NZB mice or RAW264.7 cells inversely correlated with Aim2 protein levels and the activation of caspase-1 (Fig. 5). These observations indicate that the activation of the type I IFN-signaling in *Aim2*-deficient immune or J774.A1 cells increases the expression of IFN-inducible proteins, including the p202. Moreover, these observations support the idea that increased levels of the p202 protein, a negative regulator of caspase-1 activation by Aim2 (24,25), increase the chances of survival for cells with cytosolic DNA, resulting in chronic activation of the innate immune responses that are often associated with the development of SLE.

SLE is an autoimmune disease characterized by chronic stimulation of the innate immune system by endogenous nucleic acids, resulting in increased levels of type I IFNs and associated defects in immune cells (32–34). SLE patients and murine models of the disease develop peripheral blood gene expression profile, which is characterized by an “IFN-α signature” (33,34). Moreover, the severity of SLE is correlated with high levels of type I IFNs and pathogenic auto-antibodies against the nuclear antigens (32–34). Because gender-dependent increased levels of the p202 protein in immune cells of certain strains of female mice are associated with increased production of antinuclear autoantibodies (4,17) and associated kidney pathology (35), our observations described here support the idea that cellular levels of the Aim2 protein at certain thresholds are necessary to suppress type I IFN response after sensing cytosolic DNA (either pathogen or self-derived). Given that defects in caspase-dependent cell death are associated with the development of lupus disease (36,37), our observations also support the idea that elimination of cells with cytosolic DNA through pyroptosis (caspase-1-dependent cell death) may be important to reduce the production of type I IFNs and chronic stimulation of the innate immune system.

The activation of caspase-1 in bone marrow-derived macrophages, in response to dsDNA, inversely correlated with the levels of p202 protein in three strains (B6, BALB/c, and NZB) of female mice (24). Interestingly, basal (spontaneous) activation of caspase-1 was only detected in macrophages from NZB females (and not in other strains of mice) and introduction of synthetic DNA into macrophages did not result in appreciable further increase in levels of activated caspase-1 (24). Therefore, our observations that the basal activation of caspase-1 was detectable in macrophages from NZB mice are consistent with the previous report (24). Moreover, our observations (Fig. 5) that macrophages from NZB males had higher basal levels of activated caspase-1 than age-matched females are consistent with basal and IFN-β-induced increased levels of p202 protein in females as compared to age-matched males. Collectively, these observations indicated the mouse gender-dependent differential activation of caspase-1 in immune cells by the expression levels of the Aim2 and p202 proteins.

Levels of IL-1β mRNA are reported to be significantly lower in peritoneal macrophages isolated from lupus-prone strains of mice (including the NZB mice) than nonautoimmune mice (including the B6 mice) when cultured under certain conditions (38). Given that the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

expression of pro-IL-1β is, in part, regulated by the transcription factor NF-κB (39) and defects in the activity of NF-κB in immune cells are associated with lupus disease (40), our observation that steady-state levels of pro-IL-1β are reduced in splenic cells from preautoimmune B6.*Nba2*-ABC and B6.*Nba2*-C females as compared to B6 mice (Fig. 4E) are of some interest and warrant further investigation to determine whether Aim2 and/or p202 regulate the expression of pro-IL-1β by modulating the activity of NF-κB in a particular splenic cell type (6).

Presently, it is not clear how defects in the expression of the *Aim2* gene in immune or J774.A1 cells increase steady-state levels of *Ifi202* mRNA and protein. Increased steady-state levels of IFN-β (but not IFN-γ) mRNA, STAT1 protein, and p-STAT1 in *Aim2*-deficient or knocked down cells make it likely that increased production of IFN-β through autocrine or paracrine mechanism activates the transcription of the *Ifi202* gene in cells (Fig. 6). Interestingly, gender-dependent expression of *Ifi202* in splenic B and T cells (17) makes it likely that *Aim2* expression in immune cells is also regulated in a gender-dependent manner. Consistent with this prediction, we have noted 4-fold higher levels of the Aim2 protein in purified splenic B cells ($B220^+$) from B6 male mice than the age-matched females (data not shown). However, further work will be needed to determine whether the gender-dependent factors also regulate the expression of *Aim2* in other cell types.

It has been proposed (27) that an unidentified sensor of cytosolic DNA, which has a relatively lower threshold than the Aim2 protein to sense cytosolic DNA, may induce the expression of IFN-β. Because *Aim2*-deficient cells appear to express more IFN-β (than the wild type cells) and that the Aim2 protein has the ability to homo and heterodimerize (6), it is conceivable that the Aim2 protein interacts with this cytosolic DNA sensor and negatively regulates its ability to induce IFN-β expression through the activation of IRF3 and/or NF-κB (Fig. 6). Further work will be needed to identify the molecular mechanisms by which the Aim2 protein suppresses a type I IFN response and the expression of IFN-inducible genes.

Overexpression of the human AIM2 or the murine Aim2 protein in transfected human embryonic kidney cells indicated that both proteins are detected in the cytoplasm (8,21,22). Likewise, uninduced basal levels of the p202 protein are also detected primarily in the cytoplasm of splenic B cells (13) and MEFs (18) from the B6.*Nba2* congenic mice. Interestingly, the IFN-α treatment of cells potentiates the nuclear accumulation of the p202 protein (13,18). Consistent with these observations, we found that increased levels of p202 protein in *Aim2*-deficient MEFs were detected both in the cytoplasm and nucleus (Fig. 1F). Therefore, it is likely that age and gender-dependent increased serum levels of type I IFNs in lupus-prone or *Aim2*-deficient mice potentiate the nuclear localization of the p202 protein in immune cells.

Our observations that overexpression of p202 in RAW264.7 cells decreases levels of *Aim2* mRNA and protein (Fig. 5) raise the possibility that promoter polymorphisms and gender-dependent increased levels of the p202 protein in immune cells of certain strains of female mice, such as B6.*Nba2*-ABC, down-regulate the expression of *Aim2.* Although, it remains to be determined how increased levels of p202 protein negatively regulate the levels of the *Aim2* mRNA and protein, the demonstrated ability of p202 protein to act as a transcriptional modulator for a number of transcription factors makes it conceivable that p202 protein represses the transcription of the *Aim2* gene. However, our observations do not rule out the possibility that polymorphisms in the *Aim2* gene contribute to its differential expression in certain strains of mice. Further work is in progress to elucidate the molecular mechanisms that regulate the differential expression of the *Aim2* gene in certain strains of mice.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

In conclusion, our observations provide support for our model (Fig. 6). The model predicts that levels of the Aim2 protein at certain thresholds are necessary for the suppression of a type I IFN response and the expression of the IFN-inducible proteins (including the p202). Additionally, the increased levels of the p202 protein in immune cells inhibit the formation of the Aim2 inflammasome in response to cytosolic DNA and the activation of caspase-1, thus, contributing to chronic stimulation of the innate immune responses. Given that the sequence of the murine Aim2 protein is conserved between mice and humans (6), our observations will serve basis to understand the role of the human AIM2 protein in defective innate immune responses that are associated with certain autoimmune diseases, including SLE.

Acknowledgments

This work was supported by a grant (AI066261) to D.C. A grant from the Lupus Research Institute supported work in the laboratory of L. D. E.

References

1. Choubey D, Snoddy J, Chaturvedi V, Toniato E, Opdenakker G, Thakur A, Samanta H, Engel DA, Lengyel P. Interferons as gene activators. Indications for repeated gene duplication during the evolution of a cluster of interferon-activatable genes on murine chromosome 1. J Biol Chem. 1989; 264:17182–17189. [PubMed: 2477366]
2. Choubey D, Kotzin BL. Interferon-inducible p202 in the susceptibility to systemic lupus. Front Biosc. 2002; 7:e252–262.
3. Wang C, Chatterjee G, Meyer JJ, Liu CJ, Manunath NA, Bray-Ward P, Lengyel P. Characteristics of three homologous 202 genes (*Ifi202a*, *Ifi202b*, and *Ifi202c*) from the murine interferon-activatable gene 200 cluster. Genomics. 1999; 60:281–294. [PubMed: 10493828]
4. Choubey D, Panchanathan R. Interferon-inducible *Ifi200*-family genes in systemic lupus erythematosus. Immunol Lett. 2008; 119:32–41. [PubMed: 18598717]
5. Rozzo SJ, Allard JD, Choubey D, Vyse TJ, Izui S, Peltz G, Kotzin BL. Evidence for an interferon-inducible gene, *Ifi202*, in the susceptibility to systemic lupus. Immunity. 2001; 15:435–443. [PubMed: 11567633]
6. Choubey D, Duan X, Dickerson E, Ponomareva L, Panchanathan R, Shen H, Srivastava R. Interferon-inducible p200-family proteins as novel sensors of cytoplasmic DNA: role in inflammation and autoimmunity. J Interferon Cytokine Res. 2010; 30:371–380. [PubMed: 20187776]
7. DeYoung KL, Ray ME, Su YA, Anzick SL, Johnstone RW, Trapani JA, Meltzer PS, Trent JM. Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. Oncogene. 1997; 15:453–457. [PubMed: 9242382]
8. Burckstummer T, Baumann C, Bluml S, Dixit E, Durnberger G, Jahn H, Planyavsky M, Bilban M, Colinge J, Bennett KL, Superti-Furga G. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol. 2009; 10:266–272. [PubMed: 19158679]
9. Woerner SM, Kloor M, Schwitalle Y, Youmans H, Doeberitz MK, Gebert J, Dihlmann S. The putative tumor suppressor AIM2 is frequently affected by different genetic alterations in microsatellite unstable colon cancers. Genes Chromosomes Cancer. 2007; 46:1080–1089. [PubMed: 17726700]
10. Flinn LJ. Genomic analysis of a human interferon-inducible gene family and systemic lupus erythematosus (a Ph. D. Thesis Abstract). J Interferon Cytokine Res. 2007; 27:819–834.
11. Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, Vanaja SK, Monks BS, Ganesan S, Latz E, Hornung V, Vogel SN, Szomolanyi-Tsuda E, Fitzgerald KA. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat Immunol. 2010; 11:395–402. [PubMed: 20351692]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

12. Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferon (α/β) in immunity and autoimmunity. Annu Rev Immunol. 2005; 23:307–336. [PubMed: 15771573]
13. Choubey D, Panchanathan R, Shen H, Duan X. Comment on “Development of murine lupus involves the combined genetic contribution of the *SLAM* and *FcgammaR* intervals within the Nba2 autoimmune susceptibility locus”. J Immunol. 2010; 184:4051–4052. [PubMed: 20368282]
14. Jorgensen TN, Alfaro J, Enriquez HL, Jiang C, Loo WM, Atencio S, Bupp MR, Mailloux CM, Metzger T, Flannery S, Rozzo SJ, Kotzin BL, Rosemblatt M, Bono MR, Erickson LD. Development of murine lupus involves the combined genetic contribution of the *SLAM* and *FcgammaR* intervals within the *Nba2* autoimmune susceptibility locus. J Immunol. 2010; 184:775–786. [PubMed: 20018631]
15. Jorgensen TN, Roper E, Thurman JM, Marrack P, Kotzin BL. Type I interferon signaling is involved in the spontaneous development of lupus-like disease in B6.*Nba2* and (B6.*Nba2* × NZW)F_1 mice. Genes Immunity. 2007; 8:653–662. [PubMed: 17882225]
16. Pramanik R, Jorgensen TN, Xin H, Kotzin BL, Choubey D. Interleukin-6 induces expression of *Ifi202*, an interferon-inducible candidate gene for lupus susceptibility. J Biol Chem. 2004; 279:16121–16127. [PubMed: 14764608]
17. Panchanathan R, Shen H, Bupp MG, Gould KA, Choubey D. Female and male sex hormones differentially regulate expression of *Ifi202*, an interferon-inducible lupus susceptibility gene within the *Nba2* interval. J Immunol. 2009; 183:7031–7038. [PubMed: 19890043]
18. Choubey D, Pramanik R, Xin H. Sub-cellular localization and mechanisms of nucleo-cytoplasmic distribution of p202, an interferon-inducible candidate for lupus susceptibility. FEBS Lett. 2003; 553:245–249. [PubMed: 14572632]
19. Santiago-Raber ML, Baccala R, Haraldsson KM, Choubey D, Stewart TA, Kono DH, Theofilopoulos AN. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J Exp Med. 2003; 197:777–788. [PubMed: 12642605]
20. Choubey D, Walter S, Geng Y, Xin H. Cytoplasmic localization of the interferon-inducible protein that is encoded by the *AIM2* (absent in melanoma) gene from the 200-gene family. FEBS Lett. 2000; 474:38–42. [PubMed: 10828447]
21. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 2009; 458:514–518. [PubMed: 19158675]
22. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature. 2009; 458:509–513. [PubMed: 19158676]
23. Schroder K, Muruve DA, Tschopp J. Innate immunity: cytoplasmic DNA sensing by the AIM2 inflammasome. Curr Biol. 2009; 19:R262–R265. [PubMed: 19321146]
24. Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, Hardy LL, Garceau V, Sweet MJ, Ross IL, Hume DA, Stacey KJ. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science. 2009; 323:1057–1060. [PubMed: 19131592]
25. Krieg AM. AIMing 2 defend against intracellular pathogens. Nat Immunol. 2010; 11:367–369. [PubMed: 20404848]
26. Fernandes-Alnemri T, Yu JW, Juliana C, Solorzano L, Kang S, Wu J, Datta P, McCormick M, Huang L, McDermott E, Eisenlohr L, Landel CP, Alnemri ES. The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. Nat Immunol. 2010; 11:385–393. [PubMed: 20351693]
27. Jones JW, Kayagaki N, Broz P, Henry T, Newton K, O’Rourke K, Chan S, Dong J, Qu Y, Roose-Girma M, Dixit VM, Monack DM. Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis*. Proc Natl Acad Sci U S A. 2010; 107:9771–9776. [PubMed: 20457908]
28. Rathinam VA, Hoag KA, Mansfield LS. Dendritic cells from C57BL/6 mice undergo activation and induce Th1-effector cell responses against *Campylobacter jejuni*. Microbes Infect. 2008; 10:1316–1324. [PubMed: 18725315]
29. Choubey D, Lengyel P. Interferon action: cytoplasmic and nuclear localization of the interferon-inducible 52-kD protein that is encoded by the *Ifi200*-gene from the gene 200 cluster. J Interferon Res. 1993; 13:43–52. [PubMed: 8454910]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

30. Wiesen JL, Tomasi TB. Dicer is regulated by cellular stresses and interferons. Mol Immunol. 2009; 46:1222–1228. [PubMed: 19118902]
31. Meraz MA, White JM, Sheehan KC. Targeted disruption of the *Stat1* gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell. 1996; 84:431–442. [PubMed: 8608597]
32. Crispin JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med. 2010; 16:47–57. [PubMed: 20138006]
33. Crow MK, Wohlgemuth J. Microarray analysis of gene expression in lupus. Arthritis Res Ther. 2003; 5:279–287. [PubMed: 14680503]
34. Baechler EC, Gregersen PK, Behrens TW. The emerging role of interferon in human systemic lupus erythematosus. Curr Opin Immunol. 2004; 16:801–807. [PubMed: 15511676]
35. Ichii O, Kamikawa A, Otsuka S, Hashimoto Y, Sasaki N, Endoh D, Kon Y. Overexpression of interferon-activated gene 202 (*Ifi202*) correlates with the progression of autoimmune glomerulonephritis associated with the MRL chromosome 1. Lupus. 2010; 19:897–905. [PubMed: 20167632]
36. Rathmell JC, Thompson CB. The central effectors of cell death in the immune system. Annu Rev Immunol. 1999; 17:781–828. [PubMed: 10358774]
37. Navratil JS, Ahearn JM. Apoptosis, clearance mechanisms, and the development of systemic lupus erythematosus. Curr Rheumatol Rep. 2001; 3:191–198. [PubMed: 11352787]
38. Fan H, Longacre A, Meng F, Patel V, Hsiao K, Koh JS, Levine JS. Cytokine dysregulation induced by apoptotic cells is a shared characteristic of macrophages from nonobese diabetic and systemic lupus erythematosus-prone mice. J Immunol. 2004; 172:4834–4843. [PubMed: 15067061]
39. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol. 2009; 27:693–733. [PubMed: 19302050]
40. Tsokos GC, Wong HK, Enyedy EJ, Nambiar MP. Immune cell signaling in lupus. Curr Opin Rheumatol. 2000; 12:355–363. [PubMed: 10990169]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

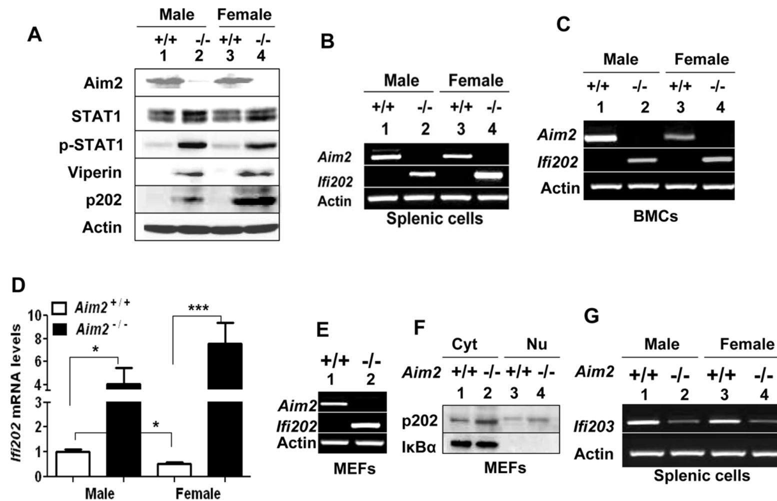

FIGURE 1. *Aim2*-deficiency stimulates the *Ifi202* expression

(**a**) Total cell lysates were prepared from splenocytes isolated from wild-type (lanes 1 and 3) and age-matched *Aim2*-deficient (lanes 2 and 4) male (lanes 1 and 2) or female mice (lanes 3 and 4) (age ~4 weeks). Extracts containing equal amounts of proteins were analyzed by immunoblotting using antibodies specific to the indicated proteins. (**b**) Total RNA was prepared from splenocytes isolated from wild-type and age-matched *Aim2*-deficient male or female mice (age ~5 weeks). Steady state levels of *Aim2* and *Ifi202* mRNA were analyzed by semi-quantitative PCR using a pair of primers specific to the indicated gene. (**c**) Total RNA was prepared from bone marrow cells isolated from wild-type and age-matched *Aim2*-deficient male or female mice (age ~5 weeks). Steady state levels of *Aim2* and *Ifi202* mRNA were analyzed by semi-quantitative PCR as described in (**b**). (**d**) Total RNA isolated from bone marrow cells from wild-type and age-matched *Aim2*-deficient male or female mice (age ~5 weeks) was analyzed by quantitative TaqMan real-time PCR using the assay specific to the *Ifi202* gene. The ratio of the test gene to *β2-microglobulin* mRNA was calculated in units (one unit being the ratio of the test gene to *β2-microglobulin* mRNA). The relative steady-state levels of *Aim2* or *Ifi202* mRNA in male wild-type mice are indicated as 1. Results are mean values of triplicate experiments and error bars represent standard deviation (* $p < 0.05$; *** $p < 0.001$). (**e**) Total RNA was prepared from MEFs isolated from wild-type and *Aim2*-deficient embryo. Steady state levels of *Aim2* and *Ifi202* mRNA were analyzed by semi-quantitative PCR using a pair of primers specific to the indicated gene. (**f**) MEFs isolated from wild-type and *Aim2*-deficient embryo were fractionated into the cytoplasmic (Cyt) and nuclear (Nu) fractions and fractions containing equal amounts of proteins were analyzed by immunoblotting using specific antibodies to p202 protein and the cytoplasmic IκBα protein, which served as a quality control for the fractionations. (**g**) Total RNA, which was isolated as described in (**b**), was analyzed for steady state levels of the *Ifi203* mRNA by semi-quantitative PCR using a pair of specific primers.

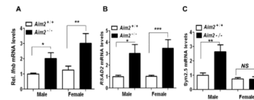

FIGURE 2. *Aim2* expression is inversely correlated with the expression of IFN-β and the IFN-inducible genes

Total RNA isolated from splenocytes from wild-type and age-matched *Aim2*-deficient male or female mice (age ~5 weeks) was analyzed by quantitative TaqMan real-time PCR using the assay specific to the *Ifnb* (**a**), *Rsad2* (**b**), or *Syn2,5* (**c**) gene. The ratio of the test gene to *β2-microglobulin* mRNA was calculated in units (one unit being the ratio of test gene to *β2-microglobulin* mRNA). The relative levels of the *Ifnb* (**a**), *Rsad2* (**b**), or *Syn2,5* (**c**) mRNA in the wild-type male mice are indicated as 1. Results are mean values of triplicate experiments and error bars represent standard deviation (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

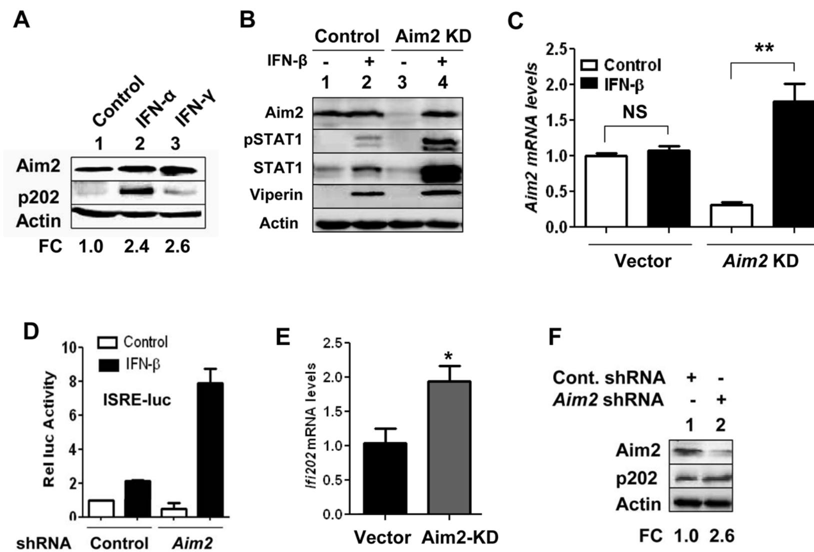

FIGURE 3. Knockdown of *Aim2* expression in J774.A1 cells potentiates the activating phosphorylation of STAT1 and the expression of IFN-inducible proteins

(**a**) Sub-confluent cultures of J774.A1 cells were either left untreated (control) or treated with IFN-α (1,000 u/ml for 14 h) or IFN-γ (10 ng/ml for 14 h). After the treatment, total cell extracts containing equal amounts of proteins were analyzed by immunoblotting using specific antibodies to the indicated proteins. (**b**) J774.A1 cells were either infected with lentivirus expressing control shRNA (lanes 1 and 2) or shAim2 RNA (lanes 3 and 4) and cells were selected in puromycin for a week. Puromycin resistant colonies were pooled and allowed to grow without the drug for three days. Total cell lysates prepared from cells either left untreated (control; lanes 1 and 3) or treated with IFN-β (lanes 2 and 4) for 18 h were analyzed by immunoblotting using antibodies specific to the indicated proteins. (**c**) J774.A1 cells stably infected with control lentivirus (Vector) or shAim2 lentivirus (Aim2 KD) were either left untreated or treated with IFN-β for 18 h. After the treatment, total RNA was analyzed by quantitative TaqMan real-time PCR using the assay specific to the *Aim2* gene. The ratio of the test gene to *β2-microglobulin* mRNA was calculated in units (one unit being the ratio of the test gene to *β2-microglobulin* mRNA). The relative steady-state levels of *Aim2* mRNA in vector untreated cells are indicated as 1. Results are mean values of triplicate experiments and error bars represent standard deviation (** $p <0.005$). (**d**) Sub-confluent cultures of J774.A1 cells (control or shAim2RNA), as described in (**a**), were transfected with the ISRE-luc-reporter plasmid (2.5 μg) along with pRL-TK (0.5 μg) reporter plasmid using FuGENE 6 transfection reagent. 24 h after transfections, cells were either left untreated or treated with IFN-β for 18 h. 40–45 h after transfections, cells were processed for dual luciferase activity. (**e**) Total RNA from stably infected with control lentivirus (Vector) or shAim2 lentivirus (Aim2 KD) J774.A1 cells was analyzed by quantitative TaqMan real-time PCR using the assay specific to the *Ifi202* gene. The ratio of the test gene to *β2-microglobulin* mRNA was calculated in units as described in (**c**). The relative steady-state levels of *Ifi202* mRNA in vector cells are indicated as 1. Results are mean values of triplicate experiments and error bars represent standard deviation (* $p <0.05$). (**f**) Extracts described in (**b**) were analyzed for levels of the p202 protein by immunoblotting. FC, indicates fold change in the p202 protein levels.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

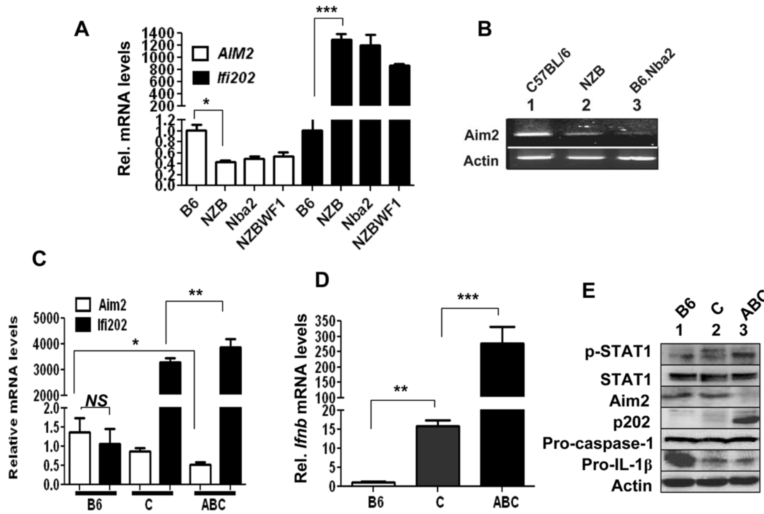

FIGURE 4. Lupus susceptible mice express reduced levels of the *Aim2* protein

(**b**) Total RNA prepared from splenocytes isolated from C57BL/6 (B6), NZB, B6.*Nba2* (Nba2), or (NZB × NZW)F_1 (NZBWF1) female mice (age ~9 weeks) was analyzed by quantitative TaqMan real-time PCR using an assay either specific to the *Aim2* or the *Ifi202* gene. The ratio of the test gene to *β2-microglobulin* mRNA was calculated in units (one unit being the ratio of the test gene to *β2-microglobulin* mRNA). The relative levels of *Aim2* or *Ifi202* mRNA in the B6 female mice are indicated as 1. Results are mean values of triplicate experiments and error bars represent standard deviation (* $p < 0.05$; *** $p < 0.001$). (**b**) Total RNA isolated in (**a**) was also analyzed by semi-quantitative PCR using a pair of primers specific to the indicated gene. (**c**) Total RNA prepared from splenocytes isolated from B6, B6.*Nba2*-C (C), or B6.*Nba2*-ABC (ABC) female mice (age ~4-months) was analyzed by quantitative TaqMan real-time PCR using assay either specific to *Aim2* or *Ifi202.* The ratio of the test gene to *β2-microglobulin* mRNA was calculated in units (one unit being the ratio of the test gene to *β2-microglobulin* mRNA in splenocytes). The relative levels of *Aim2* or *Ifi202* mRNA are indicated. Results are mean values of triplicate experiments and error bars represent standard deviation (*NS*, not significant; * $p < 0.05$; ** $p < 0.01$). (**d**) Total RNA as described in (**c**) was analyzed by quantitative TaqMan real-time PCR using assay specific to the *Ifnb* gene. The ratio of *Ifnb* mRNA to *β2-microglobulin* mRNA was calculated in units (one unit being the ratio of the *Ifnb* to *β2-microglobulin* mRNA in splenocytes). The relative levels of *Ifnb* mRNA in the B6 female mice are indicated as 1. Results are mean values of triplicate experiments and error bars represent standard deviation (** $p < 0.01$; *** $p < 0.001$). (**e**) Total cell extracts prepared from splenocytes isolated from B6, B6.*Nba2*-C (C), or B6.*Nba2*-ABC (ABC) female mice (age ~4-months) were analyzed by immunoblotting using antibodies specific to the indicated proteins.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

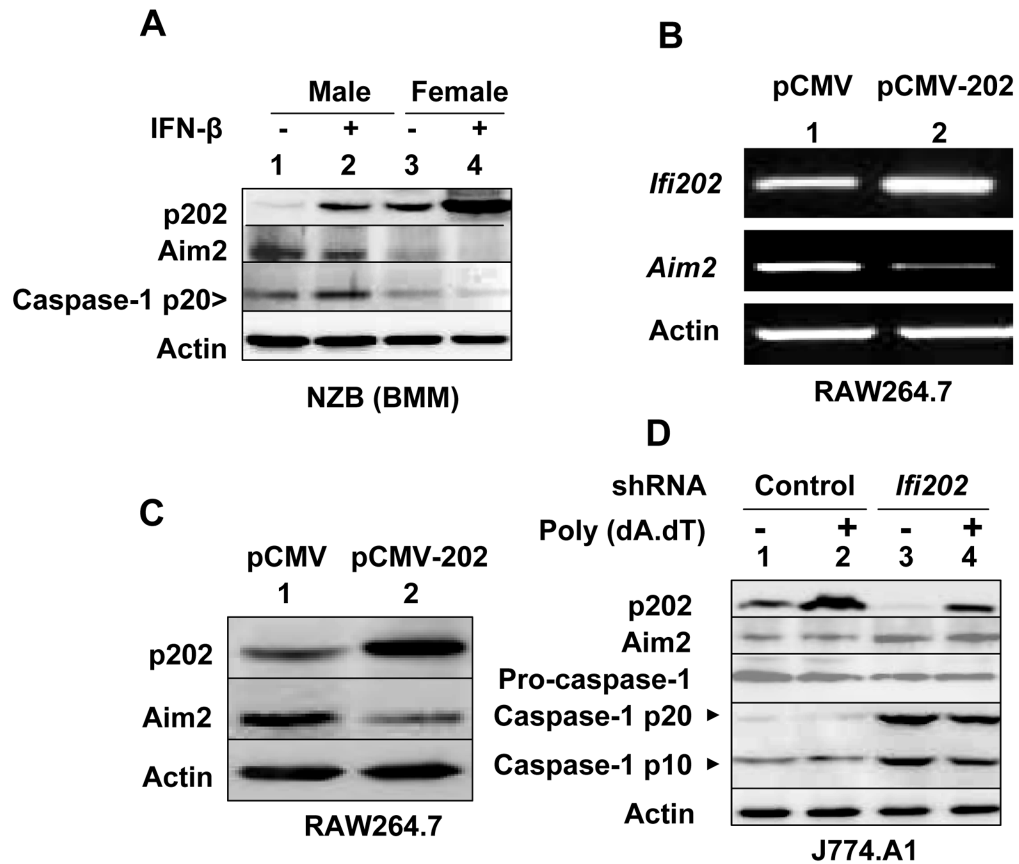

FIGURE 5. Expression levels of p202 protein are inversely correlated with the activation of caspase-1

(**a**) Purified bone marrow-derived macrophages from NZB males (lanes 1 and 2) or age-matched females (lanes 3 and 4) (age ~12 weeks) were either left untreated (lanes 1 and 3) or treated with IFN-β (lanes 2 and 4) for 15 h in the culture medium (supplemented with GM-CSF). After the treatment, total cell lysates containing equal amounts of protein were analyzed by immunoblotting using antibodies specific to the indicated proteins. Levels of the cleaved pro-caspase-1 (the p20) were detected in total cell lysates as described previously (21). (**b**) Total RNA isolated from RAW264.7 cells either stably transfected with a control vector (pCMV) or a plasmid (pCMV-202) that allowed the expression of the *Ifi202* gene was analyzed by semi-quantitative PCR using a pair of primers specific to the indicated gene. (**c**) Total cell lysates prepared from RAW264.7 cells either stably transfected with pCMV plasmid or the pCMV-202 plasmid were analyzed by immunoblotting using antibodies specific to the indicated proteins. (**d**) J774.A1 cells were either infected with lentivirus expressing control shRNA (lanes 1 and 2) or sh*Ifi202* RNA (lanes 3 and 4) and cells were selected in puromycin for a week. Puromycin resistant cells were pooled and allowed to grow without drug for three days. Total cell lysates prepared from cells either left untreated (control; lanes 1 and 3) or transfected with synthetic DNA poly (dA.dT) (lanes 2 and 4) and incubated for 18 h were analyzed by immunoblotting using antibodies specific to the indicated proteins.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

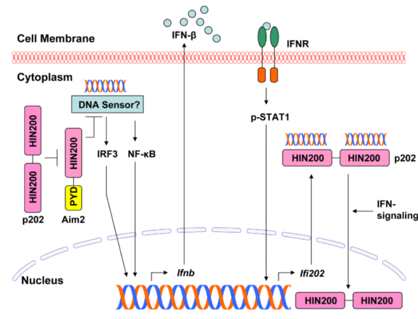

FIGURE 6.

Proposed role of Aim2 protein in cytosolic DNA-induced regulation of type I IFN responses and the expression of the IFN-inducible genes.