Self-reactive IgE exacerbates interferon responses associated with autoimmunity

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Canonically, immunoglobulin E (IgE) mediates allergic immune responses by triggering mast cells and basophils to release histamine and type 2 helper cytokines. Here we found that in human systemic lupus erythematosus (SLE), IgE antibodies specific for double-stranded DNA (dsDNA) activated plasmacytoid dendritic cells (pDCs), a type of cell of the immune system linked to viral defense, which led to the secretion of substantial amounts of interferon-α (IFN-α). The concentration of dsDNA-specific IgE found in patient serum correlated with disease severity and greatly potentiated pDC function by triggering phagocytosis via the high-affinity FcεRI receptor for IgE, followed by Toll-like receptor 9 (TLR9)-mediated sensing of DNA in phagosomes. Our 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.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the loss of immunotolerance to nucleic acids, the activation of auto-reactive lymphocytes, and the production of large quantities of self-reactive antibodies that induce tissue damage4. Renal autoantibody deposition and lymphocyte infiltration lead to nephritis, a serious complication of lupus that presents in the clinical course of up to 60% of patients2. A hallmark of SLE is the production of type I interferons in response to immunocomplexes (ICs) containing self DNA from dead cells and DNA-specific immunoglobulin G (IgG)3. There is now a mounting body of evidence indicating that plasmacytoid dendritic cells (pDCs) are the main producers of pathogenic type I interferons in SLE4. pDCs are cells of the immune system that specialize in antiviral responses5. After sensing viral nucleic acids through the Toll-like receptors TLR7 (which senses RNA) and TLR9 (which senses DNA), pDCs release up to 1,000 times more type I interferons than any other cell type releases6, which promotes the cellular expression of interferon-stimulated genes and the apoptosis of infected cells. Although TLR9 binds indiscriminately to both viral DNA and endogenous host 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) and form DNA-containing ICs; these are then internalized by pDCs via the FcγRIIa receptor for IgG7, a process that allows delivery of self DNA to TLR9 within pDCs, which triggers an aberrant antiviral response. The recognition of self DNA by TLR9 leads to recruitment of the adaptor MyD88 and then to activation of the transcription factors NF-κB and IRF7, which induce the secretion of proinflammatory cytokines (such as TNF) and large amounts of type I interferons, respectively8,9. Activation of TLR9 also induces the migration of pDCs and their ability to activate T cell and B cells, which positions pDCs at the crossroads of both innate immune responses and adaptive immune responses10.

Published evidence has demonstrated that double-stranded DNA (dsDNA)-specific antibodies of the IgE immunoglobulin class are also present in some patients with SLE11–13, and although they have been associated with the activation of basophils12,14, their role in disease pathogenesis has remained unclear. Found only in mammals, IgE is the least abundant immunoglobulin isotype and signals through two types of Fc fragment receptors: 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 to innocuous foreign proteins (allergens)15,16. In both of these cases, IgE recognizes exogenous antigens and triggers an immunological response that is associated with the degranulation of mast cells and the subsequent release of biogenic amines and lipid mediators, the production of cytokines of the TH2 subset of helper T cells (such as interleukin 4 (IL-4), IL-5 and IL-13), and eosinophilia15. Paradoxically, none of these inflammatory responses are key drivers of the pathogenesis of SLE11,17,18, and patients with SLE do not appear to be more prone to IgE-driven environmental allergies than is the general population19–21. Thus, it is plausible that self-reactive IgE in autoimmunity might have functions different from those described for IgE in helminth defense and allergy. To explore

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RESULTS
IgE-triggered secretion of interferon-α in SLE

In the SLE cohort we studied, 98 of 180 patients (54.4%) exhibited detectable concentrations of dsDNA-specific IgE, while healthy subjects, as well as patients with atopic dermatitis (a disease associated with elevated concentrations of IgE in serum), were all negative for this autoantibody (Fig. 1a). The amount of circulating dsDNA-specific IgE was increased particularly in active disease (Fig. 1b), and this correlated with small amounts of the complement component C3, a serological marker of disease activity\(^\text{13,22}\) (Fig. 1c). To investigate the role of dsDNA-specific IgE in SLE, we explored whether these autoantibodies were able to modulate the type I interferon response. Activation of FcεR on pDCs by heat-aggregated IgE inhibited influenza virus–induced secretion of interferon-α (IFN-α) (IFN-α) (Supplementary Fig. 1a), a mechanism believed to explain the impaired IFN-α response observed for pDCs in atopic patients\(^\text{23–25}\). Therefore, the production of self-reactive IgE in patients with SLE with active disease might be a protective immunological mechanism for limiting exacerbated interferon responses and for diminishing inflammation. To test this hypothesis, we stimulated peripheral blood mononuclear cells (PBMCs) with serum from dsDNA-IgE\(^±\) patients with SLE, in the presence of an IgE-blocking antibody that interferes with the binding of this immunoglobulin to its receptor. Unexpectedly, blockade of IgE did not increase IFN-α secretion by PBMCs but instead reduced its production (Fig. 1d), which indicated that IgE might have actively contributed to the aberrant interferon responses in these patients. To assess if IgE autoantibodies were associated with clinical manifestations, we conducted a multivariate regression analysis using the general linear model, with the SLE disease activity index (SLEDAI) score as the dependent variable. According to this model, the concentration of dsDNA-specific IgE was a risk factor for SLE activity independently of the concentration of dsDNA-specific IgG (Table 1). Together these results suggested that these IgE autoantibodies found in SLE potentiates type I interferon responses and represented a previously unknown culprit that contributed to the aberrant secretion of interferon that underlies the pathogenesis of SLE.

Sensing of dsDNA-specific IgE by pDCs

To gain mechanistic insight into how the self-reactive IgE found in SLE might contribute to this disease pathology, we engineered the variable regions of a previously described dsDNA-specific IgG (IgG\(^\text{D}\)) onto an IgE backbone that maintained specificity for dsDNA (IgE\(^\text{D}\)) (Supplementary Fig. 2a,b). As expected, IgE\(^\text{D}\) showed dose-dependent binding to plate-bound DNA (Supplementary Fig. 2c). IgE\(^\text{D}\) induced secretion of IFN-α by pDCs in a concentration-dependent manner when combined with DNA, but an isotype-matched control IgE (IgE\(^\text{D}\)) did not (Fig. 2a); this demonstrated that ICs formed by IgE\(^\text{D}\) and DNA were able to elicit interferon responses. Moreover, DNA-IgE ICs induced amounts of IFN-α similar to those induced by DNA-IgG ICs (Fig. 2b). Notably, the IgE-blocking antibody did not affect the production of IFN-α induced by DNA-IgG ICs in pDCs, while it completely inhibited IFN-α production in cells stimulated with DNA-IgE ICs (Fig. 2c). Together these findings demonstrated that dsDNA-specific IgE similar to that found in SLE was able to trigger the production of large quantities of IFN-α by pDCs.

To investigate the mechanisms leading to the activation of pDCs mediated by dsDNA-specific IgE, we first evaluated the cell-surface expression of both its high-affinity receptor (FcεRI) and its low-affinity receptor (FcεRII). We found that pDCs expressed only FcεRI (Fig. 3a). Ectopic expression of human FcεRI by mouse macrophages was required for a marked increase in recruitment of the DNA receptor TLR9 to particles coated with DNA-IgED ICs (Fig. 3b), which indicated that FcεRI at the cell surface facilitated the delivery of DNA-IgED into a phagosomal compartment, where the engulfed DNA could be sensed. TLR9 triggers IRF7-dependent secretion of IFN-α from mature compartments positive for the lysosome marker LAMP-1 (refs. 26,27). Therefore, we further explored the subcellular localization of DNA-IgED ICs within the cell. DNA-IgED ICs localized together with LAMP-1 in pDCs after internalization of the ICs (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 inhibitors of TLR9 (Fig. 3e). DNA-IgED 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 showed that DNA-IgE ICs engaged with FcεRI at the cell surface, which then delivered the complexes into mature compartments, where TLR9 sensed the DNA. In this intracellular compartment, TLR9 initiated a complex inflammatory response characterized by

**Figure 1** Anti-dsDNA IgE autoantibodies contribute to IFN response in SLE. (a) Enzyme-linked immunosorbent assay (ELISA) of dsDNA-specific IgE in serum from healthy donors (HD, n = 26) and patients with atopic dermatitis (AD) (n = 24) or SLE (n = 180). (b) ELISA of dsDNA-specific IgE in serum from healthy donors (n = 26) and patients with SLE, grouped by SLEDAI as having inactive disease (SLEDAI = 0; n = 25), mild disease (SLEDAI >0 to <4; n = 87) or active disease (SLEDAI 4; n = 66). (c) ELISA of dsDNA-specific IgE (presented as pg/ml × 10\(^\text{4}\)) from supernatants of human PBMCs stimulated with 20% serum from dsDNA-IgE\(^±\) patients with SLE (n = 7), assessed in the presence of an antibody that interferes with the binding of IgE to its receptors (Anti-IgE), an isotype-matched control antibody (Isotype), or no antibody; results (paired values) are presented relative to those obtained with no antibody. Each symbol (a–c) represents an individual donor; red horizontal lines (a,b) indicate the mean (± s.e.m.). *P < 0.001 (Mann-Whitney U-test (a,b) or paired t-test (d)). Data in each panel are representative of one experiment.
IgE deposits in SLE kidney biopsies

We found that 54.4% of patients with SLE in our cohort presented with dsDNA-specific IgE (Fig. 1a). We then sought to determine whether any disease subset in our cohort showed particular enrichment for these autoantibodies 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. 1a). Patients diagnosed with the most severe and common subtype of LN (diffuse proliferative nephritis; LN class IV) showed further enrichment for dsDNA-specific IgE, with a large majority of the patients (82%) having detectable levels of dsDNA-specific IgE (Fig. 1b). As previously shown for IgG,28,29 IgE deposits were present in both glomerular areas and extraglomerular areas (Fig. 1c and Supplementary Fig. 1a,b). IgE was deposited similarly in both areas, while IgG deposits were present mainly in glomerular structures (Fig. 1c and Supplementary Fig. 1a,b). pDC infiltrates were present in glomerular and extraglomerular areas, with larger infiltrates of pDCs in the glomeruli (Fig. 1c and Supplementary Fig. 1a,b). However, we did not detect substantial infiltration of basophils, a cell type that responds to IgE and has been linked to MxA, a protein produced in response to the secretion of type I interferons, was expressed mainly in the glomeruli of biopsies from patients with LN, with a distribution similar to that of pDCs (Fig. 1c and Supplementary Fig. 1a,b). This suggested that infiltrating pDCs were able to interact directly with and become activated by local ICs containing nucleotides able to trigger TLR7- and/or TLR9-dependent stimulation. As circulating IgG and IgE autoantibodies have equal access to exposed nucleic acid autoantigens shed by dead cells in tissues, the interferon response visualized as MxA positivity in the kidney biopsies was probably driven by ICs that simultaneously contained immunoglobulins of both classes. Therefore, we concluded from these experiments that IgE autoantibodies deposited in renal glomeruli during lupus could potentially contribute to local inflammatory processes.

Synergy of IgE ICs and IgG ICs

In the SLE cohort we studied, circulating dsDNA-specific IgE correlated with dsDNA-specific IgG (Fig. 6a). Notably, all patients whose serum contained dsDNA-specific IgE also tested positive for dsDNA-specific IgG, with no patient being positive for the IgE isotype alone (Fig. 6a). As exposed DNA from damaged cells is accessible to both dsDNA-specific IgG and dsDNA-specific IgE in the circulation, patients probably form pathogenic ICs that simultaneously contain both classes of immunoglobulins. That concept is consistent with the finding of deposits of both IgG autoantibodies and IgE autoantibodies in glomerular and tubular areas of biopsies from patients with LN (Fig. 1c and Supplementary Fig. 1a,b). For this reason, we investigated the effect of ICs containing DNA plus IgG and IgE on pDC responses. We found that combining both isotypes at a ratio

Table 1 Multivariate analysis of dsDNA-specific IgE and IgG as risk factors for disease activity in SLE

| Variable       | Coefficient (95% CI) | P value |
|----------------|----------------------|---------|
| Anti-dsDNA IgG | 0.00426 (0.000453 to 0.00807) | 0.029   |
| Anti-dsDNA IgE | 0.157 (0.0583 to 0.256) | 0.0020  |
| Anti-dsDNA IgG x anti-dsDNA IgE | −0.000232 (−0.000681 to 0.000218) | 0.31 |

Analysis of the independent relationship between anti-dsDNA IgE and SLEDAI by a F-test of the regression coefficient of a multiple linear regression model fit with SLEDAI as a dependent variable and the concentration of anti-dsDNA IgE and/or anti-dsDNA IgG (in µg/ml) and their interaction as independent variables, presented as regression coefficient values (with confidence interval (CI) in parentheses) and statistical significance.

The production of several proinflammatory cytokines, including the secretion of substantial amounts of IFN-α, was observed in cultures of pDCs stimulated with DNA in combination with IgG and IgE (Supplementary Fig. 4a,b). As previously shown for IgG,28,29 IgE deposits were present in both glomerular areas and extraglomerular areas. However, we did not detect substantial infiltration of basophils, a cell type that responds to IgE and has been linked to MxA, a protein produced in response to the secretion of type I interferons, was expressed mainly in the glomeruli of biopsies from patients with LN, with a distribution similar to that of pDCs (Fig. 4c and Supplementary Fig. 4a,b). This suggested that infiltrating pDCs were able to interact directly with and become activated by local ICs containing nucleotides able to trigger TLR7- and/or TLR9-dependent stimulation. As circulating IgG and IgE autoantibodies have equal access to exposed nucleic acid autoantigens shed by dead cells in tissues, the interferon response visualized as MxA positivity in the kidney biopsies was probably driven by ICs that simultaneously contained immunoglobulins of both classes. Therefore, we concluded from these experiments that IgE autoantibodies deposited in renal glomeruli during lupus could potentially contribute to local inflammatory processes.

B cell responses triggered by DNA-IgE-stimulated pDCs

We also observed areas of kidney biopsies of patients with LN in which both pDCs and B cells were present in close proximity (Fig. 5a). pDCs can induce the proliferation of B cells and the differentiation of B cells into plasma cell (PCs)30. Therefore, we investigated the ability of dsDNA-specific IgE to stimulate PC differentiation in a pDC–B cell co-culture system, using purified human pDCs and B cells. The addition of DNA-IgED ICs led to a marked increase in the number of B cells (Fig. 5b), the differentiation of B cells into PCs (Fig. 5c) and the 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 pDCs act sequentially on B cells to trigger their differentiation into PCs, with IFN-α generating plasmablasts that do not secrete immunoglobulins, and IL-6 inducing their final differentiation into immunoglobulin-secreting PCs.31 We therefore investigated whether these soluble factors were required for the generation of PCs in response to the IgE-containing ICs. Inhibiting the type I interferon receptor IFNAR or inhibiting IL-6 with blocking antibodies resulted in a marked reduction in the formation of PCs in the co-cultures treated with DNA-IgED ICs (Fig. 5e). Collectively, these results demonstrated that ICs containing dsDNA-specific IgE were able to lead to pDC-mediated activation of B cells, a mechanism that might exacerbate the B cell–driven autoimmune response in the kidneys of patients with LN.

Figure 2 DNA-IgE ICs trigger secretion of IFN-α from pDCs. (a) ELISA of IFN-α in supernatants of purified pDCs stimulated with DNA (0.5 µg/ml) in combination with increasing concentrations (horizontal axis) of monoclonal IgG0 or with isotype-matched IgE control monoclonal antibody (IgE) (key). (b) ELISA of IFN-α in supernatants of pDCs left untreated (UT) or stimulated with DNA (0.5 µg/ml), IgE0 or IgE alone (20 µg/ml each), aggregated IgE (Agg IgE) (for activation of FcεRI in the absence of DNA), or ICs formed by DNA plus IgE0 (DNA-IgE), DNA plus IgE0 (DNA-IgE0) or DNA plus IgG0 (DNA-IgG0) (positive control). (c) IFN-α in supernatants of pDCs stimulated with DNA in combination with IgG0 or IgE0 (key) and treated with increasing concentrations (horizontal axis) of blocking antibody to IgG or isotype-matched control antibody (Isotype). * P < 0.05 and ** P < 0.001 (Mann-Whitney U-test). Data are representative of three independent experiments (a,b) or are from five independent experiments (c,d). mean ± s.e.m.)
of 1:1 resulted in the formation of complexes that triggered much greater secretion of IFN-α than that triggered by complexes containing a single immunoglobulin isotype (Fig. 6b). This combination also increased secretion of the proinflammatory cytokines TNF, IL-6 and IL-8 (Supplementary Fig. 6a–c). We monitored the intracellular production of IFN-α and TNF by stimulated pDCs for 16 h and observed that the elevated concentrations of IFN-α (Fig. 6c) and TNF (Fig. 6d) were due largely to an increase in the frequency of cells secreting these cytokines. We noted the peak of production was at 6 h, when most cells were co-expressing both cytokines (Fig. 6e). pDCs exposed to DNA-IgED ICs also had a phenotype more mature than that of unstimulated cells, with robust expression of the cell-surface co-stimulatory molecules CD83 and CD86 (Fig. 6f). CD86 expression was much higher on cells treated with IgED-containing ICs than

Figure 3 IgE-containing ICs deliver DNA to TLR9 at the phagosome. (a) Flow cytometry analyzing the cell-surface expression of FcεRI or FcεRII (red lines) in pDCs, gray lines, isotype-matched control antibody. (b) Confocal microscopy (left) of the localization of green fluorescent protein (GFP)-tagged TLR9 (TLR9-GFP) in mouse macrophages expressing GFP-tagged TLR9 and the α-chain of human FcεRI (hFceRI+) or control macrophages (hFceRI−), assessed 30 min after internalization of beads coated with DNA-IgED (yellow arrows indicate ingested beads), and quantification of TLR9+ phagosomes (right). Original magnification, ×400. BF, bright field. (c) Confocal microscopy of the intracellular localization of DNA-IgED (green) and LAMP-1 (red) in human pDCs incubated with DNA-IgED for 30 min at 4 °C (0 min), followed by incubation for 120 min at 37 °C (120 min). Scale bars, 5 μm. (d) Confocal microscopy of the intracellular localization of immunostained IRF7 in human pDCs left untreated (top) or incubated for 5 h at 37 °C in the presence of DNA-IgED (bottom). Scale bar, 5 μm. (e) ELISA of IFN-α in supernatants of pDCs stimulated for 16 h with DNA-IgED in the presence of various concentrations (horizontal axis) of an oligodeoxynucleotide inhibitor of TLR9 (TLR9 inh) or a control oligodeoxynucleotide (Ctrl). **< 0.01 and ***< 0.001 (Mann-Whitney U-test). Data are representative of three independent experiments (a) or are from three independent experiments (b,e; mean and s.d. of n = 75 phagosomes per group (b) or mean and s.e.m. (e)) or one experiment representative of three experiments (c,d).
on those treated with IgG-containing ICs (Fig. 6f). We observed the same result for expression of CCR7, a chemokine receptor used by mature pDCs to migrate into lymphoid compartments[7] (Fig. 6f). Furthermore, DNA-IgED ICs induced robust migration of pDCs to ligands of CCR7 to a significantly greater degree than the migration triggered by ICs formed with IgGD (Fig. 6g). Antigen presentation is a central feature of autoimmune disorders such as SLE, and DNA-IgED 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 these co-cultures, DNA-IgED ICs and DNA-IgGD ICs produced responses similar in magnitude and acted in synergy when combined.

From these experiments, we concluded that DNA-IgG ICs were able to trigger the secretion of proinflammatory cytokines, maturation, migration and antigen presentation by pDCs. In all cases, the responses were similar to or larger than those initiated by DNA-IgG complexes. Combining both IgG and IgE in the complexes resulted

Figure 5 dsDNA-specific IgE induces pDC-mediated PC differentiation. (a) Microscopy of pDCs (stained with antibody to BDCA-2; red) and B cells (stained with antibody to CD20; green) in a kidney biopsy from a patient with LN. Scale bar, 20 μm. (b,c) Quantification of B cells (CD123+CD19+) (b) and PCs (CD123+CD19+CD27+) (c) from pDC–B cell co-cultures left untreated in medium alone (Med) or stimulated for 7 d with DNA-IgED, assessed by flow cytometry and presented as cells acquired for a fixed amount of time per well. (d) ELISA of IgM in supernatants of pDC–B cell co-cultures as in b,c at day 7 after stimulation. (e) Quantification of PCs in pDC–B cell co-cultures stimulated with DNA-IgED in the presence of medium alone (Med), blocking antibody specific to human IFNAR (Anti-IFNAR), an IL-6-specific antibody (Anti–IL-6) or an isotype-matched control antibody (Isotype, assayed by flow cytometry. \(P < 0.001\) and **P < 0.05 (Student’s t-test); NS, nonsignificant. Data are from one experiment representative of three experiments (a) or are from at least three independent experiments (b–e; mean and s.d.).

Figure 6 Synergistic stimulation of pDCs by ICs containing IgE and IgG. (a) ELISA of dsDNA-specific IgE and IgG in serum from patients with SLE (n = 98), \(r = 0.405\) and \(P < 0.0001\) (Spearman’s rank correlation test). Each symbol represents an individual donor. (b) IFN-α production by pDCs stimulated for 16 h with ICs containing DNA plus increasing concentrations (horizontal axis) of IgGD and/or IgEGD. (c,d) Frequency of cells with intracellular expression of IFN-α (c) or TNF (d) among pDCs stimulated for various times (horizontal axes) with ICs containing DNA plus IgGD and/or IgEGD (key). (e) Expression of TNF and IFN-α by pDCs left unstimulated (US) or stimulated for 6 h with ICs containing DNA plus IgGD and/or IgEGD (above plots). Numbers in quadrants indicate percent cells in each. (f) Surface expression of CD83, CD86 and CCR7 on pDCs left unstimulated or stimulated for 16 h with ICs containing DNA plus IgGD and/or IgEGD (top row), and mean fluorescence intensity (MFI) of CD83, CD86 and CCR7, relative to that of untreated cells (below). (g) Migration of pDCs in response to CCR7 ligands (CCL19 plus CCL21), left unstimulated or stimulated for 16 h with DNA-containing ICs. (h) IFN-γ in supernatants of pDCs loaded with cytomegalovirus peptide and left unstimulated or stimulated for 4 h with ICs containing DNA plus IgGD and/or IgEGD (key) and cultured for 48 h with cytomegalovirus-responsive CD8 T cells. (i) Proliferation of cytomegalovirus-specific CD8 populations after 4 d of co-culture as in h, assessed by dilution of the division-tracking dye CFSE. Numbers along right margin indicate percent divided cells. \(P < 0.01\) and **P < 0.05 (Mann-Whitney U-test). Data generated from one experiment with 98 patients with SLE (a), representative of seven independent experiments (b), four independent experiments (c–e;g; mean and s.e.m. in c,d,g), five independent experiments (f; mean and s.e.m.), three independent experiments (h; mean and s.e.m.) or three independent experiments with one donor representative of three donors (i).
Figure 7 dsDNA-specific IgE enhances pDC interferon responses through increased phagocytosis of DNA. (a) Concentration of dsDNA-specific IgG (mean value, 88.0 µg/ml) or dsDNA-specific IgE (mean value, 4.8 µg/ml) in serum from dsDNA-IgE+ patients with SLE (n = 98) (Mann-Whitney U-test). Each symbol represents an individual donor; red horizontal lines indicate the mean (± s.e.m.). (b) IFN-α in supernatants of pDCs stimulated for 16 h with ICs containing a fixed amount of DNA (0.5 µg/ml) and a fixed amount of IgG (10 µg/ml) plus increasing concentrations (horizontal axis) of IgE; results are presented relative to those of pDCs treated with DNA-IgG ICs (red dashed line). Numbers above bars (red) indicate ratio of IgG to IgE (mean value, 88.0). (c) IFN-α in supernatants of pDCs left untreated or treated for 16 h with ICs containing RNA and IgG only (red dashed line). Above bars (red), ratio of IgE to IgG. (e) Internalized DNA in pDCs treated with ICs containing DNA plus IgG and/or IgE (left margin). Scale bar, 5.0 µm. (f) Frequency of pDCs positive for phagocytosed DNA after incubation with medium alone (UT) or with DNA or with ICs as in e, quantified by flow cytometry. (g) Quantification of pDCs positive for engulfed DNA among pDCs treated with ICs formed with single immunoglobulins (Fig. 6a, c, f) or with DNA-ICs containing both IgG and IgE than when stimulated with DNA-IgG ICs (red dashed line). However, the combination of IgE and IgG induced synergistic phagocytosis when combined at a ratio of 1:1 (Fig. 7a–f). DNA was similar when either IgGD or IgED was used to form the ICs (Fig. 7e). The number of pDCs with detectable phagosomal DNA at those two ratios were 5.11-fold higher and 2.97-fold higher, respectively, than that of pDCs with detectable phagosomal DNA when ICs formed with only IgG were used (Fig. 7g). These results indicated that the enhanced secretion of IFN-α triggered by low concentrations of IgG was caused by substantially greater IC phagocytosis. This mechanism would also explain how more cells secreted pathogenic IFN-α when stimulated with DNA-ICs containing both IgG and IgE than when stimulated with ICs formed with single immunoglobulins (Fig. 6c), as the presence of dsDNA-specific IgE in the ICs lowered the threshold for triggering pDC responses.

Enhancement of IC phagocytosis by IgE

Although we detected dsDNA-specific IgE in a majority of patients with SLE, the concentration of dsDNA-specific IgE in the circulation was, on average, about 20 times lower than that of the corresponding IgG (Fig. 7a). Hence, we investigated the relative contribution of IgE to interferon responses in conditions similar to those found in patients. For this, we prepared DNA-containing ICs with a fixed amount of DNA-igG and assessed the effect of adding increasing amounts of IgE on the secretion of IFN-α. Strikingly, the addition of even small amounts of IgE to the complexes greatly enhanced the amount of IFN-α produced by pDCs (Fig. 7b). This was true even when the concentration of IgE was 100 times lower than that of IgG in the ICs, under which circumstances the presence of IgE enhanced the interferon response more than fivefold (Fig. 7b). We obtained similar results for complexes formed in vitro with U1 small nuclear ribonucleoprotein particles, an RNA-associate autoantigen found in SLE (Fig. 7c,d), which indicated that the role of IgE in SLE might not only be restricted to DNA but also expand to autoantigens sensed by TLR7. To understand mechanistically how IgE enhanced the secretion of IFN-α, we investigated its effect on the phagocytic uptake of ICs, through the use of fluorescence-labeled DNA. The frequency of pDCs with detectable uptake of intracellular DNA was similar when either IgG or IgE was used to form the ICs (Fig. 7e–f). However, the combination of IgE and IgG induced synergistic phagocytosis when combined at a ratio of 1:1 (Fig. 7a–f). This synergy was maintained even in conditions under which the amount of IgE used was 10- or 100-fold lower than that of IgG (similar to the ratios found in patients with SLE) (Fig. 7e–f). The number of pDCs with detectable phagosomal DNA at those two ratios were 5.11-fold higher and 2.97-fold higher, respectively, than that of pDCs with detectable phagosomal DNA when ICs formed with only IgG were used (Fig. 7g). These results indicated that the enhanced secretion of IFN-α triggered by low concentrations of IgE was caused by substantially greater IC phagocytosis. This mechanism would also explain how more cells secreted pathogenic IFN-α when stimulated with DNA-ICs containing both IgG and IgE than when stimulated with ICs formed with single immunoglobulins (Fig. 6c), as the presence of dsDNA-specific IgE in the ICs lowered the threshold for triggering pDC responses.

DISCUSSION

Measures to limit the exposure of host DNA to the nucleic-acid sensor TLR9 are critical for 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, which highlights the importance of maintaining TLR9 within the cell. Here we demonstrated that the DNA-IgE autoantibodies found in SLE undermined
those aforementioned safeguards by shuttling self DNA to intracellular TLR9 via the activation of cell-surface FcεRI on pDCs. This mechanism parallels that used by IgG autoantibodies that, in contrast, utilize FcγRIIa to deliver DNA into the cell.\(^\text{25-26}\) Furthermore, we have provided evidence that IgE acted in synergy with IgG to induce more engulfment of DNA-containing ICs and that IgE enhanced pDC responses even when 100- to 1,000-fold less abundant in the ICs than IgG. One potential explanation for the potent synergistic activity of IgE in the DNA-containing ICs formed with both IgG and IgE might be that FcεRIIa binds monomeric IgE with an association constant of $10^{10}$ M\(^{-1}\) (ref. 34), which is at least three order of magnitude higher than the binding of IgG to the low-affinity receptor FcγRIIa\(^\text{35}\). Therefore, the ability of IgE to enhance the phagocytic capacity of pDCs might be a critical step in promoting the further loss of tolerance in these patients with SLE. This mechanism becomes even more important given 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 might facilitate the accumulation of DNA-containing ICs in the phagosome in concentrations that facilitate elicitation of TLR9-mediated pDC responses. Indeed, we found that the combined stimulation of pDCs with IgE ICs plus IgG ICs exacerbated the inflammatory response due to an increase in the frequency of cells responding to self DNA, rather than increased production of IFN-γ or TNF per cell.

IgE is the least-abundant and least-well-understood immunoglobulin isotype in disease. Evidence obtained with animal models and statistical population trends suggests that IgE might be beneficial in fighting parasites, whereas IgE is believed to trigger harmful allergic reactions to innocuous foreign proteins\(^\text{16,36}\). 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, which results in degranulation and the subsequent release of biogenic amines, lipid mediators and TGF-β cytokines. The presence of IgE antibodies that bind endogenous host antigens in SLE has been known for some time. The characteristic IgE-mediated responses are not observed in SLE, and this has somewhat complicated elucidation of their role in a disease that is driven mainly by an aberrant antiviral response\(^\text{5}\). For example, dsDNA-IgE\(^\ast\) patients with SLE do not exhibit higher concentrations of circulating IL-4 or IL-5 than do seronegative patients or healthy control subjects\(^\text{37}\). Instead, we found that the concentration of self-reactive dsDNA-IgE in serum correlated with disease severity and was a risk factor for SLE activity, regardless of the amount of IgG antibodies that bound the same autoantigens in our patient cohort. Furthermore, we demonstrated that dsDNA-specific IgE actively contributed to SLE by enhancing the specific immunological effects, including interferon secretion, associated with this disease. Collectively, the unexpected responses described here for IgE were unique and distinct from the classical Th2-skewed responses observed in allergic disease.

That aforementioned link among IgE, autoimmunity and interferon responses revealed an unexpected concept for the physiological role of IgE in the immune system. Notably, we found that DNA-IgE ICs functionally affected pDCs by 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 population expansion of PCs. Notably, IgE acted in synergy with IgG even when present in the ICs at several orders of magnitude lower than IgG. Therefore, we propose that this robust activation of pDCs is deleterious in SLE, since the response is aberrantly generated against self nucleic acids. Alternatively, robust pDC activity would be beneficial to the host if these cells were targeting viral particles. One possibility is that IgE acts together with IgG to facilitate sensing of viruses and to deliver their pathogenic nucleic acids to TLR7 and TLR9 within the phagosome. Studies of humans and animals have identified IgE antiviral antibodies in serum\(^\text{38-40}\). These antibodies seem to be driven by viral load, as reported by a study focused on respiratory syncytial virus. Notably, the concentration of IgE antibodies directed against respiratory syncytial virus correlates with that of their IgG counterpart, as well as with circulating viral protein\(^\text{39}\). This is consistent with our observations of good correlation between anti-dsDNA IgE and anti-dsDNA IgG generated in response to autoantigens in SLE. There is also evidence suggesting that IgE directed against human immunodeficiency virus might be beneficial in controlling infection with this virus. Total IgE from patients seropositive for IgE antibodies to human immunodeficiency virus have been found to block viral production in PBMCs by mechanisms that exclude viral neutralization, which would suggest that these antibodies have antiviral properties by acting through cells of the immune system\(^\text{38}\).

Furthermore, a small study has reported that IgE directed against human immunodeficiency virus in patients is not associated with a higher incidence of allergy\(^\text{41}\), which resembles findings of similar epidemiological studies of patients with SLE who are seropositive for dsDNA-IgE. How the mechanisms we have proposed for IgE in autoimmunity extend to scenarios of viral defense warrants further investigation. Nevertheless, the data presented here expand the physiological functions of IgE in the immune system and suggest that mammals’ recent evolutionary acquisition of IgE might provide benefits to the host beyond defense against helminths.

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 here indicate previously unknown mechanisms for a pathogenic role of IgE in this 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 have reported here provides additional insight into the pathological mechanisms underlying autoimmunity and might be useful in the rational design of therapies for the treatment of diseases such as SLE.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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**Author Contributions**

J.H., J.M.R., J.L.K., V.M.L., J.L., L.S., L.I. and L.C., experimental design and conception, under the guidance of L.X., R.H., R.E. and T.M.; K.A.C., M.A. Smith and D.B.K., statistical analysis; M.P. and M.R.C., supervision of the generation and analysis of data from patients with SLE; J.H., R.K. and M.A. Sanjuan conception and direction of the project; and J.H. and M.A. Sanjuan authorship of the manuscript with input from all authors.
COMPETING FINANCIAL INTERESTS
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ONLINE 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 classification criteria for SLE34. All patients provided informed consent to participate in the study, and all patient data were made anonymous for 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 internal blood donor program of MedImmune in accordance with the guidelines of the Institutional Review Board of MedImmune. Serum samples from patients with atopic dermatitis were obtained through Bioreclamation In Vitro Technologies.

Cell culture. Human PBMCs either were obtained from healthy donors with leukopacks (SeraCare Life Sciences) or were isolated from whole blood obtained through the internal blood donor program of MedImmune through the use of CPT cell preparation tubes (BD Biosciences). In some cases, donors were pre-screened for cytomegalovirus (CMV) reactivity using through the use of phycoerythrin (PE)-conjugated antibody to (anti-) CD8 and CMV Dextramer HLA-A*0201-NLVPVVATV- allophyocytocin (ImmuDex). Human PBMCs were maintained in RPMI-1640 medium supplemented with 5% FBS (both from Life Technologies). An EasySep Human pDC Enrichment kit and an EasySep Human CD8 T cell enrichment kit (StemCell Technologies) were used for enrichment of pDCs, while total B cells were isolated with a B Cell Isolation Kit II (Milteny Biotec). The purity of the enriched cells was routinely assessed by flow cytometry with PE-conjugated anti-BDC4 (AD5-17F6; Miltenyi Biotec) and/or allophyocytocinin-conjugated anti-CD123 (7G3; BD Biosciences) for pDCs, or with PE-conjugated anti-CD8 (SK1; BD Biosciences) and peridinin chlorophyll protein (PerCP)–cyanine 5.5 (Cy5.5)–conjugated CD19 (HIB19; BD Biosciences), for B cells. Cell purity was consistently above 95%. Unless stated otherwise, enriched pDCs were maintained in X-Vivo 15 medium (Lonza) supplemented with 1% FBS (Life Technologies) and 10 ng/ml of human recombiant IL-3 (R&D Systems). Mouse macrophages expressing GFP-tagged TLR9 (a gift from Professor Dr. M. Grewal, University of Maryland, College Park, MD) were used for enrichment of pDCs, while total B cells were isolated with a B Cell Isolation Kit II (Milteny Biotec). The purity of the enriched cells was routinely assessed by flow cytometry with PE-conjugated anti-BDC4 (AD5-17F6; Miltenyi Biotec) and/or allophyocytocinin-conjugated anti-CD123 (7G3; BD Biosciences) for pDCs, or with PE-conjugated anti-CD8 (SK1; BD Biosciences) and peridinin chlorophyll protein (PerCP)–cyanine 5.5 (Cy5.5)–conjugated CD19 (HIB19; BD Biosciences), for B cells. Cell purity was consistently above 95%. Unless stated otherwise, enriched pDCs were maintained in X-Vivo 15 medium (Lonza) supplemented with 1% FBS (Life Technologies) and 10 ng/ml of human recombinant IL-3 (R&D Systems). Mouse macrophages expressing GFP-tagged TLR9 were generated as described48. TLR9-deficient macrophages were immortalized from the bone marrow of Tlr9−/− mice through use of the J2 recombinant retrovirus (carrying oncogenes encoding v-Myc and v-Raf). Bone marrow cells were infected with J2 virus after 3 d of culture in medium conditioned with L929 mouse fibroblasts. Immortalized macrophages were then retrovirally transduced to express GFP-tagged mouse TLR9 and human FcRIRz. Mouse macrophages were cultured in DMEM supplemented with 10% FBS and 1× penicillin-streptavidin (all from Life Technologies).

Vectors and constructs. Human FcERIα (NM_002001; GenBank) was initially amplified from a plasmid (GeneCoepia) and was cloned into the vector pMSCVPuro (Clontech). The fusion plasmids were used for transfection of the packaging cell line pT67 (Clontech) or Gryphon amphotropic packaging cells (Allele Biotech), by standard transfection protocols.

Monoclonal dsDNA-specific IgE. The human dsDNA-specific IgG clone E11 (IgG3) was generated as described49. For the generation of human dsDNA-specific IgE (IgE), sequence encoding the variable regions of IgG2a was cloned by digestion with restriction enzymes BssHII and SalI (New England BioLabs), followed by ligation into the IgE Orip–EBNA-1–based episomal mammalian expression vector pOE (MedImmune). Human IgE specific for metapneumovirus was generated in a similar fashion and was used as an isotype-matched control antibody (IgE2). The human dsDNA-specific IgE clone E11 was established by Balb/c mice 30 d after i.p. injection of 10 mg of pDNA-IC. After immunization, serum samples from the immunized animals were screened for antibodies to dsDNA by ELISA (Calbiotech). Monoclonal IgG2a and IgE2 were used as assay standards.

Measurement of dsDNA-specific antibodies. Human serum was screened for dsDNA-specific IgG and IgE by ELISA of dsDNA IgG (Calbiotech). For the detection of dsDNA-specific IgE, horseradish peroxidase–conjugated polyclonal antibody to human IgE (GE-80P; Immunology Consultants Lab) was used instead of the horseradish peroxidase–conjugated antibody to human IgG provided with the ELISA kit. Monoclonal IgG2a and IgE2 were used as assay standards.

Detection of C3. Human serum was screened for C3 by quantitative multiplex immunoassay (Myriad; Rules-Based Medicine).

SLE serum assay. The in vitro assay of PBMCs from humans with SLE serum was performed by transfer of PBMCs primed with IFN-α (500 U/ml; PBL Assay Science) plus GM-CSF (1 ng/ml; R&D Systems) into U-bottomed 96-well plates (1.0 × 10⁴ cells per well), followed by incubation of the cells with 20% SLE serum positive for dsDNA-specific IgE. For blockade of the interaction of serum IgE with cell-surface FcεRs, an antibody specific for the Fc portion of human IgE (E85; MedImmune) was used at a concentration of 50 µg/ml (ref. 45). A triple amino acid modification in the Fc portion of E85 prevented binding of E85 to the Fc portion of FcεR146. An isotype-matched control antibody with the same modification (clone NMGC, MedImmune) was also used as a control. Cells were incubated for 16 h at 37 °C in 5% CO2.

pDC in vitro assay. In vitro assays of pDCs were performed by transfer of enriched human pDCs into U-bottomed 96-well plates (0.5 × 10⁴ to 1.0 × 10⁴ cells per well), followed by incubation of the cells for the appropriate time at 37 °C in 5% CO2. For culture of pDCs with DNA-containing ICs, ICs were generated by mixture of 0.5 µg/ml of the plasmid pMCG-50 (which contains 50 copies of a CpG immunostimulatory sequence (CG50)) with IgG2a or IgE14 or a combination of both. For the assay with antibody blockade of IgE, human anti-IgE (E85; described above) and the relevant isotype-matched control antibody were used. For the stimulation of pDCs with influenza virus, cells were incubated for 16 h with influenza A virus (H111N1; strain A/Puerto Rico/8/1934; American Type Culture Collection) at a multiplicity of infection of 3, in the presence of increasing concentrations of aggregated IgE. For cross-linking of surface FcεR1, aggregated IgE was generated by dilution of IgE14 in 1× phosphate-buffered saline (PBS) at a concentration of 22.0 mg/ml, followed by incubation for 30 min at 65 °C. Aggregated IgE was then added in culture medium at the appropriate concentration. For the TLR9-inhibition assay, the TLR9 inhibitor (oligodeoxynucleotide TTAGGGG) or control oligodeoxynucleotide for TTAGGG (both from Invivogen) was added to the cells at the appropriate concentration. For the stimulation of pDCs with RNA-containing ICs, cells were incubated for 16 h with 2.0 µg/ml of biotinylated U1 small nuclear ribonucleoprotein particles (Arotec Diagnostics) plus 0.5 µg/ml streptavidin (Sigma-Aldrich), alone or in combination with biotinylated human IgG or biotinylated IgE (MedImmune), or both immunoglobulin isotypes. The small nuclear ribonucleoprotein particles and antibodies were biotinylated with a Biotin–XX Microscale Protein Labeling Kit (Life Technologies).

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

Detection of intracellular cytokines by flow cytometry. Enriched human pDCs were incubated for 6, 9 or 16 h at 37 °C in 5% CO2 in U-bottomed 96-well plates (2.5 × 10⁴ cells per well) with ICs generated by mixture of 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 with anti-BDC4-4 (12C2, Biolegend), were fixed and permeabilized with an Inside Stain Kit (Miltenyi Biotec), and were stained with anti-IFN-α (LT27:295; Miltenyi Biotec) and anti-TNF (cA2; Miltenyi Biotec). Intracellular production of IFN-α and TNF in BDCA-4+ cells was assessed by flow cytometry.

Labeled DNA-IC–internalization assay. Enriched human pDCs were transferred into U-bottomed 96-well plates (2.5 × 10⁴ cells per well), followed by incubation for 2 h at 37 °C in 5% CO2 with ICs generated by combination of Alexa Fluor 546–labeled CG50 plasmid DNA (Ulysia Alexa Fluor 546 Nucleic Acid Labeling Kit; Life Technologies) with IgG2a or IgE14 or a combination of both. After incubation, cells were treated with a 0.1 mg/ml DNase I solution (StemCell Technologies) for degradation of surface-bound DNA. Internalized DNA was visualized by confocal microscopy and was quantified by flow cytometry.
pDC-maturation assay. Enriched human pDCs were transferred into U-bottomed 96-well plates (5.0 × 10^4 cells per well) and were incubated at 37 °C in 5% CO2 with ICs generated by mixture of 0.5 µM CG50 plasmid DNA with IgG\textsubscript{D} or IgE\textsubscript{D} or a combination of both. Surface-marker expression was assessed by flow cytometry with PE-cyanine-fluorescent dye 594–conjugated anti-CD83 (HB15e; BD Biosciences), Alexa Fluor 700–conjugated anti-CD86 (FUN-1; BD Biosciences) and PerCP-Cy5.5–conjugated anti-CCR7 (G043H7; Biolegend).

Immunofluorescence of renal biopsies. Patients at the University of Chicago who met revised 1982 American College of Rheumatology criteria for SLE\textsuperscript{43} provided consent for collection of renal tissue samples. Those with class II, III, IV or V LN (by classification of the International Society of Nephrology or Renal Pathology Society) of renal biopsies were selected for this study. A total of 27 LN biopsies were used. Normal kidney samples (12 biopsies) from the University of Chicago Pathology Core Facility, with sample identity removed, were used as controls. 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 were stored at −80 °C. Freshly frozen sections 2–8 µm in thickness were stained with immunofluorescence-conjugated anti-CD20 (EP459Y; Abcam) or polyclonal rabbit IgG (P0202; DAKO), in combination with fluorescein isothiocyanate (FITC)-labeled anti-BDCA2 (AC144; Miltenyi), polyclonal anti-MxA (AF7946; R&D Systems), PE-labeled IgE (MHE-18; Biolegend), PE- or allophycocyanin-labeled control immunoglobulin k-chain (MOPC-21; Biolegend) or anti-CD123 (6H6; ebioscience). Basophils were detected with anti-CD11a (2D7; Biolegend). The addition of nucleic acid stain, either DAPI (4,6-diamidino-2-phenylindole; Invitrogen) or SYTOX Blue (Life Technologies), was used for visualization of nuclei, depending on the number of simultaneous fluorochromes visualized. Fluorescence-labeled species-specific secondary antibody to IgG (a11058 (Alexa Fluor 594), a21039 (Alexa Fluor 750), a11063 (Alexa Fluor 430), a11096 (Alexa Fluor 488), a31571 or 31573 (Alexa Fluor 647); Invitrogen) was applied for visualization of primary antibodies that were not directly labeled. Mouse anti-PE (EBIOPE-DLF; ebioscience) was used as needed for visualization of IgE. 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 two-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 with a 600 x oil-immersion objective with a high numerical aperture (1.25). Images were acquired by means of a photomultiplier detector and were stored in either the original LEI file format (‘Leica experimental information’) or LIF file format (‘Leica application suite advanced fluorescence’) (Leica), depending on the 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. The appropriate single-stained controls were used with each antibody to ensure that no fluorochrome spectral cross-bleeding was present. The average intensity value of immunofluorescence signal was quantified with Adobe Photoshop CS6 software as described\textsuperscript{47}. Images in the LIF file format were converted to tagged image file format (TIFF files), and regions of a fixed number of pixels were drawn with the Marquee function of Adobe software. The average signal intensity for each individual channel was measured with the Histogram function of Adobe software. Individual channels were ‘gray scaled’ before measurement.

Intracellular trafficking assay. For visualization of the trafficking of GFP-tagged TL1R9 to phagosomes, mouse macrophages were incubated for 30 min. at 37 °C and 5% CO2 with polystyrene beads (3.8 µm in diameter; Spherotech) coated with 10 µg/ml CG50 plasmid DNA and with 50 µg/ml of either IgG\textsubscript{D} or IgE\textsubscript{D}. For monitoring of the trafficking of ICs to phagosomes, enriched human pDCs were first incubated for 30 min at 4 °C with ICs generated by combination of 2 µg/ml of CG50 plasmid DNA with 50 µg/ml of Alexa Fluor 488–coupled IgE\textsubscript{D} then were incubated for 120 min at 37 °C and 5% CO2. After incubation, cells were fixed with 4% paraformaldehyde and were permeabilized with 0.05% Triton. Phagosome maturation was assessed with PE-labeled anti-LAMP-1 (eBioH4A3; ebioscience). For the assay of the nuclear translocation of IRF7, enriched pDCs were incubated for 5 h at 37 °C and 5% CO2 with ICs generated by combination of 2 µg/ml of CG50 plasmid DNA with 40 µg/ml of IgE\textsubscript{D}. After incubation, cells were fixed and permeabilized as described above and were stained with anti-IRF7 (H-246; Santa Cruz Biotechnology) and Alexa Fluor 647–coupled anti-rabbit (A-21443; Life Technologies). Nuclei were detected with DAPI (Life Technologies). Cell imaging was performed with a Leica TCS SP5 confocal system consisting of a Leica DMi6000 B inverted microscope (Leica Microsystems). Images were acquired at the appropriate time points and were analyzed with LAS AF version 2.2.1 Leica Application Suite software (Leica Microsystems).

PC-differentiation assay. B cells and pDCs were resuspended in RPMI-1640 medium supplemented with 10% FBS, 1x penicillin-streptomycin, 2 mM l-glutamine and 5 mM HEPES (all from Life Technologies), plus 55 µM β-mercaptoethanol (Sigma), and were transferred into U-bottomed 96-well plates. 7.5 × 10^3 B cells and 3.75 × 10^4 pDCs were added to each well, along with 1.5 nM mega CD40L (Enzo Biosciences). ICs generated by a combination of 2.5 µg/ml of IgE\textsubscript{D} with 1 µg/ml of CG50 plasmid DNA were added to the co-cultures. Cells were cultured for 7 d at 37 °C and 5% CO2, then were washed and were stained with FITC-conjugated anti-CD123 (7G3), Brilliant Violet 421–conjugated anti-CD19 (HB19), PerCP-Cy5.5–conjugated anti-CD27 (M-T271), PE-conjugated IgD (IA6-2) and allophycocyanin-conjugated anti-CD38 (HT2) (all from BD Biosciences). For quantification of B cells and PCs by flow cytometry, co-cultured cells were acquired for a fixed amount of time. B cells were defined as CD123\textsuperscript{+}CD19\textsuperscript{+} and PCs were defined as CD123\textsuperscript{−}CD19\textsuperscript{+}CD27\textsuperscript{−}CD38\textsuperscript{−}. Supernatants were recovered at day 7 for indirect quantification of IgM by ELISA as described\textsuperscript{48}. Antibody to the human IFN-α receptor IFNAR (9D4; MedImmune), anti-IL6 (6708; R&D Systems) or anti-IL6 (CAT6001; MedImmune)\textsuperscript{50} was 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 × 10^4 cells per well) and were stimulated for 4 h with ICs generated by combination of 0.5 µg/ml CG50 plasmid DNA with 10 µg/ml of IgG\textsubscript{D} or IgE\textsubscript{D} or 5 µg/ml of each isotype (IgG\textsubscript{D} and IgE\textsubscript{D}), in the presence of 10 µg/ml of a CMV peptide (NLVPVMAT\textsubscript{V} ProImmune). pDCs were then washed and then were incubated for 4 d with autologous CD8\textsuperscript{+} T cells (2.5 × 10^5 cells per well) in the presence of 100 IU/ml recombinant human IL-2 (Peprotech). CMV-specific CD8\textsuperscript{+} T cells were identified with CMV Dextramer HLA-A2*0201/NLVPVMAT\textsubscript{V} (Immudex). The proliferation of CMV-specific CD8\textsuperscript{+} T cells was assessed by flow cytometry with a CellTrace CFSE Cell Proliferation Kit (Life Technologies).

pDC-migration assay. pDCs (5.0 × 10^4) were transferred into the upper chamber of a 96-well HTS Transwell plate with 5-µm pores (Corning) and were stimulated with ICs generated by combination of 0.5 µg/ml CG50 plasmid DNA with 10 µg/ml of IgG\textsubscript{D} or IgE\textsubscript{D} or 5 µg/ml of each isotype (IgG\textsubscript{D} and IgE\textsubscript{D}). The temperature was adjusted to 37 °C and cells were allowed to migrate to the bottom chamber, which contained 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 were analyzed by flow cytometry.

Statistical analysis. The statistical significance of differences in the distribution of variables between groups were determined with a two-tailed Mann–Whitney U-test, except where stated otherwise. In each case, the required assumptions of the non-parametric test were met, since all data consisted of independent observations that originated from continuous distributions. The monotonic relationship between variables was evaluated with 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 F-test of the regression coefficient of a multiple linear regression model fit with SLEDAI as a dependent variable and the concentration of dsDNA-specific IgE and their interaction as independent variables. We graphically assessed normality of model residuals by examining a normal probability plot that was linear and approximated 45°. We assessed assumptions
of homoscedasticity and linearity between the variables by plotting model residuals against fitted values and observed no substantial departure from model assumptions. Results with a P value of <0.05 were considered significant. Multiple linear regression was performed with the software package SYSTAT 13.0. All other statistical analyses were performed with GraphPad Prism 6.0 software (GraphPad Software).

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