Type 1 diabetes (T1D) is an autoimmune disease that shows familial aggregation in humans and likely has genetic determinants. Disease linkage studies have revealed many susceptibility loci for T1D in mice and humans. The mouse T1D susceptibility locus insulin-dependent diabetes susceptibility 3 (Idd3), which has a homologous genetic interval in humans, encodes cytokine genes Il2 and Il21 and regulates diabetes and other autoimmune diseases; however, the cellular and molecular mechanisms of this regulation are still being elucidated. Here we show that T cells from NOD mice produce more Il21 and less Il2 and exhibit enhanced Th17 cell generation compared with T cells from NOD.Idd3 congenic mice, which carry the protective Idd3 allele from a diabetes-resistant mouse strain. Further, APCs from NOD and NOD.Idd3 mice played a central role in this differential Th17 cell development, and IL-21 signaling in APCs was pivotal to this process. Specifically, NOD-derived APCs showed increased production of pro-Th17 mediators and dysregulation of the retinoic acid (RA) signaling pathway compared with APCs from NOD.Idd3 and NOD.II21r-deficient mice. These data suggest that the protective effect of the Idd3 locus is due, in part, to differential RA signaling in APCs and that IL-21 likely plays a role in this process. Thus, we believe APCs provide a new candidate for therapeutic intervention in autoimmune diseases.

Introduction

Type 1 diabetes (T1D) is an autoimmune disease that shows familial aggregation in humans and appears to be, at least partially, genetically determined. The NOD mouse serves as a good model for human diabetes, because the disease appears spontaneously and bears pathogenic features remarkably similar to those of human T1D. Genetic analyses of T1D in NOD mice and in humans have identified many susceptibility loci. One of these loci, insulin-dependent diabetes susceptibility 3 (Idd3), encodes several genes, of which only the cytokine genes Il2 and Il21 currently have known immunological importance. NOD mice carrying the Idd3 locus from diabetes-resistant C57BL/6 mice (NOD.Idd3 mice) are protected from autoimmune diabetes and EAE (1, 2). In this genetic interval, Il2 and Il21 are inherited as a haplotype. IL-2 and IL-21 are both T cell–derived cytokines that have antagonistic effects on the development of Foxp3+ Tregs and proinflammatory Th17 cells. IL-2 promotes Treg differentiation, whereas IL-21 suppresses it; in contrast, IL-21 supports the generation of Th17 cells, while IL-2 constrains Th17 differentiation (3–7). Thus, the Idd3 genetic interval may regulate the balance between pathogenic Th17 and protective Foxp3+ Tregs through the differential effects of IL-2 and/or IL-21.

Indeed, some studies suggest that the protective effect of the Idd3 genetic interval is due to increased production of Il21, which enhances Treg function, in NOD.Idd3 congenic mice (8). In this regard, we and others reported that the defective Treg function observed in NOD mice, relative to that of NOD.Idd3 mice, is determined by differences in NOD-derived versus NOD.Idd3-derived APCs (9, 10), rather than by intrinsic defects in the Tregs from NOD mice.

Although the role of IL-2 in the protective effect of Idd3 has received much attention, considerable evidence supports a role for IL-21 as well. It has been reported that IL-21 expression is elevated in NOD mice compared with that in diabetes-resistant strains of mice and that increased IL-21 expression is the key to susceptibility to autoimmune disease (11). In support of this, II21 receptor-deficient (II21r-deficient) NOD mice were reported to be protected against autoimmune diabetes (12–14).

The recently characterized Th17 cells have been shown to be highly pathogenic, and it has been argued that this subset is the primary mediator of tissue inflammation in many autoimmune diseases (15). The involvement of Th17 cells in diabetes is not well understood, but there is a growing body of research implicating Th17 cells in its pathogenesis (16–18). Using either neutralizing anti–IL-17 mAb or recombinant IL-25, Th17 cells have been demonstrated to have a pivotal role in T1D. The Th17-blocking therapies prevented diabetes development in NOD mice with a concomitant reduction in islet-specific T cell responses (19).

Here we show that T cells derived from NOD mice have a greater propensity to differentiate into Th17 cells compared with T cells from both NOD.Idd3 mice and NOD.II21r KO mice. We further show that, in addition to intrinsic T cell differences, APCs have an important role in determining the balance of regulatory and pathogenic T cells. NOD- and NOD.Idd3-derived APCs differentially support Th17 cell polarization, and this difference in APC...
function is associated with retinoic acid (RA) signaling that may be regulated by IL-21. In the study of autoimmunity, APCs have received little attention relative to T cells. Our data suggest that APCs are key players and represent other avenues for therapeutic intervention in autoimmune diseases.

Results

NOD-derived cells have a greater potential to generate Th17 cells than NOD.ddit3-derived cells. To compare the expression of Il21 and Il2 in naive T cells from NOD and NOD.ddit3 congenic mice, real-time PCR was performed. We found that T cells from NOD mice expressed more Il21 and fewer Il2 transcripts than NOD.ddit3-derived T cells upon in vitro activation using anti-CD3 plus anti-CD28 (Supplemental Figure 1A; supplemental material available online with this article; doi:10.1172/JCI46187DS1). Accordingly, when naive T cells were activated in the presence of self APCs, IL-21 and IL-17 production was elevated in NOD cultures compared with that in NOD.ddit3 cultures (Supplemental Figure 1B). Since recent data show that IL-2 and IL-21 have pivotal and opposing roles in the development of Th17 cells, we compared the generation of Th17 cells in vitro using naive T cells from NOD and NOD.ddit3 mice. In the absence of APCs, the polarization of Th17 cells, using a combination of IL-6 and TGF-β in NOD cultures, was on average twice as efficient as that in NOD.ddit3 cultures, as indicated by a higher frequency of IL-17+ T cells (Figure 1A). This defect is in line with the increased Il21 and decreased Il2 expression by T cells from NOD mice compared with that from NOD.ddit3 mice (Supplemental Figure 1). Furthermore, Th17 polarization was enhanced in both NOD and NOD.ddit3 cultures when neutralizing anti-IL-2 mAb was added. However, even at the highest dose of anti-IL-2 used, NOD.ddit3-derived T cells remained less efficient in polarizing to Th17 cells than NOD-derived T cells (Supplemental Figure 2). Thus, differential expression of IL-2 alone cannot account for the reduced potential of NOD.ddit3 T cells to differentiate into Th17 cells.

Since we and others previously showed that APCs from NOD and congenic NOD.ddit3 mice have important effects on the suppressive activity of Tregs (9, 10) and that Tregs and Th17 cells have a reciprocal relationship (20), we examined the relative contribution of NOD- versus NOD.ddit3-derived APCs on Th17 cell differentiation. By swapping APCs, we found that NOD-derived APCs were more efficient at supporting the differentiation of Th17 cells, irrespective of the source of T cells (Figure 1B), while NOD.ddit3-derived APCs could not support polarization of Th17 cells as efficiently as NOD-derived APCs. Moreover, APCs from NOD.ddit3 mice enhanced production of IFN-γ, a prototypical cytokine of Th1 cells, even in the presence of Th17-polarizing cytokines IL-6 and TGF-β (Figure 1B). This increased production of IFN-γ in the presence of NOD.ddit3-derived APCs was coincident with reduced IL-17 production. This raises the possibility that IFN-γ induced by and/or produced by NOD.ddit3 APCs was responsible for inhibiting Th17 polarization. Indeed, the addition of neutralizing anti-IFN-γ mAb improved Th17 differentiation in all conditions and almost completely abolished the differences in Th17 differentiation observed between APCs derived from NOD and NOD.ddit3 mice (Figure 1B). This is consistent with previous reports that IFN-γ inhibits Th17 cell generation (16, 21, 22).

Figure 1
Cells from NOD mice generate Th17 cells more efficiently than cells from NOD.ddit3 mice. Naïve T cells were polarized with TGF-β and IL-6. After 3 days, cells were restimulated with PMA and ionomycin in the presence of monensin for 4 hours before cell surface and then intracellular staining. Values in each quadrant represent the percentage of 7-AAD–CD4+ cells. Cells were activated with (A) plate-bound anti-CD3 and soluble anti-CD28 or (B) with soluble anti-CD3 in the presence of APCs, with or without anti–IFN-γ mAb. Data shown are representative of 4 to 9 experiments.

Figure 2
The Treg/T effector balance is skewed toward T effectors in NOD mice. LN and spleen cells from Foxp3.gfp KI NOD (prediabetic) and NOD.ddit3 mice were stimulated with PMA and ionomycin for intracellular cytokine staining. Foxp3/GFP+ expression on CD4+ T cells was assessed in the same samples prior to restimulation. Each dot represents the ratio of Treg/cytokine+ 7-AAD–CD4+ T cells per individual mouse, and the means are indicated by the bars.
Together, these data suggest that there is an APC-driven — as well as a T cell–intrinsic — difference in Th17 cell development between NOD and NOD.Idd3 mice. APCs from NOD.Idd3 congenic mice not only impaired Th17 differentiation but also promoted IFN-γ production by T cells, even under Th17 polarization conditions, thereby further inhibiting Th17 differentiation.

Th17 cells accumulate in NOD mice and can induce diabetes upon adoptive transfer. Given that NOD.Idd3 mice are protected from T1D and that both IL-2 and IL-21 can modulate the development of induced Tregs and Th17 cells, we assessed the balance of regulatory and pathogenic T cells in these mice. We and others have previously shown that the frequency of Tregs is not significantly different between NOD and diabetes-resistant NOD.Idd3 mice (8, 9, 23, 24). However, in prediabetic mice, we found that the ratio of Tregs/Th17 cells was higher in NOD.Idd3 mice than in NOD mice in both the splenic and pancreatic draining LN compartments (Figure 2). We also looked at the development of Th1 cells by staining for IFN-γ. Treg/Th1 ratios in the pancreatic LN of 4- to 6-week-old mice were also higher in NOD.Idd3 congenic mice than those in NOD mice, but there was no difference in older mice or in the splenic compartment (Figure 2). These data indicate that the balance of Tregs/Th1 effector cells is shifted toward effector cells, particularly Th17 cells, in NOD mice.

Th17 cells have become recognized as key orchestrators of many autoimmune disease processes (15), and indeed, NOD mice have higher frequencies of IL-17+ CD4+ T cells in the pancreatic LNs as they progress toward diabetes (data not shown and ref. 17). Using T cells from mice with a TCR that is reactive to an islet antigen (BDC2.5 Tg mice), we thus directly compared the pathogenic capacity of Th1 and Th17 cells to induce T1D upon adoptive transfer. Upon transfer into either lymphopenic NOD/SCID mice or intact NOD mice, islet-specific Th1 and Th17 cells, generated in vitro, were equally efficient at inducing diabetes (Supplemental Figure 3A and ref. 18). Previous studies suggest that the diabetogenic effects of Th17 cells are due to and rely on the conversion of Th17 cells into IFN-γ-producing Th1 cells in vivo (17, 18). To address this issue, we generated islet-specific Th17 cells from IFN-γ-deficient mice and demonstrated that they were just as efficient as IFN-γ+ Th17 cells at inducing T1D upon adoptive transfer into NOD mice (Supplemental Figure 3B). Thus, Th17 cells can induce T1D independently of IFN-γ.

IL-21 signaling both in T cells and APCs contributes to the defect in Th17 cell generation and diabetes. Since the Idd3 locus regulates expression of both Il2 and Il21, and perturbation of IL21R signaling completely protected NOD mice from autoimmune diabetes (12–14), we investigated the effects of IL-21 signaling on Th17 cell development using NOD.II21r KO mice. In line with previous reports using II21r- and II21r-deficient mice on diabetes-resistant backgrounds (5–7), II21r-deficient T cells from NOD mice exhibited a defect in generating Th17 cells (Figure 3A), similar to that of NOD.Idd3-derived T cells (Figure 1A). Given our observations that IL-21 production is reduced in NOD.Idd3 mice and that NOD.Idd3-derived APCs do not promote Th17 differentiation, we next investigated the ability of NOD.II21r KO APCs to support Th17 differentiation. Like NOD.Idd3-derived APCs, NOD.II21r KO APCs did not support Th17 differentiation as well as NOD-derived APCs (Figure 3B). Furthermore, APCs from II21r-deficient NOD.Idd3 mice (NOD.Idd3.II21r KO mice) were even less efficient than NOD.Idd3-derived APCs at supporting Th17 generation (Figure 3B). These data show, for the first time to our knowledge, that IL-21 signaling in APCs conditions them for optimal Th17 generation. Moreover, these data support the hypothesis that decreased IL-21 signaling in APCs may partly underlie the disease resistance in NOD.Idd3 mice.

It is of interest that APCs from mice with either reduced IL-21 signaling (NOD.Idd3 mice) or complete loss of IL-21 signaling (NOD.II21r KO mice) were not able to support Th17 generation as well as APCs derived from NOD mice. Moreover, when NOD-derived T cells were activated under neutral conditions, they produced more IFN-γ when cocultured with APCs from NOD.Idd3 or NOD.II21r KO mice than with APCs from NOD mice (Figure 3C). Together with the observation that NOD.Idd3-derived APCs could promote IFN-γ production by T cells, even in the presence of Th17-polarizing cytokines, and that neutralization of IFN-γ resulted in enhanced generation of Th17 cells.
Given that PGE2 and IL-6 are known pro-Th17 mediators, decreased production of these mediators by NOD.Idd3-derived and NOD.II21r KO APCs is in line with a defect in Th17 cell generation regulated by APCs in these mice. In support of our hypothesis that IL-21 influences mediator production by CD11b+ APCs in NOD and NOD.Idd3 mice and that the difference in mediator production is not caused by differential II21r expression, we found that II21r mRNA was similarly expressed on CD11b+ cells ex vivo from both strains (Supplemental Figure 4A). On the other hand, II2ra expression was undetectable in CD11b+ cells ex vivo (Supplemental Figure 4A). Taken together, these data suggest that the differences in IL-21 signaling between NOD and NOD.Idd3 mice can influence the repertoire of proinflammatory mediators produced by APCs, which, in turn, affect Treg and Th17 cell generation and/or function. Thus, CD11b+ APCs may be differentially conditioned in diabetes-susceptible and diabetes-resistant mouse strains, and the observed differences in PGE2 and IL-6 production are merely products of greater underlying differences.

Gene profiling of CD11b+ cells reveals a link between altered RA signaling in APCs and Th17 differentiation. To further explore the effect mediated by the Idd3 locus on CD11b+ cells, we compared the gene profiles of NOD- and NOD.Idd3-derived CD11b+ cells using whole genome microarray analysis (GEO accession GSE24350). Comparisons between CD11b+ cells isolated from NOD and NOD.Idd3 mice yielded 213 and 335 transcripts with consistently more than 2-fold differential expression in duplicate comparisons of unstimulated and PGN-activated cells, respectively (Figure 5A).

Ingenuity Pathways Analysis software was used to highlight transcriptional networks of genes differentially expressed between NOD- and NOD.Idd3-derived CD11b+ cells. Interestingly, some genes differentially expressed after TLR2 ligation in NOD- and NOD.Idd3-derived CD11b+ cells have been reported to be downstream of RA signaling (Figure 5B). All-trans RA, a vitamin A metabolite, has been reported to regulate the reciprocal relationship between Tregs and Th17 cells, whereby RA inhibits Th17 cell generation and enhances Treg induction (28). Previously, we showed that IFN regulatory factor 4 (IRF4), which is critical for Th17 cell generation (29, 30), is downregulated by RA (31). Microarray analysis showed that Irf4 expression in CD11b+ cells from NOD mice was higher than that in NOD.Idd3 mice, both in unstimulated and PGN-stimulated cells; this was confirmed by real-time PCR (Figure 5, A and C). In support of RA possibly modulating Irf4 expression in NOD and NOD.Idd3 mice, transcripts of RA receptors were detected in both CD11b+ and T cells from these mice (Supplemental Figure 4B). Moreover, Irf4 expression was also higher in acutely activated T cells from NOD mice than in those from NOD.Idd3 mice (Supplemental Figure 4C). Together with relatively higher Irf4 expression in NOD-derived CD11b+ cells, this is consistent with increased RA signaling in NOD.Idd3 mice. In addition, we showed previously that RA inhibits Il23r expression in T cells (31). Indeed, while this probe was not included on the Affymetrix Mouse Genome
2.0 microarray, real-time PCR showed that T cells as well as CD11b+ cells from NOD mice expressed elevated levels of Il23r transcript compared with those in cells from NOD.Idd3 mice (Supplemental Figure 4D and Figure 5C).

Epoxide hydrolase 1 (EPHX1) is an enzyme that protects against oxidative stress; CXCR5 is a chemokine receptor; ficolin A (FCNA) is a lectin; and apelin receptor (APLNR) is a receptor for angiotensin. Genes encoding these proteins are reported to be regulated by RA (32–35) and were more highly expressed in NOD-derived than NOD.Idd3-derived CD11b+ cells, as determined by microarray and confirmed by real-time PCR (Figure 5, A and C). Interestingly, the expression of Irf4, Ephx1, Fcna, and Aplnr in NOD.Ildr KO CD11b+ cells showed a similar pattern to that observed in NOD.Idd3-derived CD11b+ cells (Figure 5D), suggesting a possible connection between the RA and IL-21 signaling pathways.

If differential RA signaling in NOD.Idd3- and NOD-derived APCs regulates Th17 differentiation, then increasing RA signaling in NOD-derived APCs could dampen Th17 differentiation to levels similar to those in cultures with NOD.Idd3-derived APCs. We therefore pretreated APCs from NOD mice with RA prior to coculture with naive T cells. When NOD-derived APCs were pretreated with RA, their ability to support Th17 polarization was dampened to the level observed in cultures with untreated NOD.Idd3-derived APCs (Figure 6, A and B). Furthermore, pretreatment of APCs in vivo by injecting RA into mice reduced their capacity to support generation of Th17 cells (Figure 6C). Lymphopenic mice (NOD/SCID) were used to avoid confounding effects resulting from stimulation of T cells with RA. Collectively, these data support the hypothesis that RA signaling is dysregulated in NOD mice relative to that of NOD.Idd3 mice and that this altered signaling acts on APCs to modulate Th17 differentiation in these 2 strains.

**Discussion**

Our data demonstrate that naive T cells from NOD mice exhibit a greater propensity to differentiate into Th17 cells than NOD.Idd3-derived T cells and that the balance of Tregs/Th17 cells is tipped toward pathogenic Th17 cells in vivo in NOD mice carrying the diabetes-susceptible Idd3 allele. Moreover, we show that NOD- and NOD.Idd3-derived APCs, specifically, CD11c+CD11b+ cells, differentially support Th17 differentiation, and this difference is likely...
to be regulated by IL-21. Lastly, our data suggest that RA signaling may be dysregulated in NOD mice, relative to that in NOD.Idd3 mice, and that this leads to altered function of APCs.

We showed previously that NOD-derived CD11b+ APCs inhibit Treg function (9). We now present data that show that this same APC population also promotes Th17 cell differentiation in NOD mice relative to that of NOD.Idd3 congenic mice. Indeed, we show for the first time to our knowledge that there is a defect in Th17 cell generation in NOD.Idd3 mice that is not only T cell intrinsic but also mediated by APCs. Interestingly, these defects are mirrored in APCs from NOD.Idd3 KO mice, suggesting that IL-21 signaling, in part, modulates APC function. IL-21 may act on APCs to induce production of mediators that, in turn, feedback onto T cells to further promote Th17 polarization. Indeed, PGE2 and IL-6, mediators that facilitate Th17 generation, are more highly produced by APCs from NOD mice relative to that produced by APCs from either NOD.Idd3 or NOD.Idd3 KO mice upon activation. It is plausible that IL-21 signaling not only acts directly on T cells to induce Th17 cell development and inhibit Tregs but also acts indirectly by conditioning APCs to produce mediators that further amplify proinflammatory Th17 cell differentiation and suppress Treg function. For instance, IL-6 can inhibit Treg induction (20, 36) and induce further IL-21 production (7), completing a positive feedback loop to maintain high IL-21 expression in NOD mice and boosting the inherent proinflammatory bias. In addition, under our experimental conditions, the Th17 polarization supported by NOD-derived and NOD.Idd3-derived APCs could be equalized after neutralization of IFN-γ, suggesting that IFN-γ is another possible mediator produced by APCs and/or induced in T cells by APCs to inhibit Th17 cell generation. This represents a mechanism by which APCs can skew T cell differentiation away from Th17 and ultimately protect mice from autoimmune disease in diabetes-resistant strains.

Gene profiling of CD11b+ APCs from NOD and NOD.Idd3 mice shows that expression of many genes differs, indicating that the Idd3 locus either directly or indirectly affects the development and effector functions of CD11b+ cells. IRF4, a transcription factor essential for Th17 cell development (29), is downregulated in CD11b+ cells from NOD.Idd3 mice as well as NOD.Idd3 KO mice, relative to that in cells from NOD mice. Given our recent data showing that RA inhibits Th17 cell development by inhibiting IL-6 signaling and downregulating Irf4 (31), it is conceivable that NOD.Idd3 mice have elevated RA signaling. In support of differential RA signaling in NOD and NOD.Idd3 mice, many genes reported to be regulated by RA signaling were differentially expressed by CD11b+ APCs from NOD and NOD.Idd3 mice. Since RA has been reported to tip the Treg/T effector cell balance toward regulatory cells (28, 31, 37), it is in line with NOD mice having a lower Treg/T effector ratio than NOD.Idd3 mice. This is also consistent with studies showing that RA treatment (38, 39) and vitamin A–rich diets (40) can inhibit the development of T1D. Moreover, RA and PGE2, an enhancer of Th17 cell development, are reported to be mutually antagonistic, whereby PGE2 inhibits synthesis of RA and vice versa (41, 42).

Since IRF4 induces IL-6 (43), increased Irf4 in NOD mice is in line with elevated IL-6 protein production by CD11b+ cells and consequently Th17 cell generation in these mice. While Il6 mRNA was not found to be differentially expressed in stimulated NOD and NOD.Idd3-derived CD11b+ APCs in our microarray analyses, this may simply reflect the different kinetics of IL-6 protein and mRNA expression. Given that IL-6 induces Il21 (7) and that Irf4 is required for IL-21–dependent generation and amplification of Th17 populations (30), this represents a convergence of pathways that lead to enhanced Th17 cell development. Furthermore, similar expression patterns of genes downstream of RA signaling in CD11b+ cells from both NOD.Idd3 and NOD.Idd3 KO mice suggest that IL-21 may affect RA signaling. Reduced capacity of RA-treated APCs from NOD mice to promote the generation of Th17 cells further supports a link between RA signaling and APC-driven modulation of Th17 cell development.

Earlier studies have implicated defective APCs in the development of autoimmune diabetes in NOD mice (44–46), but the molecular mechanisms responsible for the difference were not identified. Moreover, recent studies on T1D patients show a defect in APCs, in that they produce more IL-6 and IL-1 (47). This is consistent with APCs having an important role in T1D development by altering the balance of pathogenic Th17 cells and protective Tregs. We previously reported that the reduced suppressive capacity of Tregs from NOD mice was mediated by APCs (9, 10), and here we provide evidence that APCs are important regulators of proinflammatory Th17 cell development and that IL-21 signaling is pivotal to the T cell–APC cross-talk that modulates Th17 differentiation, which, in turn, determines susceptibility to autoimmunity. Most of the differences between NOD and NOD.Idd3 mice are recapitulated in NOD.Idd3 KO mice, suggesting an important role for IL-21, in addition to IL-2, in mediating the protective effects attributed to Idd3.

Clearly, RA-regulated pathways do not exist in isolation, and much of what we know about RA and its effects has been studied in other cell types. Further studies are required to elucidate how RA signaling operates in APCs and how this differs between NOD and NOD.Idd3 mice. Furthermore, understanding how IL-21 sig-
naling converges with the RA pathway to modulate APC function would uncover important avenues for regulating T cell responses and autoimmune diseases.

**Methods**

Mice and reagents. Female NOD and NOD.Idd3 (line 1098) mice were purchased from Taconic Farms as required. NOD.J21r mice were backcrossed onto the NOD background using a speed congenic approach (13). NOD.Idd3.J21r KO mice were generated by intercrossing NOD.Idd3 and NOD.J21r KO mice. Foxp3 gfp knockin (KI) reporter mice were generated as previously described (20) and then backcrossed 12 times onto NOD and NOD.Idd3 backgrounds. All experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee at Harvard Medical School.

Beads for MACS were purchased from Miltenyi Biotec. All flow cytometry reagents were purchased from BioLegend, except CD3-APC-e77 and 7-amino-actinomycin D (7-AAD), which were from BD Bioscience. Purified functional grade mAbs were obtained from BioXCell. Recombinant cytokines were purchased from R&D Systems.

**T cell activation.** CD4+CD62L+44+ naive cells were purified from prediabetic NOD and age-matched NOD.Idd3 or NOD.J21r KO mice by cell sorting using a FACSaria (BD Bioscience), after enrichment for CD4+ T cells by MACS. Naive T cells were activated with either 1 μg/ml plate-bound anti-CD3 (clone 145-2C11) and 2 μg/ml soluble anti-CD28 (clone PV-1) or with 1 μg/ml soluble anti-CD3 in the presence of self APCs irradiated with 33 Gy. Cytokine production in cell culture supernatants was determined after 48 hours.

**In vitro RA experiments.** CD11c+CD11b+ cells were further depleted by cell sorting. APCs were pre-treated with RA in vivo by injecting 0.5 mg RA in corn oil or corn oil alone i.p. into NOD/SCID mice on alternate days over 6 days; unfractionated splenocytes were used as APCs; for some experiments, CD11c+ or CD11c CD11b+ cells were further depleted by cell sorting. APCs were pre-treated with RA in vivo by injecting 0.5 mg RA in corn oil or corn oil alone i.p. into NOD/SCID mice on alternate days over 6 days; unfractionated splenocytes were used as APCs. For in vitro RA experiments, APCs (T cell–depleted splenocytes) were first pretreated with or without 10 nM RA (Sigma-Aldrich) for 20 hours. Cells were then washed, irradiated, and cultured with naive NOD–derived T cells under Th17-polarizing conditions. Polarized cells were analyzed by intracellular cytokine staining after 3 days.

**Intracellular cytokine staining.** Cells were stimulated for 4 hours with 50 ng/ml soluble anti-CD3 in the presence of self APCs irradiated with 199 μg/ml ionomycin (both from Sigma-Aldrich) in the presence of IL-2 (10 ng/ml) and IL-23 (25 ng/ml). Supernatants were assayed for cytokine production by cytometric bead array and PGE2 production using a Singleplex Multibead Kit from Assay Designs, both in accordance with the manufacturer’s instructions. For transcript profiling, CD11b+ cells were isolated by positive selection using MACS, and then CD11c CD11b+ cells were purified by flow cytometry. Purified cells were stimulated with or without PGN for 4 hours.

**Microarray analysis.** RNA was isolated using the RNAeasy Mini Kit (Qiagen), including a genomic DNA elimination column, according to the manufacturer’s instructions. cRNA probes were synthesized from RNA isolated from 2 independent experiments, and each was hybridized to Affymetrix Mouse Genome 430 2.0 microarrays at the Partners Center for Personalized Genetic Medicine. Microarray data have been deposited at the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24350; accession number GSE24350). Analysis of microarray data was performed using GenePattern software (Broad Institute). Transcripts with a coefficient of variance of greater than 0.55 for unstimulated cells and 0.7 for PGN-stimulated cells in the duplicate samples were excluded. Network analysis was performed using Ingenuity Pathways Analysis software (Ingenuity Systems Inc.).

**Statistics.** Statistical analysis was performed using unpaired, 2-tailed Student t tests, 1-way ANOVA, or Dunnett’s multiple comparison test and compared with the NOD reference group (Figure 6B). P values of less than 0.05 were considered statistically significant.

**Study approval.** The experimental methods were reviewed and approved by the Institutional Animal Care and Use Committee at Harvard Medical School.

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