

**IgE inhibits TLR-7 and -9 mediated expression of interferon-alpha by
plasmacytoid dendritic cells in systemic lupus patients**

Running title : IgE inhibits IFN- α production by pDCs in SLE

Liliane Khoryati, PhD,^{a,b} Jean-François Augusto, MD, PhD,^b Emilie Shipley, MD,^c
Cécile Contin-Bordes, PhD,^{a,b,c} Isabelle Douchet,^b Stéphane Mitrovic, MD,^{b,c} Marie-
Elise Truchetet, MD, PhD,^{a,b,c} Estibaliz Lazaro, MD, PhD,^{a,b,c} Pierre Duffau, MD,
PhD,^{a,b,c} Lionel Couzi, MD, PhD,^{a,b,c} Clément Jacquemin, PhD,^{a,b} Thomas
Barnetche, MD, PhD,^c Pierre Vacher, PhD,^{a,d} Thierry Schaefferbeke, MD, PhD,^{a,c}
Patrick Blanco, MD, PhD,^{a,b,c}, Christophe Richez, MD, PhD^{a,b,c}; on behalf of the FHU
ACRONIM.

Affiliations:

^a Université de Bordeaux, 146 rue Léo Saignat, 33076 Bordeaux, France

^b ALYSAI, CNRS-UMR 5164, 146 rue Léo Saignat, 33076 Bordeaux, France

^c Centre Hospitalier Universitaire de Bordeaux, Place Amélie Raba Léon, 33076
Bordeaux, France

^d INSERM U916, Institut Bergonié, Bordeaux, France

Corresponding authors:

Patrick Blanco and Christophe Richez

ALYSAI, CNRS UMR 5164, Bât. 1B

146 Rue Léo Saignat, 33076 Bordeaux, France

Telephone number: +33557820270

Fax number: +33556796084

e-mail: patrick.blanco@chu-bordeaux.fr

e-mail: christophe.richez@u-bordeaux.fr

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Accepted Article

Abstract

Objective: Plasmacytoid dendritic cells (pDCs) play a central role in systemic lupus erythematosus (SLE) pathogenesis through their unique ability to produce large amounts of type I interferon (IFN-I) upon Toll-like receptor (TLR) 7/9 triggering. pDCs express specific surface regulatory receptors involved in negative regulation of IFN- α secretion. These receptors use the gamma chain of high-affinity Fc receptor for immunoglobulin E (IgE), Fc ϵ RI. We hypothesized that IgE engagement of Fc ϵ RI on pDCs may impact IFN- α production in SLE patients.

Methods: Serum levels of total IgE were measured in healthy volunteers, SLE, and allergic patients. Fc ϵ RI expression on pDCs was evaluated by flow cytometry in SLE patients. Purified pDCs were incubated with monoclonal IgE for 24h, then stimulated for 18 hours with TLR agonists or immune complexes. IFN- α production by pDCs was detected by quantitative RT-PCR and ELISA. Expression of TLR7/9 and IRF7 was quantified in pDCs by quantitative RT-PCR.

Results: We observed significantly higher IgE levels in SLE patients with quiescent disease than with active disease. In SLE patients, IgE levels were inversely correlated to disease activity. IgE levels were not associated with presence of anti-nuclear IgE. Purified pDCs treated during 24h with monoclonal IgE upregulate Fc ϵ RI expression in an IgE dose-dependent manner. IgE-treated pDCs significantly decrease IFN- α secretion and downregulate CCR7 expression upon TLR7/9 and immune complex stimulation from lupus patients. IgE treatment downregulates expression of TLR7/9 and IRF7.

Conclusions: Our results support a protective role of IgE in SLE pathogenesis through the modulation of inflammatory response by pDCs.

Autoimmune diseases (AID) encompass a wide variety of illnesses in which innate and adaptive immune responses lead to autoimmune-mediated tissue damage. Epidemiological studies have established that AID have a higher prevalence in Western societies compared to developing countries (1). Developing countries are characterized by high prevalence of parasitic infections (2). Interestingly, in areas of the world where helminth infections are endemic, rates of AID, including rheumatoid arthritis (RA), type I diabetes (T1D) and systemic lupus erythematosus (SLE), remain low (3-7). Intestinal helminth parasites are large, multicellular organisms that can establish long-term chronic infections that last for years. Resistance to infection is typically mediated by a type 2 (Th2) response that is characterized by the production of cytokines such as IL-4, IL-5, and IL-13. Current hypothesis to explain helminth-mediated protection against autoimmunity postulates that the Th2 immune responses induced by helminth infections in the host limit pathogenic Th1-driven autoimmune responses. This supports recent works conducted to develop helminth-based therapies for AID (8, 9). Of note, helminth infections are not only characterized by a Th2 immune response, but also by the production of immunoglobulin E (IgE), yet the direct implication of IgE in the protection of autoimmunity through their Fc-mediated immune regulation has not been carefully addressed.

IgE is the immunoglobulin isotype that has the lowest abundance in serum with a concentration of free serum IgE ranging between 50 and 200 ng per ml of blood in healthy humans. IgE binds to IgE receptors and stabilize them on immune cells in tissues and circulation (10). There are two receptors for IgE: the high-affinity Fc receptor for IgE (Fc ϵ RI) and the low-affinity Fc receptor for IgE (Fc ϵ RII; also known as CD23) (10). When IgE binds the high affinity Fc ϵ RI, in the presence of a specific antigen, these complexes aggregate, and trigger an immunoreceptor-based tyrosine activation motif (ITAM)-mediated signal, especially in mast cells and basophils (10-12). This reaction contributes directly to the pathogenesis of allergic diseases and is implicated in parasite-induced immune reactions. Interestingly, Fc ϵ RI is also expressed by plasmacytoid dendritic cells (pDCs) (10, 12). pDCs play a crucial role against viral infections through their unique ability to secrete high amounts of type I interferon (IFN-I: IFN- α and IFN- β) upon stimulation of endosomal Toll-like Receptors (TLR) which bind pathogen nucleic acids (13). Several studies have

shown that Fc ϵ RI crosslinking downregulates the type I response by pDCs upon TLR/viral activation (14-16). Other pDC specific receptors, including blood dendritic cell (DC) antigen 2 (BDCA2)(17) and immunoglobulin-like transcript 7 (ILT7)(18), use the γ -chain of Fc ϵ RI to activate pDCs through an ITAM-mediated signaling pathway that suppresses the activation of both TLR7 and TLR9, inhibiting the production of IFN-I (19). Therefore, the Fc ϵ RI complex can be viewed as an important receptor implicated in the tight control of IFN-I secretion to prevent aberrant immune responses.

In recent years, research has revealed the fundamental implication of unabated IFN-I secretion in driving the development of different AID, including SLE (20, 21). Because of its central role, the mechanism for its induction is therefore critical, and has been the subject of many publications. Pioneering work from Ronnblom's group showed that sera from SLE patients contained an endogenous IFN-I inducer, characterized as being immune complexes (IC) (22, 23). Such ICs are internalized via the Fc γ RIIA expressed on pDCs, reach the endosome, and stimulate the relevant TLR with subsequent production of IFN-I (13, 24). Implication of a defect in negative regulators of IFN-I pathways, including the IgE/Fc ϵ RI pathway, has not been investigated in SLE.

The implication of IgE in SLE is incompletely understood. Recently (25, 26) it was shown that ICs composed of autoreactive IgE were able to trigger basophil activation in spontaneous lupus mouse models. This lead to enhanced B cell activation and autoantibody production, in parallel to the effect of IgG-containing ICs on Fc γ -expressing cells. Moreover, autoreactive IgE antibodies are prevalent in SLE patients and their levels correlate to disease activity and the presence of lupus nephritis (27). This suggests that recognition of nuclear-containing bodies by autoreactive IgE could sustain the autoimmune response in SLE. However, based on their potential ability to downregulate the type I IFN system, it is reasonable to suggest that non-autoreactive IgE could be beneficial to SLE patients, therefore extending the indication of helminth-based therapies to systemic autoimmune diseases and IFN-I-mediated disorders. In the present study, we investigated the impact of total IgE on the ability of pDCs to secrete IFN-I in the context of SLE.

Materials and methods

Study population

SLE patients (n=42) who fulfilled the American College of Rheumatology revised criteria for the disease (28) were enrolled in this study. The supplementary table 1 summarizes demographic and clinical characteristics. SLE patients with a history of allergic disorders were excluded from the study. Clinical disease activity was scored using the SLE Disease Activity Index (SLEDAI). Active patients were defined as SLEDAI score ≥ 6 . For control groups, IgE-dependent allergic patients (n=18) and healthy volunteers (n=109) were enrolled. After written informed consent was obtained, healthy subjects blood was drawn and patients' blood samples were used from routine lab analysis. This study has been approved by the local ethic committee.

Measurement of circulating antibodies

The levels of serum antibodies were measured by the immunology laboratory at CHU Pellegrin. Total IgE were measured by immunoCAP Phadiatop and IgE autoantibodies to nuclear components were detected by immunofluorescence using Hep-2-coated-slides (Antibodies Inc.). Detection of IgE antibodies was performed by adding monoclonal mouse anti-IgE-HRP for 2 h at 37 °C. Serum anti-ds DNA IgG antibodies were determined by a commercially available ELISA kit (QUANTA Lite® dsDNA, Inova Diagnostics, San Diego, CA) according to manufacturer's instructions. Anti-ds-DNA IgE antibodies were determined by an in-house ELISA in healthy subjects and in SLE patients. Optical density (OD) at $\lambda = 450$ nm minus $\lambda = 570$ nm was measured in a spectrophotometer. The minimum positive value was determined as the median OD + 2SD observed in the healthy subject population. The value of 4000 arbitrary units (AU) was attributed to the sample with the highest observed OD value.

Purification of pDCs from whole blood

Hemochromatosis blood pouches were obtained from the Etablissement Français du Sang (EFS) after written informed consent was obtained from participants. Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by Ficoll gradient centrifugation. pDCs were purified from PBMCs by immunomagnetic bead positive selection using CD304 (BDCA-4/Neuropilin-1) MicroBead kit (Miltenyi Biotec, 130-

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090-532) following the manufacturer's instructions. Purity assessed by flow cytometry was >90% and pDCs were identified as CD123⁺DR⁺BDCA2⁺.

pDC culture conditions

Purified pDCs were cultured in complete RPMI medium supplemented with IL-3 (10 ng/mL), at concentrations of 2×10^4 pDCs/0.2 mL. For IgE treatment, pDCs were incubated with monoclonal IgE (Calbiochem, 401152) for 24h (every 1IU/mL of IgE is equal to 2.4 ng/mL). For control conditions, pDCs were incubated with monoclonal IgG (Calbiochem, 400120) or with a mixture of pre-incubated monoclonal IgE and omalizumab (Ratio 1:66), for 24h. For TLR stimulation assays, pDCs were pre-treated or not with IgE for 24h then stimulated with R848 (1mg/mL; Invitrogen), CpG type A ODN 2216 (0,1 μ M; Invitrogen), or lupus immune complexes (20%), for 18h.

Generation of immune complexes

ICs were generated as described previously (29). Briefly, 1×10^6 /mL T cell line Jurkat cells were treated with soluble Fas Ligand (50 ng/mL) for 4 h at 37°C. Apoptotic bodies were harvested and incubated with sera from SLE patients or healthy volunteers for 2h at 37°C with end over end rotation.

Quantitative Real time-PCR

Total RNA was extracted from 10×10^4 pDCs using NucleoSpin[®] RNA (Macherey-Nagel). cDNA was synthesized using Oligo(dT)₁₅ Primer (Promega, C1101) and GoScript[™] Reverse Transcriptase (Promega, A5003) according to the manufacturer's instructions. Quantitative Real Time PCR was performed in Bio-Rad Thermocycler CFX384 Touch[™] using GoTaq[®] qPCR Master Mix (Promega, A6001). Beta-2 microglobulin and S18 housekeeping genes were selected for normalization and the results were based on 2^{-ddct} values. All oligonucleotide primers were obtained from Sigma and are listed in Table S1.

Cytokine measurements by ELISA

IFN- α levels were measured using an in-house ELISA which was developed using commercially available antibodies. The protocol has been previously described (30). The detection limit of the IFN- α ELISA is 78 pg/ml.

Analysis of cell surface markers by flow cytometry

DAPI was used in all assays to exclude dead cells. The phenotype of pDCs was analysed after gating on CD123⁺DR⁺BDCA2⁺ live cells. The following antibodies were used for flow cytometry assays: anti-CD123-PE, anti-HLA-DR-PE-Cy7, anti-BDCA2-FITC, anti-Fc ϵ RI-APC, anti-CD86-PE, anti-CD83-PE, and anti-CCR7-APC. Irrelevant control mAbs: mouse IgG2-PE, mouse IgG2-APC, and rat IgG2-APC.

Calcium imaging

Purified pDCs were loaded with 5 μ M Fura-PE3/AM calcium dye (Euromedex Mundolsheim, France) and incubated for 30min in Hank's Balanced Salt Solution (HBSS) at 37°C. After washing with HBSS, dye-free, single-cell [Ca²⁺]_i imaging was performed ratiometrically as described previously. (31). A stimulus was added to the medium at 200s [(monoclonal IgE 10 μ g/ml and anti-IgE 5 μ g/ml (A80-108A, Bethyl Laboratories)]. Each experiment was independently repeated 3 times, and for each experimental condition, we displayed an average of more than 20 single-cell traces. Raw data were acquired with MetaFluor and graphed in GraphPad Prism 6.00 software. Injection of the medium used for dilution does not affect intracellular calcium concentration of pDCs (data not shown).

Statistics

The normality of the variable distribution was assessed using Kolmogorov-Smirnov test (with Dallal-Wilkinson-Lilliefor P value). Paired two-tailed Student's t-test was performed for comparison between two populations, unless otherwise specified. For *in vivo* studies, when more than two populations were compared, a nonparametric One-Way ANOVA (Kruskal-Wallis test) was first performed. If significance was reached (P<0.05), an unpaired two-tailed Mann Whitney test was performed between each compared population. Statistical analysis was performed with GraphPad Prism 6.00 software.

Results

Circulating IgE levels are inversely correlated to disease activity in SLE patients.

We first compared total IgE level in the serum of three cohorts of patients: healthy volunteers, SLE patients and patients with IgE-dependent allergic disorders. Total IgE levels in SLE patients were not significantly different from the healthy volunteers, whereas, as expected, total IgE levels were significantly higher in allergic patients (Figure 1A). Within the SLE cohort, patients with quiescent disease were characterized by higher IgE levels than patients with active disease (mean $\pm$ SEM: 172 ± 62 IU/ml in patients with quiescent disease versus 95 ± 40 IU/ml in patients with active disease; $p = 0.03$) (Figure 1B). We found an inverse correlation between total IgE levels and SLE disease activity, as assessed by the SLE Disease Activity Index (SLEDAI) ($p < 0.01$, $r = -0.4$) (Figure 1C). Anti-dsDNA antibodies, serum C3 and C4 concentrations did not correlate with total IgE levels (data not shown). Some recent studies suggest that self-reactive IgE antibodies are associated with active SLE (25, 27). For this reason, we looked for the presence of IgE antinuclear antibodies (ANAs), which were present in the sera of SLE patients but did not associate with higher total IgE levels (Figure 1D). In contrast, patients with IgE ANAs scored slightly higher on the SLEDAI compared to patients without IgE ANAs but this was not significant (mean SLEDAI $\pm$ SEM: 6.9 ± 1.3 in patients with IgE ANAs versus 5.2 ± 1.5 in patients without IgE ANAs; $p = 0.46$). To determine if our results on autoreactive IgE were similar to those previously published by others (25, 27), we quantified anti-double stranded (ds) DNA IgE in a new cohort of SLE patients. We found a direct correlation between anti-ds DNA IgE levels and SLEDAI ($p = 0.002$, $r = 0.62$) (figure 1E). Interestingly, we also found a strong correlation between anti-ds DNA IgE levels and anti-ds DNA IgG levels ($p < 0.0001$, $r = 0.74$) (figure 1F). Thus, we conclude that autoreactive IgE levels directly correlate to disease activity while total IgE levels are increased in SLE patients, are independent of autoreactive IgE, and inversely correlate to disease activity.

Exposure of normal purified pDCs to IgE in vitro increases Fc ϵ RI expression, but does not impact the overall phenotype and survival of pDCs.

Cell-surface expression of the Fc ϵ RI complex is tightly regulated through the availability of specific receptor components, but also by the amount of circulating IgE (10). Accordingly, in basophils and mast cells (32-34), IgE levels positively regulate

Fc ϵ RI surface expression. Whether this observation can be extended to pDCs has not been carefully addressed. Therefore, purified pDCs from healthy donors were cultured with increasing doses of commercially available IgE for 24 hours, and then phenotyped by flow cytometry. We observed that IgE up-regulated surface expression of Fc ϵ RI on pDCs in a dose-dependent manner (Figures 2A-B). No up-regulation of Fc ϵ RI was found when we exposed pDCs to increasing doses of IgG (Figure 2C). Further inhibition of Fc ϵ RI upregulation was observed when IgE antibodies were preincubated with the anti-IgE monoclonal antibody, omalizumab, before addition to pDCs (Figure 2D). Exposure of pDCs to exogenous IgE did not modify antigen-presentation (HLA-DR), co-stimulation (CD86) or migration (CCR7) markers (Figure 2E). DAPI was used in all assays to exclude dead cells. No significant difference in cell survival was observed when pDCs were cultured with IgE compared to non-treated cells (supplementary Figure 1). We also observed that the expression of Fc ϵ RI on circulating basophils and pDCs was positively correlated to the levels of IgE in allergic patients (data not shown), as well as in patients with SLE (Figure 2F and 2G) ($r = 0.54$; $p < 0.01$ and $r = 0.85$; $p < 0.0001$, respectively). Of note, it was difficult to assess Fc ϵ RI expression on circulating pDCs in most of the active SLE patients due to undetectable or very low numbers of circulating pDCs. Taken together, these data suggest that IgE positively regulates Fc ϵ RI in pDCs.

Exposure of purified pDCs to IgE decreases their ability to secrete IFN- α , and to express maturation markers upon TLR7 and TLR9 stimulation.

pDCs express an array of surface receptors, including BDCA2, ILT7 and Fc ϵ RI, which suppress their ability to produce IFN- α in response to TLR7 and TLR9 ligands. Cross-linking of these receptors negatively regulates pDC responses (15-18). Since IgE binding to Fc ϵ RI can lead to low-level receptor aggregation and antigen-independent effects (10), we explored the impact of IgE on IFN- α production by pDCs in response to both TLR7 and TLR9 agonists. After 24 hours of culture, pDCs exposed to monoclonal IgE were stimulated with TLR7 and TLR9 agonists for 18 additional hours and IFN- α gene expression was measured by quantitative Real Time PCR. We found that mRNA coding for IFN- α was decreased in IgE-primed pDCs compared to unexposed pDCs (Figure 3A). To confirm the ability of IgE priming to inhibit IFN- α production at the protein level, we repeated the same

experiments, harvested supernatants and measured IFN- α concentration by ELISA. We observed a significant inhibition of IFN- α production when pDCs were primed by monoclonal IgE 24 hours before stimulation of TLRs (Figure 3B). Medium or monoclonal IgE alone, without stimulation of TLRs, did not induce any IFN- α production. TLR7 and TLR9-triggered pDCs upregulate activation and maturation markers including the CC-chemokine receptor 7 (CCR7) (Figures 3C). To test, whether IgE could impact pDC activation, we monitored the expression of HLA-DR, CD86 and CCR7 markers after TLRs stimulation of IgE-primed or not pDCs. We found that IgE priming of pDCs significantly impacts their ability to upregulate CCR7 (Figures 3C), without affecting co-stimulatory molecules or antigen-presenting molecules expression (Supplementary figures 2A and 2B). Taken together, these results suggest that IgE impacts the ability of pDCs to secrete IFN- α upon TLR7 and TLR9 activation as well as their ability to migrate to lymph nodes, as suggested by inhibition of CCR7 expression.

Production of IFN- α by pDCs stimulated with SLE sera is inhibited by IgE.

It has been demonstrated that immune complexes (IC) from SLE patients can promote the secretion of IFN- α by pDCs (13). We therefore set out to determine whether IgE-primed pDCs stimulated with lupus ICs would have a similar response as observed in IgE-primed pDCs stimulated with synthetic TLR ligands. First, serum from lupus patients was cultured with apoptotic bodies to generate ICs as described elsewhere (23, 35), and then exposed to IgE-primed or untreated pDCs. We observed that IgE-primed pDCs secrete lower levels of IFN- α when stimulated with SLE sera compared to untreated pDCs (Figure 4A). Interestingly, we observed that the inhibition was stronger with ICs generated from SLE sera of patients that were positive for anti-dsDNA and anti-Sm/RNP, which represent, respectively, endogenous TLR9 and TLR7 ligands. Moreover, IgE downregulated CCR7 expression (Figure 4B and 4C) but, did not impact HLA-DR or CD86 expression (supplementary figure 3A and 3B). Taken together, these data suggest that IgE blocks the ability of SLE ICs to trigger IFN- α secretion by pDCs.

IgE induces intracellular calcium activity and downregulates intracellular TLR7, TLR9 and IRF7.

Inhibition of IFN- α production induced by engagement of BDCA2, ILT7 and Fc ϵ RI at the pDC surface occurs through activation of ITAM-mediated signaling which leads to intracellular calcium mobilization in pDCs (15, 17, 18). To determine if exposure of pDCs to monoclonal IgE induces intracellular signaling, we compared the kinetics of intracellular calcium responses in pDCs treated by a monoclonal IgE or an anti-IgE. We observed that monoclonal IgE, in the absence of cross-linking, induced a significant and sustained cytosolic calcium increase, whereas cross-linking by the anti-IgE, triggered a robust, rapid and transient, but delayed peak of cytosolic calcium in pDCs (Figure 5A). This data supports the engagement of ITAM-mediated signaling in both conditions, but with a different amplitude and kinetics. Both TLR7 and TLR9 are responsible for IFN- α production by pDCs through a signaling pathway downstream of the adaptor MyD88. Interaction of MyD88 with IRF7, the master regulator of IFN- α -dependent immune responses, is required for IFN- α production (36). To determine the molecular mechanisms involved in the impaired ability of IgE-exposed pDCs to secrete IFN- α , we used quantitative Real Time PCR to measure the expression of TLR7, TLR9 and IRF7 in IgE-treated pDCs and controls. We found that the exposure of pDCs to IgE significantly decreased the expression of TLR9 and IRF7 (Figure 5A-C). This suggests that IgE down-regulation of IFN- α secretion is mediated through decreased expression of TLR7 and IRF7.

Discussion

Given the importance of IFN- α in activating a wide range of cells of the innate and adaptive immune system, its production has to be under tight control to prevent aberrant immune responses. pDCs are the main producers of IFN-I, and express different surface receptors including BDCA2 (17), ILT7 (18) and Fc ϵ RI (14-16), that modulate IFN- α production. Cross-linking of these receptors activates pDCs through an ITAM-mediated signaling pathway, but also significantly blocks the ability of the pDCs to secrete IFN-I upon TLR7 and 9 stimulation. Because IFN-I has been implicated in several autoimmune diseases including SLE, we evaluated the ability of IgE to block pDC secretion of IFN-I after TLR7 and 9 activation. In this study, we show that engagement of the Fc ϵ RI by IgE reduces IFN- α production by human pDCs, therefore suggesting a potential protective role of non-autoreactive IgE in lupus disease.

We observed that SLE patients with inactive disease had higher levels of total IgE compared to patients with active disease. Accordingly IgE level correlated with disease activity assessed by the SLEDAI. These findings support a protective role of total IgE in lupus disease. The implication of IgE in SLE pathogenesis has not been carefully studied. In agreement with our study, Charles *et al* demonstrated that autoreactive IgE recognizing nuclear components is present in SLE patients, and that their levels are associated with increased disease activity, and lupus nephritis (27). Interestingly, in this study as in ours, the levels of total IgE and autoreactive IgE are not correlated, suggesting a dual role of autoreactive versus total IgE in SLE patients.

In order to understand the potential mechanism, we focused our attention on the interaction between pDCs and IgE. IgE binding to Fc ϵ RI has been shown to stabilize Fc ϵ RI expression at the cell surface *in vitro* (37). Consistent with cell surface stabilization, mast cell and basophil Fc ϵ RI surface expression correlates to serum IgE concentration in both humans and mice (32-34, 38). However, recent studies have not demonstrated any correlation between IgE levels in blood and Fc ϵ RI surface expression on conventional DCs and monocytes, suggesting that different mechanism may be implicated in Fc ϵ RI regulation (39, 40). In our study, we observed that Fc ϵ RI expression on pDC surface was increased in the presence of IgE, in a

dose dependent manner. *In vivo*, we found, a direct correlation between levels of circulating IgE and Fc ϵ RI expression at the surface of basophils and pDCs, in SLE patients as well as in patients with asthma.

IFN- α is thought to play an important role in SLE pathogenesis with its major source being pDCs activated by RNA- or DNA-containing immune complex stimulation of TLR7 and TLR9, respectively (24). Clinical trials of IFN- α inhibition in SLE are currently in progress and the preliminary results are encouraging (41). We observed that total IgE could decrease the ability of pDCs to secrete IFN-I upon TLR7 and 9 triggering. In our study, IgE decreased SLE immune complex-induced IFN- α production by pDCs, but did not completely block this production, suggesting a more physiological effect. Upon their activation and maturation, pDCs migrates to lymph nodes where they produce IFN-I and interact with other immune cells. The CC-chemokine receptor 7 (CCR7) plays a critical role in the migration of DCs to secondary lymphoid organs (42). Interestingly, we observed that IgE-primed pDCs were unable to upregulate CCR7 expression after TLR7 and TLR9-induced activation.

Fc ϵ RI activation and signaling is thought to occur only when receptor-bound IgE is crosslinked via multivalent antigens (12, 43). However, several studies have shown that, in the absence of antigens, monoclonal IgE stimulates multiple phosphorylation events in normal mast cells (44). While monoclonal IgE does not induce neither degranulation nor leukotriene synthesis, it impacts the production of cytokines. This specific impact on cytokine production is supposed to be linked to the prolonged signaling induced by monoclonal IgE as compared to the transient and robust signaling induced by antigen and Fc ϵ RI cross-linking. Our analysis of the kinetics of intracellular calcium response to IgE in human pDCs evokes a comparable prolonged signaling, suggesting a possible specific impact on cytokine production. This ITAM-mediated pathway inhibits expression of TLR7, TLR9, and, interestingly of the transcription factor IRF7, known as the master regulator of IFN- α production. Accordingly, we found a significant decrease of TLR-7- and TLR-9-induced IFN- α production after monoclonal IgE-priming of pDCs. Similar findings have been previously described with cross-linking of Fc ϵ RI, BDCA2 or ILT-7 (19).

We believe that these observations allow the possibility of the following scenario, which parallels that observed with IgG: Autoreactive IgG are present in many autoimmune conditions and are directly involved in tissue lesion of SLE patients. Yet, polyreactive IgG are given as a treatment in the same autoimmune conditions because of the immunoregulatory properties of the Fc fragment, commonly referred to as the IgG paradox (45) (figure 6, upper panel). The pathogenic role of IgG autoantibodies has been extensively explored (46). They are known to promote immune activation through Fc receptors and complement, and mediate tissue damage. On the other hand, intravenous IgG, in which IgG aggregates have been removed, exhibit a broad spectrum of immunomodulatory activities *in vivo* and *in vitro*, and have been used empirically for lupus treatment (47). Interestingly, it has been demonstrated that the Fc portion of IgG is able to inhibit the virus-induced IFN- α production from pDCs (48). We believe that this paradox could also apply to IgE (Figure 6, lower panel). Indeed, previous work in lupus mouse models demonstrated an involvement of autoreactive IgE in lupus pathogenesis through activation of basophils, promoting a Th2 environment and subsequently the production of autoantibodies by plasma cells (25, 26). More recently, autoreactive IgE has been also shown to play a role in the activation of pDCs and the subsequent production of IFN- α (49). In contrast, our findings suggest a protective effect of total non-autoreactive IgE, through a decreased ability of Fc ϵ RI-triggered pDCs to secrete IFN- α . A study exploring associations between expression of type I interferon-inducible genes in blood (IFN-high) and total IgE levels, and autoreactive IgE levels, is ongoing.

In conclusion, this study demonstrates that total IgE modulates the inflammatory response of pDCs through downregulation of IFN- α secretion and modification of maturation marker expression. These results highlight a complementary IgE mechanism that could be involved in the protective effect of worm infection in SLE, and more generally in type I IFN-mediated disorders. This study could constitute the first step to propose helminth-based therapy in SLE patients, a strategy recently described as being effective in mouse lupus model (50, 51).

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Author contributions

All authors were involved in drafting or critically revising the article for important intellectual content, and all authors approved the final version to be published. Dr. Richez had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design

Khoryati, Augusto, Contin-Bordes, Truchetet, Lazaro, Duffau, Blanco, Richez.

Acquisition of data

Khoryati, Augusto, Contin-Bordes, Shipley, Douchet, Mitrovic, Couzi, Jacquemin, Barnetche, Vacher, Schaefferbeke, Blanco, Richez.

Analysis and interpretation of data

Khoryati, Augusto, Contin-Bordes, Shipley, Truchetet, Lazaro, Duffau, Barnetche, Vacher, Blanco, Richez.

References

1. Gonzalez LA, Toloza SM, McGwin G, Jr., Alarcon GS. Ethnicity in systemic lupus erythematosus (SLE): its influence on susceptibility and outcomes. *Lupus* 2013;22:1214-24.
2. de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* 2003;19:547-51.
3. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259-60.
4. Sotgiu S, Angius A, Embry A, Rosati G, Musumeci S. Hygiene hypothesis: innate immunity, malaria and multiple sclerosis. *Med Hypotheses* 2008;70:819-25.
5. Greenwood BM. Autoimmune disease and parasitic infections in Nigerians. *Lancet* 1968;2:380-2.
6. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 2009;373:2027-33.
7. Cooke A, Zaccane P, Raine T, Phillips JM, Dunne DW. Infection and autoimmunity: are we winning the war, only to lose the peace? *Trends Parasitol* 2004;20:316-21.
8. Harnett W, Harnett MM. Helminth-derived immunomodulators: can understanding the worm produce the pill? *Nat Rev Immunol* 2010;10:278-84.
9. Zaccane P, Cooke A. Vaccine against autoimmune disease: can helminths or their products provide a therapy? *Curr Opin Immunol* 2013;25:418-23.
10. Kraft S, Kinet JP. New developments in FcepsilonRI regulation, function and inhibition. *Nat Rev Immunol* 2007;7:365-78.
11. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008;8:205-17.
12. Kinet JP. The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu Rev Immunol* 1999;17:931-72.
13. Richez C, Blanco P, Rifkin I, Moreau JF, Schaefferbeke T. Role for toll-like receptors in autoimmune disease: the example of systemic lupus erythematosus. *Joint Bone Spine* 2011;78:124-30.
14. Gill MA, Bajwa G, George TA, Dong CC, Dougherty, II, Jiang N, et al. Counterregulation between the FcepsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells. *J Immunol* 2010;184:5999-6006.
15. Schroeder JT, Bieneman AP, Xiao H, Chichester KL, Vasagar K, Saini S, et al. TLR9- and FcepsilonRI-mediated responses oppose one another in plasmacytoid dendritic cells by down-regulating receptor expression. *J Immunol* 2005;175:5724-31.
16. Schroeder JT, Chichester KL, Bieneman AP. Toll-like receptor 9 suppression in plasmacytoid dendritic cells after IgE-dependent activation is mediated by autocrine TNF-alpha. *J Allergy Clin Immunol* 2008;121:486-91.
17. Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* 2001;194:1823-34.
18. Cao W, Rosen DB, Ito T, Bover L, Bao M, Watanabe G, et al. Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production. *J Exp Med* 2006;203:1399-405.

19. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol* 2008;8:594-606.
20. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by IFN- α in systemic lupus erythematosus. *Science* 2001;294:1540-3.
21. Crow MK. Type I interferon in the pathogenesis of lupus. *J Immunol* 2014;192:5459-68.
22. Bave U, Magnusson M, Eloranta ML, Perers A, Alm GV, Ronnblom L. Fc gamma RIIa is expressed on natural IFN- α -producing cells (plasmacytoid dendritic cells) and is required for the IFN- α production induced by apoptotic cells combined with lupus IgG. *J Immunol* 2003;171:3296-302.
23. Bave U, Vallin H, Alm GV, Ronnblom L. Activation of natural interferon- α producing cells by apoptotic U937 cells combined with lupus IgG and its regulation by cytokines. *J Autoimmun* 2001;17:71-80.
24. Marshak-Rothstein A, Rifkin IR. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. *Annu Rev Immunol* 2007;25:419-41.
25. Charles N, Hardwick D, Daugas E, Illei GG, Rivera J. Basophils and the T helper 2 environment can promote the development of lupus nephritis. *Nat Med* 2010;16:701-7.
26. Dema B, Charles N, Pellefigues C, Ricks TK, Suzuki R, Jiang C, et al. Immunoglobulin E plays an immunoregulatory role in lupus. *J Exp Med* 2014;211:2159-68.
27. Dema B, Pellefigues C, Hasni S, Gault N, Jiang C, Ricks TK, et al. Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis. *PLoS One* 2014;9:e90424.
28. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
29. Duffau P, Seneschal J, Nicco C, Richez C, Lazaro E, Douchet I, et al. Platelet CD154 potentiates interferon- α secretion by plasmacytoid dendritic cells in systemic lupus erythematosus. *Sci Transl Med* 2010;2:47ra63.
30. Yasuda K, Richez C, Maciaszek JW, Agrawal N, Akira S, Marshak-Rothstein A, et al. Murine dendritic cell type I IFN production induced by human IgG-RNA immune complexes is IFN regulatory factor (IRF)5 and IRF7 dependent and is required for IL-6 production. *J Immunol* 2007;178:6876-85.
31. Tauzin S, Chaigne-Delalande B, Selva E, Khadra N, Daburon S, Contin-Bordes C, et al. The naturally processed CD95L elicits a c-yes/calcium/PI3K-driven cell migration pathway. *PLoS Biol* 2011;9:e1001090.
32. Conroy MC, Adkinson NF, Jr., Iichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-21.
33. Saini SS, Richardson JJ, Wofsy C, Lavens-Phillips S, Bochner BS, Macglashan DW, Jr. Expression and modulation of Fc ϵ RI α and Fc ϵ RI β in human blood basophils. *J Allergy Clin Immunol* 2001;107:832-41.
34. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J Exp Med* 1997;185:663-72.

35. Blanco P, Pitard V, Viallard JF, Taupin JL, Pellegrin JL, Moreau JF. Increase in activated CD8⁺ T lymphocytes expressing perforin and granzyme B correlates with disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 2005;52:201-11.
36. Richez C, Barnetche T, Miceli-Richard C, Blanco P, Moreau JF, Rifkin I, et al. Role for interferon regulatory factors in autoimmunity. *Joint Bone Spine* 2010;77:525-31.
37. Borkowski TA, Jouvin MH, Lin SY, Kinet JP. Minimal requirements for IgE-mediated regulation of surface Fc epsilon RI. *J Immunol* 2001;167:1290-6.
38. Foster B, Metcalfe DD, Prussin C. Human dendritic cell 1 and dendritic cell 2 subsets express Fc epsilon RI: correlation with serum IgE and allergic asthma. *J Allergy Clin Immunol* 2003;112:1132-8.
39. Greer AM, Wu N, Putnam AL, Woodruff PG, Wolters P, Kinet JP, et al. Serum IgE clearance is facilitated by human Fc epsilon RI internalization. *J Clin Invest* 2014;124:1187-98.
40. Dehlink E, Baker AH, Yen E, Nurko S, Fiebiger E. Relationships between levels of serum IgE, cell-bound IgE, and IgE-receptors on peripheral blood cells in a pediatric population. *PLoS One* 2010;5:e12204.
41. Kirou KA, Gkrouzman E. Anti-interferon alpha treatment in SLE. *Clin Immunol* 2013;148:303-12.
42. Forster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol* 2008;8:362-71.
43. Beaven MA, Metzger H. Signal transduction by Fc receptors: the Fc epsilon RI case. *Immunol Today* 1993;14:222-6.
44. Kalesnikoff J, Huber M, Lam V, Damen JE, Zhang J, Siraganian RP, et al. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 2001;14:801-11.
45. Nimmerjahn F, Ravetch JV. The antiinflammatory activity of IgG: the intravenous IgG paradox. *J Exp Med* 2007;204:11-5.
46. Liu Z, Davidson A. Taming lupus-a new understanding of pathogenesis is leading to clinical advances. *Nat Med* 2012;18:871-82.
47. Schwab I, Nimmerjahn F. Intravenous immunoglobulin therapy: how does IgG modulate the immune system? *Nat Rev Immunol* 2013;13:176-89.
48. Green DS, Lum T, Green JA. IgG-derived Fc down-regulates virus-induced plasmacytoid dendritic cell (pDC) IFN α production. *Cytokine* 2004;26:209-16.
49. Henault J, Riggs JM, Karnell JL, Liarski VM, Li J, Shirinian L, et al. Self-reactive IgE exacerbates interferon responses associated with autoimmunity. *Nat Immunol* 2015.
50. Rodgers DT, McGrath MA, Pineda MA, Al-Riyami L, Rzepecka J, Lumb F, et al. The parasitic worm product, ES-62 targets MyD88-dependent effector mechanisms to suppress ANA production and proteinuria in MRL/Lpr mice. *Arthritis Rheumatol* 2014.
51. Aprahamian TR, Zhong X, Amir S, Binder CJ, Chiang LK, Al-Riyami L, et al. The immunomodulatory parasitic worm product ES-62 reduces lupus-associated accelerated atherosclerosis in a mouse model. *Int J Parasitol* 2015;45:203-7.

Figure legends

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IgE inhibits IFN- α production by pDCs in SLEKhoryati *et al*

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Lower panel. (Left) Autoreactive IgE are able to activate basophils, thereby promoting a Th2 environment and subsequently, the production of autoantibodies by plasma cells. (Right) We believe that non-autoreactive IgE are protective, through a decreased ability of Fc ϵ RI-triggered pDCs to secrete IFN- α .

Supplementary figure legends

Figure S1. IgE does not impact plasmacytoid dendritic cells survival. Purified pDCs were treated for 24h with monoclonal IgE (10 μ g/ml), then DAPI staining was determined by flow cytometry. DAPI staining is shown with stimulus-induced staining intensity. The bar represents the percentage of live cells.

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Supplementary tables

Table S1. Summary of the demographic, clinical and biological characteristics of SLE patients enrolled in the study.

The clinical manifestations have occurred at some time during the course of the disease. Jo =musculoskeletal system, Cy=cytopenia (excluding lymphopenia), Ki=renal disease, Se=seritis, and Sk=cutaneous lesions.

Patient/sex/age	Clinical manifestations	SLEDAI score	Anti-dsDNA (IU/ml)	IgE (IU/ml)
1/F/30	Jo, Sk, Cy	7	9	18
2/F/43	Jo, Sk, Ki	10	75	39
3/F/56	Jo, Sk	9	38	7
4/F/35	Jo, Sk	6	30	47
5/F/35	Jo	8	111	5
6/F/43	Jo, Sk, Cy	6	73	102
7/F/18	Jo, Sk	6	125	686
8/M/58	Jo, Sk, Cy	3	643	1118
9/F/60	Jo, Sk	2	111	5
10/F/29	Jo, Cy	2	17	21
11/F/25	Jo, Sk, Cy	2	19	26
12/F/49	Jo, Sk	0	23	35
13/M/58	Jo, Se	0	7	31
14/M/53	Jo, Se	2	250	60
15/F/25	Jo, Sk	0	5	191
16/F/39	Jo, Sk, Ki	12	1141	16
17/F/40	Jo, Sk	8	348	80
18/M/22	Jo, Ki	8	322	199
19/F/39	Jo, Sk, Se	8	516	20
20/F/18	Jo, Sk, Ki	10	452	7
21/F/29	Jo, Sk, Ki	12	398	106
22/M/45	Jo, Sk, Ki, Se	12	1071	30
23/F/43	Jo, Ki, Se	9	143	3
24/F/46	Jo, Sk, Ki, Cy	8	679	4
25/F/26	Jo, Sk, Se	8	831	5
26/F/28	Jo, Sk	8	252	634
27/M/28	Jo, Ki	6	299	7
28/F/51	Jo, Ki, Cy	8	113	27
29/F/37	Jo, Sk, Ki	11	741	21
30/F/29	Sk, Ki	8	116	28
31/F/62	Jo, Sk	4	1	30
32/F/40	Jo	0	31	227
33/F/29	Jo	4	4	52
34/F/46	Jo, Sk, Ki	0	19	31
35/F/21	Jo, Sk, Ki	0	46	95
36/F/32	Jo, Sk	4	17	27
37/M/35	Jo, Sk, Ki	2	8	157
38/F/21	Jo, Ki	4	8	76
39/F/17	Jo, Sk	0	50	93

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40/M/27	Jo, Cy	0	207	685
41/F/35	Jo, Cy	1	36	446
42/F/64	Jo	0	16	42

Table S2. Oligonucleotide primers for quantitative Real Time RT-PCR assays.

Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'
TLR9	GGACCTCTGGTACTGCTTCCA	AAGCTCGTTGTACACCCAGTCT
TLR7	TTACCTGGATGGAAACCAGCTACT	TCAAGGCTGAGAAGCTGTAAGCTA
IRF7	CCCACGCTATACCATCTACCT	GATGTCGTCATAGAGGCTGTTG
IFNalpha 1	GTGGTGCTCAGCTGCAAGTC	TGTGGGTCTCAGGGAGATCAC
Beta-2-microglobulin	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT
S18	TGCCATCACTGCCATTAAGG	TGCTTTCCTCAACACCACATG

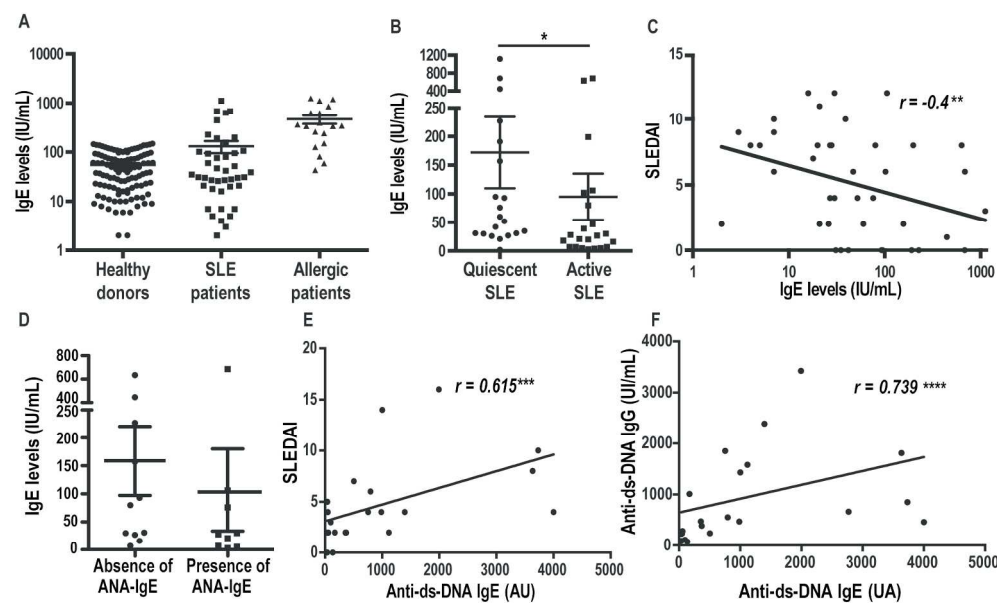


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Accep

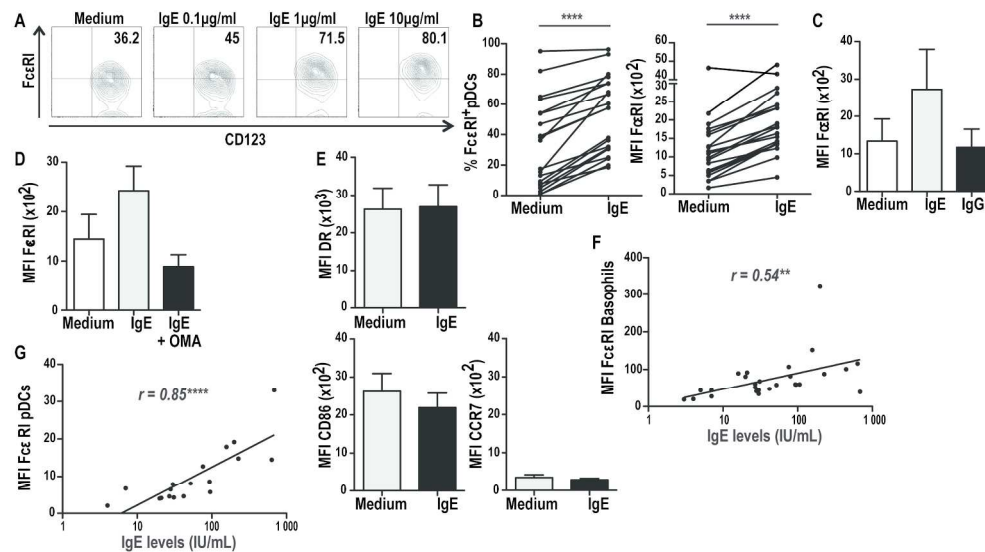


Figure 2. IgE treatment increases surface expression of FcεRI on pDCs. (A) Purified pDCs were cultured for 24h with increasing doses of monoclonal IgE (0.1, 1 and 10 μg/ml), then FcεRI expression on pDCs was determined by flow cytometry. One representative experiment of 10 independent experiments is shown. (B) Purified pDCs were treated or not treated for 24h with monoclonal IgE (10 μg/ml). FcεRI expression on pDCs surface was evaluated by flow cytometry and expressed as percentage of FcεRI positive cells or MFI. **** $P < .0001$ ($n=20$). (C) Purified pDCs were cultured for 24h with monoclonal IgE (10 μg/ml) or with monoclonal IgG (10 μg/ml). Data represent means $\pm$ SEMs ($n=4$). (D) Purified pDCs were cultured for 24 h with monoclonal IgE (10 μg/ml) or with a mixture of monoclonal IgE (10 μg/ml) and omalizumab (OMA). Data represent means $\pm$ SEMs ($n=8$). (E) HLA-DR ($n=11$), CD86 ($n=6$), and CCR7 ($n=6$) expression on pDCs. Bar plots represent means of MFI $\pm$ SEMs. (F-G) FcεRI expression (MFI) was determined by flow cytometry. Correlation between total IgE level in lupus patients and FcεRI expression on (F) basophils ($n=27$) and (G) pDCs ($n=18$) were determined. ** $P < .01$, **** $P < .0001$ by Spearman test.

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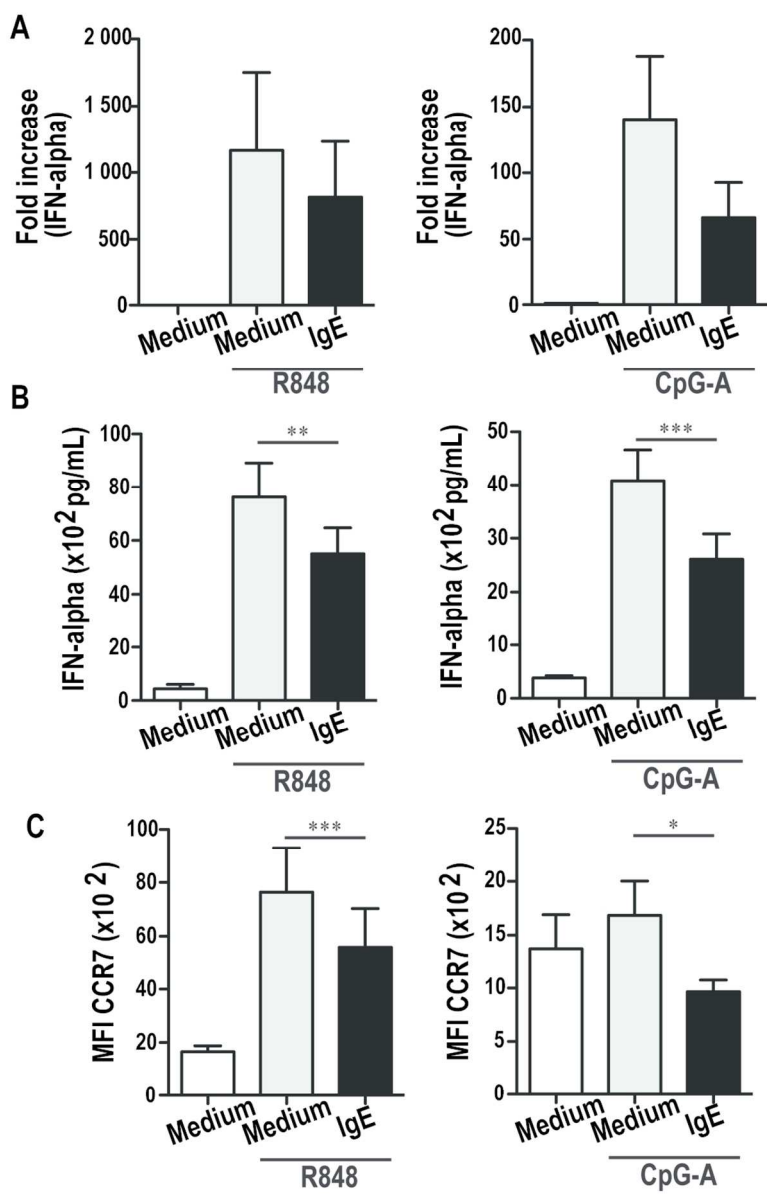


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97x143mm (300 x 300 DPI)

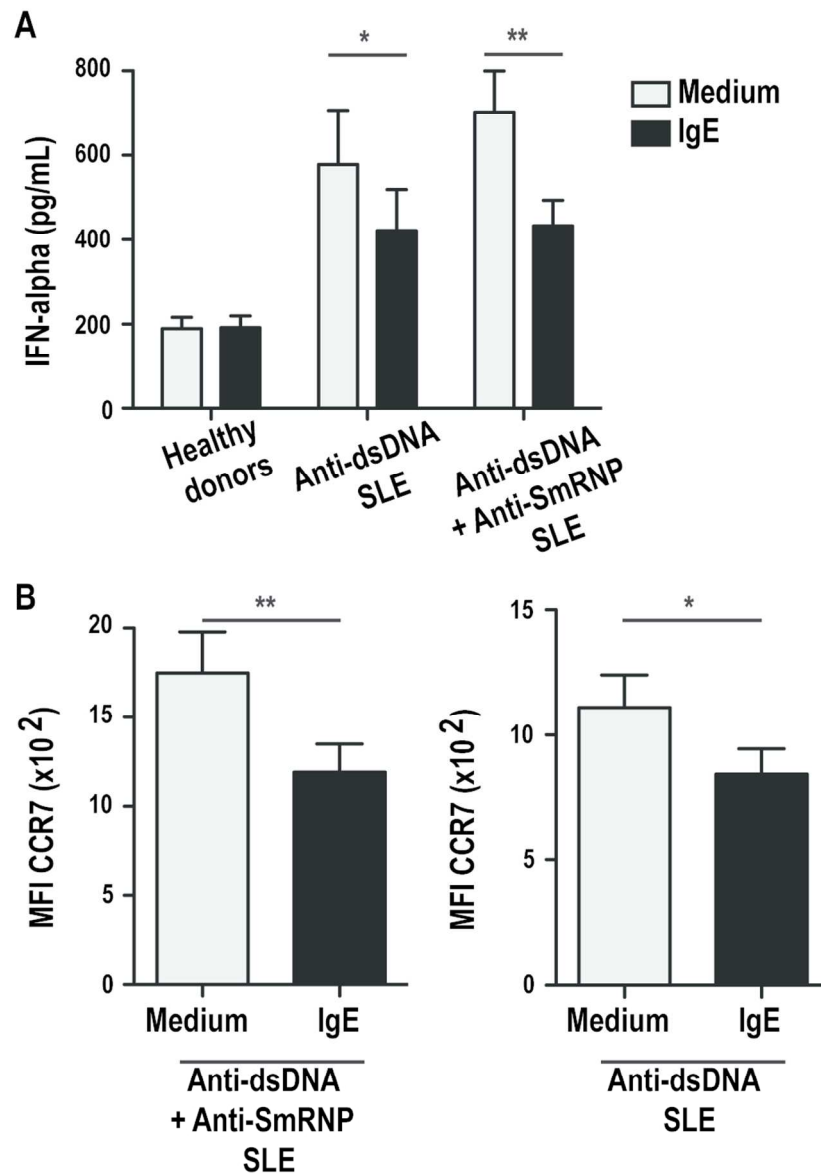


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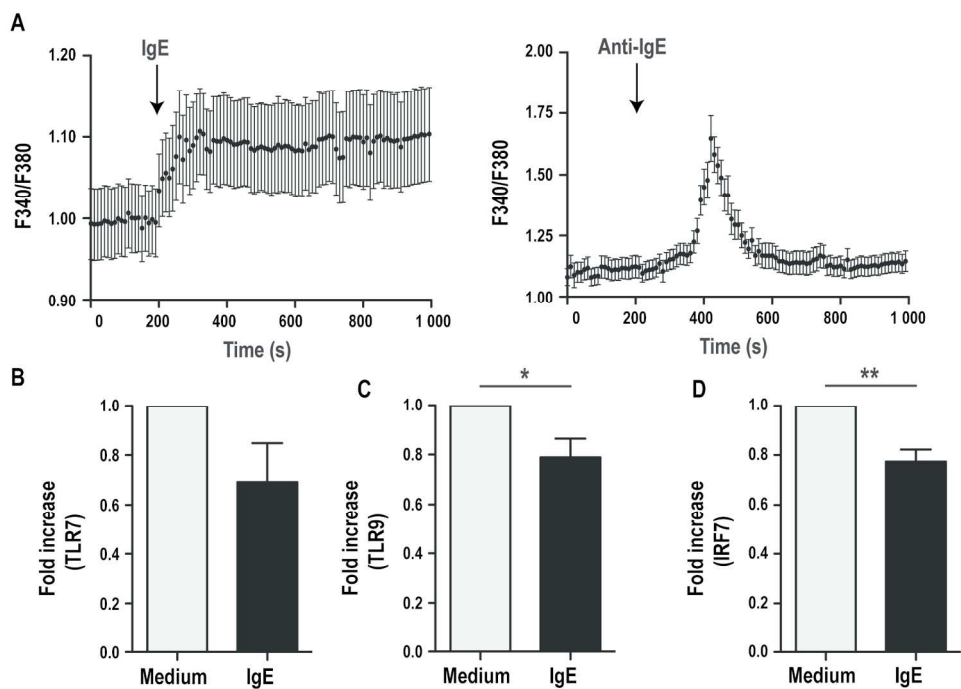


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Accel

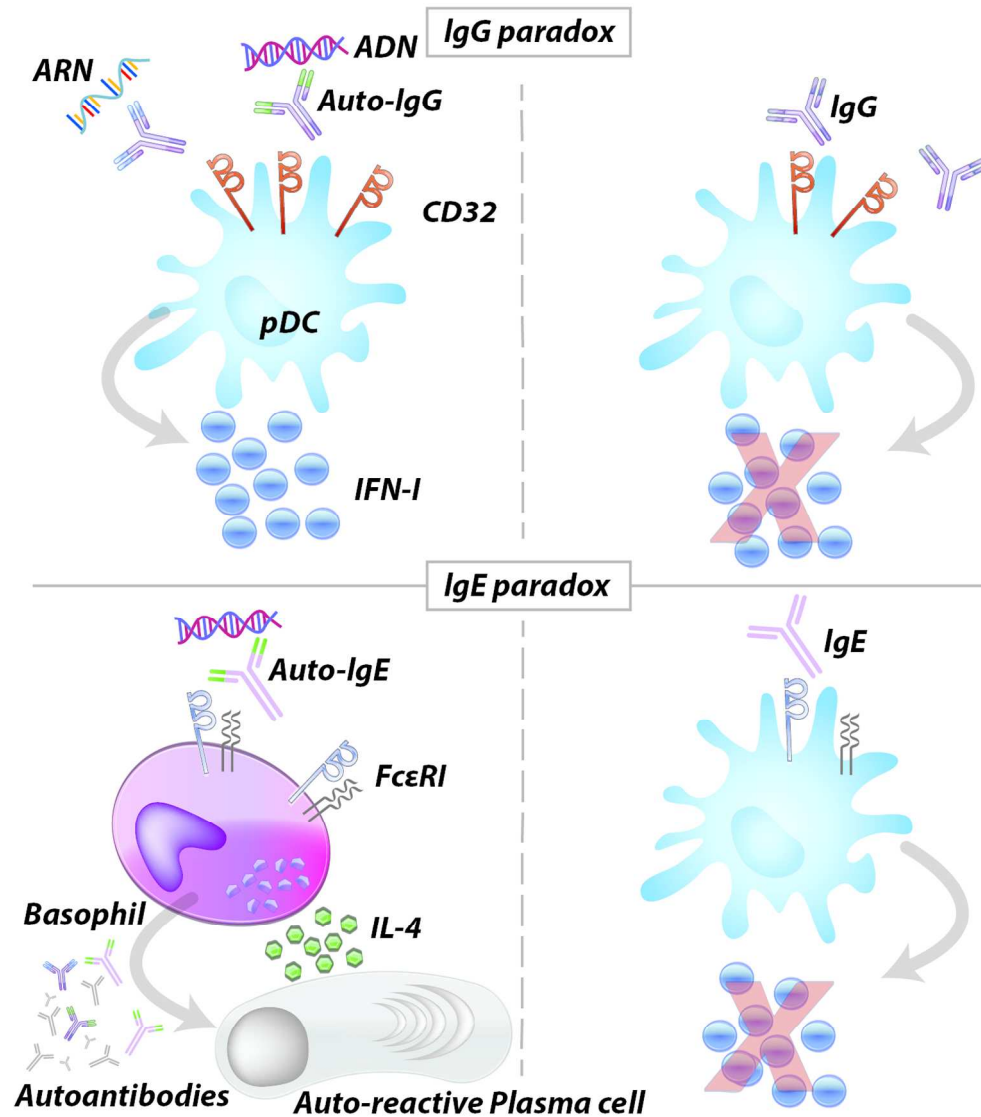


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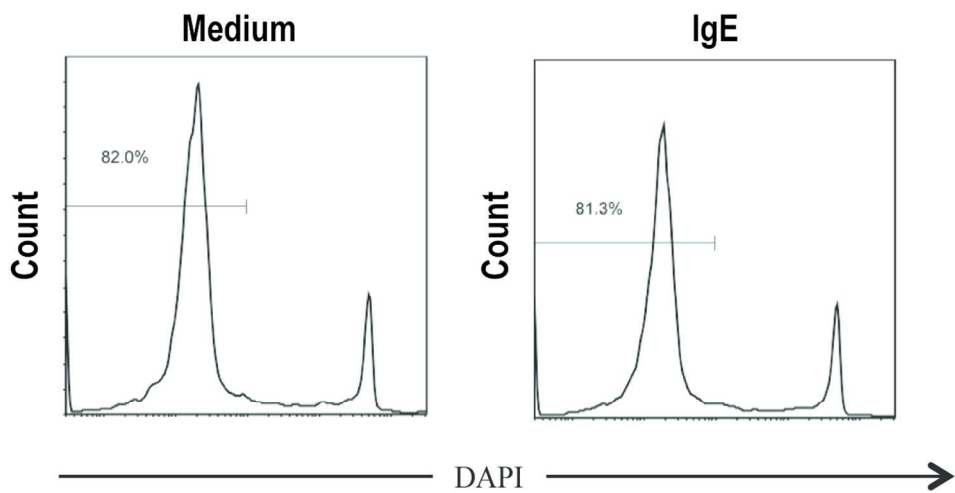


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114x60mm (300 x 300 DPI)

Accepted

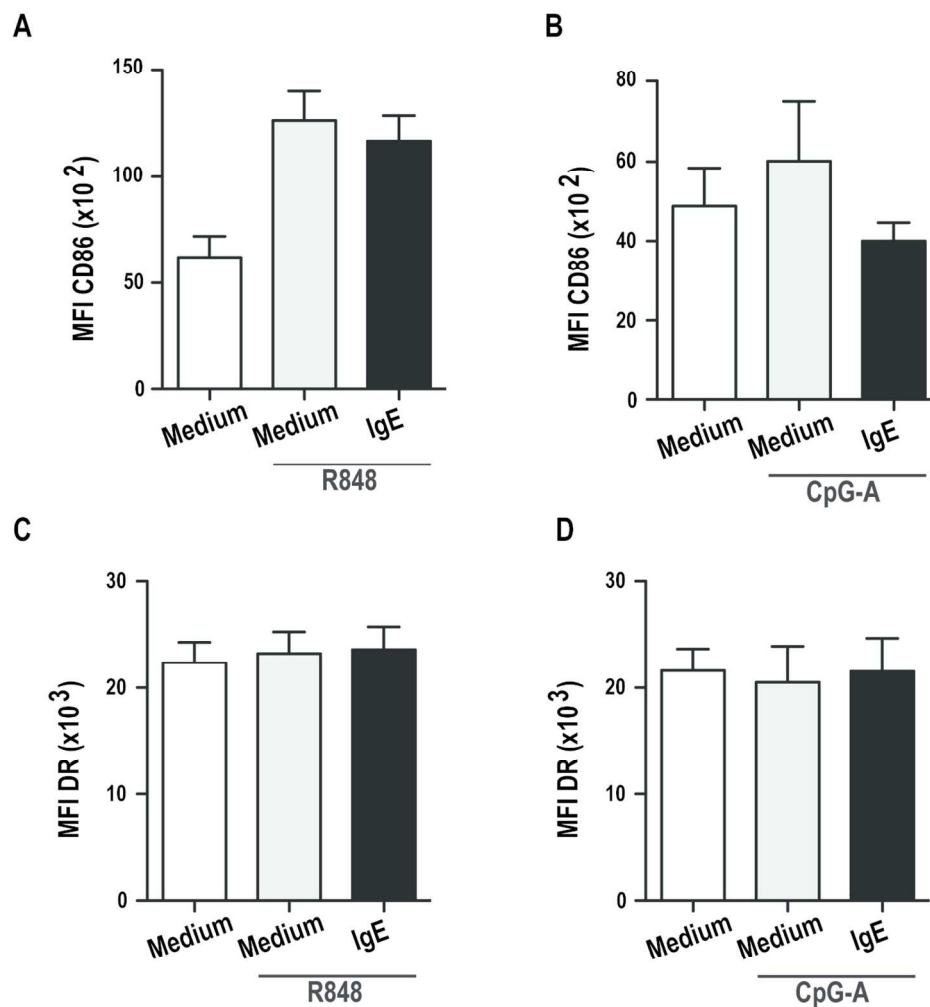


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124x133mm (300 x 300 DPI)

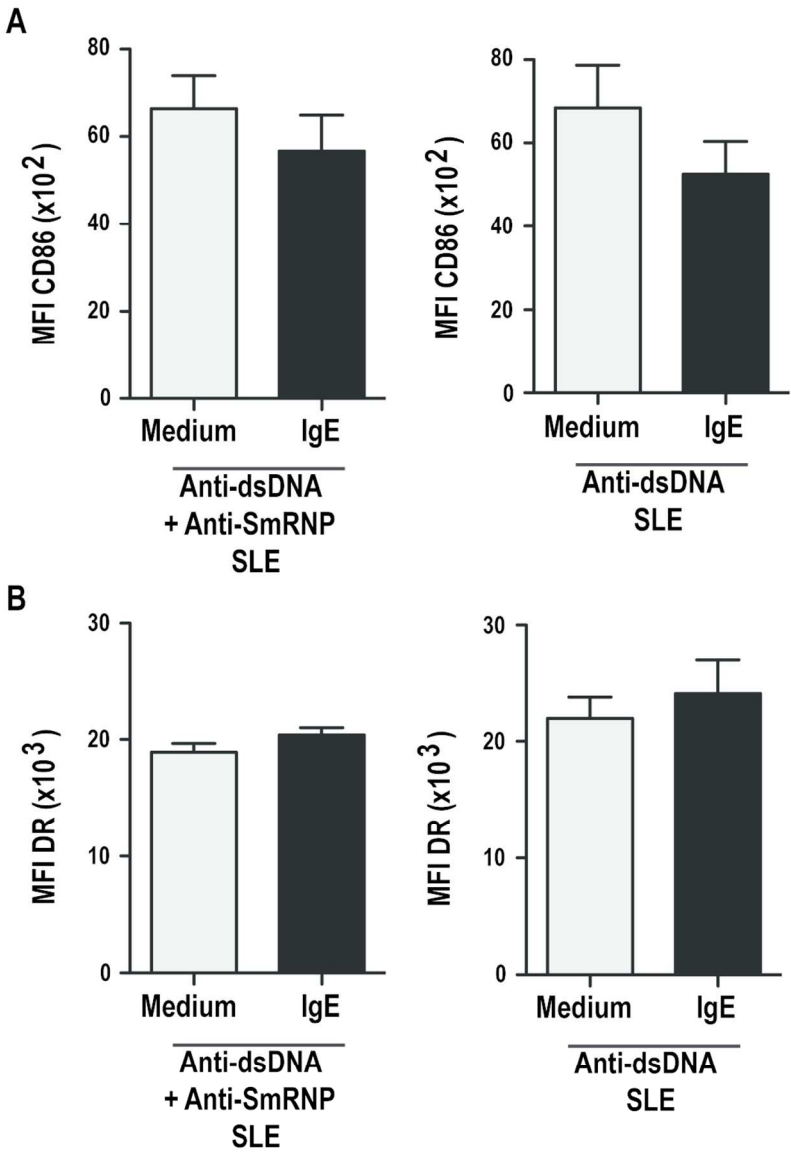


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