Published September 29, 2014

Immunoglobulin E plays an immunoregulatory role in lupus

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The (patho)physiological role of IgE in nonallergic inflammatory diseases is not well understood. Here, we explored the effect of IgE deficiency on the inflammatory response in FcγRIIB-deficient mice as well as in mice carrying both a deletion of FcγRIIB and the chromosomal translocation of *Y-linked autoimmune acceleration* (*Yaa*) that hastens and results in a more aggressive lupuslike disease in these mice. The findings show that deficiency of IgE delays disease development and severity as demonstrated by reduced autoantibody production and amelioration of organ pathologies. This was associated with decreased numbers of plasma cells and reduced levels of IgG2b and IgG3. Unexpectedly, the loss of IgE also caused a striking decrease of immune cell infiltration in secondary lymphoid organs with a marked effect on the presence of dendritic cells, monocytes, neutrophils, and eosinophils in these organs and decreased activation of basophils. The presence of autoreactive IgE in human systemic lupus erythematosus subjects was also associated with increased basophil activation and enhanced disease activity. These findings argue that IgE facilitates the amplification of autoimmune inflammation.

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Abbreviations used: ANA, antinuclear antibodies; CI, confidence interval; FMO, fluorescence minus one; MFI, mean fluorescence intensity; OR, odds ratio; RNP, ribonucleoprotein; RU, relative units; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; SLO, secondary lymphoid organs; *Yaa*, *Y-linked autoimmune acceleration*.

There is growing recognition of the presence of IgE antibodies in various diseases characterized by immune deficiencies and inflammation. The primary immune deficiencies under the umbrella of hyper-IgE syndromes such as Buckley syndrome, Job syndrome, Kawasaki syndrome, Netherton syndrome, and Wiscott-Aldrich syndrome, among others, are characterized by increased IgE and in most cases exacerbated inflammatory responses (Heimall et al., 2010). However, in some inflammatory diseases, such as systemic lupus erythematosus (SLE), total IgE levels may be normal whereas the presence of autoreactive IgE is markedly increased (Charles and Rivera, 2011). Little is known about the significance of increased total IgE or autoreactive IgE in these diseases and whether IgE contributes to the exacerbated inflammatory response and pathologies observed.

Here, we explored whether IgE contributes to the inflammation and disease pathology observed in a mouse model of spontaneous lupus. This work was based, in part, on the previous observation that a deficiency in IgE ameliorated the late life lupuslike disease phenotype observed in $lyn^{-/-}$ mice (Charles et al., 2010). However, $lyn^{-/-}$ mice first develop an atopic-like allergic phenotype that is characterized by increased levels of total IgE; thus, this model does not allow one to distinguish the contribution of allergic inflammation from that of autoimmune inflammation. To make this distinction we studied $Fc\gamma RIIB^{-/-}$ mice, which develop a lupuslike disease, and $Fc\gamma RIIB^{-/-}$ mice with the chromosomal translocation of

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The Rockefeller University Press $30.00
J. Exp. Med. 2014 Vol. 211 No. 11 2159-2168
www.jem.org/cgi/doi/10.1084/jem.20140066

Supplemental Material can be found at:
http://jem.rupress.org/content/suppl/2014/09/26/jem.20140066.DC1.html

The Journal of Experimental Medicine

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Published September 29, 2014

Y-linked autoimmune acceleration (*Yaa*; Bolland and Ravetch, 2000; Bolland et al., 2002), which accelerates the development of the lupuslike phenotype (due, in part, to a duplication of the *Tlr7* gene (Deane et al., 2007). The *FcγRIIB* locus encodes an inhibitory receptor that functions to control the activation of the many cell types that express it (Malbec et al., 1999). Polymorphisms in this gene have been associated with lupus (Li et al., 2009). In C57BL/6 mouse (whether alone or in the context of *Yaa*), loss of FcγRIIB did not cause a spontaneous allergic-like phenotype nor were the levels of total circulating IgE increased; despite the reported higher susceptibility of *FcγRIIB*$^{-/-}$ mice to type I hypersensitivity due to increased effector responses (Takai et al., 1996). However, these mice developed increased levels of autoreactive IgE. This provided a reasonable model that mimicked some features of autoreactive IgE in human SLE (Charles et al., 2010; Dema et al., 2014) and could be crossed with *Igh7*$^{-/-}$ (IgE-deficient) mice to study the role of IgE in the inflammation and lupuslike phenotype seen in *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$.*Yaa* mice. The *Igh7*$^{-/-}$ mice have been well characterized and showed no major alterations of circulating immunoglobulins in naive mice other than the loss of IgE production (Oettgen et al., 1994). The findings herein demonstrate that IgE is linked to the amplification of the inflammatory response underlying the development of the lupus-like disease in mouse models and SLE in humans.

RESULTS AND DISCUSSION

IgE deficiency ameliorates lupuslike disease

The sera from 12–16-wk-old *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$.*Yaa* mice was assessed for the presence of increased levels of autoreactive IgE. At this age, *FcγRIIB*$^{-/-}$ mice showed no obvious signs of a lupuslike phenotype although low levels of autoantibodies were present in some mice. In contrast, most of the *FcγRIIB*$^{-/-}$.*Yaa* mice had elevated levels of autoantibodies and organ pathology, including nephritis. We first explored whether these mice made autoreactive IgE by measuring the levels of dsDNA-specific IgE, the most abundant autoreactive IgE found in humans with SLE (Dema et al., 2014). This was compared with the presence or absence of antinuclear antibodies (ANA) as a determinant of disease activity. As seen in *lyn*$^{-/-}$ mice (Charles et al., 2010), all tested sera showed increased amounts of dsDNA-IgE relative to WT mice. However, as shown in Fig. 1 a, *FcγRIIB*$^{-/-}$.*Yaa* mice that trended toward higher levels of ANA-specific Ig's also showed the highest level of dsDNA-specific IgE relative to those mice that had lower levels of ANA-specific Ig's, albeit significance was not achieved. Nonetheless, this suggested an association of dsDNA-specific IgE with the high levels of these autoantibodies in mice that developed lupuslike disease. This led us to explore the effect of IgE on disease development. An almost complete penetrance of lupuslike disease with organ pathology occurs at 16 wk for *FcγRIIB*$^{-/-}$.*Yaa* and at ∼28 wk for *FcγRIIB*$^{-/-}$ mice, which results in significant mortality due to renal failure for both strains (Fig. 1 b). At this age, almost all mice were ANA^{+} for both strains. Thus, we chose to work with the respective strains at the indicated ages to assess the role of IgE in the disease. As shown in Fig. 1 b, the median survival of *FcγRIIB*$^{-/-}$.*Yaa* and *FcγRIIB*$^{-/-}$ mice was 4.8 and 7.3 mo, respectively. Crossing of *FcγRIIB*$^{-/-}$.*Yaa* and *FcγRIIB*$^{-/-}$ mice with *Igh7*$^{-/-}$ mice increased the median survival to 11 mo for *FcγRIIB*$^{-/-}$.*Yaa Igh7*$^{-/-}$ and was not defined for *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ mice as all the latter mice survived well beyond a 12-mo period. This was accompanied by decreased C3 complement (Fig. 1 c) and IgG immune complex (Fig. 1 d) deposition in the kidney of these mice as well as decreased glomerulonephritis (Fig. 1 e). Kidney function as determined by the ratio of albumin to creatinine in the urine returned to almost normal levels (Fig. 1 f). These striking findings showed that IgE contributes to the development of disease and organ pathologies.

IgE deficiency reduces autoantibody production and B cell and plasma cell numbers in lupus-prone mice

The marked absence of immune complex deposition in the kidney of *FcγRIIB*$^{-/-}$.*Yaa Igh7*$^{-/-}$ and *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ mice led us to explore whether IgE deficiency was generally affecting the production of pathogenic autoantibodies in these mice. As shown in Fig. 2 a, production of anti-dsDNA, anti-ribonucleoprotein (RNP), or anti-histone IgG was significantly decreased in *FcγRIIB*$^{-/-}$.*Yaa Igh7*$^{-/-}$ mice and in *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ mice, and there was a strong trend toward decreased responses as well. Total IgG2b and IgG3 were also decreased whereas IgG1 appeared to be elevated in *FcγRIIB*$^{-/-}$ mice regardless of whether they were IgE sufficient or deficient (Fig. 2 b). These changes suggested that the loss of IgE was broadly affecting B cell Ig production rather than narrowly or selectively impinging on autoreactive B and plasma cells. A hint that this might be the case was provided by the marked reversal of the splenomegaly and cellularity seen in the *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$.*Yaa* mice when these mice were crossed with *Igh7*$^{-/-}$ mice (Fig. 2 c). The total number of cells found in the spleen and LNs was almost completely normalized by the deficiency in IgE (Fig. 2 d). Analysis of the plasma (CD138^{+}) cells in the spleen and LNs revealed a marked decrease in their numbers in the *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ and *FcγRIIB*$^{-/-}$.*Yaa Igh7*$^{-/-}$ mice relative to their IgE-producing counterparts, and was similar to the plasma cell numbers in WT mice (Fig. 2 e). Moreover, although no significant difference was seen for CD19^{+}B220^{+} B cells in the spleen of *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$.*Yaa* relative to their IgE-deficient counterparts, a significant decrease in their numbers was detected in the LNs of these mice and there was a strong trend for a reduction in these cells in both the spleen and LNs of *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ mice (Fig. 2 f). Collectively, the findings demonstrate the influence of IgE on the B cell compartment and the production of Ig in these lupus-prone mice.

IgE deficiency normalizes T cell CD4^{+}/CD8^{+} ratios in lupus-prone mice

The decrease in numbers of B and plasma cells in the spleen and LNs of IgE-deficient lupus-prone mice suggested the possibility that T cell responses might also be affected in these mice. To explore this, we first determined the number of CD4^{+} and

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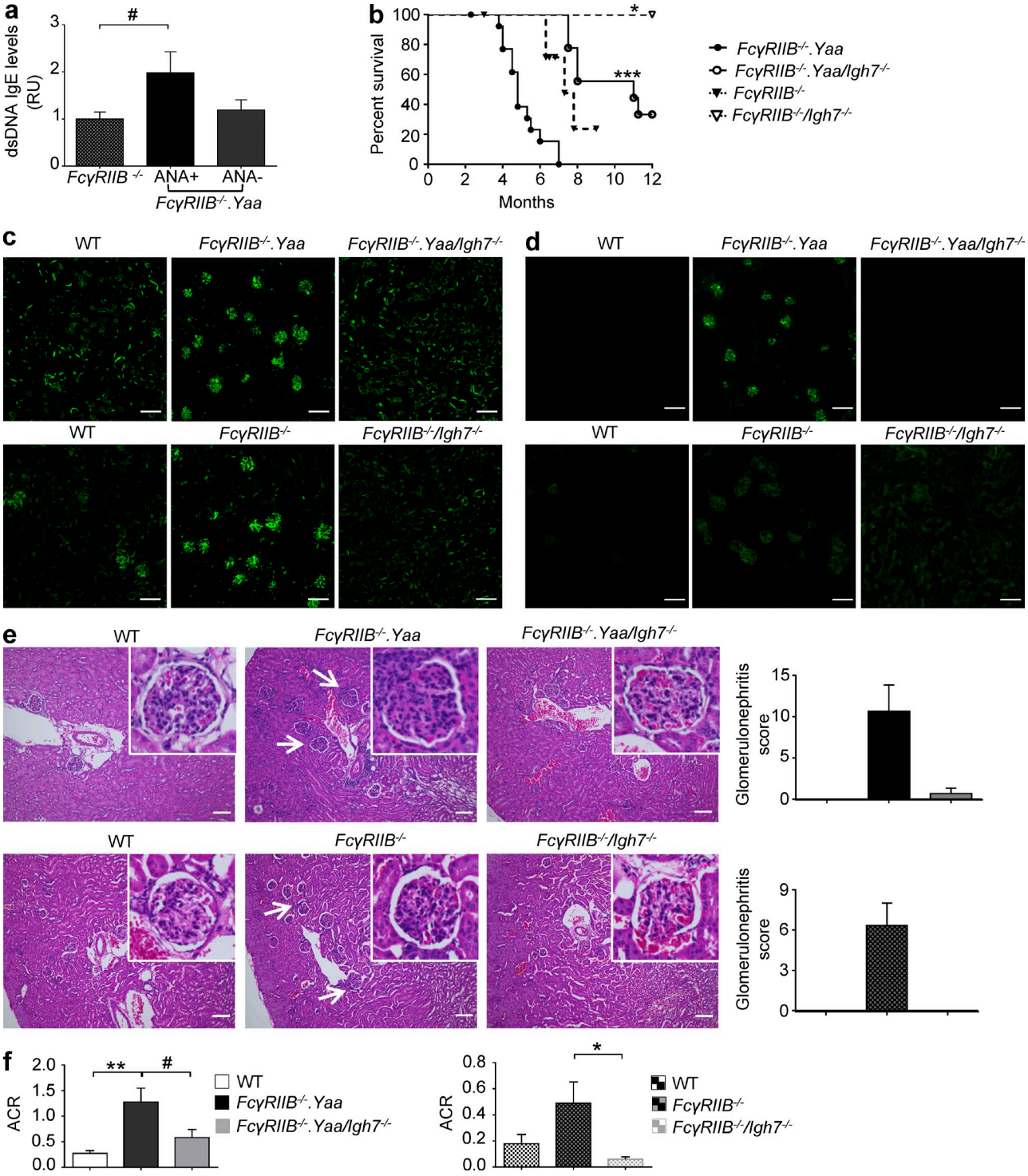

Figure 1. IgE deficiency delays onset and improves organ pathology of lupuslike disease. (a) Relative levels of dsDNA-specific IgE in serum of 12–16-wk-old mice. Data are shown as mean ± SEM ($Fc\gamma RIIB^{-/-}$, $n = 7$; $Fc\gamma RIIB^{-/-}.Yaa$ ANA$^+$, $n = 8$; $Fc\gamma RIIB^{-/-}.Yaa$ ANA$^-$, $n = 4$). #, $P \leq 0.1$. (b) Kaplan-Meier survival curve. Mantel-Cox test was used to compare $Igh7^{-/-}$ mice with the respective counterparts. ($Fc\gamma RIIB^{-/-}$, $n = 8$ vs $Fc\gamma RIIB^{-/-}Igh7^{-/-}$, $n = 6$; $Fc\gamma RIIB^{-/-}Yaa$, $n = 14$ vs. $Fc\gamma RIIB^{-/-}.Yaa\ Igh7^{-/-}$, $n = 9$). *, $P < 0.05$; ***, $P < 0.001$. (c) Complement C3 and (d) IgG deposition in the glomeruli of kidneys sections from at least 3 mice per group as determined by immunofluorescent staining. (e) H&E staining of kidneys sections from at least 3 mice per group. Graphs show renal score from blinded analysis assessing tubular and glomerular damage. (c–e). Bar, 100 µm. Glomerular lesions were defined as hypercellularity, mesangial matrix expansion, and glomerulosclerosis; tubulointerstitial lesions are defined as inflammatory infiltrates, tubular injury, and fibrosis. (f) Albumin/creatinine ratio in urine. Data are from three independent experiments with at least five mice per group. Graphs are shown as mean ± SEM. Kruskal-Wallis test with Dunn's multiple comparison test was used to compare the different strains. #, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$.

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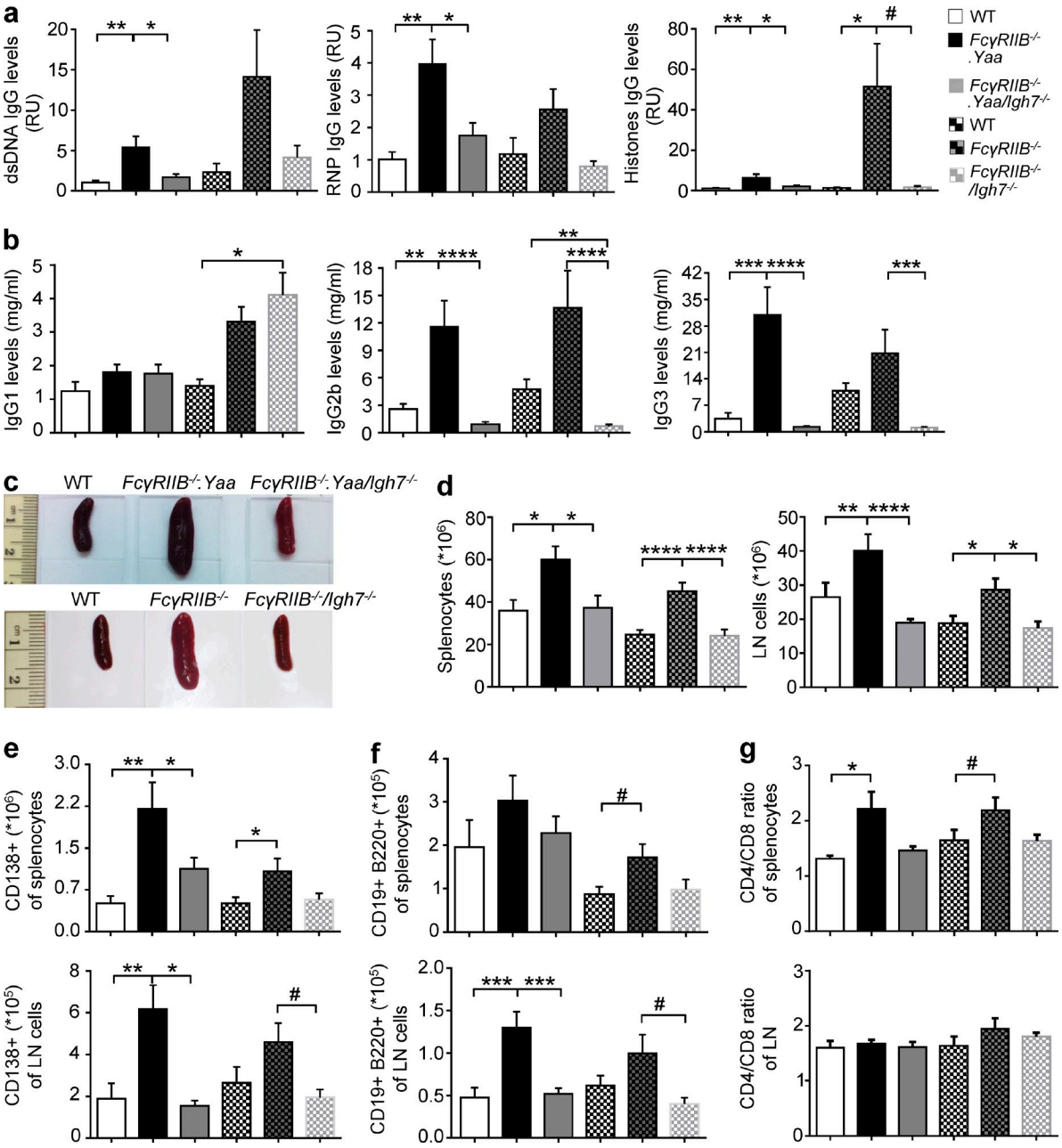

Figure 2. A decrease in autoantibody production and in B cell numbers is associated with IgE deficiency in lupus-prone mice. (a) Relative levels of dsDNA, RNP, and histone-specific IgGs in serum of the indicated strains. Data are from two independent experiments of at least four mice per group. (b) Serum levels of IgG1, IgG2b, and IgG3 in the indicated strains. Data are from three independent experiments of at least four mice per strain. (c) Size of spleen of the strains studied. (d) Cellularity of secondary lymphoid tissues (spleen, right; LN, left) of indicated strains. Data are from four independent experiments with at least four mice per group. (e and f) Absolute numbers of plasma cells ($CD138^+$) and B cells ($CD19^+$ $B220^+$) in spleen and LN. (g) CD4/CD8 ratio in spleen and LN. Data are from two independent experiments of at least four mice per group shown as mean ± SEM. One-way ANOVA with Tukey's multiple comparison test or Kruskal-Wallis test with Dunn's multiple comparison test was used to compare the different strains. #, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

$CD8^+$ T cells in the spleen and LNs of these mice. The absolute numbers of $CD4^+$ or $CD8^+$ T cells in the spleen were increased in *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice (unpublished data). A significant increase in $CD4^+/CD8^+$ T cell ratio was observed in the spleen of *FcγRIIB*$^{-/-}$*.Yaa* mice with a trend toward an increased ratio in the spleen of *FcγRIIB*$^{-/-}$ mice (Fig. 2 g). In both cases, IgE deficiency caused a decrease in this ratio to WT levels. No significant differences were observed in the LNs. Activation of both $CD4^+$ and $CD8^+$ cells, as determined by CD69 expression, was altered with a significant or strong trend for decreased activation of $CD4^+$ and $CD8^+$ cells, respectively, in the spleen of the IgE-deficient mice (unpublished data). In LNs,

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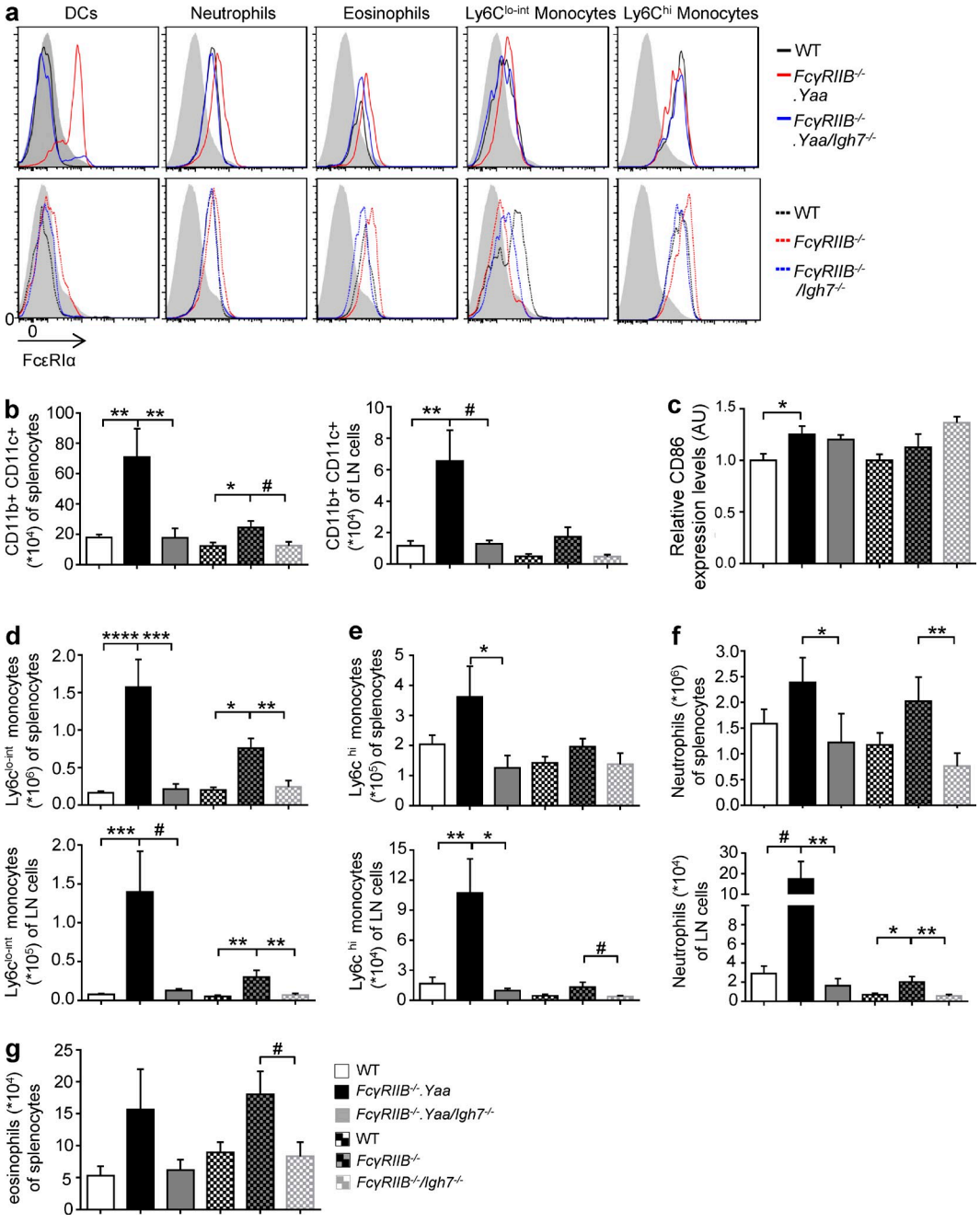

Figure 3. Modulation of FcεRI expression and the myeloid population by IgE deficiency in SLOs. (a) Representative histogram of FcεRIα expression (MFI) of DCs ($CD11b^+CD11c^+$), $Ly6c^{hi}$ monocytes ($CD11b^+CD11c^-F4/80^+Ly6G^-Ly6C^{hi}SSC^{lo}$), $Ly6C^{lo\text{-}int}$ monocytes ($CD11b^+CD11c^-F4/80^+Ly6G^-Ly6C^{lo\text{-}int}SSC^{lo}$), neutrophils ($CD11b^+CD11c^-F4/80^-Ly6G^+$), and eosinophils ($CD11b^+CD11c^-F4/80^+Ly6G^-Ly6c^+SSC^{hi}$) detected in spleen (c). Gating strategy is shown in Fig. S1. Gray histogram is fluorescence minus one (FMO). (b and c) DCs in spleen and LN (b) and relative expression of CD86 (MFI) in spleen. Absolute number of (d) $Ly6c^{lo\text{-}int}$ monocytes, (e) $Ly6c^{hi}$ monocytes, and (f) neutrophils in spleen and LNs. (g) Absolute numbers of eosinophils in spleen. Data are mean ± SEM of two independent experiments with a minimum of five mice per group. One-way ANOVA with Tukey's multiple comparison test or Kruskal-Wallis test with Dunn's multiple comparison test was used to compare the different strains. #, $P \le 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

there also appeared to be a trend toward decreased activation of $CD4^+$ and $CD8^+$ cells with IgE deficiency that was more apparent for *FcγRIIB*$^{-/-}$ relative to *FcγRIIB*$^{-/-}$*.Yaa* mice (unpublished data). These findings demonstrate that, in the absence of IgE, T cell responses appear to normalize.

Effect of IgE deficiency on nonlymphocyte immune cell responses

Given that the loss of IgE resulted in reduced numbers of T and B cells and also caused some reduction in the activation of the former, we investigated whether this deficiency also

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affected other inflammatory cell types. The α-subunit of the high-affinity receptor for IgE (FcεRI) seems to be expressed on other cell types, including dendritic cells, eosinophils, monocytes, and neutrophils (Fig. 3 a; Gould and Sutton, 2008; Hammad et al., 2010; Porcherie et al., 2011). Ly6c^{hi} (inflammatory monocytes) constitutively expressed the highest levels of FcεRI (Fig. 3 a). Changes in FcεRI expression were minor-to-modest with increased expression in dendritic cells, eosinophils, and neutrophils relative to WT numbers. Dendritic cells, eosinophils, and neutrophils showed some association between the levels of FcεRI expression and IgE sufficiency (Fig. 3 a). Analysis of dendritic cell numbers in the spleen and LNs of *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice showed that their numbers increased in the secondary lymphoid organs (SLO's) for both strains but was completely normalized by IgE deficiency (Fig. 3 b). Interestingly, although dendritic cell numbers were normalized in *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa Igh7*$^{-/-}$ mice, activation of these cells as determined by CD86 expression appeared to be unaltered (Fig. 3 c).

Two populations of monocytes (Ly6c$^{lo\text{-}inter}$ and Ly6c^{hi}, respectively) were identified from an F4/80^{+} Ly6G^{-} gated population. The numbers of resident (Ly6c$^{lo\text{-}inter}$) monocytes in the spleen and LNs was markedly increased in both *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice (Fig. 3 d). The number of resident monocytes returned to normal levels (those of WT mice) in *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa Igh7*$^{-/-}$ mice (Fig. 3 d). Similarly, whereas the levels of inflammatory monocytes (Ly6c^{hi}) trended toward an increase in *FcγRIIB*$^{-/-}$ mice and was significantly increased in *FcγRIIB*$^{-/-}$*.Yaa* mice, the apparent increases were normalized (to WT levels) in IgE deficiency (Fig. 3 e). This was also

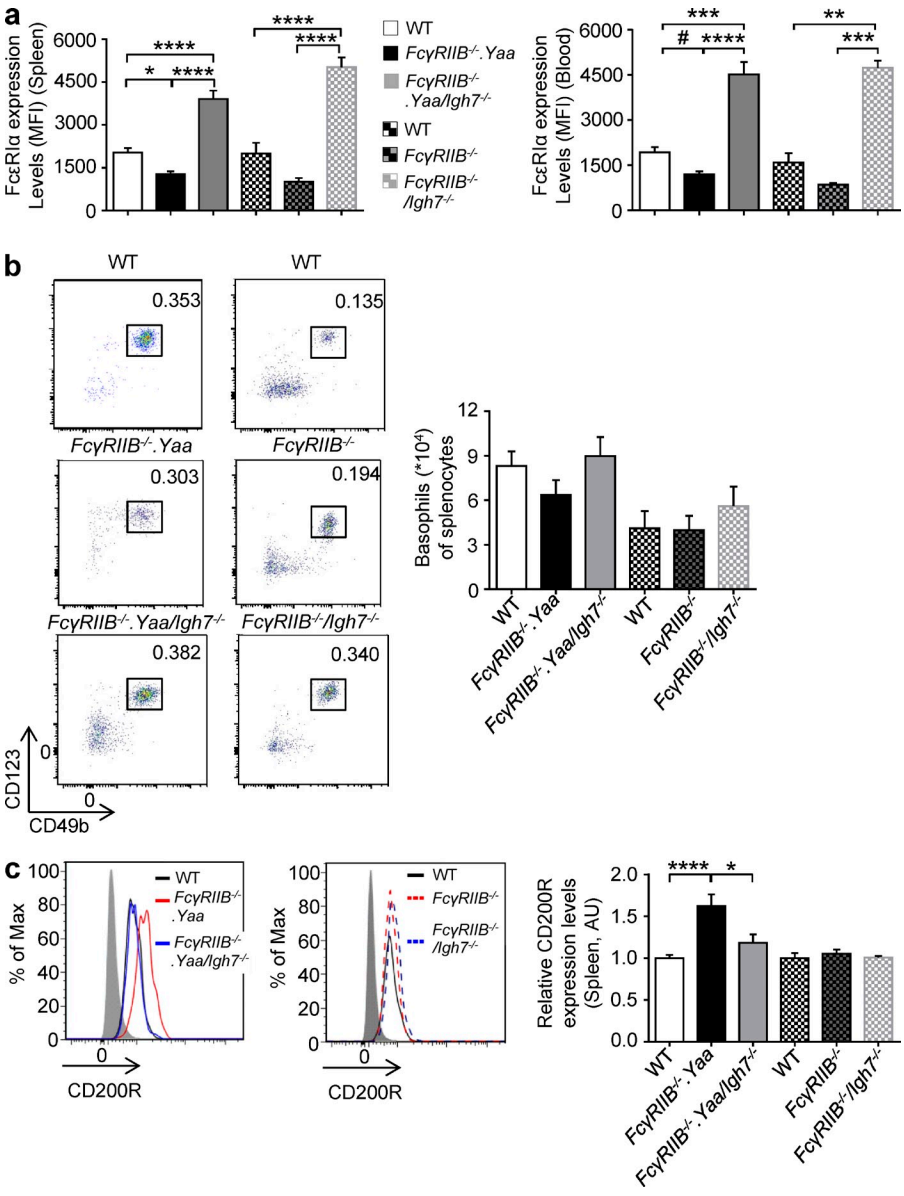

Figure 4. Basophil activation in spleen of an aggressive model of lupus-prone mice is markedly decreased by IgE deficiency. (a) FcεRIα expression (MFI) of splenic (left) and blood (right) basophils. (b) Representative dot plots (left) of gating strategy by flow cytometry. Percent of basophils in the spleen of one representative mouse is shown. Absolute numbers of basophils (FcεRIα^{+}cKit^{-}CD49b^{+} CD123^{+}) in the spleen (right) from two independent experiments of five mice per group. (c) Representative histograms of CD200R expression in spleen basophils (left). Relative CD200R expression shown as mean ± SEM (from MFI as arbitrary units, AU) in the spleen (right) from at least two independent experiments with a minimum of five mice per group. Kruskal-Wallis test with Dunn's multiple comparison test was used to compare the different strains. #, P ≤ 0.1; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

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the case when neutrophils were analyzed (Fig. 3 f). Analysis of the numbers of eosinophils in the SLO's revealed their presence only in the spleen of these mice and showed a strong trend toward increased numbers in both *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice that was reversed by IgE deficiency (Fig. 3 g). Thus, the findings demonstrate that IgE antibodies contribute to the recruitment of inflammatory cells in SLOs and suggests that IgE is involved in the inflammation seen in lupus-prone mice.

Basophils in *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice and the impact of IgE deficiency

We have previously demonstrated that basophils can participate in the amplification of the inflammatory response in *lyn*$^{-/-}$ mice by binding IgE autoantibodies (Charles et al., 2010). In this model, IgE deficiency caused a marked reduction in the numbers of basophils seen in SLO's with improvement in disease phenotype. In *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice, we did not detect sufficient numbers of basophils in LNs to study the impact of IgE deficiency in this organ. Interestingly, however, FcεRI expression on splenic and blood basophils from *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice was decreased and this was markedly reversed by IgE deficiency (Fig. 4 a), suggesting that the engagement of this receptor on basophils by IgE causes a down-regulation of its cell surface expression as described for human basophils (MacGlashan et al., 1997). We further explored if the numbers of basophils in the spleen might be altered with disease development in *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice. As shown in Fig. 4 b, splenic basophil numbers appeared to be unaltered in *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice regardless of whether these mice produced IgE or not. However, a direct assessment of their activation status by measuring the levels of CD200R (a molecule that is highly expressed upon basophil activation; Torrero et al., 2009) demonstrated a marked reduction in its expression in basophils from *FcγRIIB*$^{-/-}$*.Yaa Igh7*$^{-/-}$ mice (Fig. 4 c). Changes in splenic basophil expression of CD200R in *FcγRIIB*$^{-/-}$ *Igh7*$^{-/-}$ were likely not caused by the decreased numbers of basophils (Fig. 4 b) in the spleen of these mice. Nonetheless, basophil activation in the aggressive *FcγRIIB*$^{-/-}$*. Yaa* disease model is significantly dependent on the presence of IgE.

Relationship of autoreactive IgE with disease activity and basophil activation in human lupus

Previous work demonstrated that in human SLE the presence of autoreactive IgE was tightly linked to enhanced disease activity, increased serological markers, and lupus nephritis (Charles et al., 2010; Dema et al., 2014). Herein, we grouped human SLE subjects on whether they were positive or negative for autoreactive IgE to determine if significant differences could be observed in the association of these individual populations with markers of disease activity. As shown in Table 1, positivity for autoreactive IgE was associated with a significant increase in the proportion of individuals with active disease, increased serological markers, and lupus nephritis. Increased basophil activation in human SLE subjects (as demonstrated by enhanced CD203c expression; Bühring et al., 2004) was also previously demonstrated (Charles et al., 2010), and herein we found that increased CD203c expression is associated with mild or active SLE (Fig. 5 a). CD203c expression was also found to be significantly elevated above healthy controls in human SLE subjects that were positive for autoreactive IgE (Fig. 5 b). Moreover, in this group of subjects, CD203c expression was also significantly increased above healthy controls when individuals had active disease (Fig. 5 c). Collectively, these results implicate the presence of autoreactive IgE as a key factor in basophil activation in human SLE subjects. This is supported by our previous finding that basophils from human SLE subjects have enhanced phosphorylation

Table 1. Analysis of an SLE cohort for disease parameters when grouped by the presence (AR IgE^{+}) or absence (AR IgE^{-}) of autoreactive IgE.

	AR IgE^{-}		AR IgE^{+}		P-value, OR (CI 95%)
Disease activity *n* (%)					
Inactive (SLEDAI = 0)	52	(57.8)	31	(29.2)	
Mild (SLEDAI > 0 and < 4)	14	(15.6)	26	(24.5)	P = 0.004, 3.12 (1.42-6.84)
Active (SLEDAI ≥ 4)	24	(26.7)	49	(46.2)	P = 0.0002, 3.42 (1.77-6.63)
Hypocomplementemia *n* (%)					
No	78	(86.6)	68	(64.1)	
Yes	12	(13.4)	38	(35.8)	P = 0.0003, 3.63 (1.75-7.5)
dsDNA IgG positivity *n* (%)					
No	64	(71.1)	47	(44.3)	
Yes	26	(28.9)	59	(55.7)	P = 0.0002, 3.09 (1.70-5.60)
Nephritis *n* (%)					
Inactive	50	(79.4)	45	(60.8)	
Active	13	(20.6)	29	(39.2)	P = 0.01, 2.47 (1.14-5.34)

AR IgE, Autoreactive IgE; OR, Odds Ratio; CI, confidence interval.

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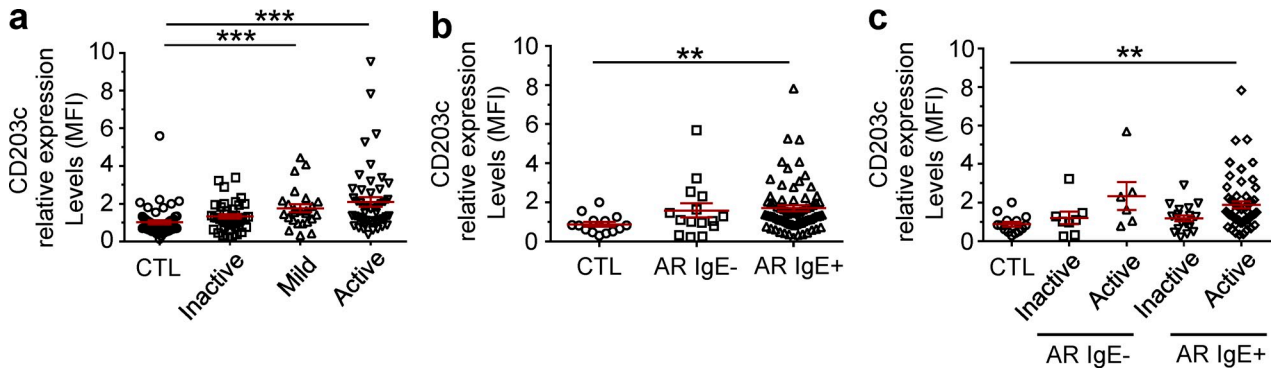

Figure 5. Activated phenotype of blood basophils from autoreactive IgE$^+$ human SLE subjects is associated with disease activity. (a) Analysis of CD203c expression levels with disease activity defined by SLE disease activity index (SLEDAI) score. Inactive, SLEDAI = 0; Mild, SLEDAI > 0 but < 4; Active, SLEDAI ≥ 4. (b and c) Analysis of CD203c expression on basophils from SLE subjects positive or negative for autoreactive IgE (2SD over or below the mean of healthy controls, respectively) ungrouped or grouped by disease activity. Kruskal-Wallis with Dunn's multiple comparisons test was used to compare the different groups. Mean ± SEM is shown. **, $P < 0.01$; ***, $P < 0.001$.

of the FcεRγ (Suzuki et al., 2013), suggesting that the activation of human basophils in lupus is mediated through engagement of FcεRI by autoreactive IgE complexes.

IgE, nonallergic inflammation, and autoimmunity

Here, we provide evidence of a role for IgE in nonallergic inflammation and demonstrate that the presence of IgE facilitates the onset of disease development in lupus-prone mice and is tightly linked to basophil activation and active human SLE. This view is strongly supported by our findings in the highly accelerated and aggressive lupuslike disease model of *FcγRIIB*$^{-/-}$*.Yaa* mice, where we found that IgE deficiency delays the onset of disease, prolonging their life span. However, *FcγRIIB*$^{-/-}$*.Yaa Igh7*$^{-/-}$ mice eventually develop a disease with many of the same phenotypic features of *FcγRIIB*$^{-/-}$*.Yaa* mice (unpublished data). Thus, although disease can develop in the absence of IgE, IgE appears to play a key role in amplifying the loss of tolerance in this model. This is supported by our human studies where individuals with increased autoreactive IgE were disproportionally linked to active disease.

Unexpectedly, our findings reveal an important and unrecognized role for IgE in the inflammatory response by promoting immune cell recruitment to SLO's. The presence of FcεRI on various immune cell types during inflammation is likely to underlie this immunoregulatory role for IgE in the inflammatory response but we cannot exclude a role for CD23 from our studies to date. Basophils, which highly express FcεRI, are known to cooperate with DC's in mounting an inflammatory response (Tang et al., 2010). Thus, as shown herein, in the absence of autoreactive IgE, basophil activation, and immune cell recruitment is dampened. Ultimately, this temporal defect is overcome as *FcγRIIB*$^{-/-}$*.Yaa Igh7*$^{-/-}$ can eventually develop disease (unpublished data). Nonetheless, the findings argue that IgE provides a microenvironment that facilitates a rapid and potent inflammatory response by promoting immune cell activation and function in SLO's. This reveals a previously unrecognized immunoregulatory role for IgE.

MATERIALS AND METHODS

Mice. All experiments were performed following the animal study protocol A010-04-03 and A012-11-01 approved by the Laboratory Animal Care and Use section in accordance of National Institutes of Health (NIH) and National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). C57BL/6 *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* mice were previously described (Bolland and Ravetch, 2000; Bolland et al., 2002) and the IgE-deficient (*Igh7*$^{-/-}$; *IgE* heavy chain locus) were generously provided by H. Oettgen (Harvard University, Cambridge, MA; Oettgen et al., 1994) and backcrossed to C57BL/6 (N10). Double-deficient and the respective WT mice were generated after crossing *FcγRIIB*$^{-/-}$ and *FcγRIIB*$^{-/-}$*.Yaa* with *Igh7*$^{-/-}$ mice for more than four generations, and all genotypes used came from the same litters. Unless otherwise stated, mice of the different genotypes (*FcγRIIB*$^{-/-}$*.Yaa*, *FcγRIIB*$^{-/-}$*.Yaa/Igh7*$^{-/-}$, WT female only) were used at 16 wk of age, whereas 28-wk-old female mice were used for *FcγRIIB*$^{-/-}$, *FcγRIIB*$^{-/-}$*/Igh7*$^{-/-}$, and corresponding WT genotypes.

Human blood samples. Blood samples were obtained from previously described cohorts of healthy controls and SLE subjects (Dema et al., 2014) as approved by the Institutional Review Board of NIAMS and by the Comité Régional de Protection des Personnes (CRPP, Paris, France). A written consent was obtained from all healthy donors and SLE patients. Parameters for human basophil identification, FcεRI, and CD203c detection were previously described (Charles et al., 2010). All sample processing was as previously described and the analysis was based on autoreactive IgE to the four most common lupus antigens (dsDNA, Sm, SSA/Ro, and SSB/La; Dema et al., 2014).

Autoantibodies and immunoglobulin measurement. The relative levels of dsDNA-specific IgE and IgG were measured as previously described (Charles et al., 2010). For measurement of anti-histone and RNP IgGs, MaxiSorp plates (Thermo Fisher Scientific) were coated with 2 µg of whole histones or RNP (ImmunoVision) in PBS overnight at 4°C. After a washing step, the plates were blocked with PBS containing 10% FBS (HyClone) and 0.05% Tween 20 (Sigma-Aldrich). Plates were incubated with serum samples diluted in blocking buffer. Horseradish peroxidase-conjugated antibody to mouse IgG (goat anti–mouse IgG-Fc fragment; Bethyl Laboratories) was used for detection. Colorimetric detection was with tetramethylbenzidine substrate (Invitrogen) and the optical density was measured at 450 nm. Total Ig levels (IgG1, IgG2b, and IgG3) were determined by ELISA kit following the manufacturer's instruction (Alpha Diagnostic International). Nuclear staining was performed to determine antinuclear Ig (ANA$^+$), after incubation of mouse sera on fixed HepG2 cells.

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Glomerulonephritis assessment and kidney function analysis. To measure C3 and IgG deposits in the kidney, frozen sections (American Histolabs) were fixed with 4% paraformaldehyde solution and blocked with PBS supplemented with 7.5% Blokhen II (Aves Labs) for 1 h. Sections were incubated with FITC-conjugated anti–mouse complement C3 (Cedarlane) and FITC-conjugated affiniPure $F(ab')_2$ fragment goat anti–mouse IgG, Fcγ-specific (Jackson ImmunoResearch Laboratories) or the respective FITC-conjugated isotype controls (FITC-rat IgG2a isotypic control; Cedarlane; FITC-goat IgG isotype control; Abcam) overnight at 4°C. The sections were washed with PBS containing 0.1% Tween 20 (Sigma-Aldrich). After washing the tissue, the slides were embedded in mounting media (Vector Laboratories, Inc.). FITC-conjugated antibodies were excited with an argon ion laser at 488 nm and fluorescence emission of >505 nm was measured with a confocal laser fluorescence microscope (LSM-780; Carl Zeiss, Inc.). Image processing was performed using ImageJ (NIH) or Photoshop (Adobe Systems) software. In these experiments, one of the two kidneys was embedded in 10% neutral buffered formalin (Sigma) and then sectioned and H&E stained by American Histolabs. Nephritis assessment and scoring from 0 to 4 (severe lesions) was established in a blinded manner by a pathologist assessing glomeruli and tubular injury. Pictures were taken on the Keyence BZ-9000E fluorescence microscope (BIOREVO).

Kidney function was assessed by measuring albumin (Bethyl Laboratories) and creatinine (R&D Systems) ratio (ACR; microgram of albumin per microgram of creatinine) in urine of the different strains.

Flow cytometry. Blood, spleen, and LNs (cervical, inguinal, axial, and brachial) were obtained from the mice. Spleen and LNs were homogenized and filtered through 70-µm cell strainer (BD Falcon) for single-cell suspensions. Red blood cells (for blood and spleen) were lysed with ammonium-chloride-potassium lysing buffer (Quality Biological, Inc.) for 5 min on ice, three times. Specific cell surface markers were detected by incubation for 25 min at 4°C with fluorescently conjugated antibody in FACS buffer (PBS, 0.1% BSA, 0.05% NaN_3) after blocking low affinity Fcγ receptors with CD16/32 antibody (BioXcell). When biotinylated antibody was used a subsequent incubation for 15 min with PerCP/Cy5.5-conjugated Streptavidin (eBioscience) was performed. Cells were stained with the Aqua dye fluorescence live/dead fixable stain kit (Invitrogen), following the manufacturer's instructions. FMO (Fluorescence minus one) strategy was used to determine if the detected fluorescence indicated positivity for a cell surface marker or not. Flow cytometry acquisition was on a FACSCanto II (BD) and analysis was done using FlowJo software (Tree Star). Antibodies used from eBioscience were as follows: PE-anti–mouse TCRβ (clone: H57-597), FITC-anti–mouse CD4 (clone: GK1.5), APC-anti–mouse CD69 (clone: H1.2F3), FITC-anti–mouse CD23 (clone B3B4), Biotin-anti–mouse CD123 (clone: 5B11), and APCeFluor780-anti–mouse MHC II (I-A/I-E; clone: M5/114.15.2). Antibodies used from BioLegend were as follows: Pacific Blue-anti–mouse CD19 (Clone: 6D5), APC/Cy7 anti–mouse B220 (clone: RA3-6B2), PE/Cy7-anti–mouse CD21/CD35 (CR2/CR1; clone: 7E9), PE-anti–mouse CD138 (Syndecan-1; clone:281-2), PerCP/Cy5.5-anti–mouse IgM (clone: RMM-1), APC-anti–mouse CD49b (pan-NK cells; clone: DX5), Pacific blue-anti–mouse CD117 (c-kit; clone: 2B8), PE-anti–mouse FcεRIα (clone: MAR-1), FITC-anti–mouse CD200R (OX2R; clone: OX-110), PECy7-anti–mouse CD62L (clone: MEL-14), PECy7-anti–mouse Ly6G (clone: 1A8), PerCP/Cy5.5-anti–mouse CD11c (clone: N418), Pacific Blue-anti–mouse CD11b (clone: M1/70), and Alexa Fluor 640-anti–mouse F4/80 (clone: CI:A3-1). Antibodies used from BD were as follows: PerCP/Cy5.5-anti–mouse CD8a (clone: 53–6.7), FITC-anti–mouse Ly6C (clone: AL-21), FITC-anti–mouse CD86 (clone: GL1), and PE-anti–mouse CD80 (B7-1; clone: 16-10A1).

Statistical analysis. One-way ANOVA with Tukey's correction test or Kruskal-Wallis with Dunn's correction test was applied when more than two groups were compared. Mean and SEM are shown on the graphs. When a different statistical analysis was performed it is indicated in the figure legend. All analysis was performed using GraphPad Prism 6.0.

Online supplemental material. Gating strategy to define mouse myeloid cell types is shown in Fig. S1. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20140066/DC1.

This research was supported by the Intramural Research Program of NIAMS and NIAID, NIH, by the French Institut National de la Santé Et de la Recherche Médicale (INSERM), by the Assistance Publique – Hôpitaux de Paris (AP-HP), and by grants from the Mairie de Paris (Emergences 2010), ANR JCJC SVSE1 2011 BASILE and the French Kidney Foundation (Fondation du Rein) to N. Charles. N. Charles and B. Dema are supported by the Laboratoire d'excellence INFLAMEX. We acknowledge the support of the Laboratory Animal Care and Use, Flow Cytometry, and Light Imaging Sections of the Office of Science and Technology, NIAMS.

The authors have no conflicting financial interests.

Submitted: 12 January 2014
Accepted: 13 August 2014

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SUPPLEMENTAL MATERIAL

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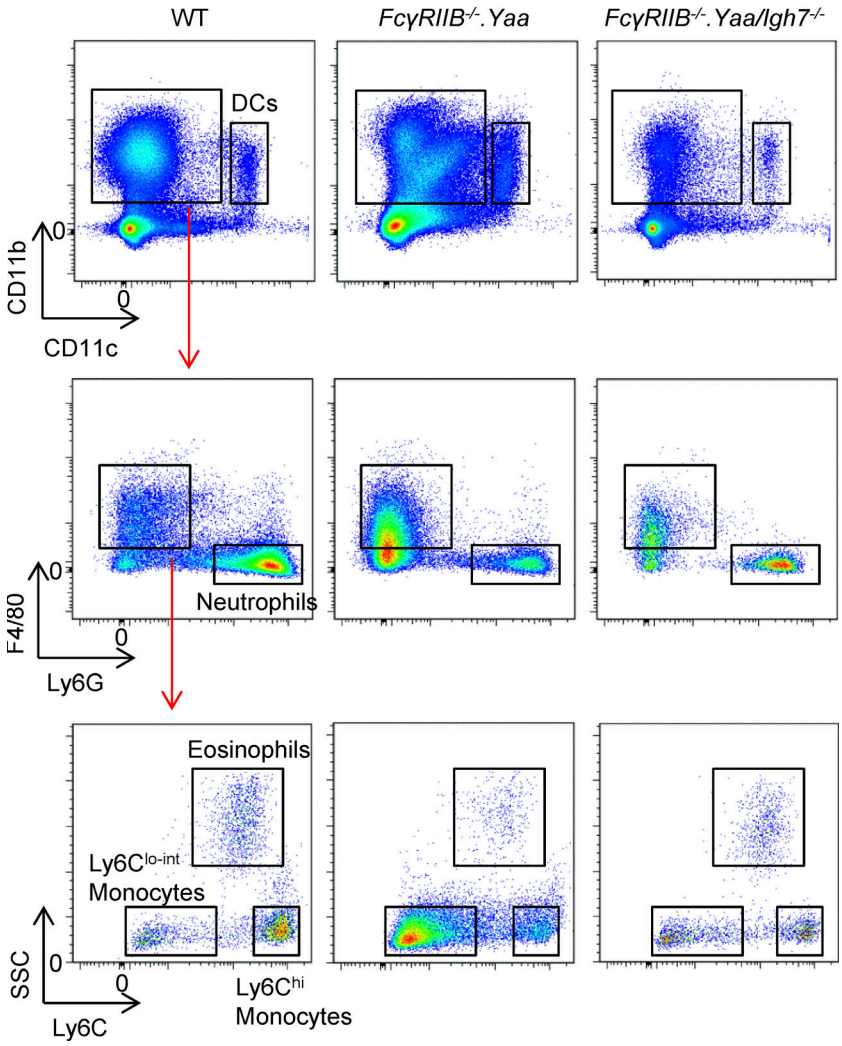

Figure S1. Representative gating strategy used to detect myeloid mouse cells by flow cytometry in three of the strains studied.