SHORT COMMUNICATION

Myeloid-Derived IL-33 Limits the Severity of Dextran Sulfate Sodium—Induced Colitis

Li-Yin Hung, Christopher F. Pastore, Bonnie Douglas, and De'Broski R. Herbert

From the Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania

Accepted for publication
November 4, 2020.

Address correspondence to
De'Broski R. Herbert, Ph.D.,
University of Pennsylvania
School of Veterinary Medicine,
Philadelphia, PA
19104. E-mail: [debroski@vet.
upenn.edu](mailto:debroski@vet.upenn.edu).

IL-33 is an IL-1 family cytokine that signals through its cognate receptor, ST2, to regulate inflammation. Whether IL-33 serves a pathogenic or protective role during inflammatory bowel disease is controversial. Herein, two different strains of cell-specific conditionally deficient mice were used to compare the role of myeloid- versus intestinal epithelial cell-derived IL-33 during dextran sodium sulfate-induced colitis. Data show that loss of CD11c-restricted IL-33 exacerbated tissue pathology, coinciding with increased tissue *Il6* levels and loss of intestinal forkhead box p3⁺ regulatory T cells. Surprisingly, the lack of intestinal epithelial cell-derived IL-33 had no impact on disease severity or tissue recovery. Thus, we show that myeloid-derived IL-33 functionally restrains colitic disease, whereas intestinal epithelial cell-derived IL-33 is dispensable. (*Am J Pathol* 2021, 191: 266–273; <https://doi.org/10.1016/j.ajpath.2020.11.004>)

IL-33 is a pleiotropic cytokine involved in a wide array of biological processes, including cardiovascular disease, inflammatory bowel disease (IBD), allergic asthma, and host protective immunity against a variety of microbial and metazoan pathogens.¹ IL-33 signaling the heterodimeric IL-1 receptor accessory protein/ST2 receptor activates mitogen-activated protein kinases and NF- κ B in a myeloid differentiation primary response 88/interleukin-1 receptor-associated kinase 1/TNF receptor-associated factor 6-dependent manner to drive cytokine and chemokine production and cellular proliferation.² As per the current paradigm, it is released following damage of structural cells (eg, epithelia, endothelia, and fibroblasts), but accumulating evidence indicates that IL-33 can be expressed by an array of myeloid cells, including macrophage, mast cell, and dendritic cell populations.^{2–5} In the context of IBD, IL-33 has been shown to have both protective⁶ and pathogenic roles⁷ through mechanisms that remain largely undefined but could be due to alteration of forkhead box p3 (Foxp3)⁺ regulatory T-cell (Treg) function.⁸ Whether endogenous IL-33 expression occurs within disparate cell lineages of hematopoietic versus nonhematopoietic origin is entirely unclear.

This study investigated the selective role of IL-33 in epithelial versus myeloid lineage cells. Data show that the cellular source of IL-33 is critical in the context of dextran

sulfate sodium (DSS) colitis. Our results show that both intestinal epithelial cells (IECs) and multiple intestinal myeloid cell populations produce IL-33 protein during DSS colitis. Although IECs were a more predominant source of IL-33, deletion in this population did not alter disease outcome and resulted in reduced production of the proinflammatory cytokine IL-6. In contrast, mice selectively deficient for IL-33 in CD11c⁺ cells developed severe intestinal pathology, significantly increased *Il6* levels, and decreased related orphan receptor γ t⁺Foxp3⁺ Treg frequencies relative to controls. These data indicate that IL-33 can serve distinct roles in DSS colitis, depending on cellular source.

Materials and Methods

Mice

IL-33^{flox/flox}—internal ribosome entry sites—green fluorescent protein (iTL IC1 C57BL/6) line was a generous gift from Dr. Paul Bryce (Northwestern University, Evanston,

Supported by NIH grants U01AI125940, AI095289, and GM083204; and the Burroughs Wellcome Fund (to D.R.H.).

Disclosures: None declared.

IL). CD11cCre [B6.Cg-Tg(Itgax-cre)1-1Reiz/J; number 008068] and VillinCre [B6.Cg-Tg(Vil1-cre)997Gum/J] mice were purchased from The Jackson Laboratory (Bar Harbor, ME; number 004586). All mice were housed under specific pathogen-free conditions at University of Pennsylvania (Philadelphia, PA). All procedures were Institutional Animal Care and Use Committee—approved (protocol 805911).

DSS-Induced Colitis Model

Mice were given autoclaved water containing 2.5% DSS (MP Biomedicals, Solon, OH) for 6 days, then regular water for another 4 to 5 days. Disease activity index was scored on the basis of grooming, piloerection, gait, posture, mobility, stool consistency, rectal bleeding, and fecal occult blood test.

Enzyme-Linked Immunosorbent Assay

Culture supernatants from restimulated mesenteric lymph nodes were stored at -80°C before analysis using an IL-6 enzyme-linked immunosorbent assay. The enzyme-linked immunosorbent assay kit was purchased from Thermo Fisher (Waltham, MA) and used according to the manufacturer's instruction.

Flow Cytometry

Flow data were acquired on LSR-Fortessa (BD Biosciences, San Jose, CA) and analyzed with FlowJo 9.9.6 (FlowJo LLC, Ashland, OR). Antibodies from Biolegend (San Diego, CA) were used: B220 (clone RA3-6B2), CD4 (GK1.5), CD11b (M1/70), CD19 (1D3), CD45 (30-F11), CD44 (IM7), CD64 (X54-5/7.1), Ly6C (HK1.4), T cell receptor β (H57/597), and IL-6 (MP5-20F3). Antibodies/reagents were from Thermo Fisher: Aqua Live/Dead viability dye, Foxp3 (FJK-16s), and NK1.1 (PK136). Anti-ROR γ t (Q31-378) was from BD Biosciences. IL-33 (396118) antibody was purchased from R&D Systems (Minneapolis, MN). For cytokine detection, lamina propria cells were incubated with brefeldin A (Sigma, St. Louis, MO) for 4 hours before staining.

Real-Time PCR

RNA from colon was isolated using NucleoSpin RNA Plus kit (Macherey-Nagel, Dueren, Germany), and cDNA was generated with Maxima H Minus RTase (Thermo Fisher). Real-time quantitative PCR was run and analyzed on CFX96 platform (BioRad, Hercules, CA). Primers were as follows: *Gapdh*, 5'-AGGTCGGTGTGAACGGATTTG-3' (forward) and 5'-TGTAGACCATGTAGTTGAGGT-3' (reverse); and *Il6* primer sequence, 5'-CCAGTTTGGTAGCATCCAT-3' (reverse) and 5'-TTCACA AGTCG-GAGGCTT-3' (forward).

Statistical Analysis

Statistical analyses were performed using Prism7 (Graph-Pad, La Jolla, CA).

Results

IL-33 Is Expressed during DSS-Induced Colitis, but Epithelial-Derived IL-33 Does Not Cause Pathology

In both ulcerative colitis and DSS-induced colitis, IL-33 is detected in inflamed tissues with expression from both epithelial and immune cells, particularly myeloid antigen-presenting cells.^{9,10} Therefore, DSS-induced colitis model was employed and animals monitored for changes in IL-33 expression in whole tissue. Mice were administered 2.5% DSS for 6 days and returned to normal drinking water to distinguish the initial injury versus repair phase of the disease¹¹ (Figure 1A). *Il33* transcript levels in whole tissues were transiently up-regulated during the peak of inflammatory tissue damage during DSS colitis (Figure 1B). To understand the contribution of IL-33 from disparate cell types, two conditional knockout mouse strains lacking IL-33 in the myeloid compartment (CD11c^{Cre} driver) or the IEC compartment (Villin^{Cre} driver) were generated. These strains were used to test whether cell source of IL-33 had a major impact on the outcome of disease caused by DSS administration (Figure 1C).

The importance of epithelial-derived IL-33 was evaluated in this model as epithelial cells are considered a major source of IL-33 and are abundant in the colon.¹² Unexpectedly, Villin^{Cre} $\times$ IL-33^{fl/fl} mice did not develop more severe disease when compared with Villin^{Cre} controls (Figure 1, D-E) despite evidence that IL-33 was selectively lost in the epithelial compartment. Tissue damage, infiltration of inflammatory cells, and colon lengths were all equivalent between the two strains (Figure 1, F-H). Interestingly, cells within mesenteric lymph nodes produced more IL-6 in the Villin^{Cre} $\times$ IL-33^{fl/fl} mice when compared with controls (Figure 1I). These findings indicated that loss of IEC-derived IL-33 did not adversely affect disease outcome in DSS-induced colitis.

Myeloid-Derived IL-33 Contributes to Tissue Repair in DSS Colitis

Although myeloid cells are considered a minor source of IL-33 in different tissue contexts, we next tested whether CD11c^{Cre} IL-33^{fl/fl} mice experienced any alterations in disease progression or severity. Compared with cohoused CD11c^{Cre} controls, CD11c^{Cre} IL-33^{fl/fl} mice exhibited significantly greater weight loss (Figure 2A) and disease activity scores (Figure 2B). At the termination of the experiment on day 10, CD11c^{Cre} IL-33^{fl/fl} mice had significantly shorter colon lengths compared with CD11c^{Cre}

Figure 1 Expression of *IL33* and the role of epithelial-derived IL-33 during dextran sulfate sodium (DSS)-induced colitis. **A:** Experimental design of DSS-induced colitis. **B:** Whole colon tissue from wild-type (C57BL/6) mice was isolated at indicated time points during colitis, and *IL33* transcript levels were assessed via real-time quantitative PCR. **C:** Schematics of green fluorescent protein (GFP)-*IL-33^{fl/fl}* allele. **D** and **E:** Weight change (**D**) and disease activity scores (**E**) of control versus *Villin^{Cre} × IL-33^{fl/fl}* mice during the course of DSS-induced colitis. **F** and **G:** Hematoxylin and eosin staining of colon tissue from control (**F**) versus *Villin^{Cre} × IL-33^{fl/fl}* (**G**). **H:** Colon length of control versus *Villin^{Cre} × IL-33^{fl/fl}* mice on the day of euthanasia. **I:** Single-cell suspensions from mesenteric lymph nodes were plated onto anti-CD3-coated or media-only plate for 72 hours, and IL-6 levels in the supernatants were measured by enzyme-linked immunosorbent assay. **H** and **I:** Each symbol represents one mouse. Representative results from three experiments. Data are shown as means $\pm$ SEM. $N = 3$ for each time point (**B**). * $P < 0.05$ compared with day 0; ** $P < 0.01$ (two-way analysis of variance). Scale bar = 20 μ m (**F** and **G**). Original magnification, $\times 600$ (**F** and **G**). DAI, disease activity index; Neo, neomycin resistance cassette; IRES, internal ribosomal entry sites.

controls (Figure 2C). Consistent with evidence that myeloid-derived inflammatory cytokines can drive disease pathology in the DSS model, colon *Il6* transcript levels were significantly higher in *CD11c^{Cre} × IL-33^{fl/fl}* colons compared with *CD11c^{Cre}* controls (Figure 2D). Flow cytometry analysis showed that IL-6 was produced by

multiple cell types, including epithelial and various myeloid cell populations. However, the increase in IL-6 production in *CD11c^{Cre} × IL-33^{fl/fl}* colons was evident only in the *CD64⁺Ly6C⁺* subset (Figure 2, E–G). Concordantly, although *CD11c^{Cre}* control colon tissues showed signs of recovery, such as restoration of epithelial architecture and

appearance of goblet cells on day 10, $CD11c^{Cre} \times IL-33^{fl/fl}$ colons remained inflamed and lacked the normal reappearance of mucus-producing goblet cells, as determined by periodic acid–Schiff histologic staining (Figure 2, H and I).

Myeloid-Derived IL-33 Is Required for Accumulation of Suppressive Tregs

Because Tregs suppress excessive cytokine responses in both mouse and human colitis^{13,14} and IL-33 both expands Treg numbers and promotes their suppressive functions,¹⁵ the impact of loss of myeloid IL-33 on intestinal Treg subsets was studied. Among Treg subsets that suppress inflammation, $Foxp3^{+}ROR\gamma t^{+}$ Tregs selectively suppress type 17 helper T-cell (Th17) responses, whereas $Gata3^{+}Foxp3^{+}$ Tregs may preferentially suppress type 2 helper T-cell responses.^{16,17} Using a flow cytometry approach, the expression of Foxp3 and ROR γt by T cells was evaluated in colon lamina propria (Figure 3A). No difference was found in $Foxp3^{+}$ or $ROR\gamma t^{+}Foxp3^{+}$ Treg populations when comparing $Villin^{Cre} \times IL-33^{fl/fl}$ and $Villin^{Cre}$ controls (Figure 3, B–E). Intriguingly, there was an increased percentage of $ROR\gamma t^{+}$ Th17 cells in $Villin^{Cre} \times IL-33^{fl/fl}$ mice (Figure 3C). Conversely, the frequency of $Foxp3^{+}ROR\gamma t^{+}$ Tregs was significantly reduced in $CD11c^{Cre} \times IL-33^{fl/fl}$ mice compared with $CD11c^{Cre}$ controls (Figure 3, E–G). Together, these data indicate that the loss of myeloid-derived IL-33 led to a significant reduction in a Th17-associated Treg suppressive response.

Discussion

This work reveals that the cellular context in which IL-33 is expressed has a dominant role over its biological function in the setting of murine colitis induced by DSS. Although treatment of IL-33 drives proliferation of IECs during the recovery phase of colitis,⁶ and can have a protective role when administered during recovery from DSS-induced colitis,¹⁸ previous studies in mouse and humans with colitis established that IL-33/ST2 signaling can have contradictory effects. IL-33 and ST2 are up-regulated in both Crohn disease and ulcerative colitis mucosa,⁹ and both $St2^{-/-}$ and $Il33^{-/-}$ mice are protected from acute DSS-induced colitis.¹⁹ Conversely, $Il33^{-/-}$ and $St2^{-/-}$ fail to recover from the disease at the chronic phase and have more severe tissue inflammation.^{6,10} This confusion may be due to multiple factors, including microbial composition, the specific type of IBD, and/or cytokine milieu.^{8,20}

Our findings potentially explain the controversy surrounding the role of IL-33 in IBD, inasmuch as the data reveal that IL-33 is expressed in broadly distinct cell lineages (Supplemental Figure S1) that, when specifically lost in a cell-lineage restricted manner, results in opposing phenotypes, particularly in different phases of this relapsing-remitting disease. This suggests that IL-33 serves an

inflammatory or anti-inflammatory role, depending on kinetics and cellular context. Further investigation is needed to understand the impact of epithelial- versus myeloid-derived IL-33 at different stages of the disease and the mechanism(s) by which it functions.

IL-6 is a key cytokine involved in the pathogenesis of IBD. Increased serum IL-6 levels positively associate with disease severity in both ulcerative colitis and Crohn disease patients,^{21,22} and treating mice with anti-IL-6 monoclonal antibody attenuates disease severity in DSS-induced colitis. Large-scale genomic studies also show that reduced IL-6 signaling is associated with lower IBD risks.²³ Consistent with previous reports, higher tissue *Il6* levels were found in $CD11c^{Cre} \times IL-33^{fl/fl}$ colons in comparison to controls during DSS-induced colitis. Besides directly activating inflammatory responses, IL-6 promotes differentiation of Th17 cells that are pathogenic in colitis.²⁴ Intriguingly, increased Th17 or higher *Il17* was absent in colon lamina propria from $CD11c^{Cre} \times IL-33^{fl/fl}$ mice. It is unclear whether effector Th17 cells have migrated out of tissue at this time. Future experiments that track the emergence and migration of effector cells in control versus $CD11c^{Cre} \times IL-33^{fl/fl}$ versus $Villin^{Cre} \times IL-33^{fl/fl}$ mice will provide more insights on how cellular sources of IL-33 can affect IL-6 production and effector T-cell populations throughout the course of DSS-induced colitis. Interestingly, the increased production of IL-6 in $CD11c^{Cre} \times IL-33^{fl/fl}$ mice mainly comes from a $CD64^{+}Ly6C^{+}$ population (Figure 2G) that also expresses CCR2 and CX3CR1 (data not shown) in colon lamina propria. Published work from Bain et al²⁵ identified a similar population ($CD64^{+}Ly6C^{+}CX3CR1^{int}CCR2^{+}$) in colon that rapidly expands on DSS treatment in a CCR2-dependent manner and expresses high levels of proinflammatory cytokines, such as inducible nitric oxide synthase, tumor necrosis factor- α , IL-1 β , and IL-6. Additional research is required to understand whether myeloid-derived IL-33 has an impact on the recruitment, retention, and inflammatory function of this population.

Tregs suppress inflammatory responses and are critical in dampening tissue damage. In the intestine, multiple Treg subsets have been identified on the basis of co-expression of effector T-helper subset–specific transcription factors along with Foxp3.²⁶ Prevailing theory posits that $Foxp3^{+}$ Tregs selectively suppress the effector populations that share lineage-specific transcription factors, such as GATA3 (type 2 helper T cell), T-box transcription factor (type 1 helper T cell), and ROR γt (Th17). Of these co-expressing populations, myeloid-derived IL-33 was selectively important for sustaining the $ROR\gamma t^{+}Foxp3^{+}$ Treg subset in this model. Although $Gata3^{+}Foxp3^{+}$ Tregs are a critical immune modulator of mucosal infection and inflammation,^{27,28} there were no significant differences of this population between control and $CD11c^{Cre} \times IL-33^{fl/fl}$ mouse strains, as both displayed low frequencies of $Gata3^{+}Foxp3^{+}$ Tregs (data not shown). Instead, DSS-mediated tissue injury generates a type 1 helper

Figure 2 Loss of CD11c-restricted IL-33 exacerbates dextran sulfate sodium (DSS)-induced colitis. **A** and **B**: Weight change (**A**) and disease activity scores (**B**) of control versus CD11c^{Cre} × IL-33^{fl/fl} mice during the course of DSS-induced colitis. **C**: Colon length of control versus CD11c^{Cre} × IL-33^{fl/fl} mice on the day of euthanasia. **D**: Levels of *Il6* transcripts in whole colon tissues from control and CD11c^{Cre} × IL-33^{fl/fl} mice on the day of euthanasia. **E**: Gating strategy to assess IL-6 levels in epithelial and myeloid cells. **F** and **G**: Percentage of IL-6⁺ cells in epithelial cells (**F**) and various subsets of myeloid cells (**G**), as gated in **E**. Periodic acid–Schiff stain of colon biopsies from control (**H**) and CD11c^{Cre} × IL-33^{fl/fl} (**I**) mice on day 10. Representative results from three experiments are shown. Each symbol represents one mouse. Data are shown as means ± SEM. **P* < 0.05, ***P* < 0.01 (*t*-test); ††*P* < 0.01 (two-way analysis of variance). Scale bar = 50 μm (**H** and **I**). Original magnification, ×600 (**H** and **I**). DAI, disease activity index; DC, dendritic cell; EpCAM, epithelial cell adhesion molecule; FSC, forward scatter; MHC, major histocompatibility complex.

Figure 3 Loss of myeloid-derived IL-33 leads to decreased related orphan receptor (ROR) γ^t regulatory T cells during dextran sulfate sodium–induced colitis. **A:** Gating strategy to identify regulatory T-cell subsets within CD4⁺ CD44⁺ T cells in colon lamina propria (LP). **B–D:** Frequencies of Foxp3⁺ (**B**), ROR γ^t ⁺ (**C**), and ROR γ^t ⁺Foxp3⁺ (**D**) in colon LP from Villin^{Cre} × IL-33^{fl/fl} mice. **E–G:** Frequencies of Foxp3⁺ (**E**), ROR γ^t ⁺ (**F**), and ROR γ^t ⁺Foxp3⁺ (**G**) in colon LP from control versus CD11c^{Cre} × IL-33^{fl/fl} mice. Each symbol represents one mouse. Representative results of three experiments are given. Data are shown as means ± SEM (**B–G**). * $P < 0.05$, *** $P < 0.005$ (t -test). TCR, T cell receptor.

T-cell/Th17—dominant microenvironment that has antagonizing effects on the Gata3⁺Foxp3⁺ Treg population.²⁷ Thus, expansion of the RORγt⁺Foxp3⁺ Treg subset would be expected in the DSS colitis context. Indeed, CD11c^{Cre} × IL-33^{fl/fl} mice had a selective reduction of intestinal RORγt⁺Foxp3⁺ Tregs compared with CD11c^{Cre} controls. This implies that IL-33 can potentially control excessive IL-17 responses following tissue injury, which has been reported in the context of allergic inflammation, where IL-33 regulates innate IL-17 production from innate lymphoid cell subsets.²⁹ However, RORγt⁺Foxp3⁺ Tregs can produce either IL-10 or IL-17, depending on the setting.³⁰ Furthermore, the maintenance of intestinal RORγt⁺Foxp3⁺ Tregs depends partially on a healthy and complex microflora,³¹ and loss of microbial complexity in IBD patients has been associated with disease pathogenesis.^{32,33} Therefore, further work will be required to decipher the importance of microbial composition and whether myeloid-derived IL-33 controls the cytokine-producing potential of Foxp3⁺ Treg subsets under basal or inflammatory conditions in distinct tissue microenvironments.

Acknowledgment

We thank Dr. Paul Bryce (Northwestern University, Evanston, IL) for IL-33flox/flox—IRES—green fluorescent protein (iTL IC1 C57BL/6) line.

Author Contributions

L.-Y.H. and D.R.H. designed the experiments and wrote the article; L.-Y.H., C.F.P., and B.D. performed experiments and analyzed data.

Supplemental Data

Supplemental material for this article can be found at <http://doi.org/10.1016/j.ajpath.2020.11.004>.

References

- Liew FY, Girard JP, Turnquist HR: Interleukin-33 in health and disease. *Nat Rev Immunol* 2016, 16:676–689
- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA: IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005, 23:479–490
- Furukawa S, Moriyama M, Miyake K, Nakashima H, Tanaka A, Maehara T, Iizuka-Koga M, Tsuboi H, Hayashida JN, Ishiguro N, Yamauchi M, Sumida T, Nakamura S: Interleukin-33 produced by M2 macrophages and other immune cells contributes to Th2 immune reaction of IgG4-related disease. *Sci Rep* 2017, 7:42413
- Hsu CL, Bryce PJ: Inducible IL-33 expression by mast cells is regulated by a calcium-dependent pathway. *J Immunol* 2012, 189:3421–3429
- Wills-Karp M, Rani R, Dienger K, Lewkowich I, Fox JG, Perkins C, Lewis L, Finkelman FD, Smith DE, Bryce PJ, Kurt-Jones EA, Wang TC, Sivaprasad U, Hershey GK, Herbert DR: Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med* 2012, 209:607–622
- Lopetuso LR, De Salvo C, Pastorelli L, Rana N, Senkfor HN, Petito V, Di Martino L, Scaldaferrì F, Gasbarrini A, Cominelli F, Abbott DW, Goodman WA, Pizarro TT: IL-33 promotes recovery from acute colitis by inducing miR-320 to stimulate epithelial restitution and repair. *Proc Natl Acad Sci U S A* 2018, 115:E9362–E9370
- Bessa J, Meyer CA, de Vera Mudry MC, Schlicht S, Smith SH, Iglesias A, Cote-Sierra J: Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. *J Autoimmun* 2014, 55:33–41
- Williams MA, O'Callaghan A, Corr SC: IL-33 and IL-18 in inflammatory bowel disease etiology and microbial interactions. *Front Immunol* 2019, 10:1091
- Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, Fiocchi C, Vecchi M, Pizarro TT: Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. *Proc Natl Acad Sci U S A* 2010, 107:8017–8022
- Sattler S, Ling GS, Xu D, Hussaarts L, Romaine A, Zhao H, Fossati-Jimack L, Malik T, Cook HT, Botto M, Lau YL, Smits HH, Liew FY, Huang FP: IL-10-producing regulatory B cells induced by IL-33 (Breg(IL-33)) effectively attenuate mucosal inflammatory responses in the gut. *J Autoimmun* 2014, 50:107–122
- Rani R, Smulian AG, Greaves DR, Hogan SP, Herbert DR: TGF-beta limits IL-33 production and promotes the resolution of colitis through regulation of macrophage function. *Eur J Immunol* 2011, 41:2000–2009
- Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, Girard JP: Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel IL-33-LacZ gene trap reporter strain. *J Immunol* 2012, 188:3488–3495
- Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F: An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999, 190:995–1004
- Bacchetta R, Passerini L, Gambineri E, Dai M, Allan SE, Perroni L, Dagna-Bricarelli F, Sartirana C, Matthes-Martin S, Lawitschka A, Azzari C, Ziegler SF, Levings MK, Roncarolo MG: Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J Clin Invest* 2006, 116:1713–1722
- Schiering C, Krausgruber T, Chomka A, Frohlich A, Adelman K, Wohlfert EA, Pott J, Griseri T, Bollrath J, Hegazy AN, Harrison OJ, Owens BMJ, Lohning M, Belkaid Y, Fallon PG, Powrie F: The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* 2014, 513:564–568
- Voo KS, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Littman DR, Liu YJ: Identification of IL-17-producing FOXP3+ regulatory T cells in humans. *Proc Natl Acad Sci U S A* 2009, 106:4793–4798
- Li L, Boussiotis VA: The role of IL-17-producing Foxp3+ CD4+ T cells in inflammatory bowel disease and colon cancer. *Clin Immunol* 2013, 148:246–253
- Grobeta P, Doser K, Falk W, Obermeier F, Hofmann C: IL-33 attenuates development and perpetuation of chronic intestinal inflammation. *Inflamm Bowel Dis* 2012, 18:1900–1909
- Sedhom MA, Pichery M, Murdoch JR, Foligne B, Ortega N, Normand S, Mertz K, Sanmugalingam D, Brault L, Grandjean T, Lefrancais E, Fallon PG, Quesniaux V, Peyrin-Biroulet L, Cathomas G, Junt T, Chamaillard M, Girard JP, Ryffel B: Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice. *Gut* 2013, 62:1714–1723

20. Nunes T, Bernardazzi C, de Souza HS: Interleukin-33 and inflammatory bowel diseases: lessons from human studies. *Mediators Inflamm* 2014, 2014:423957
21. Gross V, Andus T, Caesar I, Roth M, Scholmerich J: Evidence for continuous stimulation of interleukin-6 production in Crohn's disease. *Gastroenterology* 1992, 102:514–519
22. Hyams JS, Fitzgerald JE, Treem WR, Wyzga N, Kreutzer DL: Relationship of functional and antigenic interleukin 6 to disease activity in inflammatory bowel disease. *Gastroenterology* 1993, 104:1285–1292
23. Parisinos CA, Serghiou S, Katsoulis M, George MJ, Patel RS, Hemingway H, Hingorani AD: Variation in interleukin 6 receptor gene associates with risk of Crohn's disease and ulcerative colitis. *Gastroenterology* 2018, 155:303–306.e2
24. Mudter J, Neurath MF: IL-6 signaling in inflammatory bowel disease: pathophysiological role and clinical relevance. *Inflamm Bowel Dis* 2007, 13:1016–1023
25. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, Williams M, Malissen B, Agace WW, Mowat AM: Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol* 2013, 6:498–510
26. Koizumi SI, Ishikawa H: Transcriptional regulation of differentiation and functions of effector T regulatory cells. *Cells* 2019, 8
27. Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, Ribeiro CH, Hall JA, Yagi R, Naik S, Bhairavabhotla R, Paul WE, Bosselut R, Wei G, Zhao K, Oukka M, Zhu J, Belkaid Y: GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. *J Clin Invest* 2011, 121:4503–4515
28. Wang Y, Su MA, Wan YY: An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity* 2011, 35:337–348
29. Cai T, Qiu J, Ji Y, Li W, Ding Z, Suo C, Chang J, Wang J, He R, Qian Y, Guo X, Zhou L, Sheng H, Shen L, Qiu J: IL-17-producing ST2(+) group 2 innate lymphoid cells play a pathogenic role in lung inflammation. *J Allergy Clin Immunol* 2019, 143:229–244.e9
30. Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, Riethmacher D, Si-Tahar M, Di Santo JP, Eberl G: In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. *J Exp Med* 2008, 205:1381–1393
31. Yang BH, Hagemann S, Mamareli P, Lauer U, Hoffmann U, Beckstette M, Fohse L, Prinz I, Pezoldt J, Suerbaum S, Sparwasser T, Hamann A, Floess S, Huehn J, Lochner M: Foxp3(+) T cells expressing RORgamma t represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol* 2016, 9:444–457
32. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R: Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004, 118:229–241
33. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR: Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 2007, 104:13780–13785