Nucleic acid-containing amyloid fibrils potently induce type I interferon and stimulate systemic autoimmunity

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The immunopathophysiologic development of systemic autoimmunity involves numerous factors through complex mechanisms that are not fully understood. In systemic lupus erythematosus, type I IFN (IFN-I) produced by plasmacytoid dendritic cells (pDCs) critically promotes the autoimmunity through its pleiotropic effects on immune cells. However, the host-derived factors that enable abnormal IFN-I production and initial immune tolerance breakdown are largely unknown. Previously, we found that amyloid precursor proteins form amyloid fibrils in the presence of nucleic acids. Here we report that nucleic acid-containing amyloid fibrils can potently activate pDCs and enable IFN-I production in response to self-DNA, self-RNA, and dead cell debris. pDCs can take up DNA-containing amyloid fibrils, which are retained in the early endosomes to activate TLR9, leading to high IFNα/β production. In mice treated with DNA-containing amyloid fibrils, a rapid IFN response correlated with pDC infiltration and activation. Immunization of nonautoimmune mice with DNA-containing amyloid fibrils induced antinuclear serology against a panel of self-antigens. The mice exhibited positive proteinuria and deposited antibodies in their kidneys. Intriguingly, pDC depletion obstructed IFN-I response and selectively abolished autoantibody generation. Our study reveals an innate immune function of nucleic acid-containing amyloid fibrils and provides a potential link between compromised protein homeostasis and autoimmunity via a pDC-IFN axis.

autoimmune disease | innate immune response | disease model

The precise etiology of systemic lupus erythematosus (SLE), a heterogeneous autoimmune disease with multiple organ involvement, is unclear. SLE manifests with characteristic antinuclear antibodies (ANA), including those directed against DNA, ribonucleoprotein complex (RNP), and nucleosomes (1, 2). These autoantibodies can form immune complexes (ICs), which are deposited within the kidneys and blood vessels, and contribute critically to the pathogenesis of such diseases as lupus nephritis and vasculitis. A significant number of patients with SLE have inadequate clearing of apoptotic cell remnants, which include complex antigens containing nucleic acids. The accumulation of these autoantigens permits the eventual development of ANA. However, because self-nucleic acids and apoptotic cell debris are poorly immunogenic, the mechanism behind the initial breakdown of immune tolerance leading to systemic autoimmunity remains enigmatic.

Patients with SLE show increased levels of IFN-I in the serum and expression of IFN-inducible genes in both peripheral blood cells and affected kidneys, frequently correlating with disease flares (3–5). Administration of IFNα to patients with malignant or viral disease occasionally induces a lupus-like syndrome (5). In autoimmune-prone mice, exogenous IFNα can accelerate autoantibody production and glomerulonephritis, whereas IFNAR deficiency significantly ameliorates the disease (6–8). Functionally, IFN-I potently differentiates monocytes, matures dendritic cells (DCs), promotes B-cell differentiation and antibody production, modulates survival, proliferation, and differentiation of T cells, and primes neutrophils for death by NETosis (9–14). Therefore, IFN-I acts as a central effector molecule to promote autoimmunity.

Amyloid fibrils are stable insoluble aggregates of misfolded protein products with extensive β-sheet structures (15). Multiple aberrant polypeptides are implicated in more than 20 human pathologies (16). Amyloid and related misfolded protein species critically affect neuronal functions in the central nervous system (CNS) and participate in inflammatory responses in both CNS and peripheral organs (15, 17, 18). Previously, we characterized how misfolded amyloid precursor proteins form amyloid fibrils in the presence of DNA, RNA, and glycosaminoglycans (19). The fibrous aggregates containing nonproteinaceous cofactors displayed the biophysical and biochemical features of amyloids obtained in vitro and from patients, the latter of which are well known to harbor significant amounts of nucleic acids and/or glycosaminoglycans (20, 21).

Plasmacytoid dendritic cells (pDCs) are a unique innate immune cell population that produces high amounts of IFN-I (IFNα, -β, -ω, and -τ) upon sensing RNA or DNA by endosomal TLR7 and TLR9, respectively (22, 23). ICs of autoantibodies to chromatin and RNPs from SLE patients trigger the production of IFN-I via activation of pDCs, a process that is mediated by the Fcγ receptor (24, 25). DNA-containing neutrophil extracellular traps (NETs), production of which is accelerated by IFN-I and autoantibodies in the SLE serum, also induce IFN-I production by pDCs (13, 14). In another autoimmune condition, psoriasis, complex of antimicrobial peptide LL-37 and self-nucleic acids stimulates pDCs to secrete IFN-I (26). Given these findings on protein–nucleic acid complexes, we examined whether nucleic acid-containing amyloid fibrils can activate pDCs to induce IFN-I and its immunological effects in vivo. Our results suggest that nucleic acid-containing amyloid fibrils can function as a potent IFN-I inducer both in vitro and in vivo. Intriguingly, a healthy rodent host, in response to these complexes, developed systemic autoimmunity with features mimicking SLE.

Results

DNA-Containing Amyloid Fibrils Induce Strong IFNα/β Production by pDCs. Two prototypic amyloidogenic peptides, prion fragment and amyloid β peptide 1–42 (Aβ), bind directly to DNA (19, 21). To test whether DNA-containing prion or Aβ fibrils can activate pDCs, we complexed them with oligonucleotide CpG B. CpG B engages TLR9 in the late endosome and induces pDC to produce abundant TNFα and IL-6, but little IFN-I (22). Interestingly with CpG B, both peptides stimulated pDCs to produce elevated levels of IFNα in a dose-dependent manner, with little effect on other cytokines (Fig. S1), suggesting that DNA-containing amyloid fibrils may selectively enhance IFN-I response by pDCs.

The natural amyloidogenic peptides, such as Aβ, undergo spontaneous intermolecular rearrangement in solution to generate miscellaneous misfolding species (27). However, in fact,

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most polypeptides can adopt a native structure or form amyloid fibrils by transitioning through a precursor state (16, 28). We have characterized stabilized amyloid precursors with misfolded structures from various native proteins (19). Human serum albumin (HSA), an abundant protein with native globular structure without apparent immune stimulatory function, was chosen as a model to investigate the innate immune functions of defined amyloid fibrils. For that, four distinct structural variants of HSA were prepared: native (HSA), amyloid precursor (AP-HSA), amyloid (A-HSA), and fully denatured (D-HSA). Both native and AP-HSA are soluble, whereas A-HSA and D-HSA are insoluble precipitates. A-HSA readily bound to amyloid-specific dye Congo Red, indicating the presence of β-sheet rich amyloid fibrils (Fig. 1*A*). AP-HSA, the amyloid precursor species, which interacted mildly with Congo Red (19), formed an insoluble precipitate upon mixing with CpG B, which exhibited significantly enhanced Congo Red fluorescence (Fig. 1*A*). This result is consistent with our previous observation that amyloid precursors convert to amyloid fibrils upon binding to DNA in a sequence-independent manner (19).

After overnight culture, AP-HSA complexed with CpG B induced human primary pDCs to secrete significant levels of IFNα and slightly increased TNFα and IL-6 (Fig. 1*B*). This result was verified by FACS staining on intracellular IFNα associated with pDCs and detection of increased IFN-I gene products after stimulation by AP-HSA complexed with CpG B (Fig. 1 *C* and *D*). Despite the strong IFN-I stimulation, AP-HSA did not affect pDC maturation by CpG B (Fig. 1*D* and Fig. S2). In contrast, neither A-HSA nor D-HSA, two other misfolded variants, affected IFN-I production (Fig. 1 *B* and *C*). Because pDCs account for <1% of the mononuclear cells in the blood (23), we investigated whether CpG-containing HSA amyloid fibrils could selectively activate pDCs amid other leukocytes. AP-HSA in the presence of CpG B stimulated prominent and selective IFNα secretion from PBMCs, which depended on the function of pDCs as IFN production was abrogated by selective pDC depletion (Fig. 1*E*).

AP-HSA readily binds to genomic DNA isolated from salmon sperm, which resulted in the generation of Congo Red positive complex that displayed apple-green birefringence under polarized light, a definitive indication of amyloid formation (Fig. 1*F*). pDCs secreted significant levels of IFNα after stimulation by HSA amyloid containing salmon sperm DNA (Fig. 1*F, Lower*) and similarly by amyloid containing human genomic DNA (Fig. S3). Therefore, our data indicate that DNA-containing amyloid fibrils potently induce IFN-I by activating human pDCs.

Amyloid Fibrils Containing DNA Are Required for IFN-I Induction. Under our experimental condition, the stabilized amyloid precursor forms amyloid fibrils by complexing with DNA (19); however, DNA and AP-HSA may trigger separate signaling pathways in pDCs that synergistically heighten the IFN-I response. To examine this possibility, we cultured pDCs with DNA and AP-HSA sequentially: pDCs were cultured with DNA for 2 h, the cells were washed, and then AP-HSA was added; in a second test, the cells were cultured first with AP-HSA then incubated with DNA. pDCs produced IFNα only in the presence of both DNA and AP-HSA, a condition favoring the generation of DNA-containing amyloid fibrils (Fig. S4*A*). Furthermore, fluorescent staining with the amyloid-specific dye thioflavin S revealed the presence of amyloid aggregates inside the pDC cells after exposure to AP-HSA complexed with DNA, but not to the native HSA-DNA mixture (Fig. S4*B*).

In addition to nucleic acids, amyloid precursor proteins bind to other polyanionic cofactors, such as heparan sulfate glycosaminoglycan, and form amyloid fibrils (19). In contrast to DNA-containing amyloid fibrils, AP-HSA mixed with heparin failed to activate pDCs to induce IFN-I (Fig. S4*C*). Moreover, heparin inhibited the production of IFNα by PBMCs in response to CpG-containing HSA amyloid fibrils (Fig. S4*D*), which is consistent with its ability to compete with the formation of DNA-containing HSA amyloid fibrils (19). Therefore, among the different types of amyloid fibrils, only the nucleic acid-containing aggregates can potently induce IFN-I. A small compound polyphenol(-)-epigallocatechin

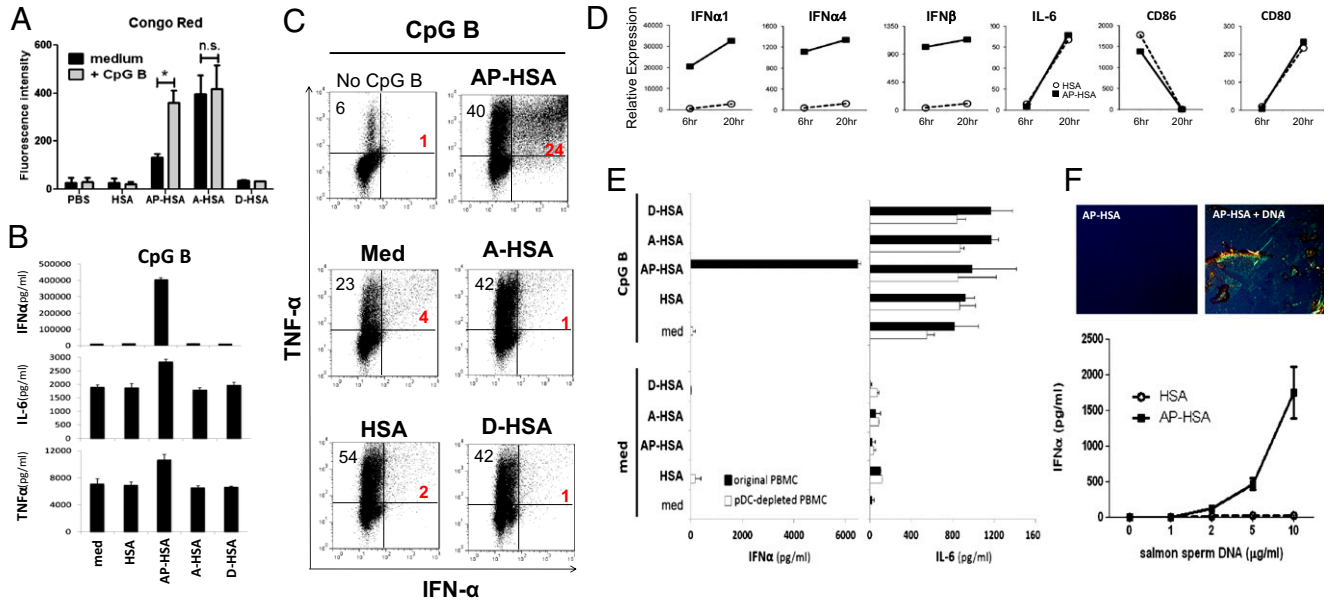

Fig. 1. DNA-containing amyloid induces prominent IFN-I production by pDCs. (*A*) Detection of β-sheet–rich structures in the HSA structural variants in the absence or presence of CpG B with Congo Red. The intensity of fluorescent emission at 646 nm was plotted (mean ± SD from three independent experiments). $*P < 0.05$. (*B* and *C*) Cytokine production by human pDCs stimulated with 1 μM CpG B with 5 μg/mL HSA variants measured by ELISA (*B*), or by intracellular FACS staining (*C*). Data shown in *B* are the mean ± SEM from a representative donor ($n > 10$). Numbers in *C* indicate the percentage of population (representative of four donors). (*D*) Gene expression by pDCs after stimulation determined by quantitative PCR analysis (levels are relative to that of a resting PBMC sample). (*E*) Cytokine production by human PBMC cultured with HSA variants (10 μg/mL) with or without CpG B. Data shown are the mean ± SEM from a representative donor ($n = 4$). (*F*) Birefringence analysis of AP-HSA in without or with salmon sperm DNA (*Upper*). IFNα secreted from pDCs cultured with HSA or AP-HSA (5 μg/mL) and salmon sperm DNA (*Lower*) (mean ± SD).

gallate (EGCG) selectively binds to amyloid precursors and interferes with the amyloid formation (29). Preincubation of AP-HSA with EGCG significantly reduced levels of IFNα produced by PBMCs stimulated by CpG-containing HSA amyloid fibrils (Fig. S4*E*). These results illustrate the functional importance of fibril formation for DNA-containing amyloid to induce IFN-I.

DNA-Containing Amyloid Uptake by pDCs Activates TLR9 in Early Endosomes. To understand how IFN-I production is selectively enhanced by DNA-containing amyloid fibrils, we examined the uptake of genomic DNAs by pDCs. In contrast to native HSA, AP-HSA significantly increased the amount of DNA associated with pDCs in a dose-dependent manner (Fig. 2*A*). The uptake of DNA-containing amyloid seems to be cell type independent, because effective internalization of DNA-containing amyloid fibrils was also observed by Jurkat cells (Fig. S5).

Because TLR signaling from different endosomal compartments leads to distinct cytokine response by pDCs (22), we then examined the subcellular localization of AP-HSA complexed with DNA in pDCs by costaining the cells with the early endosomal marker EEA1 (Fig. 2*B*) and the late endosome/lysosome marker LAMP1 (Fig. S6). After 4 h in culture, fluorescent AP-HSA and DNA were detected inside pDCs, where they remained tightly bound, as illustrated by the complete colocalization of their signals (Fig. 2*B*). Interestingly, the DNA–amyloid complex showed significant colocalization with EEA1 but not with LAMP1, suggesting the exclusive early endosome localization of the amyloid fibrils. Similarly, both Aβ and prion peptides facilitate CpG B to early ensosome (Fig. S7), which is likely responsible for the enhanced IFN-I response (Fig. S1).

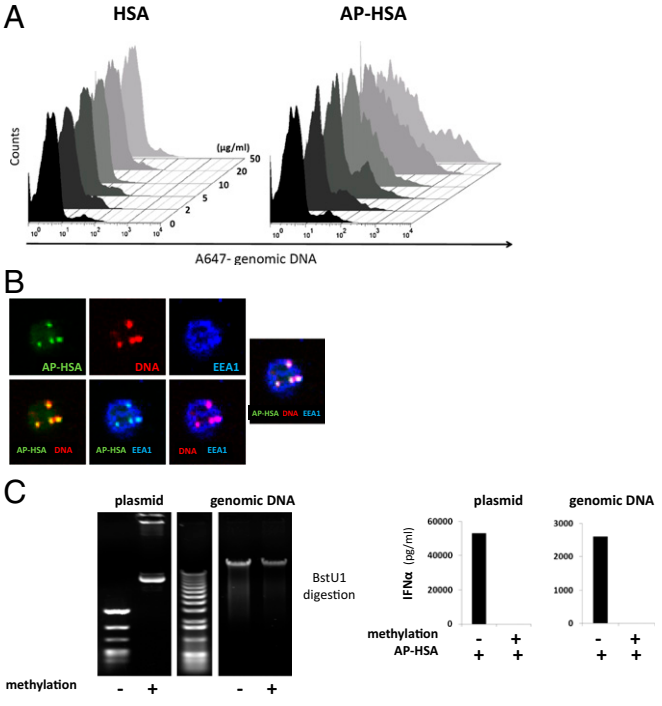

Fig. 2. DNA-containing amyloid fibrils are endocytosed into pDCs to activate TLR9. (*A*) Uptake of DNA by pDCs after incubation with HSA or AP-HSA and Alexa 647-labeled salmon sperm DNA. (*B*) Confocal analysis of pDCs containing biotinylated AP-HSA (green) and A647-labeled DNA (red). Cells were also stained for the early endosome marker EEA1 (blue). (*C Left*) Plasmid DNA and human genomic DNA, unmodified or treated with CpG methyltransferase, were digested by BstU1. Shown is the separation of DNA fragments together with a DNA ladder (center lane). (*C Right*) IFNα secretion by human pDCs stimulated by AP-HSA complexed with unmodified or methylated DNA. Shown are representative results from at least three different donors.

TLR9 recognizes the unmethylated CpG motifs present in the 2′ deoxyribose backbone of natural DNA (22, 23). In addition to TLR9, other cellular DNA sensors can participate in IFN-I response by recognizing biochemical features of DNA irrespective of unmethylated CpGs (30). To investigate the specific role of TLR9, we enzymatically methylated two DNA species, i.e., plasmid DNA and human genomic DNA, and prepared amyloid fibrils by complexing them with AP-HSA. After methylation, plasmid DNA obtained from a bacterial source became resistant to digestion by the restriction enzyme BstU1, which recognizes unmethylated CGCG sequences, whereas human DNA that contains low-frequency unmethylated CpGs showed enhanced BstU1 resistance (Fig. 2*C, Left*). When added to pDCs, amyloid containing methylated DNA completely lost its ability to induce IFN-I, demonstrating the requirement of unmethylated CpGs to trigger IFN production (Fig. 2*C, Right*). Therefore, our data collectively reveal that DNA as part of the complex amyloid is effectively taken up by pDCs and delivered to early endosomes to potently trigger TLR9-mediated IFN-I production.

Nucleic Acid-Containing Amyloid Fibrils Mediate IFN-I Response to Self-RNA and Dead Cell Debris. Similar to their interaction with DNA, AP-HSA mixed with total cellular RNA produced insoluble high molecular aggregate with enhanced Congo Red emission, fibril formation (19), and retarded migration during electrophoresis (Fig. 3*A*). Within this complex, RNA became resistant to RNase digestion, indicating a protective effect of the amyloid structure to the complexed nucleic acids. When added to pDCs, the RNA-containing amyloid fibrils induced significant levels of IFNα (Fig. 3*B*).

Upon death, cells release their cellular components and nuclear antigens. Preincubation of AP-HSA, but not other forms of HSA, with the lysates of necrotic cells stimulated purified pDCs to secrete IFNα (Fig. 3*C*). Consistently, IFNα production was detected in PBMCs stimulated by AP-HSA complexed with the cell debris (Fig. 3*D*). Such activation was sensitive to the pretreatment of the lysates with DNase and RNase, suggesting that DNA- and RNA-containing amyloid fibrils formed in the mixture are likely responsible for triggering IFN-I secretion.

Because IFN-inducing ICs implicated in SLE rely on the function of FcγR to mediate their cellular entry (5, 22), we investigated whether DNA-containing amyloid fibrils also use surface FcγRIIa (CD32) on pDCs. Although blocking CD32 significantly reduced the amount of internalized DNA and secreted IFNα induced by SLE serum, it had no effect on either the uptake of DNA-containing amyloid fibrils or the amount of IFNα secreted by pDCs after amyloid stimulation (Fig. 3 *E* and *F*). Overall, our results demonstrate that amyloid fibrils containing self-DNA, self-RNA, and dead cell debris can directly activate pDCs to produce IFN-I.

DNA-Containing Amyloid Fibrils Induce Infiltration of pDCs and IFNα/β Production in Vivo. To examine the function of nucleic acid-containing amyloid fibrils in an in vivo tissue environment, we injected mixtures of native HSA or AP-HSA together with endotoxin-free bacterial DNA into the peritoneal cavities of mice. pDCs infiltrated to the site where DNA-containing amyloid fibrils were inoculated and retained locally for days afterward (Fig. 4*A*). In contrast, few pDCs were found in the peritoneal cavities of mice injected with DNA and/or HSA. An elevated number of DCs and macrophages were also detected, but no significant difference found between the groups receiving HSA/DNA or AP-HSA/DNA. Hence, pDCs selectively infiltrate in vivo in response to DNA-containing amyloid fibrils. We next analyzed the gene expression by the peritoneal exudate cells 24 h after injection. Strikingly, inoculation of DNA–AP-HSA complex triggered the transcription of multiple IFN-I subtypes and a group of IFN inducible genes at levels significantly higher than other treatments (Fig. 4*B*).

To examine the functional involvement, we depleted pDCs by injecting mAb 120G8, an antibody recognizing the mouse pDC-specific receptor BST2 (31), i.p. 24 h before amyloid inoculation.

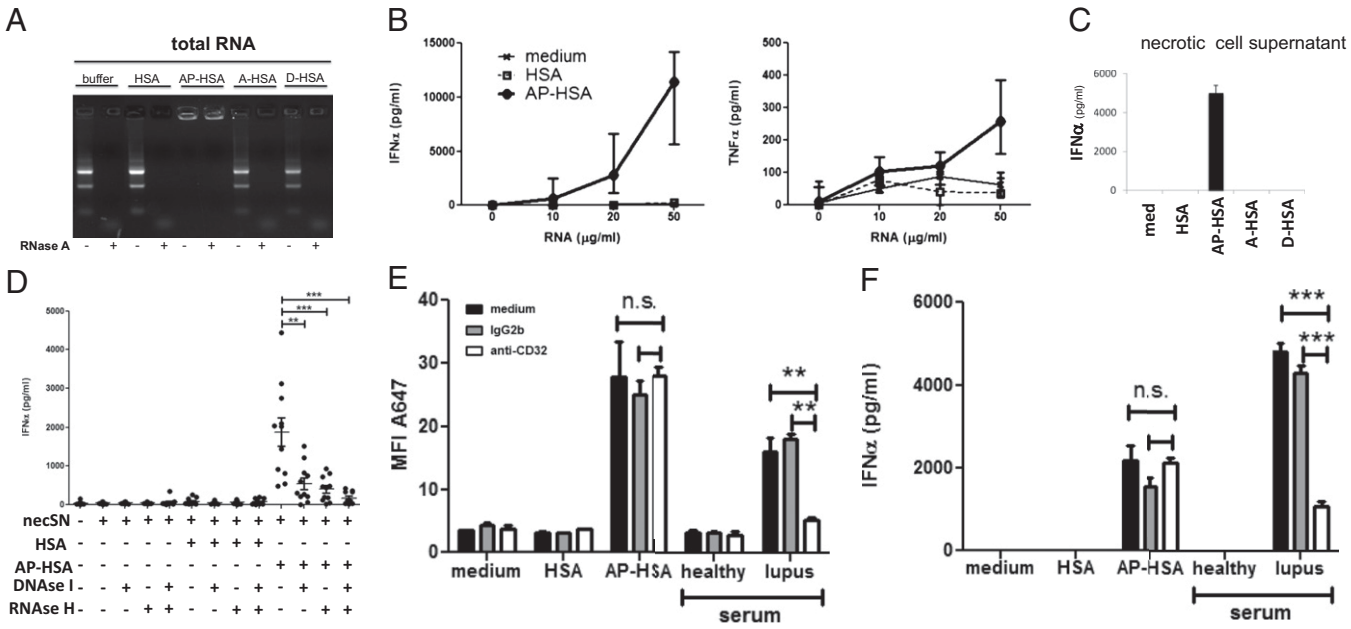

Fig. 3. Amyloid fibrils containing self-nucleic acids trigger IFN-I production by pDCs. (*A*) Gel shift analysis of human total RNA mixed with HSA structural variants in the absence or presence of RNase A. (*B* and *C*) Cytokine secreted by pDCs stimulated with HSA proteins in the presence of human total RNA (*B*; mean ± SD, four donors) or supernatants of necrotic Jurkat cells (*C*; mean ± SEM, representative of at least three donors). (*D*) IFNα secretion by PBMCs in response to necrotic Jurkat supernatants in the presence of HSA or AP-HSA ($n = 11$). In some, necrotic supernatants were treated with enzyme before mixing with HSA. *P* values were determined by a two-way ANOVA test. $^{**}P < 0.01$. (*E*) Blocking of CD32 on uptake of DNA by pDCs in the presence of HSA proteins or sera from healthy donor or SLE patient. Values of mean fluorescent intensity (MFI) are shown. (*F*) IFNα produced by pDCs in the presence of anti-CD32 blocking antibody. (*E* and *F*) Data are presented as mean ± SD ($n = 4$).

Preinjection of 120G8, but not isotype-matched control, reduced the infiltrating pDCs by more than 90% in the peritoneal cavity after DNA–AP-HSA complex inoculation (Fig. S8). As a result, the transcription of not only IFN-I genes but also IFN-inducible genes induced by amyloid fibrils was drastically decreased (Fig. 4*C*). Note that although masked in the heat map, the levels of *irf7* and *isg15*, two prominent IFN-inducible genes, were reduced after pDC depletion (Fig. S9). Interestingly, the up-regulated expression of several chemokines, such as CCL5 and CXCL9–11, was unaffected by pDC depletion, suggesting an inflammatory reaction that does not rely on the function of pDCs. Further analysis revealed an elevated transcription of IL-1β triggered by the DNA-containing amyloid fibrils, which was likewise independent of pDCs (Fig. 4*C*). Overall, our in vivo analysis confirms the in vitro human study and suggests that pDCs acutely sense and respond to nucleic acid-containing amyloid in tissues.

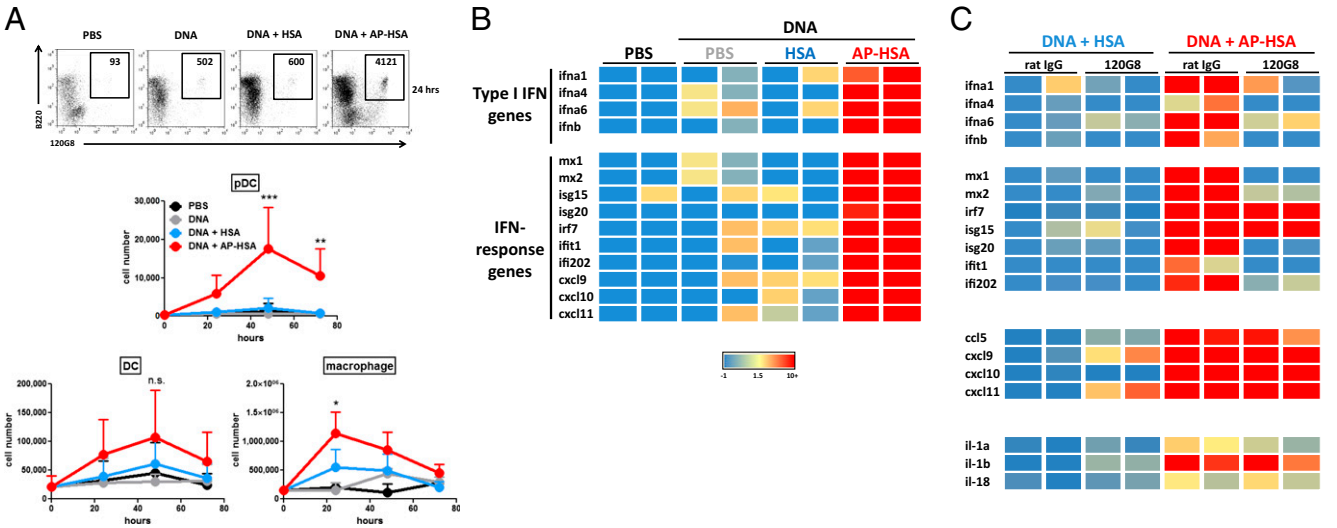

Fig. 4. DNA-containing amyloid induces pDC-mediated IFN-I response in vivo. (*A*) Number of infiltrating antigen presenting cells in the peritoneum of mice. Shown at *Top* are profiles of pDCs within $CD11c^{+}MHC\text{-}II^{+}CD11b^{-}$ population 24 h after i.p. injection. Quantification of kinetic infiltration is shown at *Bottom* (mean ± SD, four mice per time point). (*B*) Peritoneal gene expression presented as a heat map. A PBS-treated animal was used as a reference. (*C*) Gene expression by peritoneal cells 24 h after i.p. injection of HSA proteins with DNA in mice received pretreatment of antibodies (*B* and *C*) Shown are results from one experiment of at least two independent experiments with similar results.

Immunization of DNA-Containing Amyloid Fibrils Induces Autoantibodies and Proteinuria in Healthy Mice. To evaluate the long-term immune response by a healthy host to nucleic acid-containing amyloid fibrils, we immunized wild-type female BALB/c mice with such amyloid in comparison with PBS, DNA, and DNA mixed with native protein. The mice first received i.p. injection in the presence of complete Freund's adjuvant (CFA), followed at 2-wk intervals by two boost i.p. injections with IFA. No difference was detected in the total IgM and IgG levels between the experimental groups (Fig. S10). However, the sera from the mice that received DNA-containing HSA amyloid, but not from the other groups, displayed strong ANA staining on Hep-2 cells (Fig. 5*A*). Clearly visible in the nuclei of Hep-2 cells, the staining was also positive in the cytoplasm, implying a broad reactivity toward cellular antigens.

To elucidate the specificity of the ANA, we examined sera reactivity to several well-known autoantigens implicated in SLE. Interestingly, mice that received DNA-containing HSA amyloid developed significant antibody responses against single-stranded DNA (ssDNA), total RNA, Sm/RNP complex, and histone over a period of several months (Fig. 5*B*). Further analysis demonstrated that IgG1 and, to a lesser extent, IgG2a were the major Ig isotypes within the anti-ssDNA response (Fig. S11). Consistent with the negative ANA, control animals and mice immunized with DNA and HSA showed no sign of autoantibody. The autoantibodies induced by DNA-HSA amyloid are independent of the Abs against HSA, because depletion of HSA-specific Ig had no impact on the serum reactivity to the autoantigens (Fig. S12). At the time when the mice were terminated 16 wk after immunization, none of sera reacted with double-strand DNA. Because lupus nephritis is a major organ-specific pathology associated with SLE, we analyzed the renal function of the immunized mice and detected proteinuria in the group that received DNA-containing HSA amyloid (Fig. 5*C*). Furthermore, the deposition of IgG was found in the glomeruli of the kidneys from these mice (Fig. 5*D*).

Because pDC depletion resulted in diminished acute IFN-I response (Fig. 4*C*), we next investigated the role of pDCs in antibody development. Strikingly, 120G8 preinjection largely abolished the ANA response and severely affected the generation of specific autoantibodies induced by DNA-containing amyloid (Fig. 5 *E* and *F*). However, it did not affect the titer of anti-HSA antibody or the proteinuria in the immunized mice (Fig. S13). This finding suggests that pDC-IFN axis strongly influence the immune reactions leading to autoantibody development. Overall, these data collectively demonstrate that exposure of nucleic acid-containing amyloid fibrils to a nonautoimmune host can result in the development of lupus-like systemic autoimmunity.

Discussion

By forming fibrous aggregates with amyloid precursor proteins, self-nucleic acids are protected from nucleases in the environment, effectively taken up by pDCs, and then transported to the endocytic compartment. A unique membrane trafficking pathway with characteristics of endolysosomes are essential for TLR7/9 signaling and IFN production in pDCs (22, 32, 33). The nucleic acid-containing amyloid is retained in the early endosomes of pDCs, where the prolonged TLR9 activation can promote MyD88 signaling and subsequent IRF7 activation, which initiates the transcription of all IFN-I subtypes. This mechanism is analogous to other potent IFN-I inducers, i.e., type A CpG oligonucleotide and LL-37 complexed with nucleic acids (22, 26). It is unclear whether any specific pDC surface receptor mediates this process. Amyloid β fibrils effectively attach to cells by interacting with a wide array of surface receptors and directly with the phospholipid bilayer (34). Interestingly, multiple amyloidgenic peptides

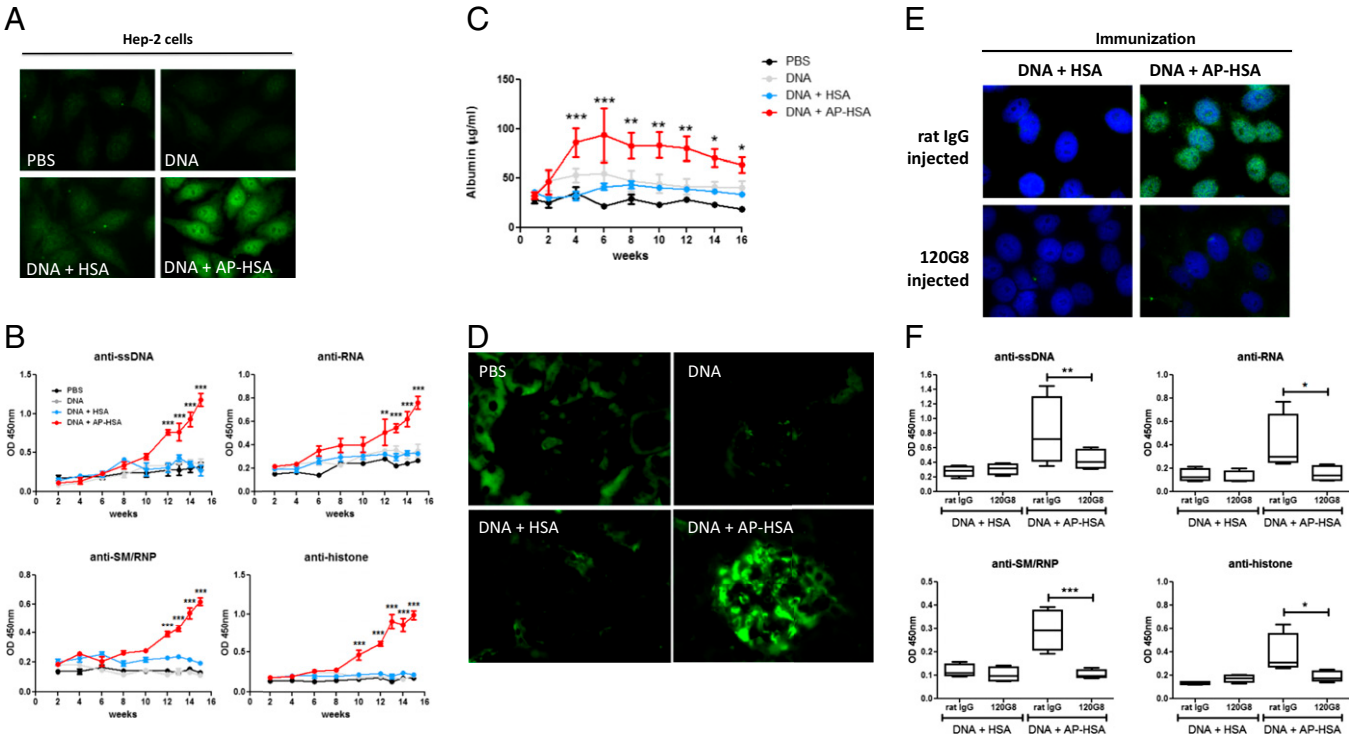

Fig. 5. BALB/c mice develop lupus-like autoimmunity after immunization with DNA-containing amyloid. (*A*) ANA reactivity from sera of mice 13 wk after immunization. (*B*) Levels of autoantibodies in the sera of the immunized mice determined by ELISA analysis (mean ± SD, five mice per group). (*C*) Levels of albumin in the urine of immunized mice (mean ± SD, five mice per group). (*D*) Detection of IgG in the kidneys from immunized mice by staining with A488-labeled anti-mouse IgG. (*A* and *D*) Shown are results with a representative mouse ($n = 5$). (*A*–*D*) Shown are results from one experiment of two independent experiments with similar results (8–12 mice per group in combination). ANA response (*E*) and levels of antigen-specific autoantibodies (*F*) from mice that received Ab pretreatment. Hep-2 cells were stained with a representative serum 9 wk after immunization (*E*). The box and whiskers plots of the data distribution of four mice per group are shown in *F*. *P* values were determined by a two-way ANOVA test.

enhance HIV attachment and entry into cells (35). Therefore, nucleic acid-containing amyloid fibrils are unusually effective to deliver nucleic acids to elicit IFN-I production by pDCs.

Despite the recognized importance of IFN-I in many autoimmune diseases, its role in the initiation phase of autoimmunity has not been fully defined. In fact, only a minor fraction of patients treated with IFNα develop ANA, and even a smaller fraction manifest with SLE (5). Distinct from human SLE, mice that develop spontaneous lupus do not exhibit significant upregulation of IFN-I. Excessive IFN-I exposure exacerbates disease only in certain lupus-prone strains but has no effect in nonautoimmune mice (6), implying that IFN-I requires certain genetic susceptibility or perhaps activation of additional pathway(s) to break immune tolerance. Here, we demonstrate the capacity of nucleic acid-containing amyloid to induce early IFN-I production upstream in a cascade of immune responses, which eventually lead to the autoantibody generation.

Aberrant IFN-I production by pDCs has been implicated in several human autoimmune disorders (4, 23). In SLE patients, the numbers of circulating pDCs are reduced, whereas increased pDC presence has been observed in the inflamed tissues (5, 22). Besides secreting IFN-I, TLR-activated pDCs promote the generation of plasma cells and antibody responses via IFN and IL-6 in vitro (10). However, how pDCs participate in systemic autoimmunity in vivo remains obscure. Here, we show the infiltration of pDCs shortly after inoculation of nucleic acid-containing amyloid. Depletion of pDCs not only abolished the IFN-I induction, but also severely and selectively diminished the development of autoantibodies against nuclear antigens. Therefore, IFN-producing pDCs play an essential role in initiating systemic autoimmunity.

Amyloid fibrils are a product of failed protein homeostasis because of germ-line mutation, erroneous transcription/translation, physical damage, or abnormal posttranslational processing (15). Because human "amylome" constitutes approximately 15% of all coding polypeptides in the genome, many "self" proteins have the potential to form amyloid (28). Amyloid depositions are frequently heterogeneous containing nonproteinaceous cofactors (15, 20, 21). Our results suggest that only the type of amyloid-containing nucleic acids is capable of inducing IFN-I through activating nucleic acid-sensing TLRs. Interestingly, protein misfolding products display another innate immune function: both fibrillar Aβ and amyloid precursor of islet amyloid polypeptide potently activate NALP3 inflammasome and induce IL-1β maturation (17, 18). We also observed that DNA-containing amyloid fibrils induced peritoneal inflammation in a pDC- and IFN-independent manner, likely due to inflammasome activation and IL-1β induction. Therefore, the protein misfolding products can activate multiple innate immune pathways in vivo.

By immunizing nucleic acid-containing amyloid fibrils, we have, in effect, created an inducible experimental lupus model. Previous attempts to immunize nonautoimmune mice with self-antigens, such as DNA, apoptotic cells, or purified nucleosome, only resulted in limited or transient autoantibody generation (36–38). Tetramethylpentadecane (TMPD) induces an array of autoantibodies and glomerulonephritis in BALB/c mice (39). Interestingly, prolonged oral administration of TMPD reportedly leads to amyloidosis (40). Our model of experimental lupus uniquely centers on the activation of pDC-IFN axis. It would be important to investigate the critical cellular players and pathways that lead to systemic autoimmunity.

Materials and Methods

Reagents. HSA structural variant proteins were prepared essentially as described (19). Wild-type BALB/cByJ mice were obtained from the Jackson Laboratory. Additional methods and detailed information can be found in *SI Materials and Methods*.

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