assessments of IL-12p40/70 production by intracellular flow cytometry. A greater percentage of WT DC produced IL-12p40/70, consistent with a role for IFNAR signaling in IL-12 production (supplemental Fig. S2F). While baseline levels of surface MHC-II, CD40, and CD86 did not significantly differ between the two DC populations, WT DC showed slightly higher expression of these molecules than did IFNAR$^{-/-}$ DC (S. Bajana and S. Kovats, unpublished data). These experiments with BM-derived MDC are consistent with our results with freshly isolated splenic DC in showing that IFNAR deficiency on the NZM 2328 background leads to reduced DC activation in response to synthetic TLR9 ligands, as well as the endogenous TLR9 ligands present in vivo.

**IFNAR-deficient NZM 2328 mice harbor reduced lymphocyte numbers, with a marked reduction in activated memory CD4$^+$ T cells**

Increased numbers of activated DC promote autoreactive B and T cell responses in murine autoimmune models (25, 56). Autocrine IFN-α and IFN-β acting via IFNAR enhance DC survival and activation, thereby increasing the ability of DC to stimulate adaptive T cell immunity and enhance humoral immunity by increasing B cell proliferation and Ig class switching (4–9). IFNAR signaling also may have direct effects in lymphocytes (57). To determine whether IFNAR signaling and/or increased numbers of activated DC led to altered numbers or phenotypes of lymphocytes in NZM 2328 mice, we compared numbers of B and T cell subsets in WT and IFNAR$^{-/-}$ mice at 5 mo of age before development of overt clinical disease, and at 8–12 mo of age, when clinical disease is apparent in WT mice. At 5 mo of age, WT mice had greater numbers of CD19$^+$ B cells ($p = 0.026$, Fig. 7A), which included an increase in CD21$^{hi}$CD23$^+$ follicular B cells (supplemental Fig. S3). However, at 5 mo of age, no differences in numbers of CD4$^+$ or CD8$^+$ T cells were found between WT and IFNAR$^{-/-}$ mice (Fig. 7, B and C). By 8–9 mo, the number of CD19$^+$ B cells was significantly increased in WT but not IFNAR$^{-/-}$ mice ($p = 0.007$, Fig. 7A), which included increased numbers of CD5$^+$ B cells (supplemental Fig. S3). These increased B cell numbers in WT mice are consistent with the increased production of anti-nuclear autoantibodies that we also observed in WT mice at this age (Fig. 3).

Notably, 8- to 9-mo-old WT mice had a significantly expanded population of activated memory CD4$^+$CD44$^{hi}$CD62L$^{lo}$ T cells, which was not present in the IFNAR$^{-/-}$ mice (Fig. 7E). Numbers of naive CD4$^+$CD44$^{low}$CD62L$^{hi}$ T cells were not different in WT and IFNAR$^{-/-}$ mice at either age 5 or 8–9 mo (Fig. 7D). These data suggest that the increased numbers of activated DC observed at 5 mo of age (before onset of clinical disease) in WT mice lead to the priming and subsequent maintenance of activated memory CD4$^+$ T cells that ultimately fuel onset and maintenance of overt clinical disease.

**Discussion**

We have shown that IFNAR deficiency on the NZM 2328 background significantly reduces the incidence of lupus nephritis, autoantibody production, and mortality. Our analyses of DC before the onset of clinical disease in 2- and 5-mo-old NZM 2328 mice show that IFNAR signaling promotes increased numbers of activated pDC in the rLN and cDC in the spleen. As disease progresses, these activated DC correlate with elevated numbers of activated effector CD4$^+$ T cells and production of pathogenic autoantibodies. These data implicate IFNAR engagement in DC as a critical factor at early preclinical stages of lupus disease.

Increased numbers of DC bearing high levels of costimulatory molecules accumulate with age in most murine models of lupus, including the MRL/lpr, NZB, (NZB × NZW)F$_1$, BXSB, (NZB × BXSB)F$_1$, NZM 2328, NZM 2410, B6.Sle1, and B6.Sle3 strains (24–31). The increased DC numbers could be due to new DC differentiation or to extended DC survival mediated by endogenous TLR ligands and IFNAR signaling. New DC arise during inflammation after differentiation from inflammatory monocytes recruited to lymphoid organs and tissues (53). Additionally, TLR9 activation induces lymphoid progenitors to switch their developmental program and differentiate to myeloid cells, including DC.
and macrophages (58). Interestingly, elevated numbers of activated DC, which accumulate due to increased new development or extended survival during chronic inflammation, have been shown to promote loss of self tolerance. An extended lifespan of activated DC can induce SLE-like autoimmunity in C57BL6/6 mice, as shown by experiments that inhibited DC apoptosis after stimulation by TLR ligands in vivo (56, 59). Similarly, lupus disease in mice with multiple extra copies of the TLR7 gene was associated with elevated numbers of DC, consistent with chronic TLR7 signaling inducing new DC development or extended DC survival (60). Our data show that in the context of the inflammation and chronic TLR signaling in NZM 2328 mice, IFNAR likely acts via one of these mechanisms to promote the increased numbers of activated cDC and pDC. Interestingly, the effect of IFNAR on activated DC numbers is specific to lupus-prone mice, since this effect of IFNAR deficiency was not observed in 129Sv mice (47).

In young (2-mo-old) mice, we identified an IFNAR-dependent population of CD40\(^{hi}\) pDC that preferentially accumulated in rLN; a general increase in rLN size was also IFNAR-dependent. This was notable since we did not observe populations of activated DC or macrophages in the spleen or BM at this age. These data suggest that an early event during preclinical stages of lupus nephritis is the recruitment of pDC to the kidney or rLN, leading to the accumulation of activated pDC at these sites. We provide evidence that IFNAR has an important role in this tissue-specific accumulation, consistent with reports that IFNAR signaling increases pDC activation, survival, and migration (4, 46). In human SLE patients, elevated numbers of pDC are observed in kidney or skin but not in blood, which also suggests that pDC are preferentially recruited to active disease sites (15, 50, 61, 62). In 5-mo-old WT NZM mice, pDC numbers in rLN increased, yet splenic pDC numbers remained comparable in NZM WT and IFNAR\(^{-/-}\) mice. The most likely explanation is that IFNAR signaling does promote pDC survival and activation in NZM 2328 mice, but the activated pDC are preferentially recruited to target tissues such as kidney and their draining lymph nodes as we have noted. It is also possible that activated splenic pDC are converted to cDC, as occurs during viral infection or upon in vivo exposure to type I IFN (63, 64).

In NZM 2328 mice, IFNAR promotes activated cDC and pDC phenotypes in vivo before development of severe lupus symptoms. In contrast, pDC and cDC in unstimulated 129Sv and 129Sv-IFNAR\(^{-/-}\) mice did not differ in low expression of CD40 or CD86 costimulatory molecules (46). This difference in costimulatory molecule expression between NZM and NZM-IFNAR\(^{-/-}\) DC is likely due to activation of DC in vivo by endogenous stimulators that occur as lupus begins to develop in NZM mice. Similar comparisons of NZM2410 to BALB/c mice (24) or B6.Sle1 and B6.Sle3 to B6 mice (25, 28) showed that DC in lupus-prone mice exhibit a hyperstimulatory phenotype before disease onset.

We found that IFNAR deficiency had a profound effect on activation of pDC and CD8\(^{+}\) LDC. During homeostasis, LDC and pDC help to maintain T cell self tolerance (53, 65). Upon bacterial or viral infection, LDC are critical for priming of naïve T cells. Activated pDC also may have a significant role in Ag presentation to T cells at sites of inflammation and in local lymph nodes (66). Thus, the IFNAR-regulated LDC and pDC activation that occurs before overt clinical disease is likely to contribute to the loss of T cell tolerance necessary to initiate the adaptive immune phase of lupus disease. Indeed, our analyses of 8- to 9-mo-old mice showed an expansion of activated memory T cells and marginal zone B cells in WT but not in IFNAR\(^{-/-}\) mice.

Our data with NZM 2328 mice are consistent with prior reports of the effect of IFNAR deficiency in spontaneous models with partial lupus phenotypes or in experimental lupus-like models. Deficiency of IFNAR in 129Sv mice inhibited autoantibody production, severe renal disease, and expression of type I IFN-stimulated genes in a chemically induced (2,6,10,14-tetramethylpentadecane) lupus model (35). NZB mice lacking the IFNAR \(\alpha\)-chain had significantly reduced anti-erythrocyte autoantibodies, hemolytic anemia, and anti-DNA Abs (32). IFNAR-deficient B6.Nba2, (B6.Nba2 \(\times\) NZW)F\(_{1}\), and 129 \(\times\) C57BL/6/lpr mice showed decreased autoantibody production and renal disease and did not develop splenomegaly or lymphadenopathy (33, 34). We now show that disease protection in these IFNAR-deficient lupus models may occur because sustained DC activation does not occur, which prevents chronic lymphocyte responses. Paradoxically, IFNAR deficiency in MRL/lpr mice worsened lymphoproliferation, autoantibody production, and end-organ disease, suggesting that type I IFNs may suppress autoimmunity induced by Fas deficiency on the MRL genetic background (36). These distinct effects of IFNAR deficiency on lupus disease in different models could relate to the various mechanisms by which DC induce tolerance. While “immature” DC lacking costimulatory molecules or inflammatory cytokines promote tolerance by failing to activate lymphocytes, in some settings activated “mature” DC are required to induce T regulatory cells (67). Thus, it is possible that disease-suppressing T regulatory cells in MRL/lpr mice are dependent on IFNAR signaling in DC, and this mechanism is less critical in NZM 2328 mice.

NZM 2328 mice exhibit a disease course and sex bias that most closely resembles human SLE. Thus, our finding that IFNAR deficiency decreases numbers of activated DC and reduces lupus severity and mortality provides additional rationale for human SLE therapy involving IFNAR blockade or anti-IFN-\(\alpha\) mAbs.

Disclosures
The authors have no financial conflicts of interest.

References

Deck, D., P. I. Kotter, R. Klein, B. Bern, and S. A. Bixler. 2006. Mono-


Blanco, A., A. K. Palucka, M. Gill, V. Pascual, and J. Banchereau. 2001. Induc-


Marshak-Rothstein, A., and I. R. Rifkin. 2007. Immunologically active autoant-


Yamashita, M., M. Hashimoto, K. Ichiyama, R. Yoshida, T. Hanada, T. Muta, S. Komune, T. Kobayashi, and A. Yoshiumi. 2007. 16:202, an IFN-α-inducible candidate gene for lupus susceptibility in NZBW/F1 mice, is a positive regulator for NF-κB activation in dendritic cells. Int. Immu-


dritic cells (DC) in aged BWF1 mice which can be matured by TNF-α into BLC/CIIC/CL13-producing DC. Eur. J. Immunol. 32: 1881–1887.


Samardzija-Kabre, M-L., R. Baccala, K. M. Haraldsson, D. Chouby, T. A. Stewart, D. H. Kono, and A. N. Theofilopoulos. 2003. Type I interferon receptor defi-


dritic cells (DC) in aged BWF1 mice which can be matured by TNF-α into BLC/CIIC/CL13-producing DC. Eur. J. Immunol. 32: 1881–1887.


Samardzija-Kabre, M-L., R. Baccala, K. M. Haraldsson, D. Chouby, T. A. Stewart, D. H. Kono, and A. N. Theofilopoulos. 2003. Type I interferon receptor defi-


dritic cells (DC) in aged BWF1 mice which can be matured by TNF-α into BLC/CIIC/CL13-producing DC. Eur. J. Immunol. 32: 1881–1887.


Samardzija-Kabre, M-L., R. Baccala, K. M. Haraldsson, D. Chouby, T. A. Stewart, D. H. Kono, and A. N. Theofilopoulos. 2003. Type I interferon receptor defi-


Corrections


The fifth header under **Results** should read “TNF-α-dependent expression of CBP is contingent on NF-κB.”

Also, the first line of the **Discussion** should read as follows: “Acetylation homeostasis is a well-preserved stoichiometrical balance of acetyltransferase and deacetylase enzymatic undertakings in normal cells that confers stability to cellular homeostasis by coordinating gene expression and repression on both a temporal and spatial basis (24).”

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0990087
97th Annual Meeting
The American Association of Immunologists
IMMUNOLOGY 2010™

The Premier Annual All-Immunology Meeting for Scientists Worldwide

Save the Date!
May 7-11, 2010
Baltimore Convention Center
Baltimore, Maryland