The Journal of Immunology

IL-17A–Producing γδ T Cells Suppress Early Control of Parasite Growth by Monocytes in the Liver

This information is current as of December 20, 2015.

Meru Sheel, Lynette Beattie, Teija C. M. Frame, Fabian de Labastida Rivera, Rebecca J. Faleiro, Patrick T. Bunn, Marcela Montes de Oca, Chelsea L. Edwards, Susanna S. Ng, Rajiv Kumar, Fiona H. Amante, Shannon E. Best, Shaun R. McColl, Antiopi Varelias, Rachel D. Kuns, Kelli P. A. MacDonald, Mark J. Smyth, Ashraful Haque, Geoff R. Hill and Christian R. Engwerda

J Immunol 2015; 195:5707-5717; Prepublished online 4 November 2015;
doi: 10.4049/jimmunol.1501046
http://www.jimmunol.org/content/195/12/5707

References This article **cites 53 articles**, 20 of which you can access for free at:
http://www.jimmunol.org/content/195/12/5707.full#ref-list-1

Subscriptions Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscriptions

Permissions Submit copyright permission requests at:
http://www.aai.org/ji/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/cgi/alerts/etoc

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.
Copyright © 2015 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

IL-17A–Producing γδ T Cells Suppress Early Control of Parasite Growth by Monocytes in the Liver

Meru Sheel,*,[1] Lynette Beattie,* Teija C. M. Frame,*,[†] Fabian de Labastida Rivera,* Rebecca J. Faleiro,*,[‡] Patrick T. Bunn,*,[§] Marcela Montes de Oca,*,[¶] Chelsea L. Edwards,*,[¶] Susanna S. Ng,*,[‖] Rajiv Kumar,*,[#] Fiona H. Amante,* Shannon E. Best,* Shaun R. McColl, Antiopi Varelias,* Rachel D. Kuns,* Kelli P. A. MacDonald,* Mark J. Smyth,* Ashraful Haque,* Geoff R. Hill,* and Christian R. Engwerda***

Intracellular infections, such as those caused by the protozoan parasite *Leishmania donovani*, a causative agent of visceral leishmaniasis (VL), require a potent host proinflammatory response for control. IL-17 has emerged as an important proinflammatory cytokine required for limiting growth of both extracellular and intracellular pathogens. However, there are conflicting reports on the exact roles for IL-17 during parasitic infections and limited knowledge about cellular sources and the immune pathways it modulates. We examined the role of IL-17 in an experimental model of VL caused by infection of C57BL/6 mice with *L. donovani* and identified an early suppressive role for IL-17 in the liver that limited control of parasite growth. IL-17–producing γδ T cells recruited to the liver in the first week of infection were the critical source of IL-17 in this model, and CCR2$^+$ inflammatory monocytes were an important target for the suppressive effects of IL-17. Improved parasite control was independent of NO generation, but associated with maintenance of superoxide dismutase mRNA expression in the absence of IL-17 in the liver. Thus, we have identified a novel inhibitory function for IL-17 in parasitic infection, and our results demonstrate important interactions among γδ T cells, monocytes, and infected macrophages in the liver that can determine the outcome of parasitic infection. ***The Journal of Immunology*, 2015, 195: 5707–5717.**

The IL-17 family has six members, including IL-17A (hereafter referred to as IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25), and IL-17F. This cytokine family signals via the cell surface IL-17R, made up of several different combinations of subunits, including IL-17RA, IL-17RB, IL-17RC, and IL-17RE. IL-17 is produced by a wide range of cells, including CD4$^+$ Th17 cells, γδ T cells, NK cells, innate lymphoid cells, and macrophages (reviewed in Refs. 1, 2). It plays important roles during chronic inflammation and autoimmune diseases (3, 4), and as such, IL-17 has become a major target for treating immune-mediated inflammatory diseases. However, the principal function of this cytokine is to provide protection against pathogens. In particular, studies in IL-17–deficient mice and humans with IL-17RA deficiency identified a critical role in host defense against extracellular bacteria and the commensal fungi *Candida albicans* (5–10). However, the role of IL-17 in parasitic diseases is less well understood.

Visceral leishmaniasis (VL) caused by the protozoan parasite *Leishmania donovani* is an organ specific disease of the liver, spleen, bone marrow (BM), and lymph nodes of humans and mice (11–14). In genetically susceptible mice, the liver is generally a site of acute infection that resolves following the formation of proinflammatory granulomas, whereas the spleen and BM become chronically infected with impaired immune responses and disruption of tissue architecture (15–17). There is also evidence of similar patterns of organ-specific disease in humans (18). Effective immunity to VL is characterized by the emergence of a strong, parasite-specific Th1 response (19–21). However, the role of Th17 cells and/or IL-17 in VL is still not clear. A study on human PBMCs isolated from people in a Sudanese village experiencing a VL outbreak over a 6-y period found that high levels of IL-17 and IL-22 following stimulation with *L. donovani* Ags most strongly associated with protection against disease (22). Support for a protective role for IL-17 comes from a study showing IL-17RA–deficient C57BL/6 mice infected with *L. infantum* harbored higher tissue parasite burdens than wild-type (WT) control mice (23). In contrast, IL-17 promoted the progression of cutaneous leishmaniasis in susceptible mice infected with *L. major* (24), whereas in IL-10–deficient mice infected with *L. major*, IL-17 was responsible for increased Th1 cell–mediated pathology (25). In humans, a negative association with parasite-induced IL-17 production by PBMCs from patients with cutaneous leishmaniasis and muco-

*QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4006, Australia; [†]School of Biomedical Sciences, University of Queensland, Brisbane, Queensland 4072, Australia; [‡]Queensland University of Technology, Institute of Health and Biomedical Innovation, Brisbane, Queensland 4059, Australia; [§]Institute of Glycomics, Griffith University, Gold Coast, Queensland 4215, Australia; [¶]School of Medicine, University of Queensland, Brisbane, Queensland 4072, Australia; [‖]School of Natural Sciences, Griffith University, Nathan, Queensland 4111, Australia; [#]Netaji Subhas Institute of Technology, New Delhi 110078, India; and **Centre for Molecular Pathology, University of Adelaide, Adelaide, South Australia 5005, Australia

[1]Current address: Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia.

ORCIDs: 0000-0003-2338-1494 (R.K.); 0000-0002-7788-5885 (F.H.A.).

Received for publication May 5, 2015. Accepted for publication October 6, 2015.

This work was supported by grants from the Australian National Health and Medical Research Council and the Australia-India Strategic Research Fund.

Address correspondence and reprint requests to Dr. Christian R. Engwerda, Queensland Institute of Medical Research, 300 Herston Road, Herston, QLD 4006, Australia. E-mail address: Christian.Engwerda@qimrberghofer.edu.au

Abbreviations used in this article: B6.IL-17$^{-/-}$, IL-17A–deficient C57BL/6; BM, bone marrow; MNC, mononuclear cell; MOI, multiplicity of infection; NOS, NO synthase; p.i., postinfection; QIMR-B, QIMR Berghofer Medical Research Institute; Sb^V, pentavalent antimony; SOD3, superoxide dismutase 3; VL, visceral leishmaniasis; WT, wild-type.

Copyright © 2015 by The American Association of Immunologists, Inc. 0022-1767/15/$25.00

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

cutaneous leishmaniasis infected with *L. braziliensis* has been reported (26, 27). Thus, these findings indicate subtle and complex roles for IL-17 in leishmaniasis that depends on the parasite species and/or sites of disease and immune cell activation.

In this study, we examined the role of IL-17 in mice infected with *L. donovani* and discovered an unexpected negative, regulatory role for this cytokine in the control of parasite growth. IL-17 produced by γδ T cells suppressed monocyte-mediated parasite control in the liver within the first 7 d of infection. However, once postinfection (p.i.) was established, IL-17 blockade had no impact on antiparasitic activity. Thus, we have identified an early γδ T cell, IL-17–dependent mechanism of immune suppression in the liver early during infection.

Materials and Methods

Inbred male and female C57BL/6 and B6.SJL.Ptprca (B6.CD45.1$^+$) mice were purchased from Animal Resources Centre (Canning Vale, Western Australia) and maintained under conventional conditions. IL-17A–deficient C57BL/6 mice (B6.IL-17$^{-/-}$ (28), B6.IL-17a^{Cre}R26R^{eYFP} (29), B6.TCRδ$^{-/-}$ (30), and B6.RAG-1$^{-/-}$ (31) mice were bred and maintained in-house at QIMR Berghofer Medical Research Institute (QIMR-B). All mice were maintained under specific pathogen-free conditions and were sex and age (6–12 wk) matched.

Chimeric mice were prepared by irradiating C57BL/6 or B6.SJL.Ptprca mice twice (2 h in between) with 550 cGy (1100 cGy total) and then engrafting with 2–5 × 10^6 fresh BM cells i.v. via the lateral tail vein. Mice were maintained on antibiotics for 2 wk after engraftment and infected with *L. donovani* 8–12 wk after receiving BM, as previously described (32).

Ethics statement

All animal procedures were approved and monitored by the QIMR-B Animal Ethics Committee, under approval number A02634M, in accordance with the Australian code of practice for the care and use of animals for scientific purposes (Australian National Health and Medical Research Council).

Parasites, infection, and assessment of parasite burden

L. donovani (LV9) were passaged and maintained in B6.RAG-1$^{-/-}$ mice, and amastigotes were isolated from the spleens of chronically infected mice. Mice were infected by injecting 2 × 10^7 amastigotes i.v. via the lateral tail vein. Mice were sacrificed by CO_2 asphyxiation, at times indicated through text, and bled via cardiac puncture. Spleens and perfused livers were removed at the times indicated, and parasite burdens were determined from Diff-Quick–stained impression smears (Lab Aids, Narrabeen, Australia) and expressed as Leishman–Donovan units (number of amastigotes/1000 host nuclei multiplied by the organ weight in grams) (33). Liver tissue also was preserved in Tissue-Tek O.C.T. compound (Sakura, Torrance, CA). Hepatic, splenic, and BM mononuclear cells (MNC) were isolated as previously described (34).

In vitro infection

Mice were euthanized by CO_2 asphyxiation, following which peritoneal lavage was conducted using 5 ml room-temperature Dulbecco's PBS (1×). Peritoneal cells were collected and washed with complete (10% [v/v] FCS containing 10 mmol L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin) DMEM, and 5 × 10^5 cells were seeded in 16-well glass chamber slides (Lab-Tek, Rochester, NY). Cells were incubated at 37°C for 24 h. After 24 h, nonadherent cells were washed and removed with complete DMEM, and LV9 amastigotes were added at a multiplicity of infection (MOI) of 10:1 (in 200 μl complete DMEM). After 1 h at 37°C, free amastigotes were removed, and cells were cultured for another 24 h p.i. On the following day, cells were washed with 1× Dulbecco's PBS, fixed, and stained. Percentage infectivity was calculated as number of parasites per 100 host macrophages. In some experiments, infected cells were cultured for 24 h with 5 ng/ml recombinant mouse IFN-γ (eBioscience, San Diego, CA) and increasing doses of recombinant mouse IL-17 (2–100 ng/ml) (eBioscience). Cell-culture supernatants were collected to measure nitrate levels using the Griess assay (35), as well as cytokines using a CBA kit (BD Biosciences, Franklin Lakes, NJ), according to the manufacturer's instructions.

In vivo mAb and drug treatments

Control rat IgG was purchased from Sigma-Aldrich (St. Louis, MO). Anti-CD4 (YTS191.1) mAb was grown in-house in 5% (v/v) FCS and RPMI 1640 containing 10 mmol L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin and purified using previously published protocols (36). Anti-NK1.1 (PK136) mAb and control mouse IgG were purchased from Bio-XCell (West Lebanon, NH). Anti–IL-17A (M210) mAb was provided by Amgen (Seattle, WA). To deplete CD4$^+$ T cells, 0.5 mg anti-CD4 mAb was administered i.p. to mice on days −1, 2, and 5 p.i. Mice were administered 0.5 mg anti-NK1.1 mAb i.p. on days −2, 0, and 3 p.i. Depletion of T cells and NK cells was confirmed using FACS staining at the termination of experiments, with >98 and 95% depletion of CD4$^+$ T cells and NK cells, respectively, in the liver and spleen on day 7 p.i. IL-17 was neutralized using 100 μg anti–IL-17A mAb i.p. on days 0, 2, 4, and 6 p.i, unless otherwise specified. IL-17E (IL-25) was blocked using 0.5 mg anti–IL-25 mAb i.p. on days 0 and 3 p.i. The pentavalent antimonial, sodium stibogluconate [Sb^V; Burroughs Wellcome], was dissolved in 0.9% saline and administered i.p. NO synthase (NOS) inhibition was performed by injecting amino guanidine (Sigma-Aldrich) at 50 mg/kg body weight in 0.2 ml 0.9% saline by i.p. injection twice daily. In all studies, control mice received the same volume of 0.9% saline delivered via the same route.

Flow cytometry and Abs

Liver and spleen MNC were prepared as described previously (33). Fluorescently conjugated mAbs against CD4 (GK1.5), CD8 (53-6.7), CD3ε (17A2), CD49β (DX5), NK1.1 (PK136), TCRβ (H57-597), TCRγ/δ (GL3), CD11b (M1/70), Ly6C (HK1.4), Ly6G (1A8), GR-1 (RB6-8C5), F4/80 (C1:A3-1), B220 (RA3-6B2), CD11c (N418), CCR6 (29-2L17), IL-17A (TC11-18H10.1), and IFN-γ (XMG1.2) were purchased from BioLegend (San Diego, CA). PE-conjugated IL-17RA/CD217 (PAJ-17R) was purchased from eBioscience. Cell-surface and intracellular staining was performed as published previously (34). Ex vivo cytokine production was measured after stimulating liver and spleen MNC with PMA (25 ng/ml) and ionomycin (500 ng/ml) in the presence of brefeldin A for 3 h at 37°C, followed by intracellular staining as previously described (36). Leukocyte populations were described as following CD4$^+$ T cells (CD4$^+$TCRβ$^+$), CD8$^+$ T cells (CD8$^+$TCRβ$^+$), NK cells (NK1.1$^+$TCRβ$^-$), monocytes (CD11b$^+$ Ly6C^{hi} Ly6G$^-$), neutrophils (CD11b$^+$Ly6C$^+$Ly6G$^+$), and γδ T cells (TCRγ/δ$^+$CD3ε^{int}). FACS was performed using a BD LSR Fortessa (BD Biosciences), and data were analyzed using FlowJo software (Tree Star).

Real-time quantitative PCR

Hepatic MNC were isolated at times indicated and collected directly into RLT buffer containing 2-ME (Qiagen, Venlo, The Netherlands) and stored at −80°C until mRNA isolation. Total RNA was isolated using RNeasy mini kit (Qiagen), according to the manufacturer's protocol. cDNA synthesis was performed in 100 μl reactions on 2.0 μg RNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Relative quantities of mRNA encoding genes of interest were determined by real-time PCR on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) using universal SYBR Green Supermix (Bio-Rad) and by the comparative threshold cycle method, according to the manufacturer's instructions. mRNA levels for NOS2 and superoxide dismutase 3 (SOD3) were normalized to the geometric mean of hypoxanthine phosphoribosyl transferase, β-actin, and Polr2a housekeeping mRNA and displayed as $2^{-\Delta CT}$. *nos2* primers were from Qiagen (Mm-Nos2_1_SG), whereas all other primers were from Sigma-Aldrich: *sod3*, forward, 5′-ATGGCTGAGGTTCTCTGCAC-3′ and reverse, 5′-ACTCAGAGGCTCTTCCTCCG-3′; *hprt*, forward, 5′ GGACTGATTATGGACAGGA-3′ and reverse, 5′-GAGGGCCACAATGTGATG-3′; *β-actin*, forward, 5′-GACGGCCAAGTCATCACTATTG-3′ and reverse, 5′-CCACAGGATTCCATACCCAAGA-3′; and *polr2a*, forward, 5′-AGCTGGTCCTTCGAATCCGC-3′ and reverse, 5′-CTGATCTGCTCGATACCCTGC-3′.

Statistical analysis

Statistical differences between groups was determined using the Mann–Whitney *U* test or a Kruskal–Wallis nonparameteric test for comparison of multiple groups as appropriate, using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, CA). The p values <0.05 were considered statistically significant. All data are presented as the mean values ± SE unless otherwise stated.

Results

IL-17 inhibits early control of parasite growth in the liver

To examine whether IL-17 influenced disease outcome following *L. donovani* infection, B6.IL-17$^{-/-}$ mice were infected and parasite burden was compared with WT C57BL/6 mice. In the liver, both mouse strains resolved infection around the same time, but B6.IL-17$^{-/-}$ mice had lower parasite burdens at day 14 p.i., relative to

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

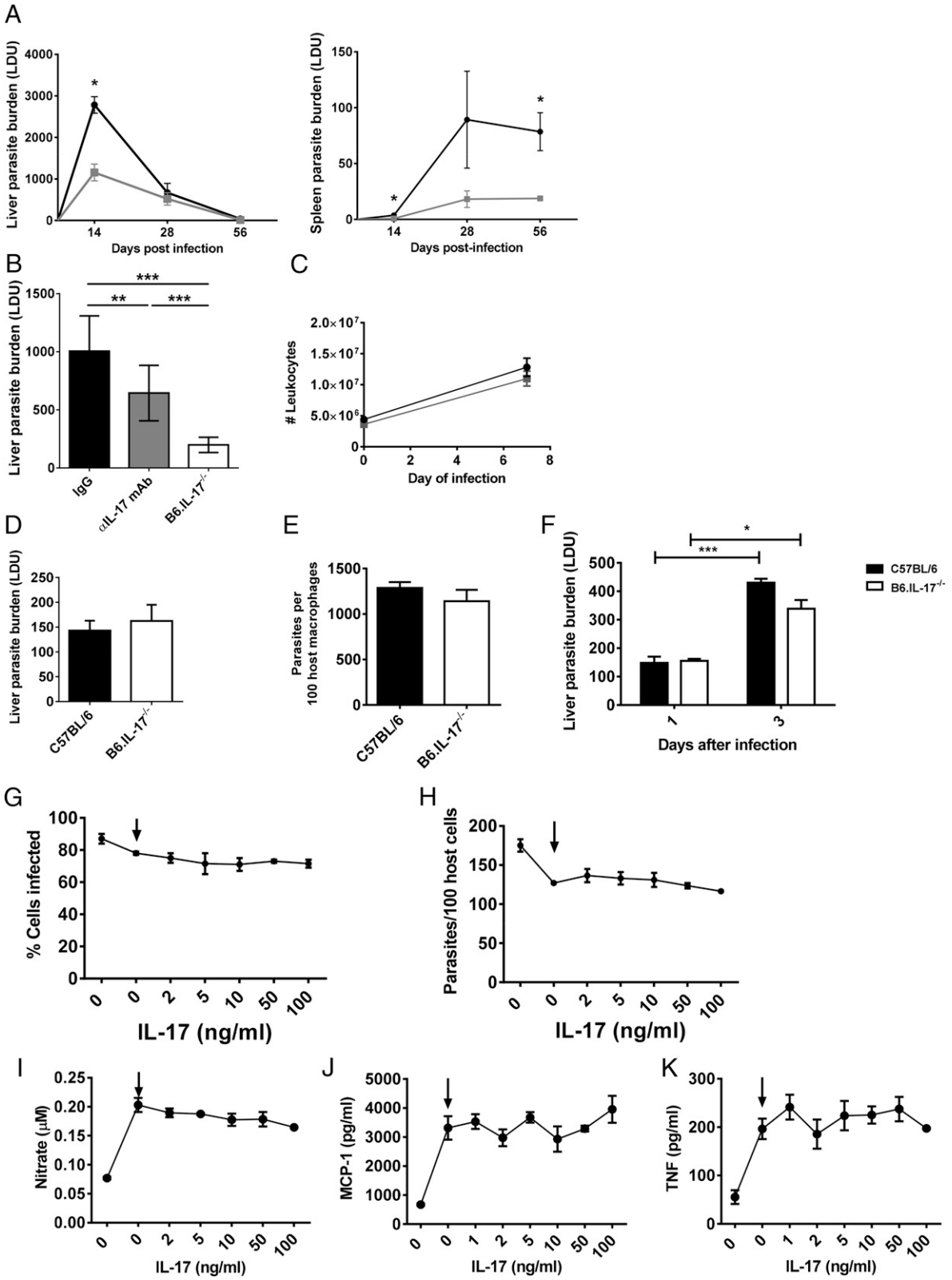

FIGURE 1. IL-17 enhances parasite growth during *L. donovani* infection. (**A**) C57BL/6 (black circles) and B6.*Il-17*$^{-/-}$ (gray squares) mice were infected with 2×10^7 *L. donovani* and liver and spleen parasite burden measured on days 14, 28, and 56 p.i. (**B**) *L. donovani*–infected C57BL/6 mice administered 100 μg anti–IL-17 mAb or control IgG on the day of infection and 2, 4, and 6 d later, prior to liver parasite burden being measured on day 7 p.i., and compared with B6.*Il-17*$^{-/-}$ mice, as indicated. (**C**) Hepatic leukocyte number was compared between C57BL/6 (black circles) and B6.*Il-17*$^{-/-}$ (gray squares) mice. (**D**) Liver parasite burden at 24 h p.i. in C57BL/6 and B6.*Il-17*$^{-/-}$ mice. Peritoneal macrophages isolated from C57BL/6 and B6.*Il-17*$^{-/-}$ mice were cultured overnight followed by in vitro infection with *L. donovani* (MOI 10:1). (**E**) Bar graph represents percent infectivity of host cells, measured as parasites per 100 host cells. (**F**) Liver parasite burdens were also compared at days 1 and 3 p.i. in C57BL/6 and B6.*Il-17*$^{-/-}$ mice. (**G**–**K**) Peritoneal macrophages isolated from C57BL/6 mice were cultured overnight followed by in vitro infection with *L. donovani* (MOI 10:1) and then cultured overnight again with indicated concentrations of IL-17. The arrow indicates the addition of IFN-γ. The percentage of infected (*Figure legend continues*)

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

controls (Fig. 1A). The increased control of parasite growth could be observed as early as 7 d p.i. (Fig. 1B), but not before day 3 p.i. (data not shown). Early hepatic control of parasite growth in the absence of IL-17 was also associated with reduced parasite loads in the spleen over the course of infection (Fig. 1A). Given the early and pronounced control of parasite growth in the liver, and relatively small changes in the spleen at early time points, we focused our attention on the liver.

We next blocked IL-17 activity in C57BL/6 mice for the first 7 d of *L. donovani* infection. Mice that received anti–IL-17 mAb had enhanced control of parasite growth, supporting results obtained from B6.IL-17$^{-/-}$ mice (Fig. 1B). We found no difference in hepatic leukocyte numbers between B6.IL-17$^{-/-}$ mice and C57BL/6 control mice prior to infection or 7 d later (Fig. 1C). Furthermore, no change in the frequency of any lymphocyte or myeloid cell subset examined was observed (data not shown). To determine whether IL-17 influenced the ability of parasites to establish infection, we measured liver parasite burden 24 h p.i. (Fig. 1D), as well as the ability of parasites to infect peritoneal macrophages from B6.IL-17$^{-/-}$ mice (Fig. 1E). Kupffer cells in the liver are the main targets for early *L. donovani* infection and have previously been shown to have innate mechanisms of parasite control that operate in the first 24 h (37). To examine whether these innate mechanisms of parasite control were impacted by IL-17 deficiency, we also compared hepatic parasite burdens between C57BL/6 controls and B6.IL-17$^{-/-}$ mice on days 1 and 3 p.i. and found that in both lines, parasite burden increased to a similar extent (Fig. 1F). Together, these experiments showed that IL-17 deficiency had no effect on parasite number in the first 3 d, indicating this cytokine was not promoting the establishment of infection or changes to innate antiparasitic mechanisms in host macrophages.

We next investigated whether IL-17 was acting directly on macrophages to inhibit classical macrophage activation pathways. Peritoneal macrophages were infected with *L. donovani*, activated by addition of IFN-γ and then IL-17 was titrated into cell cultures. We found that addition of IL-17 at any concentration tested had no effect on the frequency of infected macrophages (Fig. 1G), the number of parasites in culture (Fig. 1H), or the generation of nitrate (Fig. 1I), MCP-1 (Fig. 1J), and TNF (Fig. 1K). These data indicate that IL-17 was not acting by suppressing classical macrophage activation pathways.

To test whether IL-17 influenced established infection, and thus might be targeted for therapeutic advantage, C57BL/6 mice were infected with *L. donovani* and then treated with an IL-17–neutralizing mAb 14 d later for 7 d. Additionally, we also treated cohorts of mice with a suboptimal dose of antiparasitic drug (pentavalent antimony [Sb^{V}]), with or without IL-17 blockade (Fig. 2). Although a high dose of antiparasitic drug reduced parasite burden in the liver, IL-17 blockade, either alone or in combination with a suboptimal drug dose, had no impact on parasite growth in either the liver (Fig. 2A) or spleen (Fig. 2B). Together, these data indicate that IL-17 promotes hepatic parasite growth early in infection, but not once infection has been established.

γδ T cells are the main producers of IL-17 in the liver early during L. donovani *infection*

As a first step to identify the cellular source of the IL-17 that suppressed antiparasitic immunity, we first generated a series of

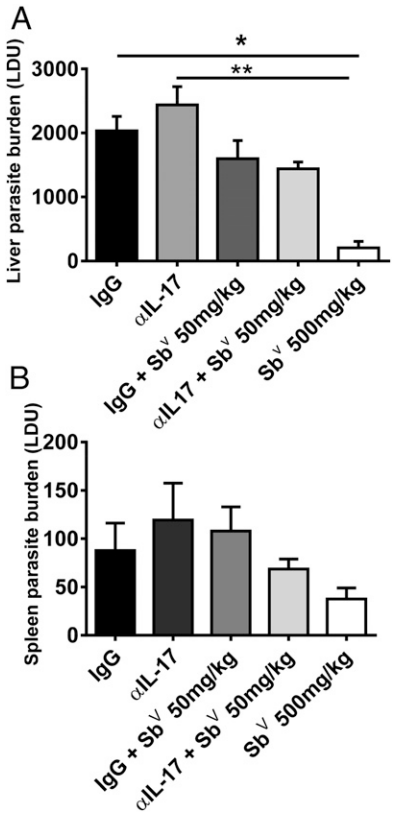

FIGURE 2. Blockade of IL-17 during established *L. donovani* infection has no therapeutic benefit. C57BL/6 mice were infected with *L. donovani* on day 0 and treated with 100 μg control IgG or anti–IL-17 Ab on days 14, 16, 18, and 20 p.i. Groups of mice received Ab alone or in combination with 50 mg/kg Sb^{V} on day 14 p.i. Liver (**A**) and spleen (**B**) parasite burden was measured on day 21 p.i. One representative experiment of two performed is shown (n = 4 to 5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $*p \leq 0.05$ and $**p \leq 0.01$ are indicated between control-treated mice and those receiving a high (500 mg/kg) dose of Sb^{V} on day 14 p.i. LDU, Leishman–Donovan units.

BM chimeric mice (Fig. 3A). Mice that received BM from B6.IL-17$^{-/-}$ donors responded to infection in a similar manner to the IL-17–deficient mice 7 d after *L. donovani* infection, regardless of whether they were B6.IL-17$^{-/-}$ or C57BL/6 control recipients, indicating the cellular source of hepatic IL-17 early in infection was hematopoietic in origin. Given the critical role of CD4^{+} T cells in controlling *L. donovani* growth in the liver (33, 38), we next investigated whether altered CD4^{+} T cell activation could account for the increased control of parasite growth observed in B6.IL-17$^{-/-}$ mice. There was no change in the number of CD4^{+} T cells producing the essential proinflammatory and antiparasitic cytokine IFN-γ (19, 20) in B6.IL-17$^{-/-}$ mice compared with WT controls (Fig. 3B). Furthermore, depletion of CD4^{+} T cells in C57BL/6 and B6.IL-17$^{-/-}$ mice had no impact on hepatic parasite burdens (Fig. 3C). Similarly, depletion of NK cells failed to alter the outcome of infection in B6.IL-17$^{-/-}$ mice (Fig. 3D).

These data suggested that CD4^{+} T cells were not the critical cellular source of IL-17 in *L. donovani*–infected mice, so we infected B6.*Il-17a*^{Cre}*R26R*^{eYFP} fate reporter mice (29) to determine which cell populations had expressed IL-17. The expected in-

cells (G), number of parasite per 100 host cells (H), as well as levels of nitrate (I), MCP-1 (J), and TNF (K) were then measured. One representative experiment of at least two performed is shown (n = 4 to 5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ are indicated. LDU, Leishman–Donovan units.

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

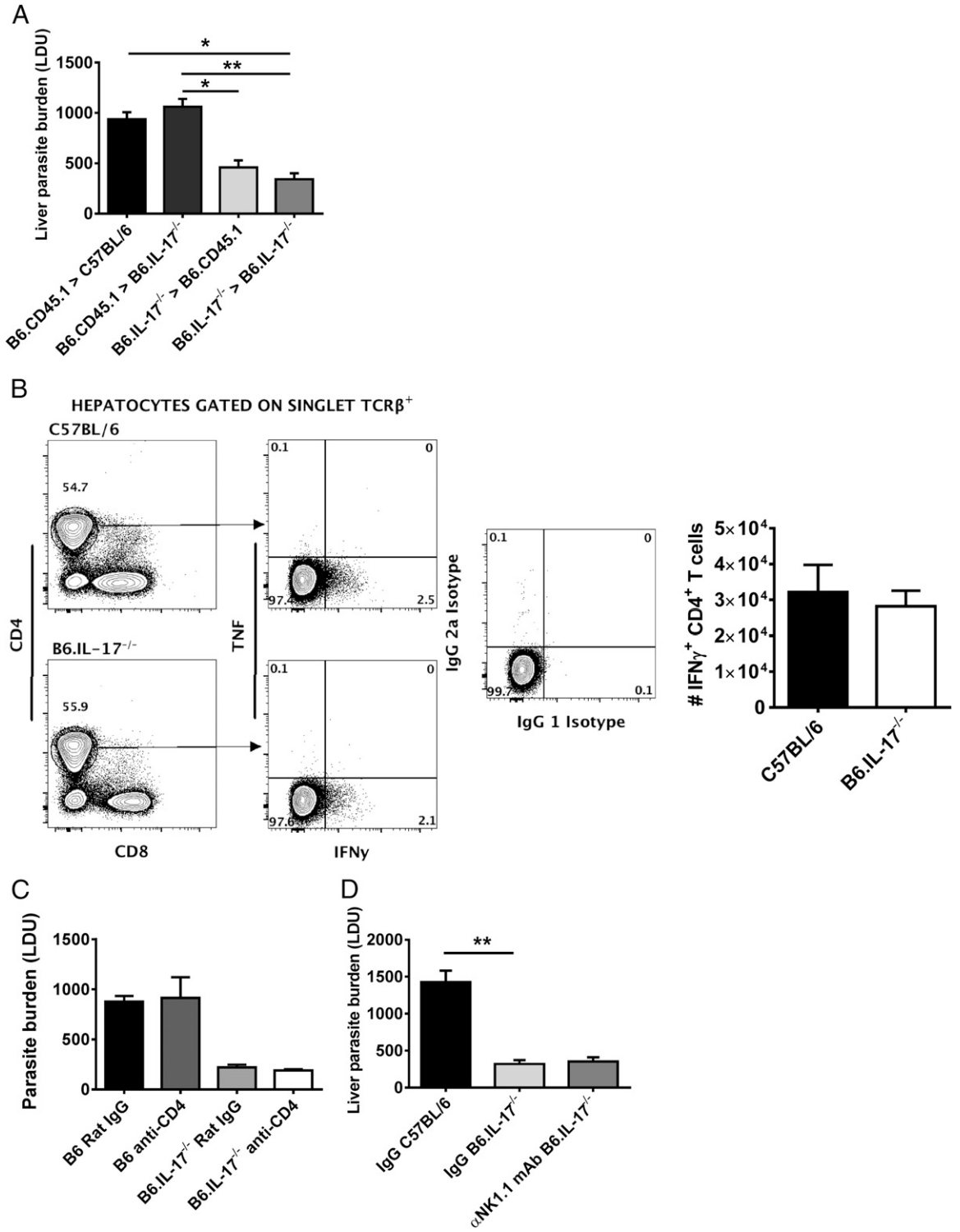

FIGURE 3. IL-17 derived from hematopoietic cells promotes the growth of *L. donovani*. (**A**) BM chimeric mice were infected with *L. donovani* and parasite burden in the liver was measured on day 7 p.i. (**B**) The number of IFN-γ–producing $CD4^+$ T cells in the livers of infected C57BL/6 and B6.*Il-17*$^{-/-}$ mice was measured on day 7 p.i. (**C**) A representative gating strategy is shown. C57BL/6 and B6.*Il-17*$^{-/-}$ mice were infected with *L. donovani* and administered 0.5 mg control IgG or anti-CD4 mAb on day −1, day 2, and day 5 p.i., prior to measuring liver parasite burden on day 7 p.i. (**D**) C57BL/6 and B6.*Il-17*$^{-/-}$ mice were infected with *L. donovani* and administered 0.5 mg control IgG or anti-NK1.1 mAb on days −2, 0, 3, and 6 p.i., prior to measuring liver parasite burden on day 7 p.i. One representative experiment of at least two performed is shown (n = 4 to 5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $*p \leq 0.05$ and $**p \leq 0.01$ are indicated. LDU, Leishman–Donovan units.

crease in hepatic MNC numbers following 7 d of *L. donovani* infection (Fig. 4A), included increased numbers of $CD4^+$ T cells (Fig. 4B) and γδ T cells (Fig. 4C), which comprised the majority of YFP-positive cells (Fig. 4D). Similar frequencies of YFP-positive cells were found in the livers of naive and infected mice, with γδ T cells accounting for ∼70% of YFP-positive cells, $CD4^+$ T cells accounting for ∼15%, and the remaining positive cells including NK cells, $CD8^+$ T cells, and other MNC populations. Importantly,

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

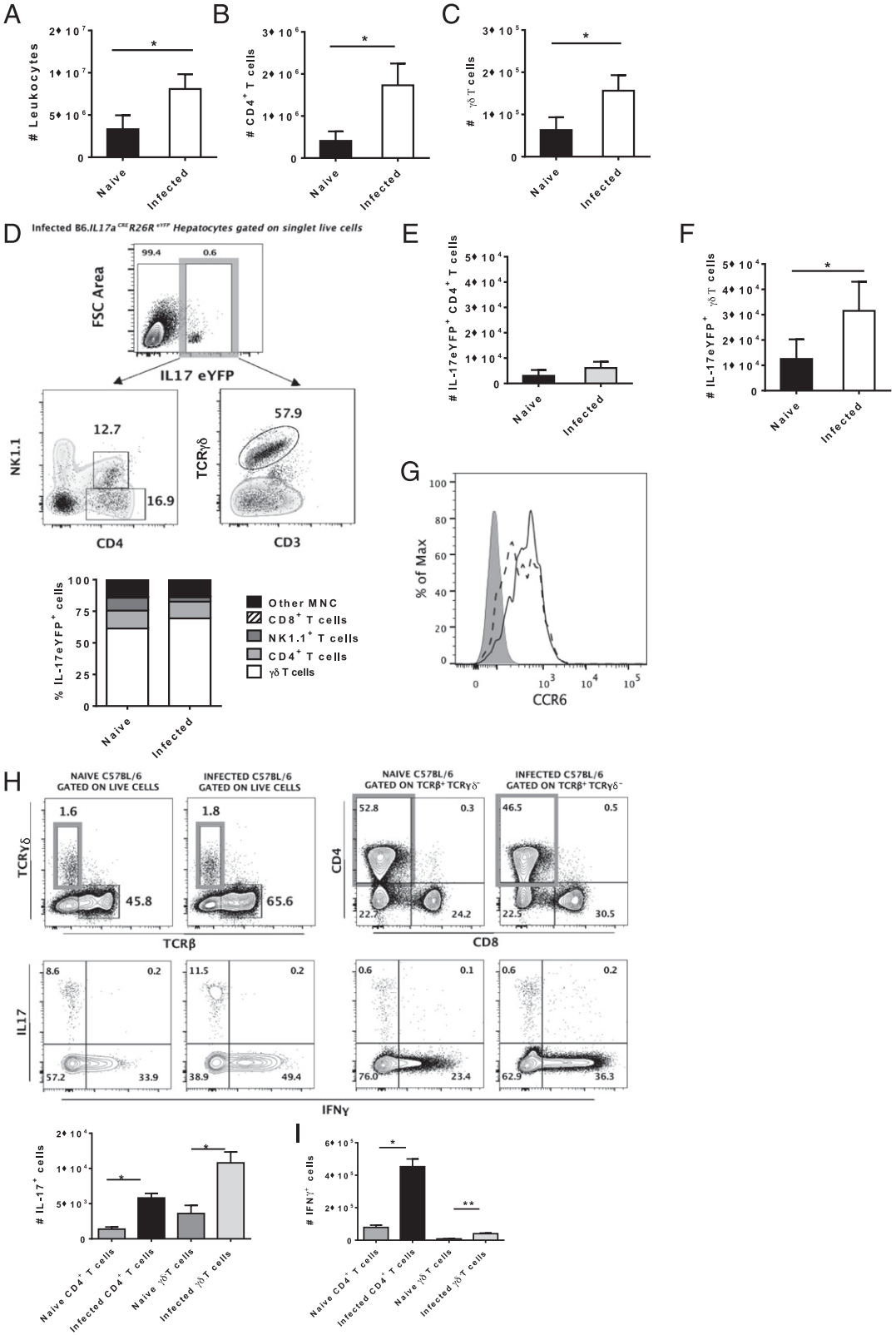

FIGURE 4. γδ T cells are the major producers of IL-17 in the liver during *L. donovani* infection. The number of leukocytes (**A**), CD4$^+$ T cells (**B**), and γδ$^+$ T cells (**C**) was measured in the liver of naive and *L. donovani*–infected B6.*Il-17a*^{Cre}*R26R*^{eYFP} mice on day 7 p.i. The frequency of IL-17eYFP$^+$ γδ T cells, CD4$^+$ T cells, NK1.1$^+$ T cells, CD8$^+$ T cells, and other MNC, as indicated, was measured at day 7 p.i. (**D**) A representative gating strategy is shown. The number of IL-17eYFP$^+$ CD4$^+$ T cells (**E**) and IL-17eYFP$^+$ γδ T cells (**F**) in livers of naive and infected mice at day 7 p.i. is also shown. (**G**) Expression of CCR6 on IL-17eYFP$^+$ γδ T cells from naive (black line) and infected (dashed line) mice was measured by FACS and compared with isotype control Ab staining (solid curve) on day 7 p.i. Hepatic MNC from naive C57BL/6 and *L. donovani*–infected C57BL/6 mice (day 7 p.i.) were stimulated with PMA and ionomycin in the presence of brefeldin A, as described in the *Materials and Methods*. (**H**) A representative gating strategy is shown. The number of CD4$^+$ T cells and γδ T cells producing IL-17 and IFN-γ (**I**) was measured. One representative experiment of at least two performed is shown (n = 3–5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $^*p \leq 0.05$ and $^{**}p \leq 0.01$ are indicated. FSC, forward light scatter.

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

although no increase in YFP-positive CD4$^+$ T cells was observed following infection (Fig. 4E), there was a significant increase in YFP-positive γδ T cell numbers (Fig. 4F). These cells expressed the chemokine receptor CCR6 (Fig. 4G), consistent with previous descriptions of IL-17–producing γδ T cells (39). The B6.*Il-17a*^{Cre} *R26R*^{eYFP} fate reporter mice may not always accurately detect IL-17–producing γδ T cells and CD4$^+$ T cells (29). Therefore, to establish that IL-17 protein was produced by γδ T cells in the liver of *L. donovani*–infected mice, we stimulated hepatic MNC ex vivo with a short burst of mitogen and measured intracellular IL-17 and IFN-γ. Again, we found the majority of IL-17 was produced by γδ T cells and that this increased following infection (Fig. 4H). In contrast, the majority of IFN-γ produced following infection came from the CD4$^+$ T cell compartment (Fig. 4I). Although similar patterns of IFN-γ production were observed in the spleen, no infection-mediated increase in IL-17–producing cells was found in this tissue at the time point examined (Fig. 5). Together, these results indicate that γδ T cells are the major source of IL-17 in the first week of *L. donovani* infection in the liver.

IL-17 produced by γδ T cells suppresses early antiparasitic immunity

To assess the role of γδ T cells early during experimental VL, C57BL/6 mice and B6.TCRδ$^{-/-}$ mice (lacking γδ T cells) were infected with *L. donovani*. Mice lacking γδ T cells had reduced parasite burdens in the livers 7 d later, compared with WT mice (Fig. 6A), suggesting a suppressive role for γδ T cells in *L. donovani* infection. However, the reduction in parasite burden in B6.TCRδ$^{-/-}$ mice was not as great as that observed in B6.IL-17$^{-/-}$ mice, suggesting there other important cellular sources of IL-17 in the liver during *L. donovani* infection. Treatment of C57BL/6 mice with anti–IL-17 mAb resulted in reduced parasite burdens in the livers (Fig. 6A), as expected. However, when TCRδ$^{-/-}$ mice were treated with this Ab, no effect on parasite burden was observed, compared with TCRδ$^{-/-}$ mice that received a control Ab (Fig. 6A). Finally, to establish whether IL-17 from γδ T cells was the cause of suppressed antiparasitic immunity and also examine the role of CCR6 in this process, we generated a series of mixed BM chimeras using WT, B6.TCRδ$^{-/-}$, B6.CCR6$^{-/-}$, and B6.IL-17$^{-/-}$ BM for engraftment. Surprisingly, mice reconstituted with 80% γδ$^{-/-}$ BM plus 20% CCR6$^{-/-}$ BM had similar parasite burdens to mice reconstituted with 80% γδ$^{-/-}$ BM plus 20% WT BM (Fig. 6B), indicating a limited or redundant role for CCR6 in γδ T cell recruitment to the liver following *L. donovani* infection. However, mice reconstituted with 80% γδ$^{-/-}$ BM plus 20% WT BM had higher parasite burdens compared with mice reconstituted with 80% γδ$^{-/-}$ BM plus 20% IL-17$^{-/-}$ BM (Fig. 6B). Therefore, because IL-17–deficient γδ T cells were only present in the latter group of chimeric mice, these results support a suppressive role for IL-17 derived from γδ T cells in the liver early during *L. donovani* infection.

The antiparasitic activity of monocytes is targeted by IL-17 in the liver early during L. donovani *infection*

Having identified γδ T cells as an important source of IL-17 following infection, we wished to determine which cells IL-17 was acting on to mediate its suppressive effect. To this end, we investigated the expression of IL-17RA. These data showed that CD11b$^+$, Ly6C^{hi} monocytes recruited to the liver in response to *L. donovani* infection had the highest expression of the IL-17RA, compared with other myeloid cells and nonmyeloid cells (Fig. 6C), indicating they were a potentially major targets for this cytokine. Moreover, mice lacking CCR2, which have limited recruitment of monocytes to infected and inflamed tissue (40, 41), failed to improve control of parasite growth following IL-17 blockade (Fig. 3E), but showed no defect in γδ T cell recruitment to the liver (136,000 ± 9,700 versus 147,500 ± 21,800 cells at day 7 p.i., for C57BL/6 and B6.CCR2$^{-/-}$ mice, respectively). We conformed that CCR2-deficient mice had a selective defect in monocyte recruitment, because at day 7 p.i., they had no change in

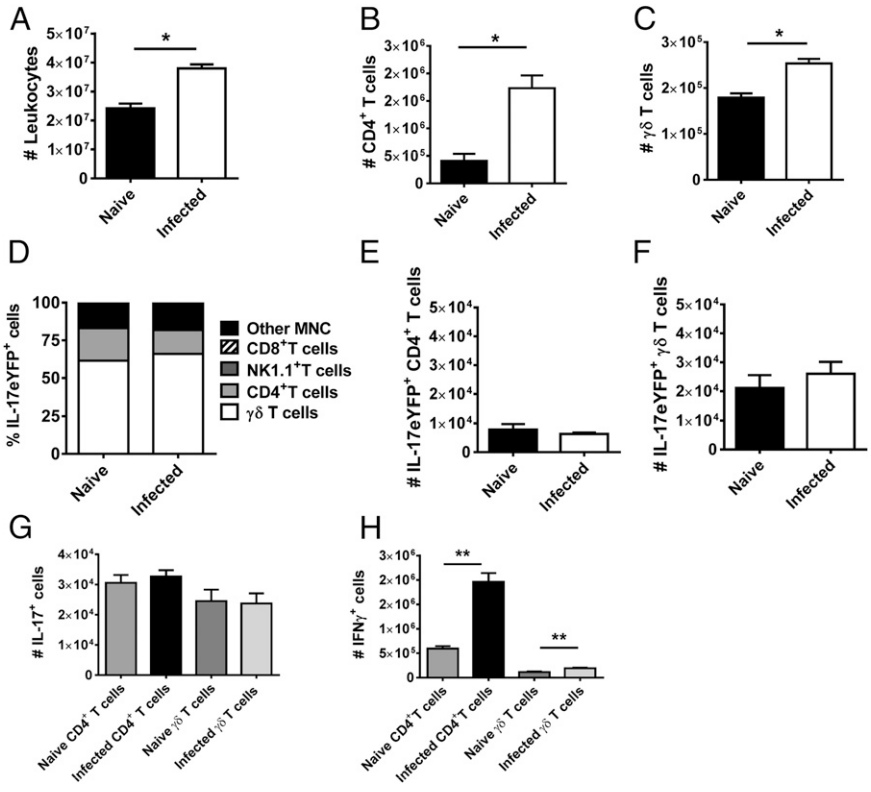

FIGURE 5. Sources of IL-17 in the spleen during *L. donovani* infection. The number of leukocytes (**A**), CD4$^+$ T cells (**B**), and γδ$^+$ T cells (**C**) were measured in the spleen of naive and *L. donovani*–infected B6.*Il-17a*^{Cre} *R26R*^{eYFP} mice on day 7 p.i. (**D**) The frequency of IL-17eYFP$^+$ γδ T cells, CD4$^+$ T cells, NK1.1$^+$ T cells, CD8$^+$ T cells, and other MNC, as indicated, was measured at day 7 p.i. The number of IL-17eYFP$^+$ CD4$^+$ T cells (**E**) and IL-17eYFP$^+$ γδ T cells (**F**) in spleen of naive and infected mice at day 7 p.i. is also shown. Splenic MNC from naive C57BL/6 and *L. donovani*–infected C57BL/6 mice (day 7 p.i.) were stimulated with PMA and ionomycin in the presence of brefeldin A, as described in the *Materials and Methods*, and the number of CD4$^+$ T cells and γδ T cells producing IL-17 (**G**) and IFN-γ (**H**) was measured. The same gating strategies employed in Fig. 4 were used. One representative experiment of at least two performed is shown (n = 3–5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $^*p \leq 0.05$ and $^{**}p \leq 0.01$ are indicated.

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

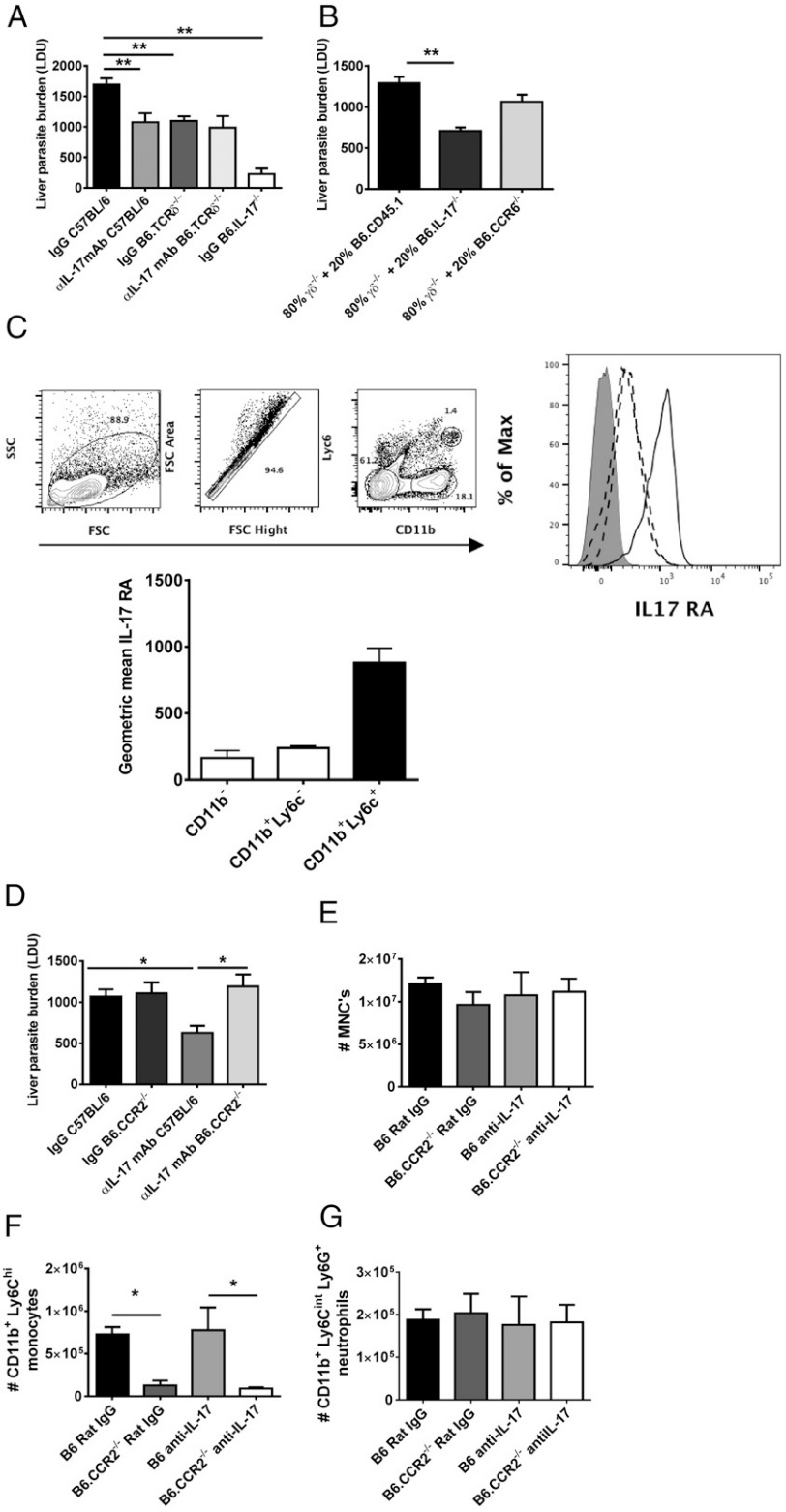

FIGURE 6. γδ T cells are a critical source of IL-17 during *L. donovani* infection. (**A**) C57BL/6, B6.*Il-17*$^{-/-}$, and B6.*Tcrδ*$^{-/-}$ mice were infected with *L. donovani* and treated with either 100 μg control IgG or anti–IL-17 mAb on days 0, 2, 4, and 6, as indicated, prior to measuring hepatic parasite burden on day 7 p.i. Mixed BM chimeras were generated by engrafting indicated BM combinations into irradiated C57BL/6 mice. (**B**) Mice were rested for 10–12 wk prior to infection with *L. donovani* and measuring parasite burden on day 7 p.i. (**C**) The geometric mean of expression of IL-17RA was measured on hepatic inflammatory monocytes (CD11b^{+}, Ly6C^{hi}; black line), all other myeloid cells (CD11b^{+}, Ly6C^{-}; dashed line), and nonmyeloid cells (CD11b^{-}; dashed line) at the same time and compared with staining of hepatic MNC with an isotype control mAb (filled curve). C57BL/6 and B6.*Ccr2*$^{-/-}$ mice were infected with *L. donovani* and administered 100 μg control IgG or anti–IL-17 Ab on days 0, 2, 4, and day 6 p.i., before measuring liver parasite burden (**D**), as well as numbers of hepatic MNC (**E**), inflammatory monocytes (**F**), and neutrophils (**G**). One representative experiment of at least two performed is shown, except for (A), performed only once (n = 5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $*p \leq 0.05$ and $**p \leq 0.01$ are indicated. FSC, forward light scatter; LDU, Leishman–Donovan units; SSC, side scatter.

total hepatic MNC (Fig. 6E) or neutrophil (Fig. 6F) number, but few inflammatory (CD11b^{+}, Ly6C^{hi}) monocytes (Fig. 6G) in the liver. Thus, the antiparasitic activity of CCR2^{+} monocytes appears to be a major target for IL-17 in the liver following *L. donovani* infection.

Increased control of parasite growth in the absence of IL-17 is independent of NO

The generation of reactive nitrogen intermediates is thought to be an important mechanism of parasite control in mice infected with *L. donovani* (42). To test whether reactive nitrogen intermediate generation and/or activity was suppressed by IL-17 in the liver after day 3 of infection, we blocked NOS in *L. donovani*–infected B6.IL-17$^{-/-}$ and C57BL/6 mice over the first week of infection. We found that NOS blockade had no effect on the increased control of parasite growth in IL-17–deficient mice (Fig. 7A). This results was not surprising because when NOS2 mRNA accumulation in hepatic MNC was measured, we found increased accumulation in infected C57BL/6 mice, but no infection-induced

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

change in IL-17–deficient mice (Fig. 7B). Interestingly, we found that hepatic MNC SOD3 mRNA levels remained elevated in B6.IL-17$^{-/-}$ mice following *L. donovani* infection, and were significantly higher than in C57BL/6 control mice (Fig. 7C). These data suggest that differences in reactive oxygen generation may offer some explanation for the early, increased resistance to infection of IL-17–deficient mice. Studies are ongoing to investigate this further.

Discussion

There is now considerable evidence to support a role for IL-17 as a proinflammatory cytokine that plays a significant role in infectious diseases and autoimmunity (43, 44). In this study, we have demonstrated that, contrary to observations made in other infectious disease models, IL-17 is detrimental in the early control of *L. donovani* infection in the liver. γδ T cells are the critical source of IL-17 in this system, and CCR2^{+} inflammatory monocytes are required for IL-17 to have its suppressive effect. These data are the first example, to our knowledge, of an inhibitory function for IL-17 in parasitic infection and point to an important interaction among γδ T cells, monocytes, and infected macrophages in the early control of VL.

The proinflammatory and host protective functions of IL-17 in other infectious disease models are well established. This includes, but is not limited to, models of *Mycobacterium tuberculosis* (45), *Salmonella* (46), and *Listeria monocytogenes* infection in which IL-17 deficiency is associated with an enhanced inflammatory response (47) and substantial neutrophil-mediated tissue pathology (48). Contrary to the data we have presented in this study, IL-17 was proinflammatory in nature in these infections, and a deficiency in IL-17 resulted in higher pathogen burdens, stemming from uncontrolled pathogen growth. γδ T cells were the predominant source of IL-17 in these models, so the differences observed in this study were not caused by a different cellular source of IL-17. More likely, the difference may lie in the role of monocytes in *L. donovani* infection, with IL-17 acting on CCR2^{+} inflammatory monocytes to enhance parasite growth. Monocytes also express the highest level of the IL-17 receptor IL-17RA in *L. donovani*–infected livers, making them the obvious target for this cytokine.

Monocytes have an established role in the formation of *L. donovani* hepatic granulomas. Mice treated with an anti–type 3 complement receptor or anti–GM-CSF Abs to block monocyte infiltration into the infected liver throughout the course of infection had significantly higher parasite burdens at 4 wk p.i., suggesting that monocytes are important for parasite control (49, 50). Although this may appear counterintuitive to our findings, these studies did not measure the effect of monocyte deficiency on the early control of *L. donovani*. We have demonstrated that the antiparasitic function of monocytes in early infection is suppressed by IL-17. For this reason, depletion studies that aim to dissect the role of monocytes early in infection will be confounded by the IL-17–mediated suppression of monocyte function, making the contribution of monocytes difficult to assess in this model. In support of this hypothesis, CCR2$^{-/-}$ mice that lack the recruitment of inflammatory monocytes to the liver show no difference in parasite burden 7 d p.i. in our hands. It is not clear how IL-17 is controlling this early antiparasitic function of monocytes, and the molecular mechanisms mediating this are the subject of further investigations.

Regardless of the mechanism of action of the monocytes in this model, there are two possible scenarios to explain the lower parasite burdens observed in the absence of IL-17. The first is that IL-17 inhibits early macrophage killing of parasites. The second more intriguing possibility is that there is negligible killing in the early phase of infection and that IL-17 enhances the growth rate of parasites within hepatic macrophages, resulting in higher parasite burdens in the IL-17–sufficient mice. Recent investigations in lesions of *L. mexicana*–infected mice suggest that parasite growth rates within lesions are much slower than previously thought and that negligible parasite death occurs (51). It is therefore conceivable that IL-17 might support growth rather than inhibit parasite killing. This is a second area for further investigation.

One particularly interesting observation of the current study is the potential interplay among monocytes, γδ T cells, and parasite-infected macrophages that has the potential to control early parasite burden in the liver. Bidirectional crosstalk between γδ T cells and monocytes has been observed in human cells in vitro in response to pathogen ligands and metabolites such as LPS and (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (52, 53). This cross-talk increased the activation of γδ T cells and resulted in differentiation of monocytes into more dendritic-like cells (53). Whether such interactions occur in this system has not yet been elucidated. Similarly, factors such as dectins, TLRs, IL-1, and IL-23 (1) that stimulate the activation of γδ T cells and the downstream effector functions of γδ T cells once they are activated, including cytokine and chemokine production, interactions with B cells, αβ T cells, and cytotoxicity (54), are all areas of interest and warrant further investigation in the context of *L. donovani* infection given the results presented in this study.

The cellular source of IL-17 in VL has not been investigated previously; however, increased levels of IL-17A in the sera of VL patients were observed, with a decrease after chemotherapeutic treatment. In parallel studies, *IL-17ra*$^{-/-}$ mice, which are deficient in IL-17 signaling, demonstrated an increase in susceptibility to chronic infection with *L. infantum* (23). In this study, we have focused on the early phase of infection with *L. donovani* and

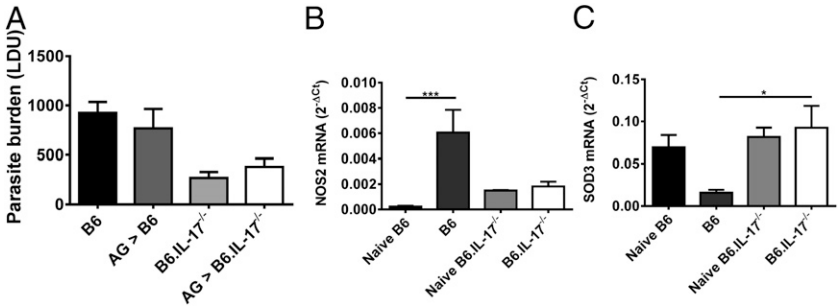

FIGURE 7. Increased control of parasite growth in the absence of IL-17 is independent of NO. (**A**) C57BL/6 and B6.*Il-17*$^{-/-}$ mice were treated with amino guanidine (AG), as indicated, to block NOS before and during infection with *L. donovani*, and liver parasite burden was measured on day 7 p.i. Hepatic MNC were isolated from C57BL/6 and B6.*Il-17*$^{-/-}$ mice before infection (n = 4/group) and 7 d p.i. with *L. donovani* (n = 5/group), and NOS2 (**B**) and SOD3 (**C**) mRNA accumulation was measured. One representative experiment of at least two performed is shown (n = 4 to 5 mice/group in each experiment). Data represented are mean ± SEM. Statistical differences of $^{*}p \leq 0.05$ and $^{***}p \leq 0.001$ are indicated. LDU, Leishman–Donovan units.

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

demonstrated that IL-17 limits the effectiveness of monocytes in controlling parasite growth and/or killing. Together, these findings point to complex roles for IL-17 in VL that are temporally, spatially, and contextually dependent.

Acknowledgments

We thank Amgen for providing anti-mouse IL-17A mAb and Brigitta Stockinger (National Institute of Medical Research, Mill Hill, London, U.K.) for generating B6.Il-17a^{Cre}R26R^{eYFP} mice. We also thank Paula Hall and Grace Chojnowski for assistance with flow cytometry and staff in the QIMR-B animal facility for animal husbandry.

Disclosures

The authors have no financial conflicts of interest.

References

1. Gaffen, S. L., R. Jain, A. V. Garg, and D. J. Cua. 2014. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat. Rev. Immunol.* 14: 585–600.
2. Korn, T., E. Bettelli, M. Oukka, and V. K. Kuchroo. 2009. IL-17 and Th17 Cells. *Annu. Rev. Immunol.* 27: 485–517.
3. Langrish, C. L., Y. Chen, W. M. Blumenschein, J. Mattson, B. Basham, J. D. Sedgwick, T. McClanahan, R. A. Kastelein, and D. J. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201: 233–240.
4. Park, H., Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y. H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6: 1133–1141.
5. Acosta-Rodriguez, E. V., L. Rivino, J. Geginat, D. Jarrossay, M. Gattorno, A. Lanzavecchia, F. Sallusto, and G. Napolitani. 2007. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat. Immunol.* 8: 639–646.
6. Aujla, S. J., Y. R. Chan, M. Zheng, M. Fei, D. J. Askew, D. A. Pociask, T. A. Reinhart, F. McAllister, J. Edeal, K. Gaus, et al. 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat. Med.* 14: 275–281.
7. Higgins, S. C., A. G. Jarnicki, E. C. Lavelle, and K. H. Mills. 2006. TLR4 mediates vaccine-induced protective cellular immunity to *Bordetella pertussis*: role of IL-17-producing T cells. *J. Immunol.* 177: 7980–7989.
8. Huang, W., L. Na, P. L. Fidel, and P. Schwarzenberger. 2004. Requirement of interleukin-17A for systemic anti-*Candida albicans* host defense in mice. *J. Infect. Dis.* 190: 624–631.
9. Khader, S. A., G. K. Bell, J. E. Pearl, J. J. Fountain, J. Rangel-Moreno, G. E. Cilley, F. Shen, S. M. Eaton, S. L. Gaffen, S. L. Swain, et al. 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8: 369–377.
10. Wu, Q., R. J. Martin, J. G. Rino, R. Breed, R. M. Torres, and H. W. Chu. 2007. IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory Mycoplasma pneumoniae infection. *Microbes Infection* 9: 78–86.
11. Engwerda, C. R., M. Ato, S. E. Cotterell, T. L. Mynott, A. Tschannerl, P. M. Gorak-Stolinska, and P. M. Kaye. 2002. A role for tumor necrosis factor-alpha in remodeling the splenic marginal zone during *Leishmania* donovani infection. *Am. J. Pathol.* 161: 429–437.
12. Smelt, S. C., C. R. Engwerda, M. McCrossen, and P. M. Kaye. 1997. Destruction of follicular dendritic cells during chronic visceral leishmaniasis. *J. Immunol.* 158: 3813–3821.
13. Veress, B., A. Omer, A. A. Satir, and A. M. El Hassan. 1977. Morphology of the spleen and lymph nodes in fatal visceral leishmaniasis. *Immunology* 33: 605–610.
14. Zijlstra, E. E., and A. M. el-Hassan. 2001. Leishmaniasis in Sudan. Visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 95(Suppl 1): S27–S58.
15. Engwerda, C. R., and P. M. Kaye. 2000. Organ-specific immune responses associated with infectious disease. *Immunol. Today* 21: 73–78.
16. Faleiro, R. J., R. Kumar, L. M. Hafner, and C. R. Engwerda. 2014. Immune regulation during chronic visceral leishmaniasis. *PLoS Negl. Trop. Dis.* 8: e2914.
17. Stanley, A. C., and C. R. Engwerda. 2007. Balancing immunity and pathology in visceral leishmaniasis. *Immunol. Cell Biol.* 85: 138–147.
18. Boelaert, M., and S. Sundar. 2014. Leishmaniasis. In *Manson'd Tropical Diseases.* J. Farrer, P. J. Hotez, T. Junghanss, G. Kang, D. Lalloo, and N. White, eds. Elsevier, Amsterdam, p. 631–651.
19. Murray, H. W., J. Hariprashad, B. Aguero, T. Arakawa, and H. Yeganegi. 1995. Antimicrobial response of a T cell-deficient host to cytokine therapy: effect of interferon-gamma in experimental visceral leishmaniasis in nude mice. *J. Infect. Dis.* 171: 1309–1316.
20. Squires, K. E., R. D. Schreiber, M. J. McElrath, B. Y. Rubin, S. L. Anderson, and H. W. Murray. 1989. Experimental visceral leishmaniasis: role of endogenous IFN-gamma in host defense and tissue granulomatous response. *J. Immunol.* 143: 4244–4249.
21. Taylor, A. P., and H. W. Murray. 1997. Intracellular antimicrobial activity in the absence of interferon-gamma: effect of interleukin-12 in experimental visceral leishmaniasis in interferon-gamma gene-disrupted mice. *J. Exp. Med.* 185: 1231–1239.
22. Pitta, M. G., A. Romano, S. Cabantous, S. Henri, A. Hammad, B. Kouriba, L. Argiro, M. el Kheir, B. Bucheton, C. Mary, et al. 2009. IL-17 and IL-22 are associated with protection against human kala azar caused by *Leishmania* donovani. *J. Clin. Invest.* 119: 2379–2387.
23. Nascimento, M. S., V. Carregaro, D. S. Lima-Júnior, D. L. Costa, B. Ryffel, M. S. Duthie, A. de Jesus, R. P. de Almeida, and J. S. da Silva. 2015. Interleukin 17A acts synergistically with interferon γ to promote protection against Leishmania infantum infection. *J. Infect. Dis.* 211: 1015–1026.
24. Lopez Kostka, S., S. Dinges, K. Griewank, Y. Iwakura, M. C. Udey, and E. von Stebut. 2009. IL-17 promotes progression of cutaneous leishmaniasis in susceptible mice. *J. Immunol.* 182: 3039–3046.
25. Gonzalez-Lombana, C., C. Gimblet, O. Bacellar, W. W. Oliveira, S. Passos, L. P. Carvalho, M. Goldschmidt, E. M. Carvalho, and P. Scott. 2013. IL-17 mediates immunopathology in the absence of IL-10 following *Leishmania major* infection. *PLoS Pathog.* 9: e1003243.
26. Bacellar, O., D. Faria, M. Nascimento, T. M. Cardoso, K. J. Gollob, W. O. Dutra, P. Scott, and E. M. Carvalho. 2009. Interleukin 17 production among patients with American cutaneous leishmaniasis. *J. Infect. Dis.* 200: 75–78.
27. Boaventura, V. S., C. S. Santos, C. R. Cardoso, J. de Andrade, W. L. Dos Santos, J. Clarêncio, J. S. Silva, V. M. Borges, M. Barral-Netto, C. I. Brodskyn, and A. Barral. 2010. Human mucosal leishmaniasis: neutrophils infiltrate areas of tissue damage that express high levels of Th17-related cytokines. *Eur. J. Immunol.* 40: 2830–2836.
28. Nakae, S., Y. Komiyama, A. Nambu, K. Sudo, M. Iwase, I. Homma, K. Sekikawa, M. Asano, and Y. Iwakura. 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17: 375–387.
29. Hirota, K., J. H. Duarte, M. Veldhoen, E. Hornsby, Y. Li, D. J. Cua, H. Ahlfors, C. Wilhelm, M. Tolaini, U. Menzel, et al. 2011. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat. Immunol.* 12: 255–263.
30. Itohara, S., P. Mombaerts, J. Lafaille, J. Iacomini, A. Nelson, A. R. Clarke, M. L. Hooper, A. Farr, and S. Tonegawa. 1993. T cell receptor delta gene mutant mice: independent generation of alpha beta T cells and programmed rearrangements of gamma delta TCR genes. *Cell* 72: 337–348.
31. Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* 68: 869–877.
32. Engwerda, C. R., M. Ato, S. Stäger, C. E. Alexander, A. C. Stanley, and P. M. Kaye. 2004. Distinct roles for lymphotoxin-alpha and tumor necrosis factor in the control of *Leishmania* donovani infection. *Am. J. Pathol.* 165: 2123–2133.
33. Bunn, P. T., A. C. Stanley, F. de Labastida Rivera, A. Mulherin, M. Sheel, C. E. Alexander, R. J. Faleiro, F. H. Amante, M. Montes De Oca, S. E. Best, et al. 2014. Tissue requirements for establishing long-term CD4+ T cell-mediated immunity following *Leishmania* donovani infection. *J. Immunol.* 192: 3709–3718.
34. Stanley, A. C., F. de Labastida Rivera, A. Haque, M. Sheel, Y. Zhou, F. H. Amante, P. T. Bunn, L. M. Randall, K. Pfeffer, S. Scheu, et al. 2011. Critical roles for LIGHT and its receptors in generating T cell-mediated immunity during *Leishmania* donovani infection. *PLoS Pathog.* 7: e1002279.
35. Engwerda, C. R., T. L. Mynott, S. Sawhney, J. B. De Souza, Q. D. Bickle, and P. M. Kaye. 2002. Locally up-regulated lymphotoxin alpha, not systemic tumor necrosis factor alpha, is the principle mediator of murine cerebral malaria. *J. Exp. Med.* 195: 1371–1377.
36. Stanley, A. C., Y. Zhou, F. H. Amante, L. M. Randall, A. Haque, D. G. Pellicci, G. R. Hill, M. J. Smyth, D. I. Godfrey, and C. R. Engwerda. 2008. Activation of invariant NKT cells exacerbates experimental visceral leishmaniasis. *PLoS Pathog.* 4: e1000028.
37. Beattie, L., R. Phillips, N. Brown, B. M. Owens, N. Chauhan, J. E. Dalton, and P. M. Kaye. 2011. Interferon regulatory factor 7 contributes to the control of *Leishmania* donovani in the mouse liver. *Infect. Immun.* 79: 1057–1066.
38. Stern, J. J., M. J. Oca, B. Y. Rubin, S. L. Anderson, and H. W. Murray. 1988. Role of L3T4+ and LyT-2+ cells in experimental visceral leishmaniasis. *J. Immunol.* 140: 3971–3977.
39. Haas, J. D., F. H. González, S. Schmitz, V. Chennupati, L. Föhse, E. Kremmer, R. Förster, and I. Prinz. 2009. CCR6 and NK1.1 distinguish between IL-17A and IFN-gamma-producing gammadelta effector T cells. *Eur. J. Immunol.* 39: 3488–3497.
40. Geissmann, F., S. Jung, and D. R. Littman. 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19: 71–82.
41. Serbina, N. V., and E. G. Pamer. 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat. Immunol.* 7: 311–317.
42. Murray, H. W., and C. F. Nathan. 1999. Macrophage microbicidal mechanisms in vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral *Leishmania* donovani. *J. Exp. Med.* 189: 741–746.
43. Jones, S. A., C. E. Sutton, D. Cua, and K. H. Mills. 2012. Therapeutic potential of targeting IL-17. *Nat. Immunol.* 13: 1022–1025.
44. Miossec, P., and J. K. Kolls. 2012. Targeting IL-17 and TH17 cells in chronic inflammation. *Nat. Rev. Drug Discov.* 11: 763–776.
45. Lockhart, E., A. M. Green, and J. L. Flynn. 2006. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 177: 4662–4669.
46. Schulz, S. M., G. Köhler, N. Schütze, J. Knauer, R. K. Straubinger, A. A. Chackerian, E. Witte, K. Wolk, R. Sabat, Y. Iwakura, et al. 2008. Protective immunity to systemic infection with attenuated *Salmonella enterica*

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015

serovar enteritidis in the absence of IL-12 is associated with IL-23-dependent IL-22, but not IL-17. *J. Immunol.* 181: 7891–7901.
47. Hamada, S., M. Umemura, T. Shiono, K. Tanaka, A. Yahagi, M. D. Begum, K. Oshiro, Y. Okamoto, H. Watanabe, K. Kawakami, et al. 2008. IL-17A produced by gammadelta T cells plays a critical role in innate immunity against listeria monocytogenes infection in the liver. *J. Immunol.* 181: 3456–3463.
48. Sieve, A. N., K. D. Meeks, S. Bodhankar, S. Lee, J. K. Kolls, J. W. Simecka, and R. E. Berg. 2009. A novel IL-17-dependent mechanism of cross protection: respiratory infection with mycoplasma protects against a secondary listeria infection. *Eur. J. Immunol.* 39: 426–438.
49. Cervia, J. S., H. Rosen, and H. W. Murray. 1993. Effector role of blood monocytes in experimental visceral leishmaniasis. *Infect. Immun.* 61: 1330–1333.
50. Murray, H. W., J. S. Cervia, J. Hariprashad, A. P. Taylor, M. Y. Stoeckle, and H. Hockman. 1995. Effect of granulocyte-macrophage colony-stimulating factor in experimental visceral leishmaniasis. *J. Clin. Invest.* 95: 1183–1192.
51. Kloehn, J., E. C. Saunders, S. O'Callaghan, M. J. Dagley, and M. J. McConville. 2015. Characterization of metabolically quiescent *Leishmania* parasites in murine lesions using heavy water labeling. *PLoS Pathog.* 11: e1004683.
52. Eberl, M., and B. Moser. 2009. Monocytes and gammadelta T cells: close encounters in microbial infection. *Trends Immunol.* 30: 562–568.
53. Eberl, M., G. W. Roberts, S. Meuter, J. D. Williams, N. Topley, and B. Moser. 2009. A rapid crosstalk of human gammadelta T cells and monocytes drives the acute inflammation in bacterial infections. *PLoS Pathog.* 5: e1000308.
54. Vantourout, P., and A. Hayday. 2013. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat. Rev. Immunol.* 13: 88–100.

Downloaded from http://www.jimmunol.org/ at Queensland Institute of Medical Research on December 20, 2015