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A T cell extrinsic mechanism by which IL-2 dampens Th17 differentiation

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Abstract

Genetic variants in *il2* and *il2ra* have been associated with autoimmune disease susceptibility in both genome-wide association studies (GWAS) in humans and in genetic linkage studies in experimental models of autoimmunity. Specifically, genetic variants resulting in a low IL-2 phenotype are susceptibility alleles while variants resulting in a high IL-2 phenotype are resistance alleles. The association of high IL-2 phenotypes with resistance has been attributed primarily to the T cell intrinsic promotion of regulatory T cell development, maintenance, and function; however, IL-2 can also act T cell intrinsically to dampen differentiation of pathogenic IL-17-producing Th17 cells. Here, we have uncovered a novel T cell extrinsic mechanism whereby IL-2 promotes both IFN-γ and IL-27 production from tissue resident macrophages which in turn dampen the differentiation of pathogenic Th17 cells.

Keywords

IL-17; T cells; Macrophages; Cytokines

1. Introduction

Single nucleotide polymorphisms (SNPs) in *Il2* as well as *Il2ra* have scored highly in genome-wide association studies (GWAS) in multiple human autoimmune diseases, including type 1 diabetes (T1D) and multiple sclerosis (MS) (1). Similarly, the *Idd3* genetic interval that encodes *Il2* has been identified as a key determinant of susceptibility in multiple experimental autoimmune diseases, including type 1 diabetes (2), experimental autoimmune encephalomyelitis (EAE) (3), and autoimmune ovarian dysgenesis (AOD) (4). NOD mice that carry the *Idd3* interval derived from diabetes-resistant C57BL/6 mice

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Conflict of interest disclosure

The authors declare no conflicts of interest related to this study.

(NOD.*Idd3* mice) are resistant to autoimmunity and T cells from NOD.*Idd3* mice produce more IL-2 than T cells from autoimmune-susceptible NOD mice (5–9). Thus, a high IL-2 phenotype is associated with resistance to autoimmunity while a low IL-2 phenotype is associated with susceptibility to autoimmunity.

The association of high IL-2 with resistance to autoimmunity has been primarily attributed to its requirement for the proper development, function, and maintenance of $Foxp3^+$ regulatory T cells (Treg) (10). However, IL-2 can also constrain the differentiation of pro-inflammatory IL-17-producing, Th17 cells (11,12). Thus, a high IL-2 phenotype would be expected to exhibit potent Treg function and dampened Th17 differentiation. Indeed, we (13), and others (5,8), have found that Treg from NOD.*Idd3* mice are more suppressive than Treg from NOD. We further found that NOD.*Idd3* T cells exhibit defective Th17 differentiation relative to NOD T cells (6). Most interestingly, we found that it was the $CD11b^+CD11c^-$ antigen presenting cells that were key determinants of both the differential Treg suppressor function and Th17 differentiation observed in NOD versus NOD.*Idd3* T cells (6,13). Together these observations raised the possibility that the T cell-derived cytokine IL-2 could act in a T cell extrinsic manner to modify the ability of $CD11b^+$ APC to support Treg suppressor function and/or inhibit Th17 differentiation. Here, we identify a mechanism by which IL-2 acts T cell extrinsically on tissue resident macrophages to dampen their ability to support Th17 differentiation.

2. Materials and Methods

2.1. Animals

6–9 week old Female NOD and NOD.*Idd3* mice were purchased from Taconic. NOD.FoxP3-GFP knock-in (KI) mice were bred and maintained at Taconic. All mice were housed in accordance with the guidelines established by the animal care and use committee at Harvard Medical School (Boston, MA).

2.2. Flow Cytometry

Single cell suspensions were stained with antibodies against CD4, CXCR3, CD11b, CD11c, MHC II, Ly-6C, and CD25 (BioLegend). For intracellular staining, cells were fixed in 4% paraformaldehyde, permeabilized in PBS 0.05% saponin and stained with antibodies against IL-27p28, IL-17A, IFN-γ, IL-10, or Tbet (BioLegend). All data were collected on a LSRII Flow Cytometer (BD Biosciences).

2.3. $CD11b^+$ stimulation assays

$CD11b^+CD11c^-$ cells were isolated from spleen by cell sorting. CpG was used at 0.5 μM, PGN 5 μg/ml, and IL-2 25 ng/ml. Cells were harvested at 4 h for RNA isolation and analyzed by real-time PCR. Culture supernatant was collected at 24 h for cytokine measurement by cytometric bead array (CBA) (BD Biosciences). For intracellular staining, cells were stimulated for 18 hrs, washed, and incubated for 4 h with Golgi Stop (BD Biosciences) prior to staining.

2.4. Th17 differentiation

Naïve $CD4^+$ $CD62L^{hi}$ $CD44^{lo}$ T cells were isolated by cell sorting and activated with soluble anti-CD3 (1 μg/ml), TGF-β (5 ng/ml), IL-6 (30 ng/ml) and irradiated T-depleted APC that had been cultured for 18 hours with CpG or CpG plus IL-2 and washed extensively prior to use. In some assays, anti-IFN-γ (XMG1.2) or anti-IL-27p28 (MM27-7B1) antibody was used at 10 μg/mL. Polarization was determined on day 3 by intracytoplasmic cytokine staining for IL-17A, IFN-γ and IL-10 after stimulation with 50ng/mL PMA and 1μg/mL ionomycin (Sigma-Aldrich) in the presence of Golgi-stop for 4 h.

2.5. Treg differentiation

Natural Treg were isolated from NOD.FoxP3-GFP KI mice by cell sorting and activated with soluble anti-CD3 (1 μg/ml) in the presence of irradiated T-depleted APC that had been cultured for 18 hours with CpG or CpG plus IL-2 and washed extensively prior to use. After 48 h, nTreg were harvested and examined for T-bet and CXCR3 expression by flow cytometry.

2.6. Real-time PCR

RNA was purified using RNeasy Plus Mini Kit (Qiagen) and cDNA synthesized using iScript (Bio-Rad). Real-time PCR was performed on either a 7900 system or ViiA 7 system (ABI) using TaqMan assays for IL-2rβ, IL-2rγc, IL-2rα, Ebi3, and IL-27 p28 (ABI). Transcript expression was normalized to β-actin.

3. Results

3.1. Tissue resident macrophages express high affinity IL-2R

Our previous work showed that the $CD11b^+CD11c^-$ antigen presenting cell population ($CD11b^+$ APC) is a major determinant of the differential Th17 differentiation in NOD vs NOD.*Idd3* mice (6). However, at that time, we did not address whether T cell-derived differences in IL-2 had a role in determining the ability of $CD11b^+$ APC to support Th17 differentiation. To address this possibility, we determined the expression of IL-2 receptor in $CD11b^+CD11c^-$ cells from both NOD and NOD.*Idd3*. We first examined the expression of IL-2 receptor beta chain (IL2Rβ) and gamma chain (IL2Rγc) mRNA and found that both IL2Rβ and IL2Rγc are constitutively expressed on $CD11b^+CD11c^-$ cells (Supplemental Fig. 1A). We examined whether this expression might change upon activation and found that although the $CD11b^+CD11c^-$ cells from NOD.*Idd3* showed an increase in IL2Rβ and IL2Rγc in some experiments, this was inconsistent across experiments and did not reach statistical significance. We further confirmed surface expression of IL2Rβ and IL2Rγc on $CD11b^+CD11c^-$ cells by flow cytometry (Supplemental Fig 1B).

We next examined the expression of high affinity IL-2 receptor alpha chain (CD25) on $CD11b^+CD11c^-$ cells. Interestingly, we found that while resting $CD11b^+CD11c^-$ cells express very little IL-2Rα mRNA, it is significantly up-regulated to similar levels in both NOD and NOD.*Idd3*-derived cells upon activation through either Toll-like receptor (TLR) 9 (CpG) or TLR2 (peptidoglycan) (Supplemental Fig. 1C). We further characterized the

population of CD11b$^+$ CD11c$^-$ cells that up-regulated CD25 as CD11b$^+$CD11c$^-$ MHC Class II$^+$ Ly6C^{low} tissue resident macrophages (Fig. 1B). Thus, tissue-resident splenic macrophages express high affinity IL-2R upon activation and are able to respond to IL-2.

3.2. IL-2 modifies CD11b$^+$ APC to suppress Th17 differentiation

IL-2 can suppress Th17 differentiation through a T cell intrinsic mechanism that requires STAT-5 and Aiolos (11,12). Our observation that activated tissue resident macrophages express high affinity IL-2 receptor led us to hypothesize that IL-2 could also act in a T cell extrinsic manner to suppress Th17 differentiation through modulation of tissue resident macrophages. Indeed, that NOD.*Idd3* T cells are high producers of IL-2 together with our finding that NOD.*Idd3*-derived CD11b$^+$ APC are defective in supporting Th17 differentiation (6) support the hypothesis that, in addition to T cell intrinsic effects, IL-2 may suppress Th17 differentiation via modulation of APC function. To test this, we examined the effect of IL-2 on the Th17-promoting ability of NOD-derived APC, which are normally effective at supporting Th17 differentiation (6). We found that while activation of NOD APC did not significantly affect Th17 differentiation, exposure to IL-2 significantly dampened the ability of activated NOD APC to drive Th17 differentiation (Fig. 2).

IFN-γ is known to suppress Th17 differentiation (14,15). Indeed, our previous data implicated IFN-γ as one of the mechanisms by which NOD.*Idd3*-derived CD11b$^+$ APC suppress Th17 differentiation (6). The immunosuppressive cytokine IL-27 also suppresses Th17 differentiation (16,17) and has been shown to induce IFN-γ and IL-10 and be protective in both EAE and type 1 diabetes (18–20). Accordingly, we examined whether IL-2 promotes IFN-γ and/or IL-27 production in CD11b$^+$ APC to suppress Th17 differentiation. We found that activated CD11b$^+$ CD11c$^-$ cells produced very little IFN-γ; however, activation in the presence of IL-2 significantly augmented IFN-γ production (Fig. 3A and Supplemental Fig. 1D). We next examined whether IL-2 similarly promotes IL-27 production in CD11b$^+$ CD11c$^-$ cells. We found that activation results in a trend towards increased p28 and EBi3 transcripts that does not reach statistical significance. However, activation in the presence of IL-2 results in a significant increase in both p28 and EBi3 (Fig. 3B). In line with these data, we found that intracellular IL-27 p28 is significantly increased only in CD11b$^+$ CD11c$^-$ cells activated in the presence of IL-2 (Fig. 3C). We further found that IL-2-driven IL-27 production is a unique property of CD11b$^+$ CD11c- cells as it is not observed in either B cells or dendritic cells (Supplemental Fig. 2).

We next addressed whether IL-2 driven IFN-γ and IL-27 were responsible for the reduced ability of IL-2-treated CD11b$^+$ APC to support Th17 differentiation. We found that neutralizing either IFN-γ or IL-27 reduced the extent by which IL-2 treated CD11b$^+$ APC suppress Th17 differentiation. However, only neutralizing both IFN-γ and IL-27 was able to fully restore Th17 differentiation to the levels observed with untreated APC (Fig. 3D). Thus, IL-2 drives both IFN-γ and IL-27 production in CD11b$^+$ APC and together these two cytokines dampen the generation of pathogenic Th17 cells.

3.3. IL-2 does not affect Tr1 and Th1 Treg induction by CD11b$^+$ APC

IL-27 has also been shown to promote the differentiation of IL-10-producing T regulatory 1 (Tr1 cells) that have an important role in suppressing autoimmunity (19,21,22). Our observation of IL-2 driving IL-27 production from CD11b$^+$ APC therefore raised the possibility that IL-2-driven increases in IL-27 in CD11b$^+$ APC may not only dampen Th17 responses but also promote the generation of protective Tr1 cells. Accordingly, we addressed whether IL-2 can modulate the ability of CD11b$^+$ APC to promote differentiation of Tr1 cells. We found that IL-2 treatment had no effect on the ability of APC to promote the generation of IL-10-producing cells from naïve T cells (Fig. 4A). In addition to Tr1 cells, Th17 cells can also produce IL-10 (23). Indeed, IL-10-producing Th17 cells constitute a non-pathogenic subset of Th17 cells. We therefore asked whether IL-2 treated APC can promote the generation of IL-10-producing non-pathogenic Th17 cells and found that IL-2 did not promote the generation of these cells (Fig. 4B). Lastly, both IFN-γ and IL-27 have recently been implicated in the promotion of Tbet$^+$CXCR3$^+$ Treg that exhibit specialized function for suppressing Th1 responses (24). Accordingly, we examined whether IL-2 stimulated APC can promote the generation of this Treg subset. We found that IL-2 did not significantly alter the differentiation of natural Treg (nTreg) to Tbet$^+$CXCR3$^+$ Treg (Fig. 4C). Collectively, our data show that IL-2 can act T cell extrinsically to dampen the ability of CD11b$^+$ APC to support pathogenic Th17 differentiation, thereby contributing to resistance to autoimmunity.

4. Discussion

Genetic variants that result in low IL-2 phenotypes have been linked with susceptibility to autoimmunity in both mice and man. In mice, the low IL-2 NOD strain is highly susceptible to T1D, EAE, and AOD while the high IL-2 NOD.*Idd3* strain is protected from disease (2–4). In humans, the SNPs that have scored in *il2r* in GWAS studies of MS result in increased production of soluble IL-2RA (25), which in effect lowers the amount of available IL-2. As IL-2 is critical for Treg function and maintenance, the association of low IL-2 phenotypes with autoimmunity has been largely attributed to a compromised regulatory T cell compartment. Our data uncover a novel T cell extrinsic mechanism by which IL-2 acts to promote tolerance over autoimmunity; IL-2 drives IFN-γ and IL-27 production in CD11b$^+$ APC to dampen pathogenic Th17 differentiation.

In addition to *Il2*, the *Idd3* interval encodes *Il21* and SNPs in *Il21* have also scored in GWAS in several human autoimmune diseases. Indeed, it has been observed that T cells from NOD and NOD.*Idd3* differ in their production of IL-21. T cells from NOD produce high IL-21 while T cells NOD.*Idd3* produce low IL-21 (7). Thus, a high IL-2/low IL-21 phenotype is associated with resistance to autoimmunity while a low IL-2/high IL-21 phenotype is associated with autoimmune susceptibility. IL-21 is known to act T cell intrinsically to suppress Treg differentiation and promote Th17 differentiation (26,27). Importantly, our previous work has shown that IL-21 can also act T cell extrinsically on CD11b$^+$ APC to promote Th17 differentiation and suppress Treg function via promotion of IL-6 and PGE2 (6). Our data now show that the IL-2 cytokine circuit also has an important T cell extrinsic component. We hypothesize that the T cell extrinsic action of IL-2 on

CD11b$^+$ tissue resident macrophages serves as a re-enforcing mechanism to stabilize T cell phenotypes that determine susceptibility versus resistance to autoimmunity. Our findings identify a novel non-T cell role for IL-2 and increase our current understanding of the mechanisms by which genetic variants that result in low IL-2 phenotypes determine susceptibility to autoimmune disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Variants in *Il2* are genetically linked to autoimmune susceptibility
- High IL-2 phenotypes are associated with resistance
- Activated tissue resident macrophages can sense IL-2
- IL-2 induces IFN-g and IL-27 in macrophages to dampen pathogenic Th17 differentiation
- IL-2 can act T cell extrinsically to influence autoimmune susceptibility

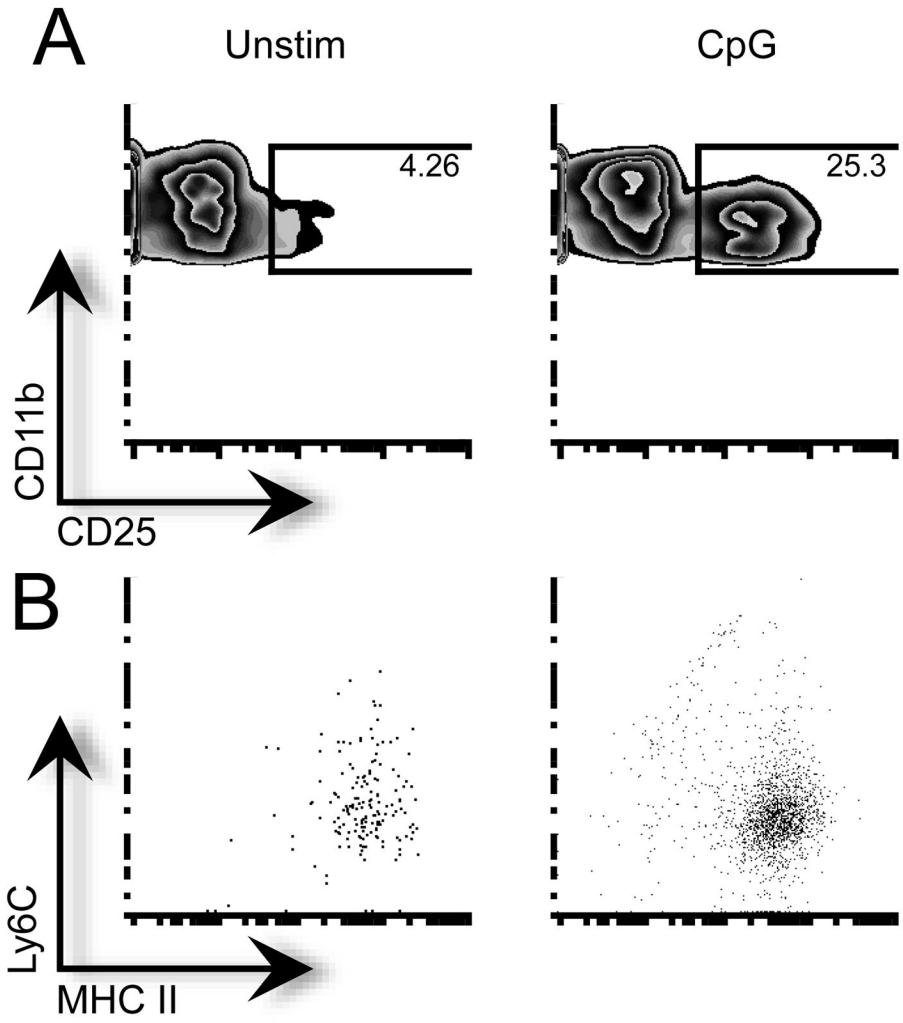

Figure 1. Tissue resident macrophages up-regulate high affinity IL-2R

$CD11b^+$ cells from NOD were cultured with media or CpG for 24 h. A) CD25 expression on $CD11b^+CD11c^-$ cells. B) Expression of MHC Class II and Ly6C on $CD11b^+CD11c^-CD25^+$ cells. Representative data are shown.

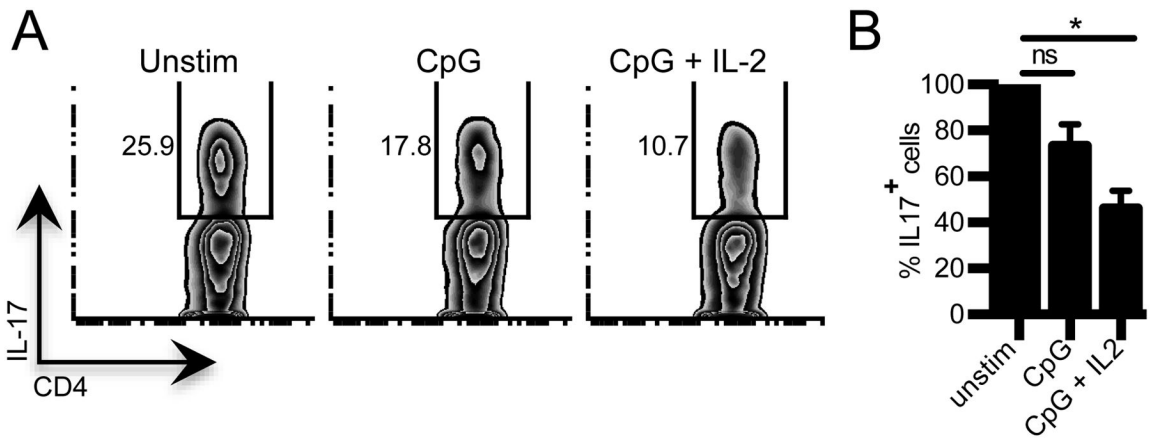

Figure 2. IL-2 modifies the ability of APC to support Th17 differentiation

Naïve NOD $CD4^+$ T cells were cultured under Th17 conditions with syngeneic T cell-depleted APC that were cultured for 18h with media (unstim), CpG, or CpG+ IL-2. Left panel, Representative flow cytometry data showing frequency of IL-17-producing cells in Th17 differentiation cultures. Right panel, summary data showing frequency of IL-17-producing T cells in Th17 differentiation cultures. Data are normalized to frequency of Th17 cells in cultures with unstim APC (n=3). *p=0.0034, one-way ANOVA, Tukey's multiple comparison test. ns=not significant.

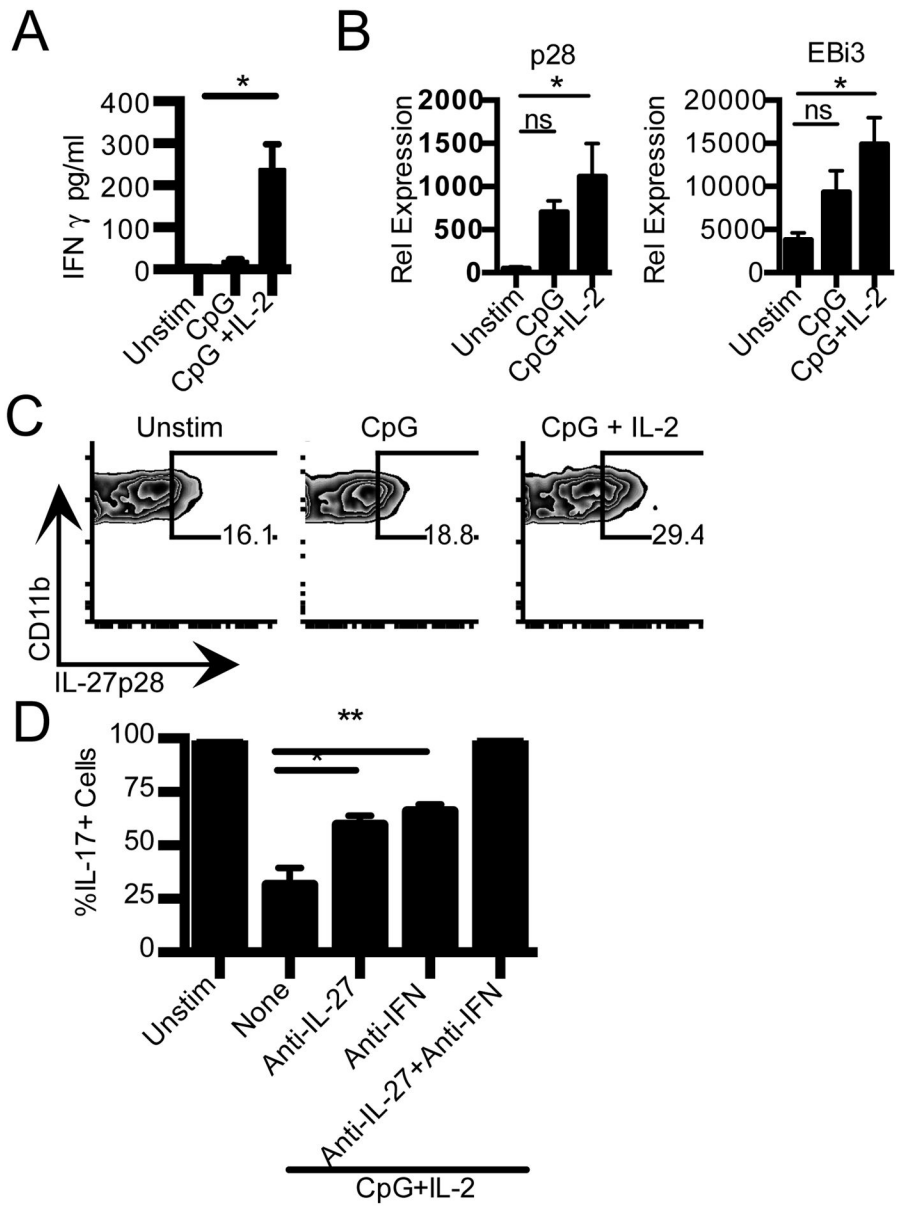

Figure 3. IL-2 promotes IFN-γ and IL-27 production from CD11b$^+$ CD11c$^-$ APC

A) CD11b$^+$CD11c$^-$ cells from NOD were stimulated with CpG, or CpG+ IL-2. IFN-γ production in culture supernatant at 24 h (n=5) was determined by CBA. *p=0.0074, t-test. B) CD11b$^+$CD11c$^-$ cells were stimulated as in (A) for 4 h. Cells were then lysed for RNA isolation and use in real-time PCR. Data are an average of 2–3 independent experiments. Left panel, IL-27 p28. Right panel, EBi3. *p<0.05, One-Way ANOVA, Tukey's multiple comparison test. C) NOD splenocytes were T-depleted and stimulated for 18h with media (unstim), CpG, or CpG+IL-2. Staining for IL27 p28 in CD11b$^+$CD11c$^-$ cells is shown. Data shown are representative of five independent experiments. D) Th17 differentiation of naïve NOD CD4$^+$ T cells cultured with syngeneic T cell-depleted APC that were cultured for 18h with media (unstim), CpG+ IL-2 in the presence or absence of anti-IFN-γ and anti-IL-27

p28 as indicated. Data are normalized to frequency of Th17 cells in cultures with untreated APC. *p=0.0334,**p=0.0148, One-Way ANOVA, Tukey's multiple comparison test.

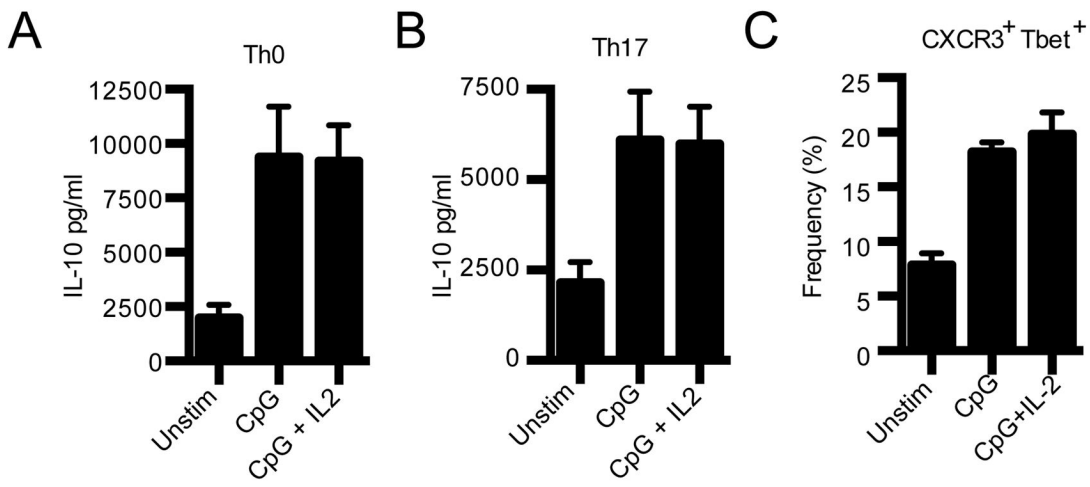

Figure 4. Generation of IL-10 producing cells by IL-2 stimulated APC

Naïve NOD CD4$^+$ T cells were cultured under neutral Th0 (A) or Th17 (B) conditions with T cell-depleted APC that were stimulated as indicated for 18h. On day 3 IL-10 in culture supernatant was determined by CBA (n=5). C) nTreg from NOD.FoxP3 KI mice were cultured with T cell-depleted syngeneic APC that were stimulated as indicated for 18h. Frequency of nTreg expressing T-bet and CXCR3 was determined by flow cytometry after 48h (n=3).