Dendritic cells suppress IgE production in B cells

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Abstract

Ig class switch recombination (CSR) is triggered by the engagement of CD40 on B cells by CD40 ligand on T cells. In addition, recent studies have shown that dendritic cells (DCs) are able to directly control the CSR of B cells through a lymphocyte stimulator protein (or B cell activation factor belonging to the tumor necrosis factor family) and a proliferation-inducing ligand. We examined in this study the regulatory role of DCs in CSR and demonstrate that DCs selectively suppress IgE production from B cells stimulated by CD40 and IL-4 through two different mechanisms: by direct cell–cell interaction or by soluble factors including transforming growth factor-β and IFN-γ. In addition, distinct DCs utilize different mechanisms: immature bone marrow-derived dendritic cells (BMDCs) and primary lung DCs strongly inhibit IgE CSR. On the other hand, LPS-induced mature BMDCs lose the ability to inhibit IgE CSR but still suppress IgE production by decreasing IgE protein expression. These results indicate novel regulatory functions of DCs on IgE production.

Introduction

Patients suffering from atopic conditions, such as asthma, allergic rhinitis and atopic dermatitis, have elevated levels of serum IgE including IgE specific for the antigens driving these diseases. The cross-linking of IgE bound to high-affinity FcRs on IgE on tissue mast cells by specific antigens results in the local release of inflammatory mediators, such as histamine and leukotrienes, which coordinately mediate the clinical manifestations of atopic diseases (1).

The level of IgE in the plasma of non-allergic individuals is 10 000- to 50 000-fold less than that of plasma IgG. Even in highly atopic individuals, the level of plasma IgE remains 1000-fold less than that of plasma IgG levels. Although IgE has a relatively short half-life in plasma, mechanisms that tightly control IgE class switch recombination (CSR) are thought to contribute to the low levels of plasma IgE (6-4). CSR replaces the heavy chain constant region $\gamma$ gene with a targeted $\gamma_1$, $\gamma_2$, or $\gamma_3$ gene by recombining the $\mu$ switch region (S$\mu$) with an $\mathrm{S}_{\gamma}1$, $\mathrm{S}_{\gamma}2$, or $\mathrm{S}_{\gamma}3$ region present in the 5′ region of the respective targeted $\gamma$ genes. Consequently, CSR allows the expression of any Ig that has the same antigen specificity with a secondary heavy chain isotype (IgG, IgA, or IgE, respectively), which exhibits different effector functions. The regulation of CSR in B cells is coordinated with germ line transcription (GLT) of $\gamma$ genes and the induction of activation-induced cytidine deaminase (AID) expression (1). Stimulation of CD40 on B cells by CD40 ligand (CD40L) on activated T cells is critical for AID induction. On the other hand, cytokines play important roles in the induction of GLT of distinct $\gamma$ genes. For example, Ce-GLT is induced by Th2-type cytokines such as IL-4 and IL-13 (5). In contrast, Th1-type cytokines such as IFN-γ and IL-21 inhibit the Ce-GLT (6, 7). Several B cell-surface receptors including the B cell receptor (BCR), CD45, CTL antigen 4 and the low-affinity FcεR CD23 and transcription factors such as B cell lymphoma 6 or inhibitor of DNA binding 2 have also been reported to suppress the Ce-GLT (4, 8-13).

Dendritic cells (DCs) are professional antigen-presenting cells that play key roles in acquired immunity predominantly by controlling T cell responses since only DCs can activate naive T cells. DC progenitors in the bone marrow (BM) give rise to blood circulating precursors that home to all of the peripheral tissues such as skin, lung and intestine where they reside as immature DCs. Immature DCs have high phagocytic capacity permitting antigen capturing and less surface co-stimulatory molecules than mature DCs. Following stimulation by pathogens, immature DCs migrate to secondary lymphoid organs and become mature DCs expressing high levels of co-stimulatory as well as MHC molecules on the surface. DCs present antigens to CD4+ T cells, which in turn regulate the differentiation of effector...
cells including antigen-specific CD8⁺ cytotoxic T cells and plasma cells (14). DCs also negatively regulate T cell responses, which is critical for preventing autoimmunity (15).

Germinal centers (GCs) where B cells undergo CSR contain specific types of DCs named germinal center dendritic cells (GCDCs) and follicular DCs (16, 17). GCDCs stimulate proliferation of CD40-activated GC B cells and drive their differentiation toward plasma cells. For example, DC-derived soluble IL-6Rα gp80 complexed with IL-6 enhances differentiation of CD40-activated memory B cells toward IgG-secreting cells (18, 19). DC-derived IL-12 in synergy with the IL-6/soluble IL-6Rα complex further supports the differentiation to plasma cells (20). Moreover, DCs directly induce CSR without T cell help through the expression of the B lymphocyte stimulator protein [BLyS, also known as B cell activation factor belonging to the tumor necrosis factor family (BAFF)] and a proliferation-inducing ligand (APRIL) (21). Although the stimulatory functions of DCs for the activation and differentiation of B cells have been well studied, the regulatory functions of DCs remain to be clarified.

In this study, we examined the regulatory functions of DCs in CSR and Ig production. We demonstrate here that DCs selectively suppress IgE production by activated B cells at two levels: CSR and protein expression.

Methods

Mice

C57BL/6 mice (6–8 weeks) were obtained from Sankyo Lab Service Company (Tokyo, Japan). Rag-2⁻/⁻ (22) and CD23⁻/⁻ (23) mice on a C57BL/6 background were obtained from Taconic (Germantown, NY, USA) and RIKEN (Tsukuba, Japan), respectively. IFN-γ⁻/⁻ (24) and IFN-γR⁻/⁻ (25) mice on a C57BL/6 background were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Rag-2⁻/⁻/IFN-γ⁻/⁻ mice were generated by crossing Rag-2⁻/⁻ and IFN-γ⁻/⁻ mice. All mice were maintained under specific pathogen-free conditions in our animal facilities. All experiments were done in accordance with our Institutional Guidelines.

BM-derived DCs

BM cells were collected from naive C57BL/6 mice. Red blood cells were depleted using ammonium chloride solution (ACK lysis buffer solution), and cells were grown in RPMI 1640 (Sigma, St Louis, MO, USA) culture medium containing 10% FCS, 1% MEM non-essential amino acids solution (GIBCO, Berlin, Germany), 1% penicillin-streptomycin (GIBCO), 1% HEPES buffer solution (GIBCO), 1% sodium pyruvate (GIBCO), 50 μM 2-mercaptoethanol (GIBCO) and 10 ng ml⁻¹ recombinant mouse granulocyte macrophage colony-stimulating factor (PEPRO TECH EC LTD, London, UK) for 6 days with medium changed every 2 days. CD11c⁺ immature bone marrow-derived dendritic cells (imBMDCs) were then separated with anti-CD11c microbeads and AutoMACS (Miltenyi Biotec GmbH, Bergisch, Germany). To obtain mature bone marrow-derived dendritic cells (mBMDCs), cells were stimulated overnight with 1 μg ml⁻¹ LPS (Sigma). Purity checked by flow cytometry using a FACS Calibur (BD Biosciences, San Jose, CA, USA) was usually >92%.

Lungs DCs

Lungs from C57BL/6 mice were minced and digested with 1 mg ml⁻¹ collagenase type IV (C138, Sigma) and 0.1 mg ml⁻¹ DNase I (Sigma) at 37°C for 30 min with vortexing every 5 min. To make a single-cell suspension, the remaining tissue was filtered through a 100-μm cell strainer. After lysing red blood cells using ACK lysis buffer solution, the remaining cells were washed with RPMI 1640 culture medium and centrifuged on a 30% Percoll density gradient. Cells at the interface were collected and washed and CD11c⁺ cells were separated with anti-CD11c microbeads and AutoMACS. Purity checked by flow cytometry using a FACS Calibur was usually >90%.

Splenic B, CD4⁺ and CD8⁺ T cells

CD19⁺ B cells, CD4⁺ T cells and CD8⁺ T cells were separated from single-cell suspension of splenocytes by AutoMACS with anti-CD19, anti-CD4 and anti-CD8 microbeads, respectively. Purity checked by flow cytometry using a FACS Calibur was usually >95%.

Primary embryonic fibroblasts

Twelve- to 16-day mouse embryos were digested with 1 mg ml⁻¹ collagenase type IV and 0.1 mg ml⁻¹ DNase I as above. Cells were plated on a culture dish and cultured for 3 days with medium changed every day. These cells were used as primary embryonic fibroblasts (PEFs) at day 4.

Co-cultivation of CD19⁺ splenic B cells with other cell types

One hundred thousand B cells and the same number of indicated cells were co-cultured and stimulated with 5 μg ml⁻¹ anti-CD40 mAb (eBioscience, San Diego, CA, USA) and 25 ng ml⁻¹ recombinant IL-4 (PEPRO TECH EC LTD) in a final volume of 200 μl in 96-well plates. After 4 days, Ig production was measured by flow cytometry and ELISA. To examine the effect of cell–cell interaction, 24-well format transwells with a 1.0-μm pore size (BD Falcon™, Franklin Lakes, NJ, USA) were used to separate B cells and DCs. All upper wells contained 4 × 10⁵ B cells and lower wells contained various samples including DCs alone or mixtures of B cells and DCs. Cells were stimulated with 5 μg ml⁻¹ anti-CD40 mAb and 25 ng ml⁻¹ recombinant IL-4 (rIL-4). After 4 days, Ig production was measured by flow cytometry and ELISA. To examine the effect of transforming growth factor-β (TGF-β), 1 μg ml⁻¹ recombinant human latency-associated peptide (LAP) (R&D Systems Inc., Wiesbaden, Germany) and/or 1 ng ml⁻¹ recombinant human TGF-β3 (PEPRO TECH EC LTD) were added to the culture.

Flow cytometry

B cells were first incubated with normal rat serum and then incubated with 5 μg ml⁻¹ biotin-conjugated anti-IgG1 mAb (BD PharMingen™, Hamburg, Germany). After washing, cells were further incubated with 2 μg ml⁻¹ FITC–anti-IgE (BD PharMingen™), 2 μg ml⁻¹ PE–anti-CD19 (BD PharMingen™) and 2 μg ml⁻¹ allopurinol-conjugated streptavidin (BD PharMingen™). Cells were analyzed by flow cytometry using a FACS Calibur. For intracellular staining, cells were fixed in 70% ethanol before staining.
ELISA

Ig concentrations were measured by an ELISA kit for IgE (Mouse IgE ELISA Quantitation Kit, BETHYL, Montgomery, TX, USA) and ELISA kits for IgM, IgA and IgG1 (Southern Biotech, Birmingham, MA, USA). The concentrations of cytokines were measured by ELISA kits for TGF-β (R&D Systems Inc.) and IFN-γ (OptEIA; BD PharMingen™).

PCR analysis

Total RNAs were purified with Trizol reagent (Invitrogen, Tokyo, Japan). Two micrograms of total RNA was used for reverse transcription. The amount of cDNAs for AID, Ce-GLT, Ig-Ce post-switch transcript (Ig-Ce-PST) and β-actin were measured by semi-quantitative PCR using the following primer pairs: AID, 5'-AAAGGGACCGCATGACCTA-3', 5'-GAAC-CAGGTGACCGGAAC-3'; Ce-GLT, 5'-CATCTGGGCATGAATTATGTTA-3', 5'-GATGCCTAAGGGGCCTCAGT-3'; Ig-Ce-PST, 5'-CTTGGGCTGTTATGTTG-3', 5'-GATGCCT-CAAGGCTGTCAGT-3'; β-actin, 5'-TGGGCCCCTGCTCAGCCACAA-3', 5'-TCTTTGATGTCACGCACGATTCC-3'.

Results

DCs suppress IgE production

We examined the effect of DCs on Ig CSR by co-culturing B cells with DCs using BMDCs. CSR was induced by stimulating splenic B cells with anti-CD40 mAb and rIL-4 to mimic the interaction between B cells and activated T cells. A combination of anti-CD40 mAb and rIL-4 but not anti-CD40 mAb alone induced IgG1 and IgE production (Fig. 1A, left panels). Co-culture of B cells with either imBMDCs or LPS-stimulated mBMDCs strongly suppressed IgE production while IgG1 production was not suppressed by these BMDCs as examined by both flow cytometry (Fig. 1A, right panels) and ELISA (Fig. 1B). On the other hand, IgA production was strongly induced by co-culture with mBMDCs (Fig. 1B) as previously reported (20). Suppression of IgE production was dependent on BMDC numbers and mBMDCs showed a stronger effect than imBMDCs as lower numbers of mBMDCs were sufficient for IgE suppression compared with imBMDCs (Fig. 1C). These results suggest that BMDCs directly suppress IgE production by B cells independent of T cells.

Fig. 1. BMDCs suppress IgE production. (A) One hundred thousand purified splenic CD19+ B cells from C57BL/6 mice were stimulated with 5 μg ml⁻¹ anti-CD40 mAb alone as a control (small panel on the lower left) or anti-CD40 mAb and 25 ng ml⁻¹ rIL-4 with or without 1 × 10⁵ purified imBMDCs or mBMDCs for 4 days. Cells were stained with anti-CD19, anti-IgG1, anti-IgE mAbs and the expression of surface IgE and IgG1 on CD19+ B cells was analyzed by FACS Calibur. Numbers indicate the percentages of IgE+ and IgG1+ cells among CD19+ B cells. (B) CD19+ B cells were cultured as described in (A) with the indicated numbers of mBMDCs. Secreted IgE, IgG1 and IgA were measured by ELISA. (C) CD19+ B cells were cultured as described in (A) with the indicated numbers of mBMDCs and imBMDCs and the expression of surface IgE and IgG1 was analyzed by FACS Calibr. The percentages of IgE+ cells among CD19+ B cells are plotted as a function of DC numbers.
IgE production is suppressed under concentrated culture conditions in vitro (26). It is thus possible that suppression of IgE production is caused by co-culture of B cells with high concentrations of any cell type. To examine this possibility, B cells were co-cultured with PEF, CD4^+ T cells, CD8^+ T cells and CD11c^-cells, which remained after CD11c^-cell separation from BM cell cultures. As shown in Fig. 2A, only DCs suppressed IgE production.

Since BMDCs enhance B cell proliferation, we next tested whether increasing B cell concentrations during culture with a fixed number of BMDCs resulted in the suppression of IgE production. At various concentrations, BMDCs enhanced IgG1 production but suppressed IgE production (Fig. 2B), indicating that DC-mediated suppression is independent of B cell concentration. These results collectively suggest that DCs actively suppress IgE production.

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The mechanism of IgE suppression by BMDCs

IgE CSR requires both Ce-GLT and AID, and IgM-Ce-PST is detected after IgE CSR. We thus examined the amounts of Ce-GLT, IgM-Ce-PST and AID mRNA in B cells stimulated with anti-CD40 mAb and IL-4 in the presence or absence of BMDCs. As shown in Fig. 3A, the amount of IgM-Ce-PST was unaffected by the presence of mBMDCs. Interestingly, the amounts of Ce-GLT and AID mRNA were even higher in B cells co-cultured with mBMDCs than those without mBMDCs. Note that the expression of surface IgE was strongly suppressed in B cells co-cultured with mBMDCs (Figs 1A and 3B). In contrast, imBMDCs suppressed IgM-Ce-PST and Ce-GLT. AID induction was unaffected by either type of BMDCs. These results indicate that imBMDCs suppress IgE CSR by blocking Ce-GLT but mBMDCs do not suppress IgE CSR.

Since CSR was unaffected by mBMDCs (Fig. 3A) yet suppressed IgE expression (Figs 1A and 3B), we examined the IgE protein levels by intracellular IgE staining in addition to surface IgE staining (Fig. 3C). Although the amounts of surface IgE were greatly reduced in B cells co-cultured with mBMDCs, IgE proteins were readily detected in the cytoplasm of these B cells by intracellular IgE staining (Fig. 3C). Note that the number of intracellular IgE^+ cells in the presence of mBMDCs was similar to that of surface IgE^+ cells treated with anti-CD40 mAb and IL-4 alone. The mean fluorescence intensity of intracellular IgE in B cells co-cultured with mBMDCs was much lower than that of untreated B cells, indicating that the amounts of IgE was suppressed at the protein level. On the other hand, neither surface IgE^+ nor intracellular IgE^+ cells were detected in B cells co-cultured with imBMDCs, which is consistent with the observation that imBMDCs suppress IgE production at the CSR level. These results collectively indicate that DCs lose the ability to suppress IgE at the CSR level upon maturation but retain the ability to reduce the IgE production at the protein level.

Next, we examined whether direct contact of BMDCs to B cells is required for IgE suppression or whether soluble factors mediate surface IgE suppression. Co-cultivation of B cells with formalin-fixed mBMDCs was able to inhibit IgE production as examined by both surface IgE expression and secretion of IgE (Fig. 3B and C), indicating that the direct contact of B cells and DCs results in the IgE suppression. When B cells were separated from mBMDCs in a transwell system, IgE production was not suppressed by a low number of mBMDCs (4 x 10^5) but higher number of mBMDCs (8 x 10^5) were able to modestly suppress IgE production (Fig. 3D), suggesting the involvement of soluble factors. Fixed imBMDCs also inhibited surface IgE expression in a contact-dependent manner. In contrast to the co-culture with unfixed imBMDCs, intracellular IgE was readily detected in B cells co-cultured with fixed imBMDCs (Fig. 3C). These results suggest that direct cell–cell contact was required for the suppression of IgE production at a protein level and that the inhibition of CSR is mediated by soluble factors.

To examine surface molecules involved in the IgE suppression mediated by direct cell–cell contact, we performed the following series of experiments. Since IgE production is negatively regulated by CD23 on B cells and CD23 binds CD11c and CD11b on mBMDCs (27), we co-cultured B cells...
from wild-type (WT) and CD23 knockout (CD23<sup>-/-</sup>) mice with mBMDCs. As shown in Fig. 4(A), mBMDCs suppressed IgE production in CD23<sup>-/-</sup> B cells as well, indicating that CD23 is not involved in the IgE suppression by mBMDCs. Furthermore, BAFF (BlyS) and APRIL expressed on the DC surface can directly act on B cells (21). We thus examined whether soluble BAFF inhibits IgE production. As shown in Fig. 4(B), BAFF alone strongly stimulates B cell proliferation as previously reported (28), but failed to suppress IgE production.

**Lung DCs suppress IgE CSR**

Although lung is a site of primary immune responses and is constantly exposed to and sensitized by allergens, lung IgE levels are always low. Thus, we questioned whether DCs suppress IgE production in the lung and used lung resident DCs as primary DCs in the next series of experiments. When B cells were co-cultured with freshly isolated lung DCs from CD19<sup>+</sup> B cells, cells were stimulated with 5 μg ml<sup>-1</sup> anti-CD40 mAb and 25 ng ml<sup>-1</sup> rIL-4 for 4 days. All upper wells contained 4 × 10<sup>5</sup> purified CD19<sup>+</sup> B cells. Lower wells contained media, 4 × 10<sup>5</sup> or 8 × 10<sup>5</sup> mBMDCs or 4 × 10<sup>5</sup> or 8 × 10<sup>5</sup> mBMDCs with or without CD19<sup>+</sup> B cells. The amounts of surface IgE and IgG1 were analyzed by FACS Calibur. The percentages of IgE<sup>+</sup> and IgG1<sup>+</sup> cells among CD19<sup>+</sup> B cells are shown.

**Immunoregulatory DCs**

Immunoregulatory DCs have been characterized as being able to stimulate T cell proliferation and inhibit IgE production. Since these DCs are able to inhibit IgE production, we used these DCs in the next set of experiments. When B cells were co-cultured with freshly isolated immunoregulatory DCs, cells were stimulated with 5 μg ml<sup>-1</sup> anti-CD40 mAb and 25 ng ml<sup>-1</sup> rIL-4 for 4 days. All upper wells contained 4 × 10<sup>5</sup> purified CD19<sup>+</sup> B cells. Lower wells contained media, 4 × 10<sup>5</sup> or 8 × 10<sup>5</sup> mBMDCs or 4 × 10<sup>5</sup> or 8 × 10<sup>5</sup> mBMDCs with or without CD19<sup>+</sup> B cells. The amounts of surface IgE and IgG1 were analyzed by FACS Calibur. The percentages of IgE<sup>+</sup> and IgG1<sup>+</sup> cells among CD19<sup>+</sup> B cells are shown.
Effects of TGF-β and IFN-γ on IgE production

We noted that the surface IgE expression was also suppressed in the upper well of the transwell system when live DCs but not fixed DCs were added to the lower well (Figs 3D and 5B), suggesting that soluble factors are involved in the suppression of CSR. To examine the nature of soluble factors inhibiting IgE production, we analyzed the production of TGF-β and IFN-γ, both of which are known to inhibit IgE CSR (4, 6, 29, 30). DCs produced TGF-β and IFN-γ in the culture supernatant when co-cultured with B cells in the presence of anti-CD40 and IL-4 (Fig. 6A). Lung DCs produced more IFN-γ and less TGF-β than BMDCs whereas BMDCs produced more TGF-β than lung DCs. IFN-γ production was not affected when B cells were derived from IFN-γ−/− mice but was greatly reduced in the co-culture of IFN-γ−/− lung DCs with WT B cells (data not shown), indicating that the majority of IFN-γ was produced by DCs. These results prompted us to test whether TGF-β and IFN-γ are factors inhibiting IgE CSR using cells derived from IFN-γ−/− mice and LAP, which neutralizes TGF-β activity (31). As expected, TGF-β suppressed IgE production but the addition of LAP neutralized this effect (Fig. 6B). IFN-γ−/− imBMDCs or LAP alone partially canceled the suppression of IgE CSR by IFN-γ and TGF-β by LAP almost completely canceled suppression of IgE CSR in B cells co-cultured with IFN-γ−/− imBMDCs (Fig. 6C). The effect of IFN-γ from B cells is negligible, because IFN-γ−/− B cells gave similar experimental results as WT B cells (Fig. 6C, lower panels). These results suggest that IgE production is suppressed at least in part by IFN-γ and TGF-β produced by DCs. Since IFN-γ also affects DC functions (32–34), we next examine if IFN-γ directly acts on B cells or functions through modification of DC functions. To this end, we employed B cells and DCs from IFN-γ−/−R+/− mice. As shown in Fig. 6(D), co-cultivation with WT imBMDCs still suppressed IgE production by IFN-γ−/−R+/− B cells, indicating that IFN-γ does not directly act on B cells. In contrast, IFN-γ−/− or IFN-γ−/− imBMDCs were unable to suppress IgE production in the presence of LAP. These results collectively show that IFN-γ suppresses IgE production by acting on DCs.

Discussion

IgE is beneficial for controlling parasite infection, but excess IgE causes allergy. After clearance of parasites, the amounts of IgE must be down-regulated quickly. One mechanism for this down-regulation is that IgE has a short half-life in the serum (12). In addition, IgE production must be tightly regulated. It is known that much more IgE is produced in vitro than in vivo from purified B cells (1). This indicates that IgE production is suppressed by other cell types in vivo. Our study shows that DCs are a potential candidate for an IgE-negative regulator in vivo. We demonstrated here that DCs directly suppress IgE production from B cells stimulated with anti-CD40 mAb and IL-4, whereas DCs enhance IgG1 CSR as previously reported (21). It is possible that IgE production is surveyed by resident DCs in the secondary lymphoid organs as immature DCs constantly migrate to peripheral lymph nodes (35). It has been reported that the basal or tonic signal through the BCR is critical for the survival of peripheral B cells and that B cells are eliminated from body shortly after BCR ablation (36). The suppression of surface IgE expression specifically eliminates IgE-positive cells after IgE CSR. In fact, IgE production is terminated faster than IgG1 production after a single immunization (12).

As shown here, imBMDCs and primary lung DCs that show an immature phenotype strongly suppress IgE CSR by
blocking Ce-GLT but not AID expression. Upon maturation, BMDCs lose their ability to suppress the IgE CSR but still down-regulate IgE production at a protein level. Our results also show that there are two different mechanisms for IgE suppression: direct cell–cell interaction and soluble factors such as TGF-β and IFN-γ. The suppression of IgE production at a protein level involves direct cell-to-cell interaction rather than soluble factors since co-cultivation of B cells with mBMDCs reduced IgE production only slightly in a transwell system (Fig. 3D). Direct cell–cell interaction reduced the surface IgE but IgE protein was readily observed in the cytoplasm. Percentages of intracellular IgE-positive cells are higher than that of B cells without DCs, suggesting that mBMDCs enhance IgE CSR as previously reported (21) but suppress IgE surface expression. The identity of surface molecules involved in the suppression of cell-surface IgE expression is unknown at the moment and is the subject of future studies.

Lung DCs and imBMDCs suppress IgE CSR through both cell–cell interaction and soluble factors. Lung DCs produce TGF-β and IFN-γ, which have been reported to suppress IgE CSR (4, 6, 29, 30). Indeed, IgE suppression by DCs was abolished by neutralizing TGF-β with LAP in an IFN-γ-deficient background. Therefore, imBMDCs and lung DCs inhibit IgE CSR at least in part through production of IFN-γ and TGF-β and/or activate latent TGF-β. As shown in Fig. 6, our results suggest that IFN-γ suppresses IgE production by modifying DC functions rather than directly acting on B cells. It has been shown that stimulation of DCs through CD40 induces IL-12 production that further induces IFN-γ production by DCs in an autocrine manner (37, 38). IFN-γ is also important for DC functions. IFN-γ stimulates DCs to produce IL-12 and type I IFNs and production of IL-12 and type I IFNs are significantly reduced in IFN-γ−/− as well as IFN-γR1−/− DCs (32, 33). Incubation of DCs with IFN-γ down-regulates the TLR2-promoting capacities of DCs (32). In addition, IFN-γ plays a role in maturation of DCs in an autocrine manner (34). These results indicate that the activation of DCs through Toll like receptors and CD40 is partly dependent on
Although the DC-derived cytokines directly involved in the suppression of IgE production by B cells remain to be identified, production of such cytokines may be enhanced by IFN-γ. Since CD40L expression is induced on activated CD4+ T cells, it is likely that DCs produce IFN-γ in the secondary lymphoid organs where B and T cells are activated and interact with each other. In this context, it is of note that nematode infection, which is a strong inducer of IgE response, suppresses IL-12 production from DCs (39–42). Suppression of DC-derived IFN-γ may explain strong IgE induction by nematode infection.

Recently, it was reported that natural IgE is produced in the absence of MHC class II cognate help (43). Although CSR to IgE is conventionally viewed as an event highly dependent on T cell help, serum IgE concentrations are known to be spontaneously and dramatically increased in nu/nu mice, MHC class II-deficient mice, CD4-deficient mice with reduced T cell numbers or in the absence of cognate T cell help, demonstrating the presence of T cell-independent IgE CSR (43). Interestingly, CD4+ T cells are required for functional maturation of DCs (44). CD4+ T cell deficiency may lead to the impairment of DC maturation and hyper-IgE production. Although several reports showed that DCs are required for IgE production and the maintenance of the allergic condition (45, 46), DCs seem to control IgE production at the same time.

Our study sheds light on the role of DCs in the regulation and surveillance for B cells and in the prevention of hyper-IgE production. Our findings also suggest a novel approach to regulate B cell function by DCs in allergic diseases.

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**Abbreviations**

AID activation-induced cytidine deaminase  
APRIL a proliferation-inducing ligand  
BAFF B cell activation factor belonging to the tumor necrosis factor family  
BCR B cell receptor  
BLYS  B lymphocyte stimulator protein  
BM bone marrow  
BMDC bone marrow-derived dendritic cell  
CD40 ligand  
CSR class switch recombination  
DC dendritic cell  
GC germinal center  
GCDC germinal center dendritic cell  
GLT germ line transcription  
imBMDC immature bone marrow-derived dendritic cell  
LAP latency-associated peptide  
mBMDC mature bone marrow-derived dendritic cell  
PEF primary embryonic fibroblast  
PST post-switch transcripts  
ril recombinant IL  
TGF transforming growth factor  
WT wild type

**References**


