Essential requirement for IRF8 and SLC15A4 implicates plasmacytoid dendritic cells in the pathogenesis of lupus

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In vitro evidence suggests that plasmacytoid dendritic cells (pDCs) are intimately involved in the pathogenesis of lupus. However, it remains to be determined whether these cells are required in vivo for disease development, and whether their contribution is restricted to hyperproduction of type I IFNs. To address these issues, we created lupus-predisposed mice lacking the IFN regulatory factor 8 (IRF8) or carrying a mutation that impairs the peptide/histidine transporter solute carrier family 15, member 4 (SLC15A4). IRF8-deficient NZB mice, lacking pDCs, showed almost complete absence of anti-nuclear, anti-chromatin, and anti-erythrocyte autoantibodies, along with reduced kidney disease. These effects were observed despite normal B-cell responses to Toll-like receptor (TLR) 7 and TLR9 stimuli and intact humoral responses to conventional T-dependent and -independent antigens. Moreover, *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice, in which pDCs are present but unable to produce type I IFNs in response to endosomal TLR ligands, also showed an absence of autoantibodies, reduced lymphadenopathy and splenomegaly, and extended survival. Taken together, our results demonstrate that pDCs and the production of type I IFNs by these cells are critical contributors to the pathogenesis of lupus-like autoimmunity in these models. Thus, IRF8 and SLC15A4 may provide important targets for therapeutic intervention in human lupus.

Extensive evidence suggests that type I IFNs are major pathogenic effectors in lupus-associated systemic autoimmunity. A well-documented pattern of expression of type I IFN-inducible genes occurs in peripheral blood mononuclear cells of patients with systemic lupus erythematosus (SLE) (1–3), and reduced disease is observed in some lupus-predisposed mice that either lack the common receptor (IFNAR) for these cytokines (4, 5) or have been treated with IFNAR-blocking antibody (6). Consequently, attention has focused on defining the cell subsets and signaling processes involved in type I IFN production, the mechanisms by which these mediators accelerate disease, and approaches to interfere with these pathogenic events.

Early in vitro studies showed that type I IFN production can be induced in normal blood leukocytes by SLE autoantibodies complexed with nucleic acid-containing apoptotic/necrotic cell material, and further work demonstrated that this activity is sensitive to RNase and DNase digestion (7, 8). These results were integrated in a more comprehensive scheme following the demonstration that type I IFN induction by these complexes is mediated by the engagement of endosomal Toll-like receptors (TLRs) (9–11). Similarly, antigenic cargo containing nucleic acids was found to promote B-cell proliferation in a TLR9- or TLR7-dependent manner, with this effect enhanced by type I IFN signaling (9, 12, 13). The contribution of nucleic acid-sensing TLRs to systemic autoimmunity was further corroborated by studies in lupus-predisposed mice lacking or overexpressing TLR7 and/or TLR9 (14-20), and in *Unc93b1* (*3d*) mutant mice in which signaling by endosomal TLRs is extinguished (21).

The cell population involved in type I IFN production in response to lupus-related immune complexes corresponds to natural IFN-producing cells (22, 23). These cells, known as plasmacytoid DCs (pDCs), are the most potent producers of type I IFNs, a functional characteristic attributed to constitutive expression of TLR7, TLR9, and IRF7 and likely signaling from a unique intracellular compartment (24–27). The involvement of pDCs in lupus is further suggested by the reduced frequency of these cells in patient blood together with increases in afflicted organs, presumably caused by the attraction of activated pDCs to inflammatory sites (10). Similar increases have been noted in inflammatory tissues of patients with Sjögren's syndrome (28), rheumatoid arthritis (29, 30), dermatomyositis (31), and psoriasis (32).

Collectively, these results suggest that pDCs, acting through type I IFN hyperproduction, are major pathogenic contributors to lupus. Whether the participation of these cells is obligatory remains to be documented in vivo, however. Here, using congenic lupus-predisposed mice lacking pDCs (as well as other DC subsets) owing to IRF8 deficiency, or exhibiting pDC-specific defects in endosomal TLR signaling and type I IFN production owing to *Slc15a4* (*feeble*) mutation, we provide strong evidence that pDCs are indeed required for disease development, and this effect appears to be mediated by hyperproduction of inflammatory cytokines, most likely type I IFNs.

Results

Absence of pDCs and CD8α$^+$ DCs in IRF8-Deficient NZB Mice. Studies in normal background mice have shown that IRF8, a hematopoietic cell-specific transcription factor, is essential for the development of pDCs (33–35). Accordingly, assessment of *Irf8*$^{-/-}$ NZB mice at a young age (3 mo) showed almost complete absence of pDCs (CD11c^{low}CD11b$^-$B220$^+$Siglec-H$^+$PDCA1$^+$) in spleen (Fig. 1*A*), and this correlated with undetectable in vivo production of type I IFNs in response to CpG-DNA (Fig. 1*B*). Moreover, CD8α$^+$ conventional DCs (cDCs) were absent and CD4$^-$CD8$^-$ cDCs were reduced in *Irf8*$^{-/-}$mice (Fig. 1*C*), consistent with the levels of IRF8 expression in these subsets of normal mice (35). Total cDC numbers were unaffected, however, likely owing to compensatory increases in CD4$^+$ cDCs (Fig. 1*C*), which do not express IRF8 (35). As noted previously with *Irf8*$^{-/-}$ normal background mice (36, 37), IRF8-deficient NZB mice also showed expansion of marginal zone and CD21^{low}CD23$^+$ follicular and transitional B cells, but total B-cell and T-cell numbers were unchanged (data not shown).

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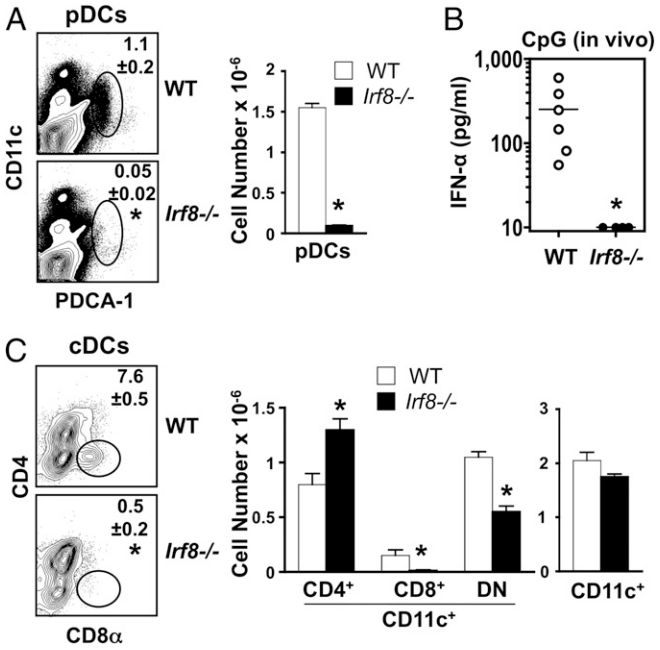

Fig. 1. Absence of pDCs and CD8α$^+$ cDCs in young IRF8-deficient NZB mice. Mutant and WT NZB mice (age 3 mo; n = 3–6/group) were analyzed for cellular differences in spleen and in vivo responses to CpG challenge. (*A*) pDC frequency and numbers. Spleen cells were assessed by flow cytometry using anti-CD11c and anti-PDCA-1 antibodies. Similar results were obtained when cells were stained with antibodies to B220, SiglecH, and CD11b (data not shown). (*B*) In vivo type I IFN production in response to TLR9 engagement. Serum IFN-α levels in CpG-challenged mice were determined by ELISA. (*C*) cDC frequency and numbers. Spleen cells were analyzed by flow cytometry after gating on CD11c$^+$ cells to identify CD4$^+$, CD8$^+$, and CD4$^-$CD8$^-$ cDC subsets. Numbers within the flow cytometry plots correspond to average frequencies of the indicated subsets. Error bars in graphs indicate SD. Asterisks indicate statistical significance ($P < 0.05$).

Reduced Autoimmunity in IRF8-Deficient NZB Mice. At age 11 mo, serum levels of polyclonal IgM and IgG, as well as IgG1 and IgG2a, were similar in *Irf8*$^{-/-}$ and WT NZB mice, whereas IgG2b was slightly increased and IgG3 was decreased in the mutant mice (Fig. 2*A*). Strikingly, however, autoantibody production was almost completely suppressed in the IRF8-deficient mice. Thus, IgG anti-erythrocyte autoantibodies, which are typically detectable in 100% of WT mice by age 7–8 mo, were undetectable in *Irf8*$^{-/-}$ mice even at 11 mo (Fig. 2*B*). Moreover, IgG2a anti-chromatin autoantibodies (the dominant subclass in WT NZB mice) and IgG anti-nuclear autoantibodies (ANA) were reduced to background levels, and IgM anti-chromatin autoantibodies were decreased substantially (Fig. 2 *C* and *D*). Mirroring these serologic changes, kidney disease was significantly ameliorated in *Irf8*$^{-/-}$ mice, as evidenced by reductions in both glomerulonephritis scores (Fig. 2*E*) and immune (IgG2a, C3) deposits (Fig. 2*F*).

Splenomegaly and accumulation of T cells and B cells, observed with aging in WT controls, were also decreased in *Irf8*$^{-/-}$ NZB mice (Fig. 3 *A* and *B*). The reduction in T-cell numbers affected both CD4$^+$ and CD8$^+$ subsets and was accompanied by a decrease in activated CD4$^+$ T cells (Fig. 3 *C* and *D*), whereas the reduction in B cells was primarily in the CD21$^-$CD23$^-$ subset (Fig. 3*E*), which includes the so-called "age-associated" B cells (38, 39). In contrast, the expanded population of marginal zone B cells detected in young *Irf8*$^{-/-}$ mice was retained with aging (Fig 3*E*), whereas the frequency of peritoneal B1 cells was unchanged. Thus, IRF8 deficiency in NZB mice was associated with decreased humoral and histological disease manifestations, along with reduced activation/expansion of T cells and B cells.

Defective cDC Responses to Endosomal TLR Ligands in IRF8-Deficient NZB Mice. Because engagement of endosomal TLRs is thought to be a major mechanism of DC activation in lupus, we also examined the response of cDCs to TLR7 and TLR9 ligands. As noted above, *Irf8*$^{-/-}$ NZB mice did not produce detectable amounts of IFN-α after CpG-DNA injection, indicating that the cDC subtypes present in these mice could not compensate for the absence of pDCs in response to this TLR9 stimulus. Furthermore, CD11c$^+$ cDCs derived from GM-CSF–differentiated bone marrow (BM) cells showed reduced CD86 up-regulation and IL-6 production after in vitro stimulation with TLR7 or TLR9 ligands in the presence or absence of IFN-α (Fig. 4). Thus, in addition to pDC deficiency, *Irf8*$^{-/-}$ NZB mice exhibited defective cDC responses to endosomal TLR stimulation.

B-Cell Responses to TLR Ligands and Exogenous Antigens Are Not Compromised in IRF8-Deficient NZB Mice. IRF8 is known to affect B-cell differentiation and germinal center formation (40), changes that might contribute to reduced autoimmunity in IRF8-deficient mice. To address this possibility, we assessed in vitro B-cell responses to TLR or BCR engagement and in vivo antibody responses to exogenous antigens. In vitro responses of purified *Irf8*$^{-/-}$ B cells to TLR7 and TLR9 ligands in the presence or absence of IFN-α were largely conserved, as indicated by efficient up-regulation of CD86 and H2-K^d, as well as production of IL-6 and IL-10 (Fig. S1 *A*–*C*). Similarly, activation by anti-IgM and anti-CD40 cross-linking was comparable in mutant and WT B cells (Fig. S1 *D* and *E*). Moreover, immunization of WT and *Irf8*$^{-/-}$ NZB mice with a thymus-independent antigen (TNP-LPS) or a thymus-dependent antigen (TNP-KLH) produced anti-hapten antibody responses of similar titers and affinities (Fig. S2). Thus, despite the numerical changes, B-cell responses to endosomal TLR ligands and to conventional exogenous antigens were largely uncompromised in IRF8-deficient NZB mice, suggesting that reduced autoimmunity likely is not due to B-cell functional defects.

Feeble Mutation of Slc15a4 Prevents Autoimmunity in Congenic C57BL/6-*Fas*^{lpr} Mice. To directly examine how pDCs contribute to systemic autoimmunity, we developed congenic C57BL/6-*Fas*^{lpr} mice carrying the *feeble* mutation of *Slc15a4*. In normal background mice with this mutation, development of both pDCs and cDCs is intact, but cytokine production, particularly type I IFNs,

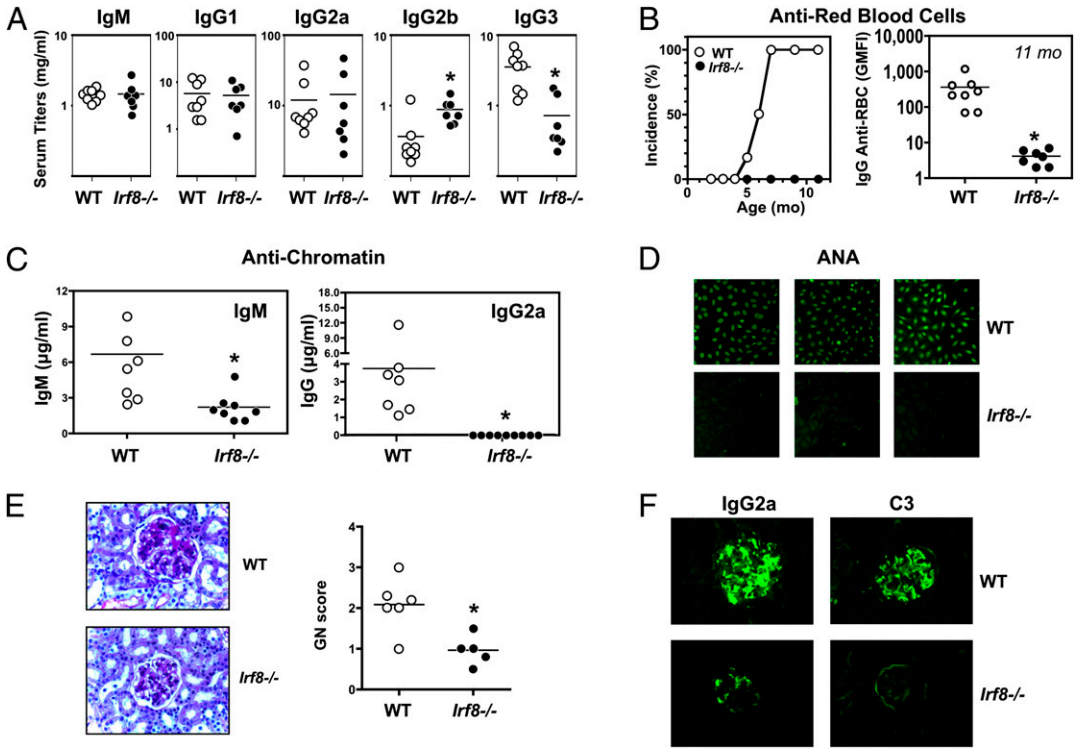

Fig. 2. Reduced autoimmunity in IRF8-deficient NZB mice. Mutant and WT NZB mice (age 11 mo; $n = 5$–8/group) were examined for serologic and histological disease characteristics. (*A*) Polyclonal serum Ig levels. (*B*) Anti-erythrocyte autoantibodies. Individuals with a geometric mean fluorescence intensity >50 were considered positive for anti-RBC autoantibodies. (*C*) Anti-chromatin autoantibodies. (*D*) ANAs, with representative individuals shown. (*E*) Representative PAS-stained kidney sections and glomerulonephritis scores. (*F*) Kidney deposits (IgG2a and C3), with representative immunofluorescence images shown. Asterisks indicate statistical significance ($P < 0.05$).

in response to TLR7 or TLR9 ligands is absent only in pDCs (27). Accordingly, in *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice, pDCs were present at normal frequencies in spleen (Fig. 5*A*) and differentiated efficiently after Flt3L treatment of BM cells, but did not produce type I IFNs in response to TLR7 or TLR9 ligands both in vivo and in vitro (Fig. 5*B*). In contrast, B cells from *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice showed normal in vitro proliferation to TLR9 stimulation (Fig. 5*C*) and efficient humoral responses to T-dependent and T-independent antigens (Fig. S3). Remarkably, at age 8 mo, these mutants had significantly reduced IgM anti-chromatin and an almost complete absence of IgG anti-chromatin autoantibodies and ANA (Fig. 5 *D* and *E*). In addition, the mutants had significantly decreased hypergammaglobulinemia (8.4 ± 2.9 mg/mL IgG vs 23.6 ± 14.8 mg/mL in WT controls; $P < 0.05$) and extended survival (Fig. 5*F*). Reductions were also observed in lymphadenopathy and splenomegaly (Fig. 6*A*), with corresponding numerical decreases in cDCs, T cells ($CD4^+$ and $CD8^+$), and B cells ($CD21^-CD23^-$), whereas pDCs and double-negative T cells were reduced, but not significantly (Fig. 6 *B* and *C*). These findings strongly indicate that pDCs, likely through type I IFN hyperproduction, are major contributors to the pathogenesis of systemic autoimmunity.

Discussion

We have used two types of genetic modification in mouse models of spontaneous lupus-like disease to define the in vivo role of pDCs in the pathogenesis of systemic autoimmunity. In the first instance, we created NZB mice deficient for IRF8, a transcription factor that regulates the development of pDCs, among other effects, whereas in the second instance, we created C57BL/6-*Fas*^{lpr} mice carrying a mutation in SLC15A4, a peptide/histidine transporter critical for endosomal TLR signaling and type I IFN production by pDCs. The evidence with the *Irf8* mutants strongly suggests that pDCs are required for lupus development, whereas the results with the *Slc15a4* mutants infer that the contribution of these cells is mediated by endosomal TLR-dependent production of proinflammatory cytokines, primarily type I IFNs.

The biological effects of IRF8, also known as IFN consensus binding protein, have been investigated extensively (40–42). IRF8 is an IFN-inducible transcription factor expressed in pDCs, $CD8\alpha^+$ cDCs, $CD4^-CD8\alpha^-$ cDCs, macrophages, and B cells. IRF8 forms heterodimeric complexes with several molecular partners and binds to various IFN stimulatory response elements, thereby governing expression of a large set of genes with broad effects in innate and adaptive immune functions. Nonetheless, a dominant characteristic of mice lacking IRF8 is the absence of pDCs.

We found that the age-associated splenomegaly and expansion of T and B cells typical of NZB mice were largely abrogated in *Irf8*$^{-/-}$ congenics, accompanied by significant decreases in $CD4^+$ T-cell activation, autoantibody production, and kidney disease. Interestingly, reductions encompassed not only the classical ANA, but also the dominant antierythrocyte response of this strain. The reductions in antinuclear specificities, particularly in the IgG isotype, likely are related to the absence of pDCs and minimal, if any, endosomal TLR-dependent induction of type I IFNs by self-nucleic acids and related immune complexes. This is consistent with previous findings with lupus-predisposed mice deficient in type I IFN or endosomal TLR signaling, which showed decreases in these autoantibodies and other disease manifestations (11).

In terms of the role of pDCs in anti-RBC responses, although RBCs lose nuclei and mitochondria and thus lack DNA that might engage TLR9, these cells contain RNA species that might engage TLR7. Alternatively, the initial trigger for the anti-RBC response might be provided by uptake of nucleated erythroid precursors or of expelled nuclei, which are surrounded by an intact plasma membrane displaying phosphatidylserine as an "eat-me" signal (43–45). Any one of these processes could pro-

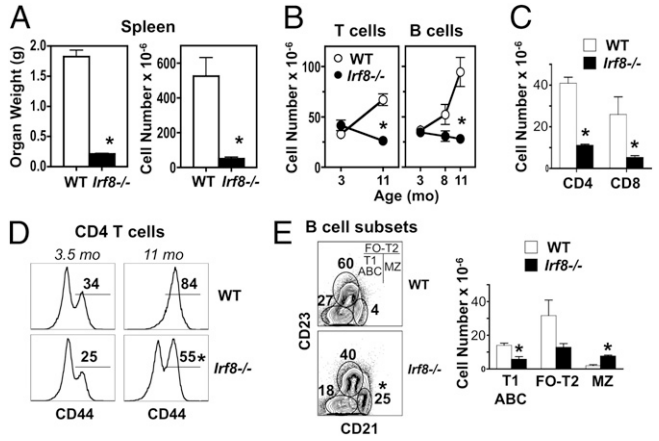

Fig. 3. Reduced T-cell and B-cell expansion in older IRF8-deficient NZB mice. Mutant and WT NZB mice (age 8–11 mo; $n = 3$/group) were analyzed for cellular changes in spleen and peritoneal cavity. (*A*) Spleen weight and cellularity at 11 mo. (*B*) T-cell and B-cell numbers in spleen at age 11 mo. Cells were analyzed by flow cytometry after gating on TCRβ$^+$ (T cell) or B220$^+$ (B cell) populations. (*C*) CD4$^+$ and CD8$^+$ T-cell subsets in spleen at age 11 mo. (*D*) Frequency of activated CD4$^+$ T cells in spleen at age 11 mo. Gated CD4$^+$ T cells were assessed for expression of CD44. (*E*) B-cell subsets in spleen at age 8 mo. Gated B220$^+$IgM$^+$ cells were analyzed for transitional T2 and follicular (T2-FO), marginal zone (MZ), and T1 immature and age-associated (T1-ABC) B cells. Numbers within flow cytometry plots correspond to average frequencies of the indicated subsets. Error bars in graphs indicate SD. Asterisks indicate statistical significance ($P < 0.05$).

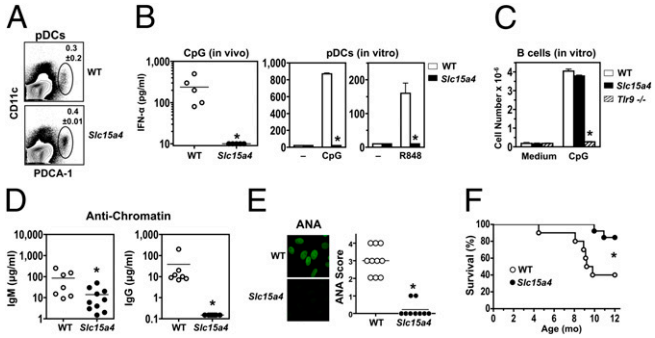

Fig. 5. Reduced autoimmunity in *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice. Mutant and WT mice were analyzed for pDC development and function at age 4 mo ($n = 3$–5/group), disease manifestations at age 8 mo ($n = 7$–10/group), and survival ($n = 10$–13/group). (*A*) Frequency of pDCs in spleen. Cells were assessed by flow cytometry using anti-CD11c and anti–PDCA-1 antibodies. Similar results were obtained using antibodies to B220, SiglecH, and CD11b (data not shown). (*B*) In vivo and in vitro type I IFN production in response to endosomal TLR stimulation. Serum IFN-α levels in CpG-challenged mice, and IFN-α production by BM-differentiated pDCs stimulated with CpG or R848, were determined by ELISA. (*C*) In vitro B-cell proliferation in response to TLR9 engagement. Purified B cells from mutant and WT mice were stimulated in vitro with CpG. B cells from *Tlr9*$^{-/-}$ C57BL/6 mice were used as negative controls. (*D*) Anti-chromatin autoantibodies. (*E*) ANA. (*F*) Survival. Error bars in graphs indicate SD. Asterisks indicate statistical significance ($P < 0.05$).

vide both antigenic cargo and nucleic acids for efficient activation of pDCs and antigen presentation by cDCs.

It is of interest that, in contrast to reductions or even absence of autoantibodies, responses to conventional T-dependent and T-independent antigens were of normal magnitude and quality in *Irf8*$^{-/-}$ NZB mice, as reported for normal background mice with B-cell–specific conditional *Irf8* deletion (37). A possible explanation for this finding is that with lupus-associated autoantigens, adjuvanticity is provided by endosomal TLR engagement by self-nucleic acids, whereas adjuvanticity of complete (CFA) and incomplete (IFA) Freund's adjuvants, or alum in response to conventional exogenous antigens is mediated by endosomal TLR-independent pathways (46–48).

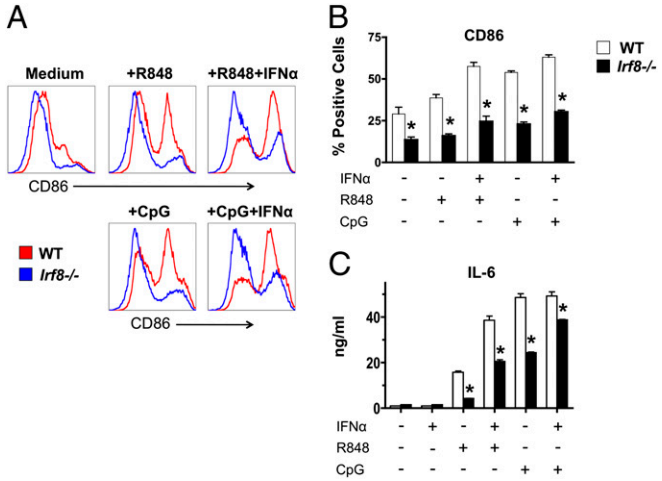

Fig. 4. Defective in vitro responses by cDCs from IRF8-deficient NZB mice. cDCs were differentiated from BM cells from WT and mutant mice (age 3 mo; pools of 2–3 mice) in the presence of GM-CSF, then stimulated with endosomal TLR ligands in the presence or absence of IFN-α. (*A* and *B*) CD86 upregulation defined by flow cytometry after gating on CD11c$^+$ cells. (*C*) IL-6 production determined by ELISA. One representative of two independent experiments is shown. Error bars in graphs indicate SD of samples analyzed in triplicate. Asterisks indicate statistical significance ($P < 0.05$).

We have attributed the disease-reducing effects of IRF8 deficiency to the absence of pDCs. However, IRF8-deficient mice also lacked CD8α$^+$ cDCs and had diminished in vitro responses to endosomal TLR stimulation in the retained cDC subsets. These modifications also may contribute to disease reduction. CD8α$^+$ cDCs have several functional properties of potential relevance to autoimmunity, including ingestion of materials from dying cells, antigen cross-presentation, and production of IL-12, a cytokine involved in Th1 differentiation (49). Regardless, however, these cells are unlikely to promote disease, because they do not express TLR7, the engagement of which appears to be a major pathogenic contributor in several lupus-predisposed mouse strains (14, 15, 21), and do not produce significant amounts of type I IFNs in response to ligands or viruses that engage endosomal TLRs or cytosolic sensors for nucleic acids (50–54). Similarly, the partial defects in TLR7 and TLR9 responses by the IRF8-deficient CD4$^+$ and CD4$^-$CD8α$^-$ cDCs are likely insufficient to explain disease reduction, given that these subsets have been reported to produce meager amounts of type I IFNs when stimulated with endosomal TLR ligands, including lupus-associated nucleic acid-containing immune complexes (53). Nonetheless, considering our present findings and previous findings of others demonstrating disease reduction in lupus-prone mice lacking all DC subsets (including pDCs) (55), targeted deletion/inactivation and/or reconstitution of specific cDC subsets is needed to definitively clarify the exact role of cDCs, as opposed to pDCs, in systemic autoimmunity.

An additional open question is whether modifications in the B-cell compartment caused by IRF8 deficiency also might contribute to disease reduction, given that ablation of IRF8 has been shown to affect B-cell differentiation and germinal center organization, as well as to induce expansion of marginal zone and follicular B cells (40). However, as reported previously (37) and reproduced here with IRF8-deficient NZB mice, humoral responses to exogenous antigens remain unmodified. Furthermore, we have shown that *Irf8*$^{-/-}$ B cells mounted normal in vitro responses to TLR7 and TLR9 ligands, which could be enhanced with IFN-α. Our results suggest that B-cell activation by endo-

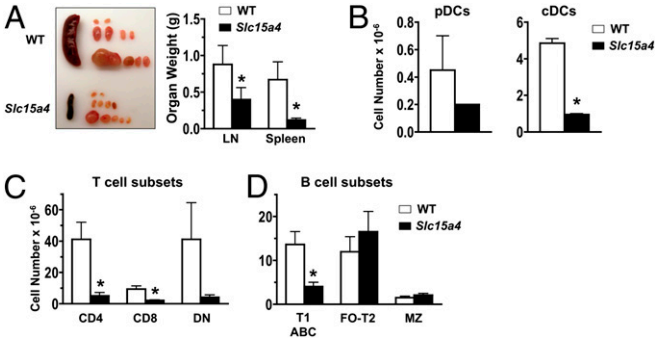

Fig. 6. Cellular changes in *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice. Spleen and LNs of mutant and WT mice were examined at age 8 mo (n = 3/group). (*A*) Spleen and LN organ weights. (*B*) pDC and cDC numbers in spleen. Flow cytometry was used to identify pDCs (PDCA-1^{+}CD11c^{low}) and cDCs (CD11c^{+}). (*C*) T-cell numbers in spleen. CD4^{+}, CD8^{+}, and CD4^{-}CD8^{-} (double-negative) T cells were identified by flow cytometry after gating on TCRβ^{+} cells. (*D*) B-cell numbers in spleen. Spleen cells were examined by flow cytometry as in Fig 3*E*. Error bars in graphs indicate SD. Asterisks indicate statistical significance ($P < 0.05$).

somal TLRs, although necessary, by itself is not sufficient to drive autoimmunity, with type I IFN hyperproduction resulting from the concomitant engagement of these TLRs in pDCs, required as well. This scheme of endosomal TLR engagement in both B cells and pDCs fits our previously outlined model for the induction of systemic autoimmunity (56).

Combined with the foregoing considerations, the data from IRF8-deficient mice provide strong support for an indispensable in vivo disease-promoting effect of pDCs. However, IRF8 deficiency prevents us from stating a definite conclusion as to whether pDCs contribute to disease solely through hyperproduction of type I IFNs or other functions, such as antigen presentation (57). The recent identification of the mutant strain *feeble*, in which defective expression of the peptide/histidine transporter SLC15A4 leads to absence of endosomal TLR signaling and severely reduced production of proinflammatory cytokines, including type I IFNs, specifically in pDCs (27), has provided an experimental tool to address this question. We found that lupus-predisposed C57BL/6-*Fas*^{lpr} mice carrying this mutation exhibited striking reductions in disease manifestations and extended survival, despite efficient B-cell responses to both endosomal TLR stimuli and conventional exogenous antigens. These findings support the critical role of type I IFN production by pDCs in systemic autoimmunity. Disease reduction in *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice, as well as in *Unc93b1* mutant C57BL/6-*Fas*^{lpr} mice (21), is at variance with the reported disease enhancement in IFNAR-deficient MRL-*Fas*^{lpr} mice (58). There is no clear explanation for this variance, but possibilities include genetic contributions beyond the *Fas* defect, exemplified by differences in disease severity between these strains (59), and complete absence of signaling in *Ifnar1*-deleted mice versus pDC-specific absence of type I IFN production in *Slc15a4* mutant mice. Creation of MRL-*Fas*^{lpr} mice congenic for the *Slc15a4* mutation (and possibly the *Unc93b1* mutation) will likely clarify this issue.

The exact mechanism by which SLC15A4 deficiency specifically compromises endosomal TLR signaling and proinflammatory cytokine production in pDCs remains to be defined. Nevertheless, the defect seems to be related not to inefficient uptake of TLR ligands or secretion of type I IFNs, but rather to inefficient signaling by endosomal TLRs, given that intracellular *Ifna* transcripts and corresponding proteins could not be detected in stimulated mutant pDCs even in the presence of exogenous IFN-α (27). A reported function of SLC15A4 is to transport, in a low pH-dependent manner, free histidine from the endosome to the cytosol (60, 61), a process that might facilitate the cathepsin-mediated cleavage of endosomal TLRs required for efficient signaling (62–65). Alternatively, SLC15A4 may be localized in a unique pDC-associated lysosome-related organelle, where it contributes to the establishment of an optimal microenvironment required for the assembly of functional TLR signaling complexes and hyperproduction of type I IFNs (27, 66).

In summary, based on our studies with *Irf8*-deleted and *Slc15a4* mutant mice, we report strong evidence that pDCs are essential for the initiation of abnormal innate immune responses leading to systemic autoimmunity, likely through high production of type I IFNs. These events appear to be instrumental for DC activation, efficient antigen presentation by DCs and B cells, and production of disease-inducing isotype-switched autoantibodies. Similar processes are likely involved in the pathogenesis of human SLE; interestingly, *IRF8* and *SLC15A4* have been identified as susceptibility loci in certain populations with this condition (67, 68). Our present results further suggest that pharmacologic inhibition of IRF8, and particularly interference with the effects of SLC15A4 on pDCs, may be of utility in the treatment of SLE and other inflammatory conditions in which type I IFNs are major pathogenic effectors.

Materials and Methods

Mice. NZB mice and C57BL/6-*Fas*^{lpr} mice were obtained from Jackson Laboratory or The Scripps Research Institute Animal Facility. C57BL/6 mice deficient for IRF8 (*Irf8*$^{-/-}$) or TLR9 (*Tlr9*$^{-/-}$) or carrying the *feeble* mutation of the *Slc15a4* gene have been reported (27, 35), and congenic *Irf8*$^{-/-}$ NZB and *Slc15a4* mutant C57BL/6-*Fas*^{lpr} mice were generated by standard marker-assisted breeding as described previously (4). The WT and *Irf8*$^{-/-}$ NZB mice used in this study were littermates generated by crossing heterozygous *Irf8*$^{+/-}$ mice. All mice were housed under specific pathogen-free conditions. The experimental protocols were performed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by The Scripps Research Institute's Animal Care and Use Committee.

Statistical Analysis. Group comparisons were analyzed using the unpaired two-tailed Student t test. Survival was analyzed by Kaplan–Meier plots and the log-rank test. $P < 0.05$ was considered significant.

Additional Methods. Procedures for in vivo and in vitro studies, cell preparations, flow cytometry, analysis of ANA and anti-erythrocyte autoantibodies, kidney pathology, and immunohistology are described in *SI Materials and Methods*.

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