Antibody-mediated blockade of the IL23 receptor destabilizes intratumoral regulatory T cells and enhances immunotherapy

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Contributed by Harvey Cantor; received January 19, 2022; accepted March 31, 2022; reviewed by Tobias Holderried and Khashayarsha Khazaie

Regulatory T cells (Treg) can impede antitumor immunity and currently represent a major obstacle to effective cancer immunotherapy. Targeting tumor-infiltrating regulatory Treg while sparing systemic Treg represents an optimal approach to this problem. Here, we provide evidence that the interleukin 23 receptor (IL23R) expressed by tumor-infiltrating Treg promotes suppressive activity. Disruption of the IL23R results in increased responsiveness of destabilized Treg to the IL12 cytokine, the production of γ-interferon, and the recruitment of CD8 T cells that inhibit tumor growth. Since the Treg destabilization pathway that is initiated by IL23R blockade is distinct and independent from the destabilization pathway coupled to glucocorticoid-induced TNFR-related protein (GITR) activation, we examined the impact of the coordinate induction of the two destabilization pathways on antitumor immune responses. Combined GITR and IL23R antibody treatment of mice inoculated with MC38 tumors resulted in robust and synergistic antitumor responses. These findings indicate that the delineation of independent Treg destabilization pathways may allow improved approaches to the development of combination immunotherapy for cancers.

T regulatory cells | Treg conversion | MC38 colon carcinoma | mAb treatment | combination therapy

Author contributions: A.E.W., J.M.S., H.-J.K., and H.C. designed research; A.E.W., J.M.S., S.D., L.A., H.N., Y.Q., X.S., and O.O. performed research; A.E.W., J.M.S., S.D., L.A., H.N., Y.Q., X.S., O.O., H.-J.K., and H.C. wrote the paper.

Reviewers: T.H., Rheinische Friedrich-Wilhelms-Universität Bonn; and K.K., Mayo Clinic Minnesota.

Competing interest statement: Author H.C. and reviewer T.H. were listed as coauthors in 2020 (PMC7031256), but this does not represent a financial or personal competing interest, since the contribution of H.C. was limited to the provision of a mutant mouse model in 2013.

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This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2200757119/-/DCSupplemental.

Published April 28, 2022.

Significance

Regulatory T cells rely on active processes to maintain a suppressive phenotype inside a tumor, leading to increased tumor burden and worse cancer outcomes. Here, we report a pathway to interfere with regulatory T cell (Treg) stability by disrupting the expression of the interleukin 23 receptor. This approach increases Treg responsiveness to interleukin 12, leading to increased production of gamma-interferon and more efficient antitumor immune responses. The combined engagement of independent pathways to destabilize Treg through the interleukin 23 receptor and the glucocorticoid-induced TNFR-related protein receptor has a synergistic impact on the Treg phenotype and promotes antitumor immune responses. These findings expand our understanding of regulatory T-cell biology and offer tools for cancer immunotherapy.
Results

IL23R Marks Suppressive, Tumor-Infiltrating Treg. We asked whether IL23R expression by tumor-infiltrating Treg marks a Treg subpopulation with increased stability and suppressive activity. Analysis of tumor-infiltrating cells into MC38 colon carcinoma revealed a significant fraction of IL23R<sup>+</sup> tumor-infiltrating Treg (Fig. 1A). A comparison with IL23R<sup>−</sup> Treg from the same tumors indicated that IL23R<sup>+</sup> Treg expressed increased levels of CD44 and the Helios transcription factor (Fig. 1 B and C), which have been correlated with Treg suppressive function and stability (3, 20, 21). To determine whether the expression of IL23R by intratumoral Treg might be linked to a stable, suppressive phenotype, Treg from FoxP3-YFP reporter mice were sorted (SI Appendix, Fig. S1) and incubated with an anti-IL23R antibody. We noted that this antibody and the control anti-GITR antibody, but not other antibodies directed against Treg surface receptors, reduced FoxP3 expression (Fig. 1D) and increased IFNγ expression (Fig. 1E). Taken together, these results suggested that IL23R marked a suppressive subset of tumor-infiltrating Treg and that targeting this receptor in vivo might destabilize Treg and enhance antitumor immune responses.

IL23R-Deficient Treg Display Increased IL12 Sensitivity and STAT4 Activation. We generated conditional knockout (KO) IL23R-Flox mice (22) after crossing to mice expressing a FoxP3 promoter-driven Cre recombinase (IL23RFlox FoxP3Cre) and backcrossing for 10 generations. Mice with a Treg-specific IL23R loss or blockade might enhance the IL12 responsiveness of Treg and thus alter their functional phenotype (SI Appendix, Fig. S4). Flow cytometric analysis of tumor-infiltrating Treg from WT and IL23RFlox FoxP3Cre mice confirmed their IL23R-deficient status (Fig. 2E) and supported the hypothesis that these IL23R-deficient Treg display increased IL12 sensitivity. We also noted increased IFNγ expression, a downstream effector of IL12 signaling, by both Treg (Fig. 2F) and tumor-infiltrating CD8<sup>+</sup> T cells from IL23RFlox FoxP3Cre tumors. Moreover, we observed increased IL12R expression (Fig. 2H) and increased phosphorylated STAT4 (pSTAT4) activation (Fig. 2I) by IL23R-deficient Treg. Finally, we noted increased responsiveness to IL12 by activated IL23R<sup>−</sup> Treg, as judged by increased pSTAT4 after incubation in IL12 for 24 h (Fig. 2J). Together, these results suggest that tumor protection mediated by IL23R-deleted Treg may reflect increased IL12 responsiveness and associated IFNγ secretion by tumor-infiltrating Treg, leading to the recruitment of activated CD8<sup>+</sup> T cells.

Impact of Enhanced Treg IL12 Signaling on the TME. We asked whether the increased tumor protection from IL23R<sup>−</sup> Treg depended in part on their recruitment of activated CD8<sup>+</sup> T cells. The adoptive transfer of 5 × 10<sup>5</sup> Treg from IL23R<sup>−</sup> or IL23R<sup>WT</sup> mice with 1 × 10<sup>9</sup> CD8<sup>+</sup> and CD25<sup>+</sup> CD4<sup>+</sup> T cells from WT donors into TCRα<sup>−</sup> mice indicated that the stronger antitumor responses of IL23R<sup>−</sup> Treg hosts (Fig. 3A) was associated with increased IFNγ production by tumor-infiltrating IL23R<sup>−</sup> Treg (Fig. 3B), as well as increased activation of intratumoral CD8<sup>+</sup> T cells (Fig. 3C). Enhanced CD8<sup>+</sup> T-cell activation was required for tumor protection since the transfer of IL23R<sup>−</sup> Treg alone into Rag2<sup>−/−</sup>γc<sup>−/−</sup> hosts did not reduce tumor burden (Fig. 3D). Taken together, these results suggest that Treg destabilization after the disruption of surface IL23R expression promotes IL12R/STAT4–dependent IFNγ responses (SI Appendix, Fig. S4) and improved antitumor immune responses.

Full Deletion of IL23R Mimics the Treg-Specific Deletion Phenotype. In addition to tumor-infiltrating Treg, IL23R may also be expressed by gut-associated Th17 cells and possibly Treg. We therefore asked whether full IL23R targeting would phenocopy Treg-specific targeting. Analysis of IL23R<sup>KO</sup> mice (23)
Fig. 2. Treg-specific IL23R ablation protects against MC38 carcinoma. (A) Tumor growth curves after MC38 challenge, as in Fig. 1, in mice bearing a Treg-specific IL23R deletion (IL23RFlox FoxP3Cre) and congenic controls (IL23RWT FoxP3Cre). (B) IL23R-deficient Treg and congenic controls from tumor-infiltrating and spleen samples were subjected to bulk next-generation RNA sequencing. Significant DEGs in tumor samples were not differentially expressed by spleen Treg from the same mice. (C) The top 10 KEGG pathways among these significant DEGs are enriched in inflammatory- or immune-relevant biological processes. TLR, Toll-like receptor; EBV, Epstein–Barr virus. (D) Volcano plot of all genes contributing to any immune-relevant biological process selected from the significant DEGs and manually annotated as inflammatory/Th1-favoring (orange) or regulatory/Th2-favoring (blue). Log2 fold change (log2FC) versus -log10 P value is shown. See also SI Appendix, Fig. S2 for details on this annotation step. (E–I) Flow cytometry analysis of tumor-infiltrating Treg from mice in (A), including (E) Treg IL23R deficiency, (F) Treg and (G) CD8 T-cell IFNγ, (H) Treg IL12Rβ2 expression, and (I) Treg STAT4 activation. Fluorescence minus one (FMO) negative controls are included. (J) Activated STAT4 staining of Treg sorted from C57BL/6 or IL23RKO mice and cocultured with IL12 for 24 h. Tumor growth and flow cytometry data are pooled from three independent experiments (n = 10 to 12/group). *P < 0.05, **P < 0.01, ****P < 0.0001.
Synergy between Antibody-Mediated Targeting of IL23R and GITR.

Since the pathway leading to Treg destabilization follows the GITR pathway initiated by agonistic GITR treatment (3, 4), we asked whether the combined targeting of both pathways might have a synergistic impact on Treg and improve tumor protection. We analyzed tumor growth after separate and combined administration of anti-IL23R and agonistic GITR antibody. The administration of IL23R or GITR antibodies alone slowed tumor growth, while the combined administration of lower doses of each antibody completely protected mice from tumor growth (Fig. 5A). We then carried out a dose–response analysis of each antibody alone and in combination. We observed reduced efficacy of both monotherapies compared to combined treatment, consistent with a synergistic effect (Fig. 5B). From this point onward, we used a larger tumor inoculum to allow characterization of the TME (Fig. 5C) and to investigate overall tumor survival (Fig. 5D). We observed a significant improvement in tumor clearance and survival in combination-treated animals compared to either monotherapy.

Investigation of the TME at an early timepoint (to allow for tumor-infiltrating cells to be harvested from combination-treated animals) revealed a Treg destabilization and CD8 T-cell activation phenotype similar to that observed in Fig. 2, including enhanced IFNγ (Fig. 5E) and IL12R (Fig. 5F) expression by Treg and IFNγ expression by CD8 T cells (Fig. 5G). We also noted increased Fas expression by tumor-infiltrating CD8 T cells (Fig. 5H), with the highest Fas expression observed after

(Fig. 4A) or mice treated with the anti-IL23R antibody (Fig. 4B) indicated that protection against MC38 growth was retained in both cases. In the latter case, the protective effects of the anti-IL23R antibody were similar to those observed in IL23Rfl FoxP3Cre mice (Fig. 4B).

In addition to confirming efficacy, we tested for potential side effects of IL23R disruption using WT and IL23RKO mice aged 12 mo. We did not observe evidence of autoimmunity in IL23RKO mice compared to age-matched WT controls, as judged by the production of antinuclear antibodies (Fig. 4C) and comparisons of Peyer’s patches and lamina propria of WT, IL23Rfl FoxP3Cre, and IL23RKO mice aged 12 mo, which did not uncover alterations in their gut immune populations (Fig. 4 D and E). In contrast, mice carrying a Treg-specific Blimp deletion displayed impaired Treg function (24).
A 2×10^4 MC38 (normal dose)

B

C

D

E

F

G

H

Fig. 5. IL23R antibody treatment combined with GITR activation to promote tumor immunity. (A) Tumor growth curves in C57BL/6 mice treated intraperitoneally with anti-IL23R (100 µg/treatment), anti-GITR (100 µg/treatment), isotype controls (100 µg/treatment), or a combination of both IL23R and GITR antibodies (50 µg each/treatment), as in Fig. 4. (B) Tumor inhibition of each antibody monotherapy and combination therapy, shown as a percentage of isotype-treated mice, where 0% inhibition indicates tumors that grew equal to isotype treatment and 100% inhibition indicates no tumor growth. (C) Tumor growth curves as in (A) using a higher dose (5 × 10^5) of MC38. (D) Survival analysis of high-dose-challenged mice as in (C). (E–H) Flow cytometry analysis of tumor-infiltrating Treg high-dose challenged mice including those with (E) Treg IFNγ expression, (F) Treg IL12Rβ2 expression, (G) CD8 T-cell IFNγ expression, and (H) CD8 T-cell Fas expression. Data are pooled from three independent experiments (n = 14 to 15/group). *P < 0.05, **P < 0.01, ***P < 0.001; ns, non significant. Iso, isotype.

### Discussion

Here we show that IL23R is a tumor-selective marker of suppressive Treg and that targeting it either alone or in combination with agonistic GITR treatment offers tumor protection that reflects increased IL12R-coupled STAT4 activation, IFNγ production by intratumoral Treg, and recruitment of activated CD8 T cells to growing tumors. This IL23R-dependent Treg destabilization pathway is distinct from GITR-linked inhibition of Helios-dependent STAT5 activation, leading to reduced FoxP3 expression by destabilized Treg (3, 4, 21). These considerations led us to determine whether the engagement of both the IL23R–STAT4 and GITR–STAT5 pathways might have a synergistic impact on Treg destabilization and tumor immunity, which proved to be the case (Fig. 5).

The observation that combined antibody targeting of IL23R and GITR provides synergistic protection against tumor growth may reflect a complementary impact on antitumor immune responses. We have shown previously that GITR activation destabilizes Treg through diminished Helios expression (3), resulting in reduced STAT5 activation and an impaired Treg suppressive phenotype (4). We have also noted that decreased Helios leads to a Th1-like phenotype and the expression of high-affinity, self-reactive T-cell receptors (4). Conversely, IL23R interference responds to environmental IL12 and leads to increased STAT4 activation by Treg, which can polarize the immune microenvironment toward type 1 immunity through the production of cytokines, including IFNγ. Since the TME that develops in IL23R-deficient mice is associated with enhanced effector CD8 T-cell activity (Fig. 3), we suggest that the activation of a STAT4–IFNγ pathway secondary to IL23R blockade may promote a type 1 immune microenvironment. It is likely that Treg → Th1-like conversion through a GITR–Helios–STAT5 pathway combined with changes in the TME secondary to the engagement of an IL23R–STAT4–IFNγ pathway may exert a synergistic impact on the TME, leading to increased CD4 and CD8 effector responses and more efficient tumor clearance.

This may also explain why IL23R monotherapy was less effective against high doses of MC38 in our experiments. Agonistic GITR antibodies directly destabilize Treg through the loss of Helios (3), while IL23R blockade sensitizes Treg to be destabilized by environmental signals such as IL12. Therefore, altering the tumor microenvironment by altering the tumor inoculum could be expected to have a stronger impact on IL23R monotherapy than on GITR monotherapy, which we observed. In addition, the Th1-supporting microenvironmental changes induced by IL23R monotherapy may be insufficient to keep pace with the more aggressive MC38 regimen unless it is supported by additional Treg destabilization and CD8 activation through IL23R and GITR combination therapy.
We have shown previously that Helios-deficient Treg induced by GITR treatment up-regulate the expression of the Ii2rb2 gene but not Ii2rb1 gene (4) (SI Appendix, Fig. S5). Increased IL12Rβ2 expression in GITR-treated Treg may not suffice for enhanced IL12R formation without a commensurate increase in IL12Rβ1 availability provided by IL23R blockade. Although IL23R blockade does not lead to increased Ii2rb1 transcription, the release of the IL12Rβ1 receptor chain may promote high-affinity IL12 receptor expression when combined with free IL12Rβ2. Coordinate treatment with anti-GITR and anti-IL23R Abs yielding increased availability of both components of the high-affinity IL12 receptor may account for improved IL12 sensitivity of Treg in combination therapy compared to either monotherapy (Fig. 5F).

The combination approach summarized above leading to Treg destabilization and establishment of a type 1 immune microenvironment is relevant to ongoing attempts to harness Treg therapies in the clinic. Although GITR agonism is exceptionally effective at improving cancer outcomes in murine cancer models, it has been less impressive in human trials to date. Although ongoing clinical studies in human cancers may be more promising (8, 25), GITR monotherapy may induce activation as well as CD8 T-cell exhaustion in humans (8). This outcome is similar to our observation that although GITR monotherapy strongly induces CD8 T-cell IFNγ production, it also induces CD8 T-cell exhaustion (Fig. 5). In contrast, GITR/IL23R combination therapy induced robust CD8 T-cell IFNγ production by activated CD8 T cells that expressed lower levels of T-cell exhaustion markers. We hypothesize that the improved CD8 T-cell response to combination treatment reflects IFNγ production by intratumoral Treg that potentiates effector CD8 T-cell responses without substantial CD8 T-cell exhaustion.

Collectively, the mechanism of Treg destabilization described here in the context of tumor immunotherapy has broader implications for our understanding of both Treg and Th17 biology. The finding that IL23R blockade can influence IL12 signaling expands our understanding of the role of the receptor in Treg and Th17 biology. These findings open the possibility that the IL23–IL17 axis that regulates gut immunity may be sensitive to IL12 as well as IL23 targeting and suggest approaches that target the IL12R and IL23R in the context of inflammatory bowel disease and cancers. In view of the current unrealized potential of Treg destabilization secondary to anti-body monotherapy, approaches that utilize complementary pathways to improve cancer outcomes through Treg targeting may open avenues of research into reshaping the tumor microenvironment through Treg intervention.

Materials and Methods

Animal Models and Cell Isolation. C57BL/6 (WT), B6.129S1-Stat3tm1Xyfu/J (Stat3lox), and B6.129(Cg)-Foxp3tm4(YFP/icre)Ayr/J (Foxp3Cre) mice were obtained by the Dana Farber Cancer Institute (DFCI) Institutional Animal Care and Use Committee (IACUC) guidelines. For in vitro analysis or flow cytometry, cells were isolated from spleens by mechanical dissociation. Tumor-infiltrating leukocytes were isolated by treatment with collagenase, dispase, and DNase I for 1 h at 37 °C, followed by mechanical dissociation and density centrifugation. Cells from the lamina propria and Peyer’s patches were isolated using the OctoMACS tissue dissociator (Miltenyi) following the manufacturer’s instructions.

Flow Cytometry. For conventional cytometry, single-cell suspensions were prepared as above and stained using Zombie fixable viability dyes (Biolegend) in 1× PBS. Surface antibody staining using antibodies indicated in Table 1 was performed in 1× PBS supplemented with 1% FBS and 1 mM EDTA. Intracellular staining was performed using the Foxp3 Staining Buffer set (Thermo) following the manufacturer’s instructions. All samples were acquired on a Fortessa X20 (BD) and analyzed using the R statistical programming language. Samples for Treg sorting were first depleted of red blood cells, B cells, and CD8 T cells by magnetic selection (StemCell), then stained as above and sorted on an Aria III (BD). All sorted Treg came from mice with a Foxp3-YFP reporter, so Treg were gated based on Foxp3 expression (SI Appendix, Fig. S1).

For high-dimensional cytometry, samples were stained as above for viability and surface dyes, then split into 24 wells and stained with a separate phycoerythrin (PE)-conjugated antibody as indicated in Table 1. Imputation was performed using the InfinityFlow R package following the developer’s instructions (26), except that output files were concatenated in silico and output events were set to 10,000 per well (240,000 events per imputed file). Concatenated cytometry files were analyzed using the CATALYST (27) and Seurat (28) R packages.

For phospho-STAT staining, single-cell suspensions were stained with fixable viability dye as above, then fixed with 1% paraformaldehyde and permeabilized in ice-cold 90% methanol. For 10 min. Following fixation and

| Target | Fluorochrome | Source |
|--------|--------------|--------|
| CD45   | BV785        | Biolegend 103149 |
| TCRb   | BV605        | Biolegend 109241 |
| CD4    | BV435        | BD 563790 |
| F4/80  | FITC         | Biolegend 123108 |
| CD11b  | PerCP-Cy5.5  | Biolegend 108706 |
| Foxp3  | BV421        | Biolegend 126419 |
| CD8a   | BV650        | Biolegend 100742 |
| B220   | BV711        | Biolegend 103255 |
| CD3    | BVU737       | BD 741788 |
| NK1.1  | APC          | Biolegend 108710 |
| CD25   | APC-Cy7      | Biolegend 101918 |
| CD62L  | APC-R700     | BD 553219 |
| CD11c  | PE-Dazzle594 | Biolegend 117348 |
| TCRgd  | PE-Cy7       | Biolegend 118124 |
| pSTAT3 | APC         | BD 560392 |
| pSTAT4 | AlexaFluor 647 | BD 558137 |
| IL12Rb1 | PE          | R&D FAB1998P |
| CD6    | PE           | Biolegend 146403 |
| CCR6   | PE           | Biolegend 129804 |
| Fas    | PE (CD95)    | Biolegend 152607 |
| Ly108  | PE           | Biolegend 134606 |
| GITR   | PE           | Biolegend 126310 |
| CD48   | PE           | Biolegend 102707 |
| PD-1   | PE           | Biolegend 135206 |
| CD54   | PE           | Biolegend 116108 |
| IL23R  | PE           | Biolegend 150904 |
| TNF    | PE           | Biolegend 506306 |
| Perforin | PE        | Biolegend 154406 |
| IL17A  | PE           | Biolegend 506904 |
| IL12Rb2 | PE          | Miltenyi 130–125225 |
| CD130  | PE           | Biolegend 149404 |
| CD98   | PE           | Biolegend 128208 |
| CD69   | PE           | BD 553237 |
| Ki67   | PE           | Biolegend 151209 |
| Blimp1 | PE           | BD 564268 |
| IFNg   | PE           | Biolegend 505808 |
| IL10   | PE           | Biolegend 505008 |

Table 1. Antibodies used for flow cytometry

https://doi.org/10.1073/pnas.2200757119
permeabilization, cells were stained using surface and intracellular markers together in 1× PBS supplemented with 1% FBS and 1 mM EDTA.

**In Vitro Treg Destabilization.** Single-cell suspensions were prepared as above and Treg were isolated by sorting YFP+ CD4 T cells from Foxp3<sup>+</sup> mice. Sorted Treg were cultured in complete RPMI supplemented with 5 ng/mL IL2, anti-CD3/CD28 beads (Miltenyi) according to the manufacturer’s instructions, and 10 μg/mL of the indicated antibody for 24 h in an incubator at 37 °C and 5% CO<sub>2</sub>.

**Tumor Models.** MC38 colon carcinoma (ATCC) was cultured in complete RPMI and passed when 70% confluent. Cells were expanded for eight passages and then frozen in multiple aliquots so that all experiments were performed with cells from the same passage number. For a given experiment, cells were thawed and expanded for 2 days, then harvested using trypsin and EDTA (Gibco) and washed twice in PBS before use in any experiments.

Flank tumors were induced by subcutaneous injection of 2 × 10<sup>5</sup> (unless otherwise indicated) MC38 cells in 100 μL 1× PBS. Mice were shorn and cleaned at the injection site to ensure good subcutaneous injection and to allow early detection of small tumors. Tumor growth was monitored every 72 h until palpable tumors were detected, then tumors were measured every 48 h with calipers. Tumor measurements were undertaken by a researcher blind to experimental conditions. Mice were humanely euthanized by CO2 inhalation when any measured diameter exceeded 16 mm or after reaching humane endpoints as defined by the DFCI IACUC. Tumor growth curve plotting and statistical analysis were performed using the R statistical programming language.

**Treg RNA Sequencing.** Treg from IL23R<sup>-/-</sup> and congenic controls were sorted as described above and subjected to low-input RNA sequencing by GeneWiz. The resulting fasta files were aligned to the GRCh38 primary assembly and feature-counting using Rsubread (29). The resulting sample-wise expression table was analyzed using edgeR (30) with default parameters. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using pathfindR (31), using mmu KEGG as the input gene set. Manual annotation of Th1 and Treg gene functions among significant DEGs was performed by generating a list of all significant DEGs contributing to immune-relevant KEGG pathways, blinding their differential expression state, and then performing a literature search for their function in Treg or T cells. The differential expression status of all genes was only revealed after the literature searches were completed. The results of this literature search are presented in SI Appendix, Fig. S2.

**Antibody Treatment.** MC38 flank tumors were induced as above. Once tumors were palpable on any mouse, mice were treated with three doses of indicated antibody every 48 h beginning when first palpated (100 μg/injection in 100 μL PBS unless otherwise indicated). The antibodies used were anti-IL23R clone 12B2B64 (Biolegend), anti-GITR clone DTA-1 (BioCell), or Rat IgG2b isotype control (BioXCell). Mice were stratified into one of four treatment groups randomly by initial tumor size (i.e., mice with the four largest tumors were randomly assigned each to a different antibody treatment, then the next four largest, and so on).

**Autoantibody Enzyme-Linked Immunosorbent Assay.** Blood was collected from the saphenous vein of aged mice, according to IACUC-approved procedures. Antinuclear antibodies were measured using an ANA enzyme-linked immunosorbent assay kit (BioVision) and a plate-based spectrophotometer (BioRad). Analysis was performed using the R statistical programming language.

**Statistical Analysis** Unless otherwise indicated, statistical analysis was performed using nonparametric, unpaired tests: the Wilcoxon rank-sum test or Kruskal-Wallis one-way test followed by Wilcoxon posthoc tests with Holm correction for multiple comparisons. P values are indicated by asterisks: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. For high-dimensional flow analysis, statistical analysis was performed using the Seurat package with default options, after constructing a single-cell sequencing object using CATALYST, with options set for flow cytometry data instead of the CyTOF defaults, as indicated by the developers. For tumor growth curve analysis, statistical comparisons were made on the area under the curve of each tumor curve. Tumor growth is plotted as mean ± SEM, with a locally estimated scatterplot smoothing (loess) regression line showing the imputed continuous growth rate.

**Data Availability.** Raw and processed RNA-seq data have been deposited in Gene Expression Omnibus (accession GSE197287). All other data pertaining to the paper are presented here. Code for the analysis of flow cytometry data in R is available at GitHub, https://github.com/NKInstitute/flowGate. All study data are included in the article and/or SI Appendix.

**ACKNOWLEDGMENTS.** We thank V. Kuchroo and P. Rosentiel for the provision of key mouse models, A. Luoma and F. Alvarez-Calderon for flow cytometry support, the DFCI Animal Resource Facility for veterinary support, E. Betch for technical support with InfinityFlow, and A. Angel for assistance with manuscript preparation and editing. This work was supported in part by research grants from the National Institute of Allergy and Infectious Diseases (NIAID) of the NIH under award numbers R01AI037562 and R01AI048125 (H.C.); the U.S. Army Medical Research and Materiel Command (W81XWH) (H.C.) and a DCI Accelerator Award (H.C.); the AAI Intersect Fellowship and Claudia Adams Barr Program in Innovative Basic Cancer Research (A.E.W.); the NRSF Fellowship (NIN/NIAID T32AI074549) (J.M.S.); and the CRI-Irvington Fellowship (H.N.).

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