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Self-reactive IgE exacerbates interferon responses associated with autoimmunity

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Summary

Canonically, IgE mediates allergic immune responses by triggering mast cells and basophils to release histamine and Type 2 helper cytokines. Here, we report that in human systemic lupus erythematosus, IgE antibodies specific for double-stranded DNA activate plasmacytoid dendritic cells (pDCs), an immune cell type linked to viral defense, leading to the secretion of substantial amounts of interferon-α. The concentrations of dsDNA-specific IgE found in patient serum correlated with disease severity and greatly potentiated pDC functions by triggering phagocytosis via FcεRI followed by Toll-like receptor 9-mediated DNA sensing in phagosomes. These findings expand the known pathogenic mechanisms of IgE-mediated inflammation beyond those found in allergy and demonstrate that IgE can trigger interferon responses capable of exacerbating self-destructive autoimmune responses.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the loss of immune tolerance to nucleic acids, activation of autoreactive lymphocytes, and the production of large quantities of self-reactive antibodies that induce tissue damage[1]. Renal

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Author Contributions

Experimental design and conception were performed by J.H., J.M.R, J.L.K., V.M.L., L.S., J.L., L.I. and L.C. under the guidance of L.X., R.H., R.E, and T.M. Statistical analysis was performed by K.A.C. D.K. and M.A.Sm. SLE patient data generation and analysis were supervised by M.P. and M.R.C. J.H., M.A.S. and R.K. conceived and directed the project. J.H. and M.A.S. wrote the manuscript with input from all authors.

autoantibody deposition and lymphocyte infiltration lead to nephritis, a serious complication of lupus that presents in the clinical course of up to 60% of patients[2]. A hallmark of SLE is the production of type I interferons (IFN-I) in response to immune complexes (ICs) containing self-DNA from dead cells and DNA-specific IgG[3]. There is now a mounting body of evidence pointing to plasmacytoid dendritic cells (pDCs) as the main pathogenic IFN-I producers in SLE[4]. pDCs are immune cells that specialize in antiviral responses[5]. Upon sensing viral nucleic acids through TLR7 (RNA) and TLR9 (DNA), pDCs release up to 1000 times more IFN-I than any other cell type[6], promoting the cellular expression of IFN-stimulated genes and the apoptosis of infected cells. Although TLR9 binds indiscriminately to both viral and host endogenous DNA, its intracellular localization within endo-lysosomal compartments prevents the recognition of self-DNA. In SLE, DNA-specific autoantibodies bind to endogenous DNA (released from damaged cells) forming DNA-ICs, which are then internalized by pDCs via the Fc-gamma receptor IIa (FcγRIIa)[7], a process that allows delivery of self-DNA to TLR9 within pDCs, triggering an aberrant antiviral response. Recognition of self-DNA by TLR9 leads to the recruitment of the adaptor protein myeloid differentiation primary response gene 88 (MyD88) and then to the activation of nuclear factor κB (NF-κB), and interferon regulatory factor 7 (IRF7), which induce the secretion of proinflammatory cytokines (such as TNF) and the secretion of large amounts of IFN-I respectively[8, 9]. TLR9 activation also induces cell migration and their ability to activate T cell and B cells, which positions pDCs at the crossroads of both innate and adaptive immune responses[10].

Recent evidence demonstrates that double-stranded DNA (dsDNA)-specific antibodies of the IgE immunoglobulin class are also found in some SLE patients[11, 12, 13] and although they have been associated with basophil activation[12, 14], their role in disease pathogenesis remains unclear. Found only in mammals, IgE is the least abundant immunoglobulin isotype and signals through two types of Fc-epsilon receptor (FcεR), the high-affinity receptor FcεRI and the low-affinity receptor FcεRII. IgE provides protection against parasitic worms (helminths), but also triggers vigorous harmful, even deadly, allergic reactions against innocuous foreign proteins (allergens)[15, 16]. In both of these cases, IgE recognizes exogenous antigens and triggers an immunological response that is associated with mast cell degranulation and the subsequent release of biogenic amines, lipid mediators, the production of Th2 cytokines (such as IL-4, IL-5, and IL-13), and eosinophilia[15]. Paradoxically, none of these inflammatory responses are key drivers of SLE pathogenesis[11, 17, 18] and SLE patients do not appear to be more prone to IgE-driven environmental allergies than the general population[19, 20, 21]. Thus, it is plausible that self-reactive IgE in autoimmunity may present with different functions than those described for IgE in helminth defense and allergy. To explore this, we investigated the potential roles of DNA-specific IgE in SLE pathogenesis.

Results

IgE triggers IFN-α secretion in SLE

In the SLE cohort we studied, 98 out of 180 (54.4%) of patients exhibited detectable concentrations of dsDNA-specific IgE, while healthy individuals, as well as patients with atopic dermatitis (a disease associated with elevated serum IgE concentrations) were all

negative for this autoantibody (Fig. 1a). Amounts of circulating dsDNA-specific IgEs were particularly increased in active disease (Fig. 1b), and correlated with low amounts of complement component 3 (C3), a marker of serologic disease activity[13, 22] (Fig. 1c). To investigate the role of dsDNA-specific IgE role in SLE, we explored whether these autoantibodies could modulate the IFN-I response. Activation of FcεR on pDCs by heat-aggregated IgEs inhibited influenza-induced secretion of IFN-α (Supplementary Fig. 1), a mechanism that is believed to explain the impaired IFN-α response observed for pDCs in atopic patients[23, 24, 25]. Therefore, the production of self-reactive IgE in SLE patients with active disease may be a protective immune mechanism to limit exacerbated interferon responses and reduce inflammation. To test this hypothesis, we stimulated peripheral blood mononuclear cells (PBMCs) with serum from SLE patients positive for dsDNA-specific IgE in the presence of an IgE-blocking antibody that interferes with the binding of this immunoglobulin to its receptor. Surprisingly, blockage of IgE did not increase IFN-α secretion by PBMCs but instead reduced its production (Fig. 1d), indicating that IgE could actively contribute to the aberrant IFN-responses in patients. To assess if IgE autoantibodies are associated with clinical manifestations, we conducted a multivariate regression analysis using General Linear Model (GLM) with SLEDAI score as the dependent variable. According to this model, the concentration of dsDNA-specific IgE was an SLE activity risk factor independent of dsDNA-specific IgG concentration (Table 1). Together, these results suggest that IgE autoantibodies found in SLE potentiated IFN-I responses and represent a newly identified culprit contributing to the aberrant secretion of interferon that underlies SLE pathogenesis.

dsDNA-specific IgE are sensed by pDCs

To gain mechanistic insights as to how the self-reactive IgE found in SLE may contribute to this disease pathology, we engineered the variable regions of a previously described dsDNA specific IgG (IgG^D) onto a IgE backbone that maintained the specificity for dsDNA (IgE^D) (Supplementary Fig 2a–b). As expected, IgE^D showed dose-dependent binding to plate-bound DNA (Supplementary Fig. 2c). IgE^D, but not an isotype control IgE (IgE^I), induced IFN-α secretion by pDCs in a concentration-dependent manner when combined to DNA (Fig. 2a), demonstrating for the first time that ICs formed by IgE^D and DNA can elicit interferon responses. Moreover, IgE-DNA ICs induced amounts of IFN-α similar to those formed with IgG-DNA ICs (Fig. 2b). Importantly, the IgE-blocking antibody did not affect the IFN-α production induced by DNA-IgG ICs in pDCs, while it completely inhibited IFN-α production in cells stimulated with DNA-IgE ICs (Fig. 2c). Taken together, these findings demonstrate that dsDNA-specific IgE similar to those found in SLE are capable of triggering large quantities of IFN-α by pDCs.

To investigate the mechanisms leading to dsDNA-specific IgE-mediated activation of pDCs, we first evaluated the cell-surface expression of both the high-affinity and the low-affinity FcεR. We found that pDCs only express the high-affinity FcεRI (Fig. 3a). Ectopic expression of human FcεRI in mouse macrophages was required for a marked increase in the recruitment of the dsDNA receptor TLR9 to particles coated with DNA+IgE^D ICs (Fig. 3b), indicating that FcεRI at the cell surface facilitates the delivery of DNA+IgE^D into a phagosomal compartment where the engulfed DNA can be sensed. TLR9 triggers IRF7-

dependent secretion of IFN-α from mature LAMP1$^+$ compartments[26, 27]. Therefore, we further explored the subcellular localization of DNA+IgE^D ICs within the cell. Upon internalization, DNA+IgE^D ICs colocalized tightly with LAMP1 in pDCs (Fig. 3c). This was associated with translocation of IRF7 to the nucleus (Fig. 3d), and with secretion of IFN-α that was completely blocked by TLR9 inhibitors (Fig. 3e). DNA+IgE^D ICs also triggered secretion of the proinflammatory cytokines TNF, IL-8 and IL-6 in a TLR9-dependent manner (Supplementary Fig. 3a–c). These findings show that DNA-IgE ICs engage with FcεRI at the cell surface, which then delivers the complexes into mature compartments where TLR9 senses the DNA. In this intracellular compartment, TLR9 initiates a complex inflammatory response that is characterized by the production of several proinflammatory cytokines, including the secretion of substantial amounts of IFN-α.

IgE deposits in SLE kidney biopsies

We found that 54.4% of patients in our cohort presented with dsDNA-specific IgE. We then asked whether these autoantibodies were particularly enriched in any disease subset within our cohort and found that they were most frequent in patients with kidney manifestations. 70% of patients diagnosed with lupus nephritis (LN) had detectable concentrations of dsDNA-specific IgE (Fig. 4a). They were further enriched in patients diagnosed with the most severe and common subtype of lupus nephritis (diffuse proliferative nephritis or LN class IV), with a large majority (82%) of the patient presenting detectable levels of dsDNA-specific IgE (Fig. 4b). As previously shown for IgG[28, 29], IgE deposits were found in both glomerular and extraglomerular areas. The IgE deposits were similarly distributed between both areas while larger deposits of IgG were found in glomerular structures (Fig. 4c, **and** Supplementary Fig. 4a–b). pDCs infiltrates were found in glomerular and extraglomerular areas with larger infiltrates of pDCs in the glomeruli (Fig. 4c, **and** Supplementary Fig. 4a–b). However, we did not detect significant infiltration of basophils, a cell type responding to IgE and recently linked to SLE[12], in neither LN nor normal kidneys (data not shown). Elevated expression of MxA, a protein produced in response to secretion of IFN-I, was also found mostly in the glomeruli of patient biopsies with a similar distribution to that of pDCs (Fig. 4c **and** Supplementary Fig. 4a–b). This suggests that infiltrating pDCs could directly interact and become activated by local ICs containing nucleotides capable of triggering TLR7- and/or TLR9-dependent pDC activation. As circulating IgG and IgE autoantibodies have equal access to exposed nucleic acid autoantigens shed by dead cells in tissues, the interferon response we visualize as MxA positivity in the kidney biopsies are likely to be driven by immune complexes that simultaneously contain both immunoglobulins. Therefore, we conclude from these experiments that IgE autoantibodies deposited in lupus renal glomeruli could potentially contribute to local inflammatory processes.

DNA-IgE stimulated pDC trigger B-cell responses

We also observed areas of the kidney biopsies of patients where both pDCs and B cells were present in close proximity (Fig 5a). pDCs can induce B cell proliferation and differentiation into plasma cell (PCs)[30]. Therefore, we investigated the capacity of dsDNA-specific IgE to stimulate PC differentiation in a pDC-B cell co-culture system, using purified human pDCs and B cells. Addition of DNA+IgE^D ICs led to a marked increase in B cell numbers (Fig. 5b), differentiation into PCs (Fig. 5c), and secretion of IgM (Fig. 5d). B cell responses were

dependent on the presence of pDCs in the co-cultures, as stimulation of B cells alone with the ICs did not result in any increase in cell number or PC formation (Supplementary Fig. 5a–c). During infection, two of the cytokines secreted by pDC act sequentially on B cells to trigger differentiation into PCs, with IFN-α generating non-Ig-secreting plasmablasts and IL-6 inducing their final differentiation into Ig-secreting PCs[30]. We, therefore, tested whether these soluble factors were required for the generation of PCs in response to the IgE-containing ICs. Inhibiting the IFN-I receptor (IFNAR) or IL-6 with blocking antibodies resulted in a marked reduction in the formation of PCs in the co-cultures treated with DNA +IgE^D ICs (Fig. 5e). Collectively, these results demonstrate that ICs containing dsDNA-specific IgE can lead to pDC-mediated B cell activation, a mechanism that could exacerbate the B cell-driven autoimmune response in the kidney of LN patients.

IgE and IgG immune complexes synergize

In the SLE cohort studied, circulating dsDNA-specific IgE was found to correlate with dsDNA-specific IgG (Fig. 6a). Interestingly, all patients whose serum contained dsDNA-specific IgEs also tested positive for dsDNA-specific IgGs, with no patient being positive for the IgE isotype alone. As exposed DNA from damaged cells are accessible to both dsDNA-specific IgGs and IgEs in the circulation, patients are likely to form pathogenic ICs that simultaneously contain both classes of immunoglobulins. This concept is consistent with the presence of both IgG and IgE autoantibody deposits in glomerular and tubular areas in the biopsies of patients (Fig. 4c, **and** Supplementary Fig. 4a–b). For this reason, we investigated the effect of IgG and IgE DNA-containing ICs on pDC responses. We found that combining both isotypes at a 1:1 ratio formed complexes that triggered much greater secretion of IFN-α than the complexes containing a single immunoglobulin isotype (Fig. 6b). The combination also increased the secretion of the proinflammatory cytokines TNF, IL-6, and IL-8 (Supplementary Fig. 6a–c). We followed the intracellular production of IFN-α and TNF on stimulated pDCs for 16 h and observed that the elevated IFN-α (Fig. 6c) and TNF concentrations (Fig. 6d) were largely due to an increase in the percentage of cells secreting them. Peak of production was seen at 6 h, when most cells were co-expressing both cytokines (Fig. 6e). pDCs exposed to the DNA+IgE^D ICs also had a more mature phenotype, with robust expression of the cell surface co-stimulatory molecules CD83 and CD86 (Fig. 6f). CD86 expression was much higher on cells treated with IgE^D-containing ICs when compared with those treated IgG^D ICs. The same was observed for the expression of the C-C chemokine receptor type 7 (CCR7), a critical chemokine receptor utilized by mature pDCs to migrate into lymphoid compartments[31] (Fig. 6f). Furthermore, DNA+IgE^D ICs induced robust migration of pDC to CCR7 ligands to a significantly greater degree than those triggered by complexes formed with IgG^D (Fig. 6g). Antigen presentation is a central feature of autoimmune disorders like SLE, and DNA+IgE^D ICs were able to trigger antigen-specific secretion of IFN-γ (Fig. 6h) and T cell proliferation (Fig. 6i) in pDC-T-cell co-cultures. In both of these assays DNA+IgE^D ICs and DNA+IgG^D ICs produced similar responses in magnitude and synergized when combined.

From these experiments, we conclude that DNA-containing IgE ICs are capable of triggering secretion of proinflammatory cytokines, maturation, migration and antigen presentation in pDCs. In all cases the responses were comparable or larger than those initiated by DNA-IgG

complexes. Combining both IgG and IgE in the complexes resulted in synergistic secretion of inflammatory cytokines, including IFN-α, in pDCs. This was not due to greater secretion per cell, but rather to a larger number of cells responding to the immune complexes, indicating that the presence of IgE in these complexes lowers the threshold for triggering pDC responses.

IgE enhances IC phagocytosis

Although dsDNA-specific IgE were detected in a majority of SLE patients, their concentrations in circulation were, on average, about 20 times lower than the corresponding IgG (Fig. 7a). Hence we investigated the relative contribution of IgE to interferon responses in conditions similar to those found in patients. To this end, we prepared DNA-containing ICs with a fixed amount of DNA+IgG^D and tested the effect of adding increasing amounts of IgE^D on IFN-α secretion. Strikingly, the addition of even small amounts of IgE^D to the complexes greatly enhanced the amount of IFN-α produced by pDCs. This was true even when the relative concentration of IgE versus IgG in the ICs was 100 times lower, under which circumstances, the presence of IgE magnified the interferon response more than 5 fold (Fig. 7b). Similar results were obtained when the complexes were formed *in vitro* with U1 small nuclear ribonucleoprotein particles (U1 snRNPs), a RNA-associated autoantigen found in SLE[32], indicating that the role of IgE in SLE may not only be restricted to DNA but expand to autoantigens sensed by TLR7 (Fig. 7c–d). To understand mechanistically how IgE enhances the secretion of IFN-α we investigated its effect on the phagocytic uptake of ICs using fluorescently-labeled DNA. The percentage of pDCs with detectable intracellular DNA uptake was similar when either IgG^D or IgE^D were used to form the ICs. However, the combination of IgE^D and IgG^D induced synergistic phagocytosis when combined at a 1:1 ratio (Fig. 7e–f). This synergy was even maintained at conditions under which the amount of IgE^D used was 10 or 100-fold lower than that of IgG^D (similar to ratios found in patients). As compared to complexes formed with only IgG^D, the number of pDCs with detectable phagosomal DNA at those two ratios were 5.11- and 2.97-fold higher respectively (Fig. 7g). These results indicate that the enhanced secretion of IFN-α triggered by low concentrations of IgE^D is caused by substantially increased IC phagocytosis. This mechanism also explains how the presence of dsDNA-specific IgE in the ICs lowers the threshold for triggering pDCs responses, which results in more cells secreting pathogenic IFN-α in SLE patients.

Discussion

Measures to limit host-DNA exposure to the nucleic acid sensor TLR9 are critical to maintaining tolerance to self. One such protective measure is the compartmentalization of TLR9 to intracellular membranes, which restricts this receptor's access to circulating free DNA, and thus limits inappropriate responses to self. Notably, cell surface expression of TLR9 on hematopoietic stem cells results in lethal autoinflammatory disease in mice[33] highlighting the importance of maintaining TLR9 within the cell. Herein we demonstrate that the DNA-IgE autoantibodies found in SLE undermine these aforementioned safeguards by shuttling self-DNA to intracellular TLR9 via cell surface FcεRI activation in pDCs. This mechanism parallels that used by IgG autoantibodies which in contrast utilizes FcγRIIa to deliver DNA inside the cell[7, 26]. Furthermore, we provide evidence that IgE markedly

synergizes with IgG to induce larger engulfment of DNA-ICs and that IgE enhances pDC responses even when found 100–1000 fold less represented in the immune complexes than IgG. One potential explanation for the potent synergistic activity of IgE in the DNA-ICs that were formed with both IgG and IgE is that FcεRI binds monomeric IgE with an association constant, Ka, of 10^{10} M^{-1} [34] which is at least 3 order of magnitude higher than binding of IgG to low affinity FcγRIIa[35]. Therefore, the ability of IgE to enhance pDC phagocytic capacity may be a critical step in promoting the further loss of tolerance in these SLE patients. This mechanism becomes even more important in light of the limited phagocytic potential of pDCs and the presence of nucleases in acidic organelles that digest and limit the amount of DNA available to be sensed by TLR9. Thus IgE may facilitate the accumulation of DNA-ICs at the phagosome at concentrations that facilitate eliciting TLR9-mediated pDC responses. Indeed, we found that the combined stimulation of pDCs with IgE + IgG immune complexes significantly exacerbated the inflammatory response due to an increase in the percentage of cells responding to self-DNA as opposed to increased production of IFN-α or TNF per cell.

IgE is the least abundant and less understood immunoglobulin isotype in disease. Evidence in animal models and statistical population trends suggest that IgE may be beneficial in fighting parasites, whereas IgE is believed to trigger harmful allergic reactions against innocuous foreign proteins[16, 36]. In both of these cases, IgE recognizes exogenous antigens and triggers an immunological response that is associated with the activation of mast cells and basophils, resulting in degranulation and the subsequent release of biogenic amines, lipid mediators and T_H2 cytokines. The presence of IgE antibodies that bind endogenous host antigens has been known for some time in SLE. The characteristic IgE-mediated responses are not observed in SLE and this has somewhat complicated elucidating their role in a disease that is mainly driven by an aberrant antiviral response[5]. For instance, dsDNA-IgE-positive SLE patients do not exhibit significantly higher concentrations of circulating IL-4 or IL-5 compared with seronegative patients or healthy controls[37]. Instead, we found that serum self-reactive dsDNA-IgE concentrations correlate with disease severity and are a SLE activity risk factor, irrespective of the amounts of IgG antibodies that bind the same autoantigens in our patient cohort. Furthermore, we demonstrate that dsDNA-specific IgEs actively contribute to SLE by enhancing the specific immune effects, including interferon secretion, associated with this disease. Collectively, the unexpected responses described here for IgE are unique and totally distinct compared to the classical T_H2-skewed responses observed in allergic disease.

This novel link between IgE, autoimmunity and interferon responses reveals an unexpected concept for the physiological role of IgE in the immune system. Notably, we found that DNA-containing IgE ICs functionally impact pDCs via inducing maturation, antigen presentation, cellular migration and the secretion of IFN-α and other proinflammatory cytokines. In addition, these ICs also triggered pDC dependent B-cell responses and the expansion of Plasma cells. Importantly, IgE synergized with IgG even when found in the ICs at several orders of magnitude lower than IgG. Therefore, we propose that this robust pDC activation is deleterious in SLE, since they are aberrantly generated against self-nucleic acids. Alternatively, robust pDC activity would be beneficial to the host if these cells were targeting viral particles. One intriguing possibility is that IgE collaborates with IgG to

facilitate sensing of viruses and deliver their pathogenic nucleic acids to TLR7 and TLR9 within the phagosome. Studies in humans and animals have identified IgE antiviral antibodies in serum [38, 39, 40]. These antibodies appear to be driven by viral load as reported for a study focused on respiratory syncytial virus (RSV). Interestingly, the concentration of IgE anti-RSV antibodies correlated with their IgG counterpart as well as with circulating viral protein F[39]. This is consistent with our observations of a good correlation between anti-dsDNA IgE and anti-dsDNA IgG that are generated in response to autoantigens in SLE. There is also evidence suggesting that IgE directed against HIV may be beneficial for controlling the infection. Total IgE from seropositive IgE anti-HIV patients have been found to block viral production in PBMCs by mechanisms that exclude viral neutralization, which suggest that these antibodies have antiviral properties by acting through immune cells[38]. Furthermore, a small study reported that anti-HIV IgE in patients was not associated with higher incidence of allergy[41], which resembles findings in similar epidemiological studies in SLE for dsDNA-IgE seropositive patients. How the mechanisms we propose for IgE in autoimmunity extend to scenarios of viral defense warrants further investigation. Nevertheless, the data presented herein expand the physiological functions of IgE within the immune system and suggest that the mammal's recent evolutionary acquisition of IgE might provide benefits to the host beyond helminth defense.

In conclusion, the aberrant recognition of self-nucleic acids is central to SLE as it triggers an inflammatory response that ultimately leads to autoimmunity and tissue damage. The data presented herein, provide novel mechanisms for a pathogenic role of IgE in the disease and explain how the appearance of self-reactive IgE can exacerbate the inflammatory pathways that contribute to self-inflicted damage. The previously unrecognized link between IgE and the interferon pathway that we report here provide additional insights into the pathological mechanisms underlying autoimmunity and may be useful in the rational design of therapies for the treatment of diseases such as SLE.

On-line methods

Patients and diagnostic criteria

The Hopkins Lupus Cohort is approved by the Johns Hopkins University School of Medicine Institutional Review Board on a yearly basis. All patients met the revised American College of Rheumatology (ACR) classification criteria for SLE[42, 43]. All patients provided informed consent to participate in the cohort and all patient data were anonymized for the purpose of this analysis. Serum samples from 180 patients were studied, including 161 (89%) females and 19 (11%) males. Of these, 97 (54%) were African-American, 72 (40%) were Caucasian and 11 (6%) were of other ethnicities. Serum and blood samples from healthy donors were obtained through the MedImmune LLC internal blood donor program in accordance with the guidelines of the Institutional Review Board of MedImmune LLC. Atopic dermatitis serum samples were obtained through Bioreclamation IVT.

Cells culture

Human PBMCs from healthy donors were either obtained from leukopacks (SeraCare Life Sciences) or isolated from whole blood obtained through the MedImmune LLC internal

blood donor program using CPT tubes (BD Biosciences). In some cases, donors were pre-screened for cytomegalovirus (CMV) reactivity using CD8-PE (clone SK1, BD Biosciences) and CMV Dextramer HLA-A*0201-NLVPMVATV-APC (Immudex). Human PBMCs were maintained in RPMI 1640 supplemented with 5% FBS (both from Life Technologies). EasySep Human pDC Enrichment kit and EasySep Human CD8 T cell Enrichment kit (StemCell Technologies) were used to enrich pDCs while total B cells were isolated using the B Cell Isolation Kit II (Miltenyi Biotec). The purity of the enriched cells was routinely assessed by flow cytometry using BDCA4-PE (clone AD5-17F6, Miltenyi Biotec) and/or CD123-APC (clone 7G3, BD Biosciences) for pDCs, CD8-PE (clone SK1, BD Biosciences) and CD19-PerCP-Cy5.5 (clone HIB19, BD Biosciences) for B cells. Cell purity was consistently above 95%. Unless otherwise mentioned, enriched pDCs were maintained in X-Vivo 15 media (Lonza) supplemented with 1% FBS (Life Technologies) and 10 ng/ml of human recombinant IL-3 (R&D Systems). Mouse macrophages expressing TLR9-GFP were generated as previously described[44]. Briefly, TLR9-deficient macrophages were immortalized from bone marrow of *Tlr9*−/− mice using the J2 recombinant retrovirus (carrying v-myc and v-raf oncogenes). Bone marrow cells were infected with J2 virus after 3 days of culture in L929 conditioned medium. Immortalized macrophages were then retrovirally transduced with mouse TLR9-GFP and human FcεRIα. Mouse macrophages were cultured in DMEM supplemented with 10% FBS and 1X Penicillin/Streptavidin (all from Life Technologies).

Vectors and constructs

Human FcERIa (GenBank #NM_002001) was initially amplified from a plasmid (GeneCoepia) and cloned into the pMSCVPuro vector (Clontech). The fusion plasmids were used to transfect the pT67 packaging cell line (Clontech), or the Gryphon amphotropic packaging cells (Allele Biotech), using the standard transfection protocol.

Monoclonal dsDNA-specific IgE

The human dsDNA-specific IgG clone E11 (IgG^D) was generated as previously described[26]. To generate the human dsDNA-specific IgE (IgE^D), E11 variable regions were cloned by restriction digestion using BssHII and SalI enzymes from New England Biolabs and ligated into an IgE Orip/EBNA-1-based episomal mammalian expression vector pOE (MedImmune LLC). A human IgE specific for metapneumovirus (hMPV) was generated in a similar fashion to be used as an isotype control (IgE^I).

dsDNA-specific antibody measurement

Human serum was screened for detection of dsDNA-specific IgG and IgE using dsDNA IgG ELISA (Calbiotech). For detection of dsDNA-specific IgE, an anti-human IgE-HRP (Immunology Consultants Lab Inc.) was used instead of the anti-human IgG-HRP provided with the ELISA kit. Monoclonal IgG^D and IgE^D were used as assay standards.

Complement component 3 (C3) detection

Human serum was screened for detection of C3 by quantitative multiplexed immunoassay (Myriad, Rules-Based Medicine).

SLE serum assay

Human PBMC *in vitro* assay with SLE serum was performed by transferring primed PBMCs (500 U/ml IFN-β, from PBL Assay Science + 1 ng/ml GM-CSF, from R&D Systems) into U-bottom 96-well plates (1.0 x 10^6 cells/well), and incubating the cells with 20% SLE sera positive for dsDNA-specific IgE. To block serum IgE interaction with cell-surface FcεRs, the human anti-IgE clone E85 (MedImmune LLC) specific for the Fc portion on human IgEs was used at a concentration of 50 μg/ml[45]. A mutation (TM) in the Fc portion of E85 prevented binding of the antibody to FcγRs[46]. A human IgG-TM isotype (MedImmune LLC) was used as control. Cells were incubated at 37°C in 5% CO_2 for 16 hours.

pDC in vitro assays

pDC *in vitro* assays were performed by transferring enriched human pDCs into U-bottom 96-well plates (0.5–1.0 x10^4 cells/well), and incubating the cells at 37°C in 5% CO_2 for the time period indicated in the figure legend. For pDC culture with DNA-ICs, ICs were generated by mixing 0.5 μg/ml of the pMCG-50 plasmid, a plasmid containing 50 copies of a CpG immunostimulatory sequence (CG50)[26], with IgG^D, IgE^D or a combination of both, as mentioned in the figure legend. For the anti-IgE blocking assay, the human anti-IgE clone E85 and the relevant isotype control were used as indicated previously. For the stimulation of pDCs with influenza virus (Flu), cells were incubated with influenza A virus (strain A/Puerto Rico/8/1934 H1N1, American Type Culture Collection) at an MOI =3 for 16 h in the presence of increasing concentrations of aggregated IgE. For surface FcεRI cross-linking, aggregated IgE were generated by diluting IgE^I in 1X phosphate-buffered saline (PBS) at a concentration of 22.0 mg/ml and heated for 30 min. at 65°C. Aggregated IgEs were then added in culture medium at the concentration mentioned in the figure legend. For the TLR9 inhibition assay, TLR9 inhibitor ODN TTAGGG or control ODN for TTAGGG (both from Invivogen) were added to the cells at concentrations specified in the figure legend. For the stimulation of pDCs with RNA-containing ICs, cells were incubated with 2.0 μg/ml of biotinylated U1 snRNP (Arotec Diagnostics) + 0.5 μg/ml streptavidin (Sigma-Aldrich), alone or in combination with biotinylated human IgG or biotinylated IgE (Medimmune LLC), or both immunoglobulin isotypes, as indicated in the figure legend, for 16 h. snRNP and antibodies were biotinylated using the Biotin-XX Microscale Protein Labeling Kit (Life Technologies).

Cytokine measurement

IFN-α in culture supernatants was measured using the multisubtype IFN-α ELISA kit (PBL Biomedical), IL-6, IL-8 and TNF were measured using Human ProInflammatory-4 Ultra-Sensitive Kits (Meso Scale Discovery) and IFN-γ was measured using LEGEND MAX™ Human IFN-γ ELISA Kit (Biolegend).

Intracellular cytokine detection by flow cytometry

Enriched human pDCs were incubated for 6, 9 or 16 hours at 37°C in 5% CO_2 in U-bottom 96-well plates (2.5 x10^4 cells/well) with ICs generated by mixing 0.5 μg/ml of CG50 plasmid DNA with a total of 20 μg/ml antibodies. 0.5 μg/ml of Brefeldin A (Sigma Aldrich) was added to the cells 3 h before the end of the incubation. The cells were then stained for

BDCA-4 (clone 12C2, Biolegend), fixed and permeabilized using Inside Stain Kit (Miltenyi Biotec), and stained for IFN-α (clone LT27:295) and TNF (clone cA2), both from Miltenyi Biotec. Intracellular production of IFN-α and TNF was assessed in BDCA-4 positive cells by flow cytometry.

Labeled DNA-ICs internalization assay

Enriched human pDCs were transferred in U-bottom 96-well plates (2.5 x 10^4 cells/well) and incubated at 37°C in 5% CO_2 for 2 h with ICs generated by combining Alexa Fluor 546-labeled CG50 plasmid DNA (Ulysis™ Alexa Fluor® 546 Nucleic Acid Labeling Kit, Life Technologies) with IgG^D, IgE^D or a combination of both, as indicated in the figure legend. After incubation, cells were treated with a 0.1 mg/ml DNase I solution (StemCell Technologies) to degrade surface-bound DNA. Internalized DNA was visualized by confocal microscopy and quantified by flow cytometry.

pDC maturation assay

Enriched human pDCs were transferred in U-bottom 96-well plates (5.0 x 10^4 cells/well) and incubated at 37°C in 5% CO_2 with ICs generated by mixing 0.5 μM of CG50 plasmid DNA with IgG^D, IgE^D or a combination of both. Surface marker expression was assessed by flow cytometry using CD83-PE-CF594 (clone HB15e), CD86-AF700 (clone FUN-1) from BD Biosciences and CCR7-PerCP-Cy5.5 (clone G043H7), from Biolegend.

Immunofluorescence on renal biopsies

Patients at the University of Chicago who met revised 1982 American College of Rheumatology criteria for SLE[43] were consented for obtaining renal tissue samples. Those who displayed International Society of Nephrology/Renal Pathology Society class II, III, IV, or V LuN on renal biopsy were selected for this study. A total of 27 LN biopsies were utilized. De-identified normal kidney (12 biopsies) samples were used as controls from the University of Chicago Pathology Core Facility. The study protocol was previously approved by the University of Chicago Institutional Review Board. Each diagnostic biopsy sample consisted of at least two tissue cores with smaller portions submitted for immunofluorescence and electron microscopy. At the time of procurement, renal biopsies were immediately frozen in optimum cutting temperature medium (Tissue-Tek) and stored at −80°C. 2–8 μm fresh-frozen sections were stained with immunofluorescent antibodies against either CD20 (Abcam, clone EP459Y) or IgG (rabbit, DAKO, polyclonal), in combination with with BDCA2 (FITC-labeled, Miltenyi, clone AC144), MxA (R&D Systems, polyclonal), IgE (PE-labeled, Biolegend, clone MHE-18), control Igk (PE or APC-labeled, Biolegend, clone MOPC-21), or CD123 (eBioscience, clone 6H6). Basophils were detected using the 2D7 clone (Biolegend). Addition of either DAPI (Invitrogen) or SYTOX Blue (Life Technologies) nucleic acid stains was used for nuclear visualization, depending on number of simultaneous fluorochromes visualized. Fluorescently labeled species-specific secondary anti–immunoglobulin G (IgG) antibodies (Invitrogen) were applied for visualization of primary antibodies that were not directly labeled. Anti-PE antibody (mouse, eBioscience, clone EBIOPE-DLF) was used as necessary for IgE visualization. When required, direct labeling of antibodies was accomplished with Zenon immunofluorescent antibody labeling kits (Invitrogen). Images were captured with either a TCS SP2 Leica laser

scanning confocal microscope or a Leica SP5 Tandem Scanner Spectral 2-photon confocal microscope, fitted with a motorized stage at the University of Chicago Integrated Light Microscopy Facility. Four-, five- and six-channel immunofluorescence was performed at ×600 magnification and a high–numerical aperture (NA, 1.25) oil-immersion objective. Images were acquired by means of a PMT detector and stored in either original LEI or LIF file formats (Leica) depending on microscope used with a bit depth of 8, no binning, and a total size of 512 × 512 pixels, with each pixel corresponding to 0.27 μm. Appropriate single-stained controls were used with each antibody to ensure that no fluorochrome spectral cross-bleeding was present. Average intensity value of immunofluorescence signal was quantified using Adobe Photoshop CS6 software as previously described[47]. LIF file format images were converted to Tagged Image File Format (TIFF) and regions of fixed number of pixels were drawn using the Marquee function. The average signal intensity for each individual channel was measured using the Histogram function. Individual channels were gray scaled prior to measurement.

Intracellular trafficking assays

To visualize TLR9-GFP trafficking to the phagosomes, mouse macrophages were incubated for 30 min. at 37°C and 5% CO_2 with polystyrene beads (3.8 μm, Spherotech) coated with 10 μg/ml CG50 plasmid DNA and with 50 μg/ml of either IgG^D or IgE^D. To follow ICs trafficking to the phagosomes, enriched human pDCs were incubated first with ICs generated by combining 2 μg/ml of CG50 plasmid DNA with 50 μg/ml of Alexa Fluor 488-coupled IgE^D at 4°C for 30 min. and then transferred at 37°C and 5% CO_2 for 120 min. After incubation, cells were fixed using 4% paraformaldehyde and permeabilized using 0.05% Triton. Phagosome maturation was assessed using PE-labeled LAMP1 antibody (clone eBioH4A3, eBioscience). For the IRF7 nuclear translocation assay, enriched pDCs were incubated at 37°C and 5% CO_2 for 5 hours with ICs generated by combining 2 μg/ml of CG50 plasmid DNA with 40 μg/ml of IgE^D. After incubation, cells were fixed and permeabilized as previously described and stained for IRF7 using clone H-246 (Santa Cruz Biotechnology) and Alexa Fluor 647-coupled anti-rabbit (Life Technologies). Nucleus was detected using DAPI (Life Technologies). Cell imaging was performed using a Leica TCS SP5 confocal system consisting of a Leica DMI6000 B inverted microscope (Leica Microsystems). Images were acquired at time points noted in the figure legends and were analyzed using the LAS AF version 2.2.1 Leica Application Suite software (Leica Microsystems).

Plasma cell differentiation assay

B cells and pDCs were resuspended in RPMI 1640 medium supplemented with 10% FBS, 1x penicillin/streptomycin, 2mM L-glutamine, 5mM HEPES (all from Life Technologies) and 55 μM β-mercaptoethanol (Sigma) and transferred into U-bottom 96-well plates. 7.5 x 10^4 B cells and 3.75 x 10^3 pDCs were added to each well, along with 1.5 nM mega CD40L (Enzo Biosciences). ICs generated by combining 2.5 μg/mL of IgE^D with 1 μg/mL of CG50 plasmid DNA were added to the co-cultures. Cells were cultured for 7 days at 37°C and 5% CO_2, washed and stained with CD123-FITC (clone 7G3), CD19-BV421 (clone HIB19), CD27-PerCPCy5.5 (clone M-T271), IgD-PE (clone IA6-2) and CD38-APC (clone HIT2), all from BD Biosciences. For flow cytometry assessment of B cell and plasma cell numbers,

co-cultured cells were acquired for a fixed amount of time. B cells were defined as $CD123^{-}$ $CD19^{+}$ and plasma cells were defined as $CD123^{-}CD19^{+}$ $CD27^{hi}$ $CD38^{hi}$ cells. Supernatants were recovered at Day 7 for indirect quantification of IgM by ELISA as described previously[48]. Anti-human IFN-α receptor (IFNAR) antibody (MedImmune LLC)[49] anti-IL6 antibody (clone 6708, R&D Systems), or an anti-IL6 antibody (MedImmune LLC)[50] were used at final concentrations of 10 μg/mL.

pDC-T cell co-culture

pDCs from donors positive for CMV reactivity were transferred into 96-well plates (2.5 x 10^4 cells/well) and stimulated with ICs generated by combining 0.5 μg/mL CG50 plasmid DNA with 10 μg/mL of IgG^D, IgE^D, or 5 μg/mL of each isotypes (IgG^D + IgE^D) for 4 hours in the presence of 10 μg/mL of a cytomegalovirus (CMV) peptide (NLVPMVATV, ProImmune). pDCs were then washed and incubated for 4 days with autologous CD8 T cells (2.5 x 10^5 cells/well) in the presence of 100 IU/ml recombinant human IL-2 (Peprotech). CMV-specific CD8 T cells were identified using CMV Dextramer HLA-A*0201/ NLVPMVATV (Immudex). CMV-specific CD8 T cell proliferation was assessed by flow cytometry using CellTrace™ CFSE Cell Proliferation Kit (Life Technologies).

pDC migration assay

pDCs (5.0 x 10^4) were transferred into the upper chamber of a 5 μm-pore HTS Transwell®-96 well plate (Corning) and stimulated with ICs generated by combining 0.5 μg/mL CG50 plasmid DNA with 10 μg/mL of IgG^D, IgE^D, or 5 μg/mL of each isotypes (IgG^D + IgE^D). Cells were transferred to 37°C and allowed to migrate to the bottom chamber containing 0.5 μg/mL of CCL19 and 0.5 μg/mL of CCL21 (both from R&D Systems). Cells in the lower chamber were harvested after 16 h and analyzed by flow cytometry.

Statistical analysis

Statistical differences in the distribution of variables between groups were determined using the two-tailed Mann-Whitney *U* test, unless stated differently in the figure legend. In each case the required assumptions of the non-parametric test were met since all data consisted of independent observations originating from continuous distributions. The monotonic relationship between variables was evaluated using the Spearman's rank correlation test. The test was deemed appropriate since all variables originated from independent observations and underlying continuous distributions. The independent relationship between dsDNA-specific IgE and SLEDAI was assessed by conducting an F-test on the regression coefficient of a multiple linear regression model fit with SLEDAI as a dependent variable and the concentration of dsDNA-specific IgE, dsDNA-specific IgG, and their interaction as independent variables. We graphically assessed residuals and observed no serious departures from model assumptions of normality of residuals, linear relationship between variables, and homoscedasticity. Results were considered significant for $P < 0.05$. Multiple linear regression was performed in SYSTAT 13.0. All other statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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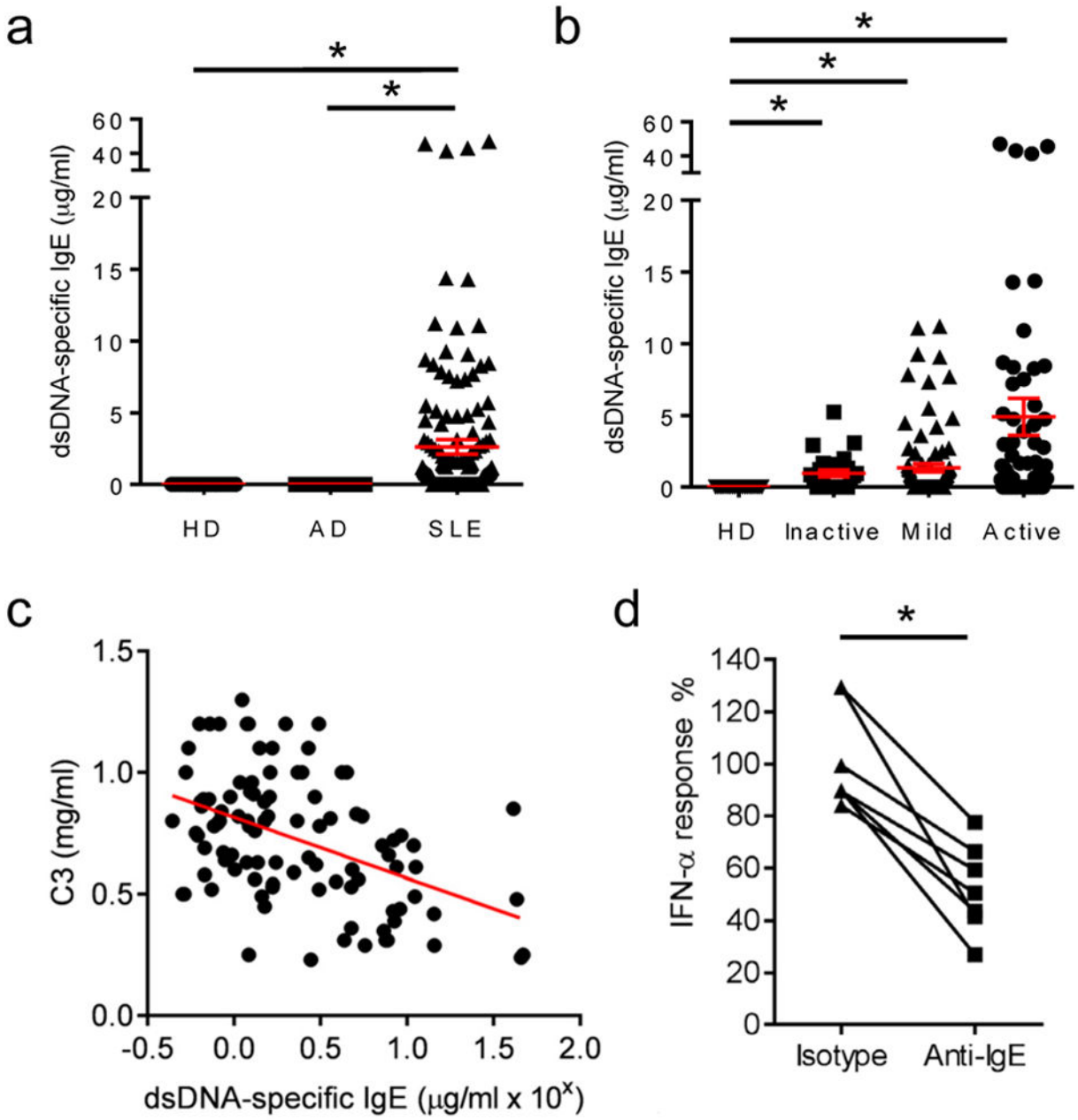

Figure 1. Anti-dsDNA IgE autoantibodies contribute to IFN response in SLE

(a) Serum concentrations of dsDNA-specific IgE in SLE patients (n = 180), atopic dermatitis patients (AD, n = 24) and healthy donors (HD, n = 26) as measured by ELISA. **(b)** SLE patients were grouped by SLE Disease Activity Index (SLEDAI). Concentrations of dsDNA-specific IgEs in SLE patients is shown for healthy donors (HD, n = 26), inactive (SLEDAI = 0, n = 25), mild (SLEDAI > 0 to <4, n = 87) and active (SLEDAI ≥ 4, n = 66) disease. Mean ± s.e.m. for each group is shown in red. **(c)** Serum concentrations of dsDNA-specific IgEs and complement component 3 (C3) from SLE patients (n = 98), as measured by ELISA and quantitative multiplexed immunoassay, respectively. Correlation was evaluated using the Spearman's rank test (r=−0.345, p<0.0001). **(d)** Human PBMCs were stimulated with 20%

serum from patients that had tested positive for dsDNA-specific IgE ($n = 7$) in the presence of an antibody that interferes with the binding of IgE to its receptors (anti-IgE), an isotype control antibody (Isotype), or no antibody. IFN-α was then measured in supernatants. Paired data are presented as percentage of response compared to the no antibody treatment. * $P < 0.001$.

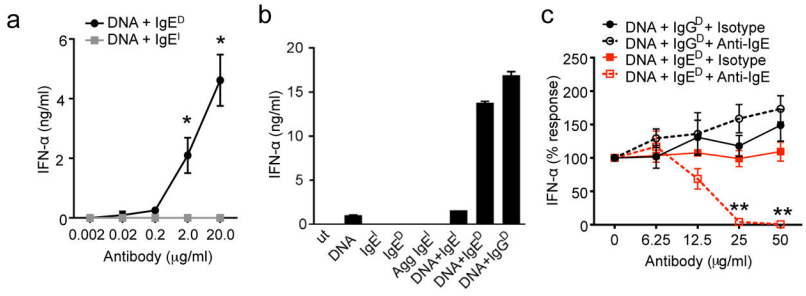

Figure 2. DNA-IgE ICs trigger IFN-α secretion from pDCs

(a) Purified pDCs were stimulated with increasing concentrations of monoclonal IgE^D or with monoclonal isotype IgE control (IgE^I) in combination with 0.5 μg/ml DNA. Data are presented as mean ± s.d. and are representative of three independent experiments. * $P < 0.05$. **(b)** IFN-α in supernatants of pDCs untreated (ut) or stimulated with 0.5 μg/ml DNA, 20 μg/ml of IgE^D or IgE^I alone or in combination, as measured by ELISA. IgG^D + DNA was used as a positive control. Aggregated IgE^I (Agg IgE^I) was used to activate FcεRI in the absence of DNA. Data are presented as mean ± s.d. and are representative of three independent experiments. **(c)** pDCs were stimulated with DNA in combination with IgG^D or IgE^D as indicated. Cells were additionally treated with increasing concentrations of the blocking anti-IgE antibody or an isotype control (Isotype). Data are presented as mean ± s.e.m. from five independent experiments. ** $P < 0.001$.

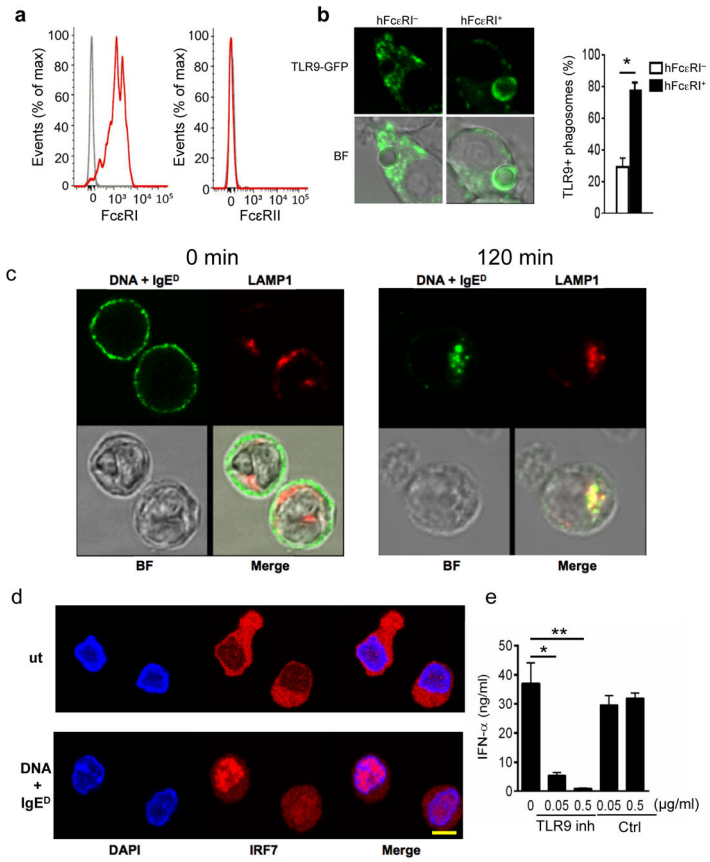

Figure 3. IgE-containing ICs deliver DNA to TLR9 at the phagosome

(a) Flow cytometry analysis of cell surface expression of FcεRI and FcεRII (red line) in pDCs compared isotype control (grey line). Data are representative of 3 independent experiments. **(b)** Mouse macrophages expressing TLR9-GFP and the alpha chain of human FcεRI (hFcεRI$^+$) were fed with beads coated with DNA+IgE^D. TLR9-GFP localization in hFcεRI$^+$ and control macrophages (hFcεRI$^-$) was visualized by confocal microscopy 30 min. after bead internalization. Yellow arrows point to ingested beads. TLR9$^+$ phagosomes were quantified (n = 75 phagosomes per/group from three independent experiments). Data are presented as mean ± s.d. **(c)** Human pDCs were incubated with DNA+IgE^D for 30 min. at 4°C (0 min) and then transferred to 37°C for 120 min. (120 min.). DNA+IgE^D (green) and LAMP1 (red) intracellular localization was visualized by confocal microscopy and frames from one representative experiment out of three are shown. BF = bright field. Scale bars, 5μm. **(d)** Human pDCs were incubated for 5 h at 37°C in the presence of DNA+IgE^D or untreated (ut). IRF7 intracellular localization was visualized by immunostaining using confocal microscopy. Frames from one representative experiment out of three are shown. **(e)** pDCs were stimulated with DNA+IgE^D in the presence of 0.05 or 0.5 μg/ml of TLR9 oligodeoxynucleotide (ODN) inhibitor (TLR9 inhib) or control ODN (Control). After 16 h, IFN-α was measured by ELISA in supernatants. Data are presented as mean ± s.e.m. from three independent. * $P < 0.01$, ** $P < 0.001$.

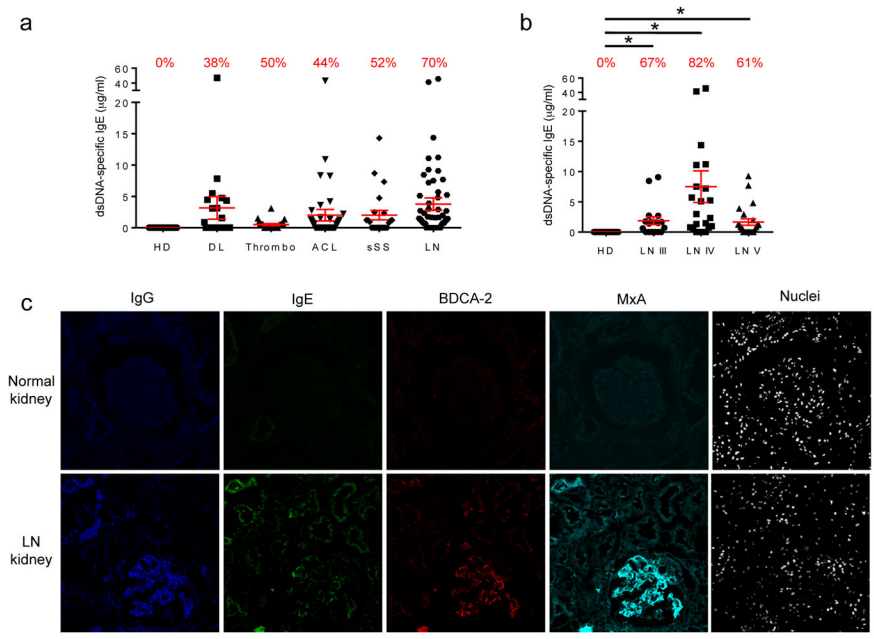

Figure 4. Deposition of IgE autoantibodies in lupus nephritis

(a) Serum concentration of dsDNA-specific IgE was measured in subgroups of SLE patients by ELISA. The subgroup tested were HD ($n = 26$), Discoid Lupus (DL, $n = 25$), Lupus-associated Thrombocytopenia (Thrombo, $n = 23$), Acute Cutaneous Lupus (ACL, $n = 47$), Lupus-associated Secondary Sjogren's (sSS, $n = 22$), and Lupus Nephritis (LN, $n = 63$). Data are presented as mean ± s.e.m. The percentage of patients positive for dsDNA-specific IgE (% pos) for each disease subgroup is indicated in red. **(b)** Prevalence of dsDNA-specific IgE in focal LN or Class III (LN III; $n = 18$), diffuse LN or Class IV (LN IV; $n = 22$), and membranous LN or Class V (LN V; $n = 23$). Data are presented as mean ± s.e.m. * $P < 0.001$. The Percentage of patients positive for dsDNA-specific IgE is indicated in red for each LN subgroup. **(c)** Immunofluorescence stainings in frozen kidney biopsies. Representative staining of IgG (blue), IgE (green), pDCs (BDCA-2, red), and IFN-α-induced protein MxA (light blue) in normal and LN kidney biopsies. Yellow arrows indicate glomerular area. Images are representative for 11 LN and 5 normal kidneys biopsies.

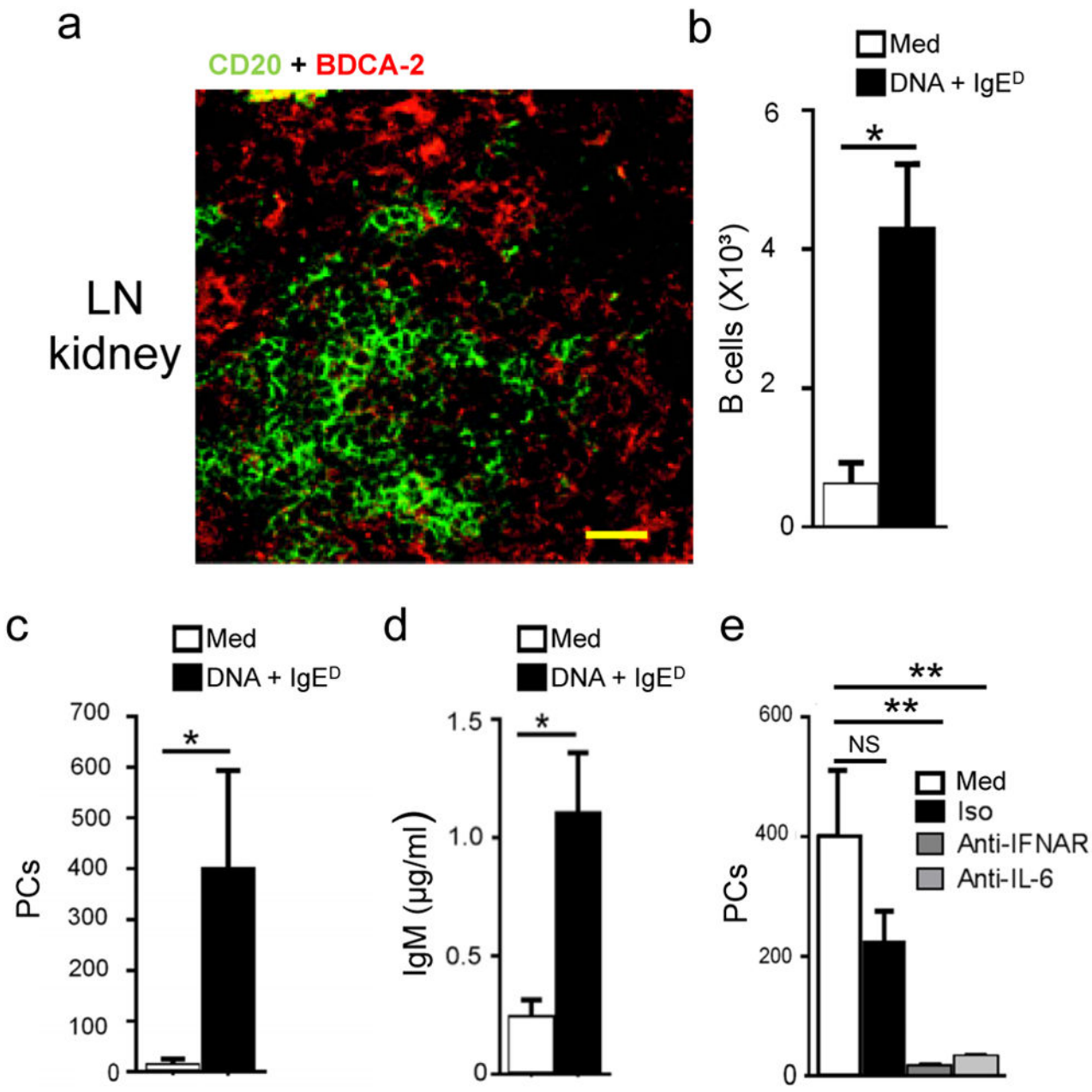

Figure 5. dsDNA-specific IgE induces pDC-mediated PC differentiation

(a) pDCs (BDCA-2, red) and B cells (CD20, green) were found in close proximity in the tubular area in kidney in 2 out of 3 LN biopsies examined. A representative frame is shown. Scale bar, 20 µm. **(b–e)** Flow cytometry analysis of B cells and PC numbers from pDC/B cell co-cultures left untreated in media (Med) or stimulated for 7 days with DNA+IgE^{D}. B cells were defined as CD123^{-} CD19^{+} cells and PCs were defined as CD123^{-}CD19^{+} CD27^{hi} CD38^{hi} cells. B cell **(b)** and PC cells **(c)** from pDC/B cell co-cultures were quantified as the number of cells acquired for a fixed amount of time per well. **(d)** IgM in pDC/B cell co-culture supernatants was measured by ELISA at day 7 after stimulation with DNA+IgE^{D}. **(e)** Flow cytometry analysis of PC numbers in pDC/B cell co-cultures stimulated with DNA

+IgE^{D} in the presence of a blocking antibody specific to human IFNAR (anti-IFNAR), an IL-6-specific antibody (anti-IL-6) or an isotype control (Iso). Data are presented as mean ± s.d. of at least three independent. For the pDC-B cell co-culture experiments, means were compared by using Student's t-test. * $P < 0.001$; ** $P < 0.05$.

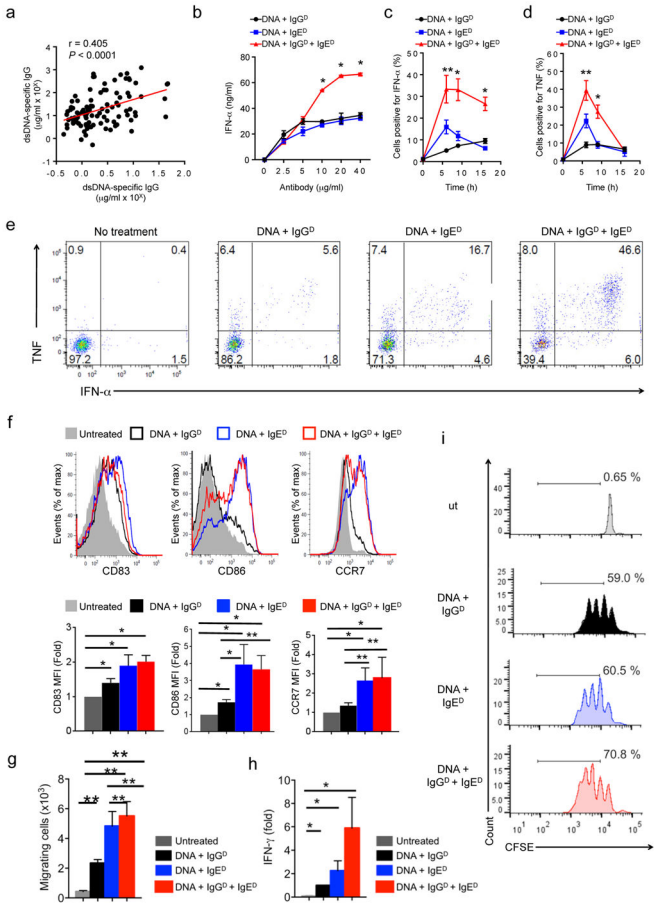

Figure 6. Synergistic stimulation of pDCs by IgE and IgG immune complexes

(a) Serum concentrations of dsDNA-specific IgE and IgG in SLE patients ($n = 98$). Correlation was evaluated using the Spearman's rank test. **(b)** IFN-α production by pDCs stimulated for 16 h with DNA-ICs containing increasing concentrations of IgE^D and IgG^D. Data are presented as mean ± s.d. and are representative of seven independent experiments. Intracellular expression of IFN-α **(c)** and TNF **(d)** in pDCs stimulated with DNA-ICs. Data are presented as mean ± s.e.m. of four independent experiments. **(e)** Representative plots of IFN-α and TNF expression 6 h after pDC stimulation. **(f)** Surface expression of CD83, CD86 and CCR7 on pDCs stimulated for 16 h with DNA-ICs. Representative histograms are shown (top panels) and fold increase of mean fluorescence intensity (MFI) over untreated cells are presented as mean ± s.e.m. of five independent experiments (lower panels). **(g)** pDCs migration in response to CCR7 ligands (CCL19/CCL21) was assessed after stimulation with DNA-ICs for 16 h.. Data are presented as mean ± s.e.m. of migrating pDC numbers from four independent experiments. **(h)** IFN-γ in supernatants of cytomegalovirus (CMV) peptide-loaded pDCs stimulated with DNA-ICs for 4 h and co-cultured with CMV-responsive CD8 T cells for 48 h. Data are presented as mean ± s.e.m. of three independent experiments. **(i)** Proliferation of CMV-specific CD8 population after 4 days of co-culture. Histograms displayed are from one representative donor out of three. * $P < 0.01$, ** $P < 0.05$.

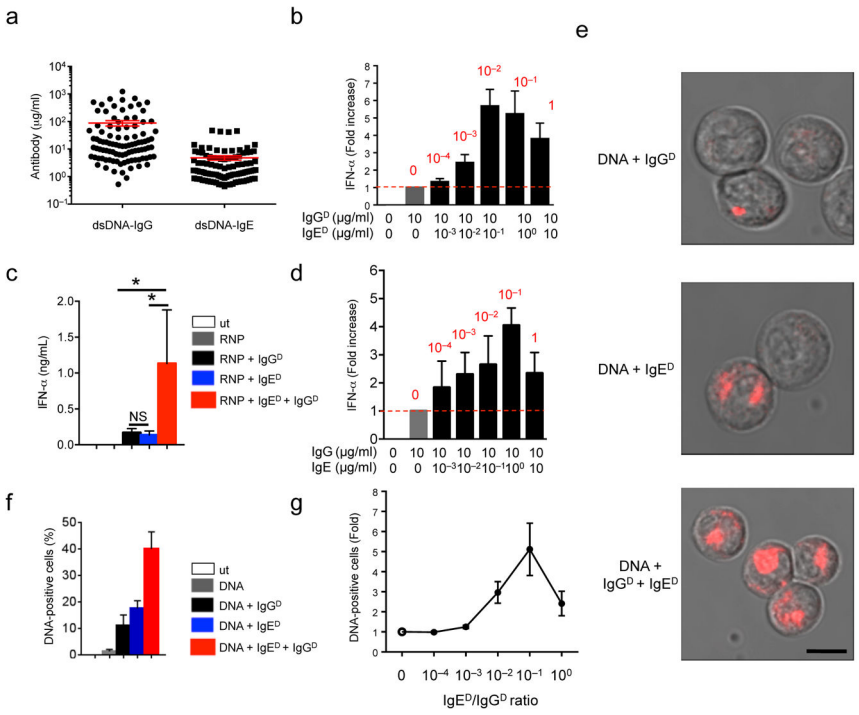

Figure 7. dsDNA-specific IgE enhance pDC interferon responses through increased DNA phagocytosis

(a) Serum concentration of dsDNA-specific IgG and dsDNA-specific IgE in SLE patients that tested positive for dsDNA-specific IgE (n = 98). Mean values were 88.0 μg/ml for DNA-IgG and 4.8 μg/ml for DNA-IgE. Mean ± s.e.m are represented in red. **(b)** IFN-α in supernatants of pDCs stimulated with DNA-ICs containing a fixed amount of IgG^D (10 μg/ml) and DNA (0.5 μg/ml) and increasing concentrations (as indicated in x-axis) of IgE^D for 16 h. The ratio IgE^D/IgG^D in the immune complexes for each of the condition is indicated in red above each bar. Data are presented as fold increase over pDCs treated with ICs containing IgG^D and DNA (red dotted line) and are mean ± s.e.m. of three independent experiments. **(c)** IFN-α in supernatants of pDCs treated with by biotinylated RNP (RNP) and biotinylated IgG, IgE or IgG + IgE in the presence of streptavidin to form RNA-containing immune complexes. Cells were incubated with the ICs for 16 h. Data are presented as mean ± s.e.m. of four independent experiments. **(d)** IFN-α in supernatant of pDCs treated with similar RNA immune complexes containing a fixed amount of IgG (10 μg/ml) and DNA (0.5 μg/ml) and increasing concentrations of IgE as indicated. The ratio IgE^D/IgG^D in the immune complexes for each of the condition is indicated in red above each bar. Data are represented as fold increase over the IFN-α obtained from cells treated with RNA-ICs containing IgG only (red dotted line). Data are presented as mean ± s.e.m. of five independent experiments. **(e)** Internalized DNA in pDCs treated with DNA-ICs. Representative frames from three independent experiments are shown. Scale bar = 5.0 μm. **(f)** Engulfment of DNA was quantified by FACS and the percentage of cells that were positive for phagocyted DNA is represented. Data are presented as mean ± s.e.m. from seven independent experiments. **(g)** DNA-ICs containing the indicated IgE^D/IgG^D ratio were fed to pDCs. Percentage of pDCs positive for engulfed DNA was measured by FACS. Data

represent fold increase over cells incubated with DNA+ IgG^D (open circle) and are presented as mean ± s.e.m. of five independent experiments. * $P < 0.05$.

Table 1

Multivariate analysis of dsDNA-specific IgE and IgG as disease activity risk factors in SLE using a General Linear Model. The results of this model indicate that dsDNA-specific IgE is an independent risk factor for disease activity in SLE. Regression coefficient values and statistical significance are represented.

Variable	Coefficient (95% CI)	p-value
anti-dsDNA IgG (μg/ml)	.00426 (.000453,.00807)	.029
anti-dsDNA IgE (μg/ml)	.157 (.0583,.256)	.0020
anti-dsDNA IgG (μg/ml)* anti-dsDNA IgE (μg/ml)	−.000232 (−.000681,.000218)	.31

The independent relationship between anti-dsDNA IgE and SLEDAI was assessed by conducting and F-test on the regression coefficient of a multiple linear regression model fit with SLEDAI as a dependent variable and the concentration of anti-dsDNA IgE, anti-dsDNA IgG, and their interaction as independent variables (Further details in Experimental Procedures section). CI = Confidence Interval