IL-23 Is Required for Neutrophil Homeostasis in Normal and Neutrophilic Mice[1]

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IL-23 is secreted by macrophages and dendritic cells in response to microbial products and inflammatory cytokines. IL-23 is a heterodimer composed of the unique IL-23p19 subunit linked to the common p40 subunit that it shares with IL-12. IL-23 is implicated in autoimmune diseases, where it supports the expansion of IL-17A-producing CD4$^+$ Th17 cells. IL-23 also regulates granulopoiesis in a neutrostat regulatory feedback loop through IL-17A-producing neutrophil regulatory (Tn) cells, most of which express $\gamma\delta$ TCR. This homeostatic system is disrupted in mice lacking adhesion molecules like β_2-integrins (*Itgb2$^{-/-}$*) which have defective neutrophil trafficking and neutrophilia. To test the role of IL-23 in the homeostatic regulation of circulating neutrophil numbers, we measured blood neutrophil numbers in p40-deficient (*IL12b$^{-/-}$*) mice and found them reduced compared with wild-type mice. *IL12b$^{-/-}$Itgb2$^{-/-}$* mice, lacking β_2-integrins, IL-12, and IL-23 showed significantly blunted neutrophilia compared with *Itgb2$^{-/-}$* mice. Treatment of both *IL12b$^{-/-}$* and *IL12b$^{-/-}$Itgb2$^{-/-}$* mice with IL-23, but not IL-12, restored circulating neutrophil counts. Serum levels of IL-17A were readily detectable in *Itgb2$^{-/-}$* mice, but not in *IL12b$^{-/-}$Itgb2$^{-/-}$* mice, suggesting that IL-17A production is reduced when IL-23 is absent. Similarly, tissue mRNA expression of IL-17A was reduced in *IL12b$^{-/-}$Itgb2$^{-/-}$*mice compared with *Itgb2$^{-/-}$* controls. The total number of CD3$^+$ IL-17A-producing Tn cells were significantly reduced in the spleen and lamina propria of *IL12b$^{-/-}$Itgb2$^{-/-}$* mice, with the largest reduction found in $\gamma\delta^+$ T cells. Our results suggest a prominent role of IL-23 in the regulation of granulopoiesis and the prevalence of IL-17A-producing Tn cells. ***The Journal of Immunology,*** **2007, 179: 8274–8279.**

Interleukin-23 is a cytokine involved in the pathogenesis of many autoimmune diseases including experimental autoimmune encephalitis (EAE),[3] rheumatoid arthritis, and inflammatory bowel disease (1–8). Before the discovery of IL-23, these diseases were considered to be primarily Th1-dependent and IFN-γ-driven through the production of IL-12 by activated macrophages (9, 10). IL-12 and IL-23 are heterodimers (p40p35 and p40p19, respectively) secreted by macrophages and dendritic cells (11). Studies using either p40-deficient (*IL12b$^{-/-}$*) mice or p40-blocking Abs disrupt the activities of both cytokines simultaneously, but this was not always recognized (12, 13). In the case of EAE, only since the generation of p35- and p19-deficient mice has the pathogenic role of IL-23 and not IL-12 become evident (1). This was further confirmed by anti-IL-23 therapy, which has been shown to ameliorate EAE, whereas anti-IL-12 Abs exacerbate the disease (1, 14–16). In inflammatory bowel disease, a genome-wide association study found a significant association between IL-23 receptor (IL-23R) polymorphisms and Crohn's disease (17). Therefore, IL-23 and/or its receptor are now attractive targets for the treatment of autoimmune diseases.

IL-23 mediates its proinflammatory effects through the activation of effector memory T cells that secrete IL-17A (18). There are three types of IL-17A-secreting T cells found in normal wild-type (WT) mice; $\gamma\delta^+$ T cells are the most abundant, followed by CD4$^+$CD8$^-$$\alpha\beta^{high}$ T cells (also termed Th17 cells) and the least abundant CD4$^-$CD8$^-$$\alpha\beta^{low}$ T cells, the role of which remains ill-defined (19, 20). IL-17A-producing $\gamma\delta^+$ T cells have been implicated in host response to *Mycobacterium tuberculosis* and *M. bovis* infections, with the Vδ1$^+$ T cell subset playing an important role in protection against *Escherichia coli* infections (21–23). Stimulation of purified $\gamma\delta^+$ T cells with IL-23 alone but not IL-6 or TGF-β enhances the production of IL-17A, and Ab blockade of IL-23 (p19) or the p40 subunit reduces IL-17A release in vitro in response to a variety of proinflammatory stimuli (21–23).

Th17 cells are distinct from Thl and Th2 cells (24, 25). Naive CD4$^+$ T cells only polarize into Th17 cells if Th1 (INF-γ and IL-12) and Th2 (IL-4) cytokines or transcription factors T-Bet and GATA-3 are blocked or removed, and if IL-6, IL-21 and TGF-β are present (24–32). The role of IL-23 in the activation and maintenance of IL-17A-expressing T cells remains controversial. Naïve CD4$^+$ T cells do not express IL-23R receptor and consequently, IL-23 does not have any effects on their differentiation or IL-17A production (26, 27, 29). We and others have shown that exogenously applied IL-23, but not IL-12, can increase the expression

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalitis; WT, wild type; BM, bone marrow; rm, recombinant mouse; MLN, mesenteric lymph node; LP, lamina propria. Tn, neutrophil regulatory T cell; Th17, IL-17 producing cox + T cells; 16-23R, 16-23 receptor; G-CSF, granulocyte-colony stimulating factor.

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and production of IL-17A from effector T cells (18, 19). Recently, a more complex role for IL-23 in vivo was demonstrated, with IL-6 and IL-21 up-regulating IL-23R expression on naive $CD4^+$ T cells, and IL-23, in combination with TGF-β, inducing the differentiation of Th17 cells (30–32). Upon activation, Th17 cells release the cytokines IL-17A, IL-17F, IL-22, TNF-α and IL-6, all of which can induce inflammatory responses (33, 34).

The IL-23/IL-17 axis regulates granulopoiesis via a neutrostat regulatory feedback loop (19, 20). Transgenic overexpression of IL-17A in murine livers elevates neutrophil counts, bone marrow (BM) and splenic colony forming units and the release of granlocyte-colony stimulating factor (G-CSF) from BM stromal cells (35, 36). In normal WT and neutrophilic mice, a positive correlation is found between neutrophil numbers and serum levels of IL-17A and G-CSF (37).

CD18 is the common β_2 subunit of the four β_2 integrins: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), CD11c/CD18, and CD11d/CD18. Mice with a targeted null mutation in the *Itgb2* gene lack all β_2 integrins and are neutrophilic, due to the inability of neutrophils to traffic into nonlymphoid tissues and altered hematopoiesis (19, 38). This was demonstrated by the transfer of WT neutrophils into *Itgb2*$^{-/-}$ mice, which lowered neutrophil counts to normal WT levels and reduced serum IL-17A levels and tissue expression of IL-23, but not IL-12 (19, 37). Phagocytosis of apoptotic neutrophils by *Itgb2*$^{-/-}$ macrophages or dendritic cells reduced IL-23 expression in these cells in vitro, thus closing the feedback loop (19).

To test the role of IL-23 upstream of IL-17A in the regulation of granulopoiesis in vivo, we generated *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice as a tool to determine the effects of IL-23 on granulopoiesis and IL-17A expression and secretion, and the prevalence of IL-17A -producing Tn cells in vivo. We found that IL-23 deficiency profoundly affects IL-17A expression and secretion, and thereby granulopoiesis in mice.

Materials and Methods

Animals and treatments

Mice lacking CD18 integrin (*Itgb2*$^{-/-}$) (38) or p40 (*IL12b*$^{-/-}$) (39) and WT C57BL/6 mice (The Jackson Laboratory) were used between 8 and 16 wk of age and kept in specific pathogen-free conditions in a barrier facility. Mice deficient in both CD18 and p40 were generated by breeding *Itgb2*$^{+/-}$ with *IL12b*$^{-/-}$ mice, and all mice were on a C57BL/6 background at least for ten generations. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Virginia. For Ab blockade of p40 (30517; R&D Systems), 100 μg of Ab or rat IgG1 isotype control (R&D Systems) per mouse was injected i.v. into WT mice and blood neutrophil counts assessed after 48 h. Some p40-deficient mice were injected with 2 μg of recombinant mouse (rm) IL-12, rmIL-23 (both R&D Systems) or saline as a control and blood neutrophil counts were assessed after 24 and 48 h via tail bleed into EDTA coated capillary tubes. Blood samples were then analyzed by automatic analyzer (Hemavet 850, CDC Technologies) and confirmed by Kimura-stained manual counts using a hemocytometer.

Recombinant proteins and Abs

The following mAbs (all reagents from BD Pharmingen, unless otherwise indicated) were used: Purified or Allophycocyanin-conjugated anti-CD3ε (145-2C11), allophycocyanin CY7 or PerCP-conjugated anti-CD4 (RM4–5), anti-CD16/CD32 (2.4G2; The Lymphocyte Culture Center, University of Virginia), purified anti-CD28 (37.51), FITC or allophycocyanin conjugated anti-TCR β-chain (H57–597), FITC or biotin-conjugated anti-$\gamma\delta$ TCR (GL3), PE-conjugated anti-IL-17A (TC11–18H10.1), and PerCP-conjugated streptavidin was used for the detection of the biotin-conjugated primary Ab.

Flow cytometry

Animals were anesthetized with an i.p. injection of a mixture containing ketamine hydrochloride (Sanofi Winthrop Pharmaceuticals, 125 mg/kg), xylazine (TranquiVed, Phoenix Scientific, 12.5 mg/kg), and atropine sulfate (Fujisawa USA, 0.025 mg/kg) then euthanized by cervical dislocation. Single cell suspensions of the spleen, mesenteric lymph node (MLN) and Peyer's patches were obtained by straining tissues through a 70-μm screen (BD Falcon). RBC were lysed and white blood cells washed twice with PBS-FBS. Lamina propria (LP) lymphocytes

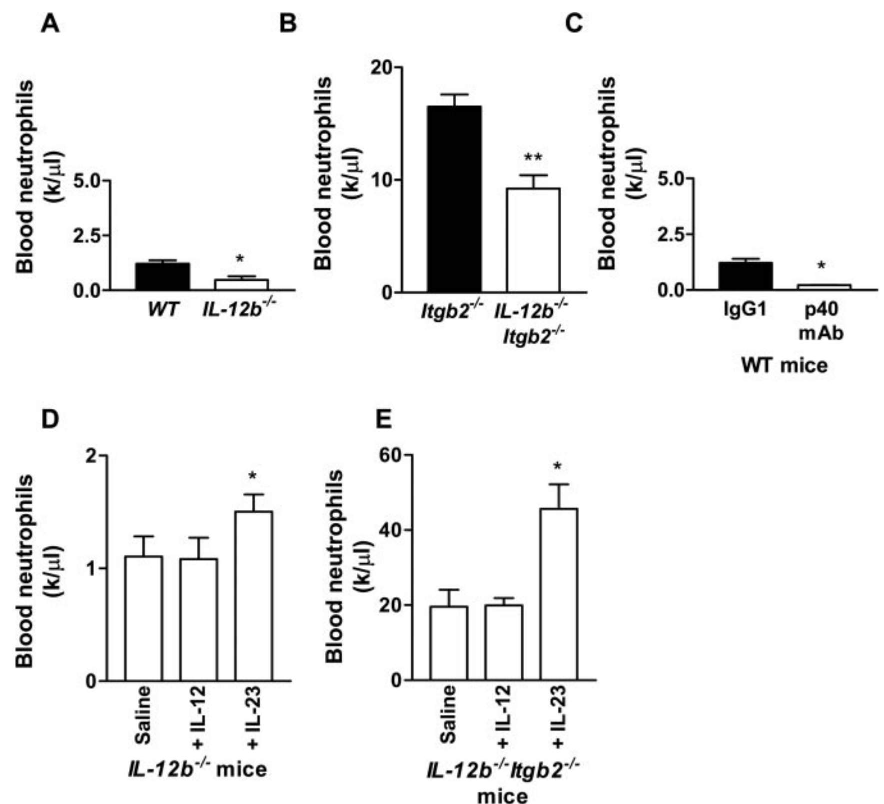

FIGURE 1. p40-deficient mice have impaired granulopoiesis. Circulating blood neutrophil levels (1000 cells per μl; k/μl) were assessed in (*A*) WT and *IL12b*$^{-/-}$ mice or (*B*) *Itgb2*$^{-/-}$ and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice (*, $p < 0.05$; **, $p < 0.01$ by unpaired students *t* test). *C*, Blood neutrophil counts were assessed in WT mice following treatment with a p40 Ab or IgG1 isotype control for 48 h (**, $p < 0.01$ by paired students *t* test). *IL12b*$^{-/-}$ mice (*D*) or *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice (*E*) were reconstituted with rmIL-23, rmIL-12, or saline and circulating neutrophil levels were analyzed at 24 h (*, $p < 0.05$ by paired Student's *t* test).

were prepared as previously described (40). Where indicated, cells were cultured in RPMI 1640 containing 10% FBS, 1× nonessential amino acids (Invitrogen Life Technologies), 10 mM HEPES, 2 mM L-glutamine (Invitrogen Life Technologies), 1 mM sodium pyruvate (Invitrogen Life Technologies), and 1% penicillin/streptomycin for 5 h with 10 ng/ml PMA (Sigma-Aldrich), 500 ng/ml calcium ionophore (Sigma-Aldrich), and GolgiStop (BD Pharmingen).

Following stimulation, cells were resuspended at 10^7/ml. Fcγ II/III receptors were blocked with 0.5 μg anti-CD16/CD32 and the cell suspension was incubated with an optimal concentration of mAbs for 20 min at 4°C in staining buffer (5% FBS in PBS) and washed. When biotinylated mAbs were used, streptavidin-PerCP was added for 20 min at 4°C, and cells washed twice in staining buffer. Intracellular staining was performed using Fix & Perm cell permeabilization reagents (Caltag Laboratories) according to the manufacturer's instructions. Flow cytometry analysis was performed on a Becton Dickinson FACSCalibur dual laser and data were analyzed using FlowJo software (Tree Star). Gates were set by isotype controls.

Cytokine quantification

IL-17A, CXCL1, and G-CSF protein in serum or cell culture supernatants were measured by DuoSet ELISA kits (R&D Systems) according to the manufacturer's directions. CCL2, IL-6, and IL-12 were measured using BD cytometric bead array, mouse inflammation kit (BD Biosciences) according to the manufacturer's instructions. Experimentally determined detection limits were calculated by adding two SDs to the mean OD value of replicate blanks.

mRNA quantification

RNA was extracted from tissue following homogenization in TRIzol (Invitrogen Life Technologies), according to the manufacturer's instructions. Reverse transcription and PCR steps were performed using QuantiTect SYBR Green RT-PCR Kit (Qiagen) as previously described (19). IL-17A primer sequences were as follows: sense: ATCCCTCAAAGCTCAGCGT GTC and anti-sense: GGGTCTTCATTGCGGTGGAGAG. One microgram of total RNA was used for all tissues. Values were determined using the iCycler iQ Real-Time Detection System Software v3.0 (Qiagen). The corresponding values were normalized to 18s RNA and then normalized to individual WT mouse organs as the calibrator control (always equal to 1), thereby expressing the values as relative quantification values.

Statistical analysis

Data were expressed as mean ± SEM. Statistical significance between groups was set at $p < 0.05$ using a two-tailed paired or unpaired t test.

Results

p40-deficient mice are neutropenic

To analyze the effects of IL-23 in vivo, we used mice lacking both IL-12 and IL-23 (*IL12b*$^{-/-}$) and investigated circulating neutrophil numbers. p40-deficient mice had 60% lower neutrophil numbers ($p < 0.05$) compared to healthy WT mice (Fig. 1*a*). As previously described, *Itgb2*$^{-/-}$ mice have severely elevated neutrophil counts (38), IL-23 message, and IL-17A-producing Tn cells (19). When *IL12b*$^{-/-}$ mice were crossed with mice suffering from severe neutrophilia (*Itgb2*$^{-/-}$) to create *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice, neutrophil numbers were significantly reduced compared to *Itgb2*$^{-/-}$ mice ($p < 0.01$) but still remained elevated compared WT mice (Fig. 1*b*). The *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice showed no additional pathology beyond the phenotype previously found in *Itgb2*$^{-/-}$ mice (data not shown) (38).

To confirm these findings, we used an Ab against p40 in WT mice, which severely reduced neutrophil numbers compared to the isotype control, confirming previous studies (Fig. 1*c*) (19). The neutropenia seen in *IL12b*$^{-/-}$ mice was reversed by reconstitution with rmIL-23, but not rmIL-12 (Fig. 1*d*). This reversal was transient with neutrophil numbers returning to baseline levels 48 h after treatment (data not shown). Reconstitution of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice with rmIL-23 significantly raised neutrophil counts after 24 h (Fig. 1*e*). RmIL-12 did not affect neutrophil counts compared to the saline controls. Thus, IL-23 and not IL-12

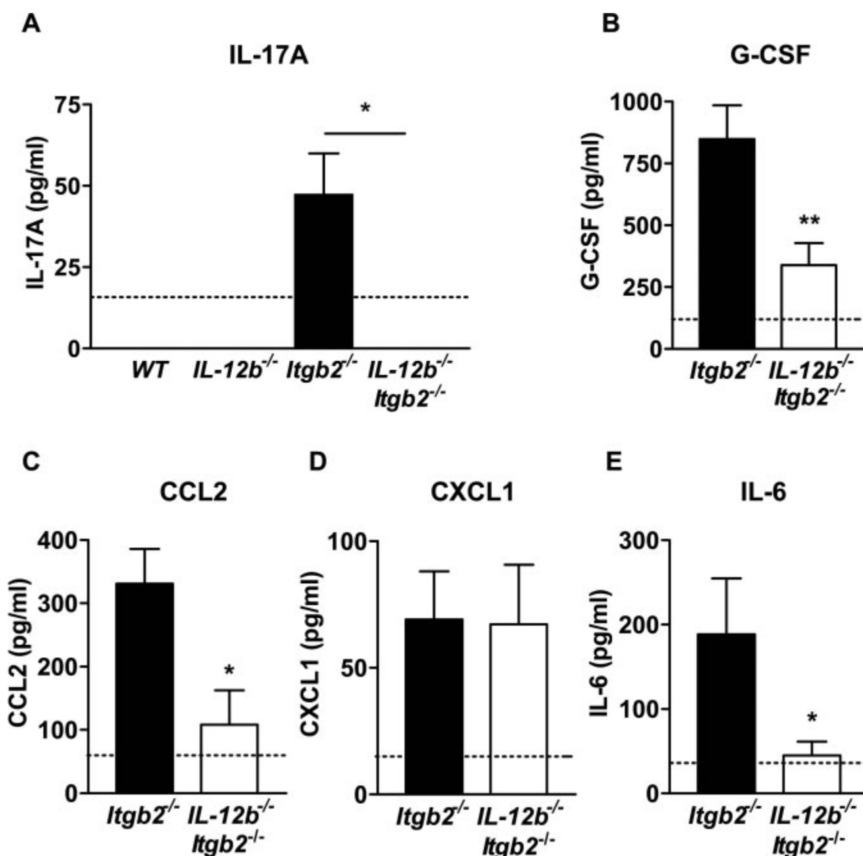

FIGURE 2. p40 deficiency affects circulating cytokine levels. Serum levels of IL-17A (detection limit; 15.7 pg/ml indicated by dashed line) (*A*), G-CSF (detection limit; 125 pg/ml) (*B*), CCL2 (detection limit; 62.5 pg/ml) (*C*), CXCL1 (detection limit; 15.7 pg/ml) (*D*), and IL-6 (detection limit; 31.3 pg/ml) (*E*) levels were assessed in WT, *IL12b*$^{-/-}$, *Itgb2*$^{-/-}$, and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice (*, $p < 0.05$; **, $p < 0.01$ by unpaired Student's t test).

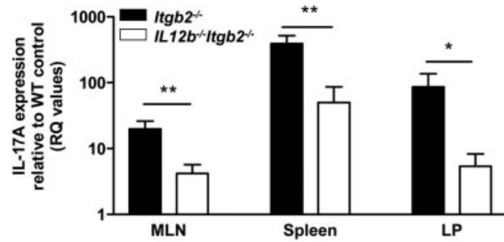

FIGURE 3. IL-17A mRNA expression is reduced in p40-deficient mice in vivo. mRNA expression of IL-17A was assessed in the MLN, spleen, and LP of *Itgb2*$^{-/-}$ and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice. Values are expressed on a log scale as relative quantification (RQ) values as determined by normalization of IL-17A to 18s RNA and expressed as fold increase compared with IL-17A expression (=1) in the respective WT mouse organs (*, $p < 0.05$; **, $p < 0.01$ by unpaired Student's *t* test).

is responsible for the regulation of granulopoiesis in p40-deficient mice on a normal or neutrophilic background.

p40 subunit regulates circulating levels of IL-17A

One of the major downstream cytokines of IL-23 is IL-17A (19). In this study, we analyzed the effects of p40 deficiency on circulating IL-17A levels in normal (WT), neutropenic (*IL12b*$^{-/}$ $-$), and neutrophilic (*Itgb2*$^{-/-}$ and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$) mice. IL-17A was not detectable by ELISA in the serum of WT mice, as

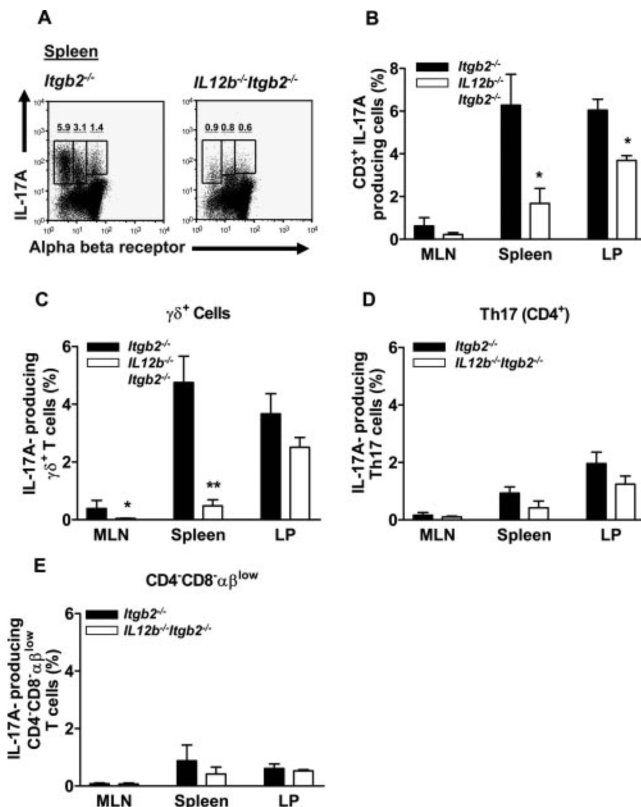

FIGURE 4. The proportion of IL-17A-producing Tn cells is reduced in p40-deficient mice. Single cell suspensions were prepared from the MLN, spleen, and LP of *Itgb2*$^{-/-}$ and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice and stimulated with a mixture of PMA, ionomycin, and GolgiStop for 5 h. *A*, Representative plots of the spleen from *Itgb2*$^{-/-}$ and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice. Total number of IL-17A-producing T cells (*B*), IL-17A-producing $\gamma\delta^+$ T cells (*C*), CD4$^+$ Th17 cells (*D*), and CD4$^-$CD8$^-$$\alpha\beta^{low}$ T cells (*E*), as a percentage of all CD3$^+$ T cells (*, $p < 0.05$; **, $p < 0.01$ by unpaired Student's *t* test).

previously reported (37), nor was it detectable in the serum of *IL12b*$^{-/-}$ mice. High levels of IL-17A were found in the serum of *Itgb2*$^{-/-}$ mice (37) however, IL-17A was not detectable in the serum of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice (Fig. 2*a*). This suggests that p40 is involved in regulating circulating levels of IL-17A in neutrophilic mice.

Cytokines downstream of IL-17A (G-CSF, CCL2, CXCL1, and IL-6) were analyzed in the sera of *Itgb2*$^{-/-}$ and *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice (Fig. 2, *b, c, d,* and *e*). G-CSF and CCL2 levels were significantly reduced ($p < 0.01$ and $p < 0.05$ respectively) in *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice, as was IL-6 ($p < 0.05$). CXCL1 was not affected by the p40 deficiency.

IL-17A expression and the number of IL-17A-producing Tn cells are reduced in p40-deficient mice

To explore whether a deficiency in IL-23 could affect the basal expression of IL-17A in neutrophilic mice, IL-17A mRNA expression was assessed in various organs of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ and *Itgb2*$^{-/-}$ mice. IL-17A expression was reduced in the MLN, spleen, and LP of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice compared to *Itgb2*$^{-/-}$ mice (Fig. 3). The IL-17A message levels were roughly mirrored by the percentage of IL-17A-producing Tn cells which were reduced in the MLN, spleen, and LP of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice compared to *Itgb2*$^{-/-}$ mice (Fig. 4, *a* and *b*). Culture of splenocytes on plate-absorbed anti-CD3, with soluble anti-CD28, with and without IL-23 for 3 days also demonstrated a significant reduction in the proportion of IL-17A-producing Tn cells from *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice compared to *Itgb2*$^{-/-}$ mice (data not shown). Analysis of the Tn cell subsets ($\gamma\delta^+$, CD4$^-$CD8$^-$$\alpha\beta^{low}$, and Th17) revealed that the number of IL-17A-producing $\gamma\delta^+$ Tn cells was significantly reduced in the MLN and spleen of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice compared to the control (Fig. 4*c*). The proportion of CD3$^+$ T cells that were Th17 cells or CD4$^-$CD8$^-$$\alpha\beta^{low}$ T cells were not significantly affected by the p40 deficiency (Fig. 4, *d* and *e*). This indicates that the expansion of IL-17A-producing $\gamma\delta^+$ cells in vivo is likely to depend on the presence of IL-23.

Discussion

The present data demonstrate a key role for IL-23 in regulating granulopoiesis. Both normal and neutrophilic (*Itgb2*$^{-/-}$) mice showed reduced circulating neutrophil counts when deficient in p40. The effect of the p40-containing cytokines, IL-23 and IL-12, on regulating neutrophil numbers was further established by Ab blockade against p40, which induced neutropenia in WT mice. Injection of rmIL-23 but not rmIL-12 into p40-deficient mice restored circulating neutrophil numbers. Circulating levels of IL-17A were not detectable in mice lacking p40. Concomitantly, expression of IL-17A mRNA was reduced in *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice, which correlated with a reduction in the percentage of IL-17A-producing Tn cells by flow cytometry. Thus, IL-23 plays a prominent role in the maintenance of IL-17A-producing Tn cells and therefore neutrophil homeostasis in vivo.

Adhesion-molecule-deficient mice demonstrate a neutrophilic phenotype, with elevated serum levels of IL-17A and G-CSF (37). These mice also contain expanded populations of IL-17A-producing Tn cells (19, 20). The neutrophilia seen in these mice is not due to the passive accumulation of neutrophils in the vasculature, nor due to alterations in the marginating pool found in the lung, but is primarily caused by increased granulopoiesis (37, 41). A reduction in neutrophil apoptosis may also contribute to neutrophilia in *Itgb2*$^{-/-}$ mice (42). Previous studies have shown that IL-17A can act directly on BM stromal cells to produce G-CSF, up-regulate the cell surface expression of stem cell factor, as well as stimulate

myeloid progenitor cell proliferation (36). Consistent with the IL-23-IL-17-G-CSF neutrophil-regulatory feedback loop (19), p40-deficient mice have a significant reduction in the number of hemopoietic progenitor cells in the bone marrow compared to WT mice (43). Mice lacking p40 also have reduced circulating neutrophil counts, which can be rescued by treatment with IL-23, but not IL-12. Taken together, these results indicate that IL-23 is a key regulator of granulopoiesis in mice.

In vitro studies have shown that IL-23 stimulates the production of IL-17A from memory $CD4^+$ T cells but not naive $CD4^+$ T cells (18). Initial reports describing the distinct lineage of Th17 cells showed that naive $CD4^+$ T cells could expand and secrete higher quantities of IL-17A when treated with IL-23 in the presence of blocking Abs against INF-γ and/or IL-4 (24, 25). However, Th17 cell polarization only occurred in the presence of IL-6 and TGF-β, and not in the presence of IL-23 (26, 27, 29). Furthermore, IL-23 did not induce the expression of RORγt, which was the first described transcriptional regulator of Th17 cells (44). Some in vitro studies have shown that IL-23 can expand existing effector memory populations of Th17 cells following polarization (26, 27). However, Bettelli et al. (29), did not find an expansion of the polarized Th17 pool following treatment with exogenous IL-23, nor did they find a reduction using anti-p40 blocking Abs. Therefore, it appeared that naive Th17 cells must first be polarized into a precursor Th17 cell which express IL-23R, and then IL-23 may act on these cells to further expand and/or maintain the cell population. IL-6 treatment of naive $CD4^+$ T cells can induce the expression of IL-23R, as can IL-21 (30–32). IL-6 can activate Stat3, which in turn activates RORγt and IL-21R expression. At this stage, the cells can be differentiated into Th17 cells independently IL-6, through the combination of IL-21 or IL-23 and TGF-β (30–32).

In the aforementioned studies, the polarization of Th17 cells was performed in vitro, a situation that cannot recapitulate the complex in vivo environment of secondary lymphoid tissues. In this study, we report a reduction in the number of IL-17A-producing cells, and IL-17A expression at the message, protein, and cellular levels in p40-deficient mice. In contrast to the in vitro findings, we find that IL-23 does support the polarization, survival and activation of IL-17A-producing Tn cells in vivo. Because Th17 cells show little response to IL-23 (29) and show little change in *IL12b*$^{-/-}$ mice (see Fig. 4), it is likely that $\gamma\delta^+$ T cells are the main Tn cell subset that respond to IL-23. The p40 deficiency also caused a reduction in serum levels of both IL-6 and IL-17A. Thus, it is possible that IL-23 may regulate the polarization of Th17 cells indirectly by making IL-6 available. Indeed, IL-6 is an indispensable ingredient in the cytokine mixes that promote the commitment of T cells to the IL-17A-producing phenotype (26, 27, 29).

Because IL-23 primarily acts on memory T cells enhancing the secretion of IL-17A (18), it is not surprising that the secretion of IL-17A was not detectable in the sera of *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice. This is consistent with the reduction in the number of IL-17A-producing Tn cells in *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice by 60%. The p40 deficiency also reduced serum levels of IL-6, CCL2, and G-CSF. In collagen-induced arthritis models, p40- or p19-deficient mice have reduced IL-17A and IL-6 mRNA expression compared with WT mice (4). p19 blocking Abs also reduce the secretion of IL-6, IL-17A, CCL2, and CXCL1 in colon homogenates of inflammatory bowel disease models and IL-17A in the sera of EAE mouse models (16, 45). Conversely, in CNS and colitis autoimmune models, IL-23 treatment increases the mRNA expression of IL-17A, CCL2, and IL-6 in $CD4^+$ T cells and the colon (3, 5).

IL-23 also plays a protective role in the host response to intracellular and extracellular bacterial and fungal infections through the regulation of proinflammatory cytokines and chemokines (46–49). Taken together, these studies indicate that multiple cytokine pathways are influenced by IL-23, which regulate inflammatory processes as well as controlling the production and release of neutrophils into the systemic circulation. The reduction in serum levels of both IL-17A and G-CSF in *IL12b*$^{-/-}$*Itgb2*$^{-/-}$ mice is consistent with the idea that IL-23 is the main upstream regulator of both of these proteins in vivo (19).

It is possible that polarization studies of Th17 cells in vitro do not faithfully replicate the in vivo situation, and as such, the role of IL-23 may have been underestimated. Most likely, $\gamma\delta^+$ T cells are the IL-17A-producers that respond most to IL-23. These cells have not been studied in vitro previously. The pathogenic role of IL-23 in autoimmune disease is well understood; however, the role of IL-23 in host response to infection by controlling granulopoiesis needs further definition. Our study shows a dominant role of IL-23 in the regulation of granulopoiesis and the prevalence of IL-17A-producing Tn cell subsets.

Disclosures

The authors have no financial conflict of interest.

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