IL-33 promotes innate lymphoid cell-dependent IFN- γ production required for innate immunity to *Toxoplasma gondii*

Authors

Joseph T. Clark¹, David A. Christian¹, Jodi A. Gullicksrud¹, Joseph A. Perry¹, Jeongho Park^{1,2}, Maxime Jacquet^{1,3}, James C. Tarrant¹, Enrico Radaelli¹, Jonathan Silver⁴, Christopher A. Hunter^{1*}

Address

¹Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA

² Kangwon National University College of Veterinary Medicine and Institute of Veterinary Science, Chuncheon, Korea

³ Liver Immunology, Department of Biomedicine, University Hospital of Basel and University of Basel, Basel, Switzerland

⁴Department of Respiratory Inflammation and Autoimmunity, AstraZeneca, Gaithersburg, MD, USA

*Corresponding Author

Abstract

IL-33 is an alarmin required for resistance to the parasite *Toxoplasma gondii*, but its role in innate resistance to this infection is unclear. *T. gondii* infection promotes increased stromal cell expression of IL-33 and levels of parasite replication correlate with IL-33 release. In response to infection, a subset of innate lymphoid cells (ILC) emerges composed of IL-33R⁺ NK cells and ILC1s. In Rag^{-/-} mice, where NK cells and ILC1 provide an innate mechanism of resistance to *T. gondii*, the loss of IL-33R reduced ILC responses and increased parasite replication. Furthermore, administration of IL-33 to Rag^{-/-} mice resulted in a marked decrease in parasite burden, increased production of IFN- γ and the recruitment and expansion of inflammatory monocytes associated with parasite control. These protective effects of exogenous IL-33 were dependent on endogenous IL-12p40 and the ability of IL-33 to enhance ILC production of IFN- γ . These results highlight that IL-33 synergizes with IL-12 to promote ILC-mediated resistance to *T. gondii*.

Introduction:

Toxoplasma gondii is an intracellular parasite of public health significance [1]–[3]. Resistance to this organism is initiated by dendritic cell production of IL-12, which promotes NK and T cell secretion of IFN- γ [4]–[6]. IFN- γ in turn induces multiple anti-microbial mechanisms, which include the activation of macrophages to express iNOS, which are required to limit parasite replication [7]–[12]. Previous studies have shown that mice deficient in the adapter molecule MyD88 have increased susceptibility to *T. gondii* associated with reduced production of IL-12 and IFN- γ [13],[14]. Since MyD88 is a major adapter required for Toll Like Receptor (TLR) signaling, this increased susceptibility is consistent with a role for TLR-mediated recognition of this pathogen [9],[10]. However, TLR1, TLR2, TLR4, TLR6, TLR9 and TLR11 are individually not required for early resistance to *T. gondii* [15]–[18], and there is a MyD88-independent mechanism of parasite recognition [19]–[21]. Moreover, administration of IL-12 to MyD88^{-/-} mice does not restore the ability to produce IFN- γ , and NK and T cell expression of MyD88 is required for optimal production of IFN- γ and resistance to *T. gondii* [22],[23]. Thus, MyD88 has a critical role in resistance to *T. gondii*, but the events that engage this adapter molecule are unclear.

Members of the IL-1 family of cytokines, including IL-1 α/β , IL-18, and IL-33, utilize distinct receptor sub-units but share downstream signaling machinery that includes MyD88. These cytokines impact a wide range of immune cells, and influence many facets of the innate immune system [24]. Mice that lack T and B cells have helped define the impact of cytokines on innate mechanisms of immunity to a wide variety of pathogens [25]–[28]. For example, these models were important to identify the role of IL-12 in promoting NK cell production of IFN- γ required for resistance to *Listeria monocytogenes* and *T. gondii* [29]–[31]. It is now appreciated that NK cells and ILC1 populations are both relevant sources of IFN- γ that contribute to resistance to *T. gondii* [32],[33]. Although IL-1 and IL-18 synergize with IL-12 to promote NK cell production of IFN- γ [34]–[36], the role of endogenous IL-1 during toxoplasmosis is secondary to those of IL-12 [17],[34],[37] while endogenous IL-18 is not

required for parasite control but rather contributes to the immune pathology that can accompany this infection [35],[38]–[41].

IL-33 is a cytokine that is constitutively expressed by endothelial and epithelial cells, and in current models the death of these cells leads to release of IL-33 that acts as a damage associated molecular pattern (DAMP) or alarmin to activate immune cell populations [42]–[45]. While there are open questions about whether this cytokine can also be secreted [46],[47], the rapid oxidation of IL-33 inactivates this cytokine and ensures that its activity is restricted to local sites of tissue damage [48]. IL-33 and the IL-33R ST2 are most prominently associated with amplification of TH2 CD4⁺ T cells, activation of ILC2 and resistance to helminths [42],[49]–[53], immune regulation by Treg cells [54],[55], and a number of metabolic and para-immune functions mediated by ILC2 and regulatory T cells [56],[57]. Consistent with its ability to promote TH2-type responses, IL-33 can antagonize inflammation mediated by TH1/TH17 cells during experimental allergic encephalomyelitis (EAE) [58]–[60] and suppresses pathological TH1 responses during visceral Leishmaniasis [61]. However, during infection with LCMV or MCMV, IL-33 promotes the expansion of NK cells and T cells and their production of IFN- γ , and loss of the IL-33R results in a delay in viral clearance [62]–[64]. In contrast, perhaps one of the most striking phenotypes of mice that lack IL-33R is that they succumb to chronic toxoplasmosis, associated with reduced astrocyte responses required for protective T cell responses [65],[66]. Nevertheless, the acute stage of toxoplasmosis is associated with the ability of *T. gondii* to infect and lyse epithelial and endothelial cells [67]–[71], but whether these events lead to the release of IL-33 or if this affects the innate response to *Toxoplasma* is unknown. The present study reveals that parasite replication during acute toxoplasmosis is associated with release of IL-33, and Rag^{-/-} mice that lack the IL-33R have defects in ILC production of IFN- γ and impaired parasite control. Furthermore, administration of IL-33 to Rag^{-/-} mice enhanced ILC production of IFN- γ associated with the expansion of a population of Ly6c^{hi} CCR2⁺ inflammatory monocytes and a marked reduction in parasite burden.

Together, these results highlight that infection-induced release of IL-33 synergizes with IL-12 to promote ILC-mediated resistance to *T. gondii*.

Materials and Methods

Mice

C57BL/6NTac (Taconic #B6-F), Rag1^{-/-} (B6.129S7-Rag1^{tm1Mom}/J) (Jackson #002216), and Rag2^{-/-}Il2rg^{-/-} (Rag2^{tm1Fwa}Il2rg^{tm1Wjl}) (Taconic #4111) mice were purchased from their respective vendors. IL-33^{-/-} (Il33^{tm1.1Arte})(Jackson #350163) mice were provided by MedImmune (now AstraZeneca). IL33^{fl/fl}-eGFP (B6(129S4)-Il33^{tm1.1Bryc}/J), originally generated by Paul Bryce were obtained locally from Dr. De'Broski Herbert, IL-33R^{-/-} *Il1rl1*^{-/-} mice, originally derived by Andrew McKenzie (University of Cambridge) (16) and back-crossed to C57BL/6 by Peter Nigrovic (Harvard University), were provided by Edward Behrens at Children's Hospital of Philadelphia. IL-33R^{-/-} Rag1^{-/-} mice were generated by crossing the knockouts described above. Mice were housed in a specific pathogen free environment at the University of Pennsylvania School of Veterinary Medicine and treated according to protocols approved by the Institutional Animal Care and Use Committee at the University. Male and Female (age 8–12 weeks at start of experiment) mice were used for all experiments.

Parasites and Infection

The ME49 strain of *T. gondii* was maintained by serial passage in Swiss Webster mice and used to generate banks of chronically infected CBA/ca mice, which were a source of tissue cysts for these experiments. Pru-derived transgenic parasites and CPS parasites were maintained in cultured human fibroblasts in DMEM supplemented with 10% FBS. For CPS parasites, supplemental uracil was also added to media. Mice were infected intraperitoneally with 20 cysts (ME49), or 1x10⁴ tachyzoites (Pru), or 2x10⁵ tachyzoites (CPS). Soluble toxoplasma antigen was prepared from tachyzoites of the RH strain as described previously [72]. For quantitative PCR (qPCR), DNA was isolated from tissues using the DNEasy DNA isolation kit (Qiagen) followed by qPCR measuring the abundance of the *T. gondii* gene B1

115 using the primers 5'-TCTTTAAAGCGTTCGTGGTC-3' (forward) and 5'-GGAAGTGCATCCGTTCATGAG-3'
116 (reverse).

117 **Histology**

118 For IHC detection of *T. gondii* and iNOS, tissues were fixed in 10% formalin solution and then paraffin
119 embedded and sectioned. Sections were deparaffinized, rehydrated, Ag retrieved in 0.01 M sodium
120 citrate buffer (pH 6.0), and endogenous peroxidase blocked by 0.3% H₂O₂ in PBS. After blocking with
121 2% normal goat serum, the sections were incubated either with anti-*Toxoplasma* Ab, anti-iNOS Ab or
122 isotype control. The sections were then incubated with biotinylated goat anti-rabbit IgG (Vector,
123 Burlingame, CA), and ABC reagent was applied (Vectastain ABC Kit; Vector Labs). Then DAB substrate
124 (Vector Labs) was used to visualize specific staining according to manufacturer's instructions, and slides
125 were counterstained with hematoxylin. To quantify parasite burden in the peritoneal exudate, 100,000
126 cells were used to prepare cytopspins. Cells were methanol fixed and then stained with the Protocol
127 Hema-3 Stain Set, and the ratio of infected cells to total cells in a field of view was calculated. For whole
128 tissue mount immunofluorescence staining, omenta were harvested from mice and fixed in 1% PFA
129 overnight at 4°C. After rinsing, tissue was blocked using 10% BSA, 0.5% normal rat serum (Invitrogen),
130 and 1 µg/ml 2.4G2 (BD) in PBS for 1 hr at room temperature. Omenta were next incubated in PBS
131 containing primary antibodies at 4°C for 3 days and subsequently rinsed with PBS overnight.
132 Immunofluorescence combining IL-33 (R&D AF3626) and CD45 (Biolegend 30-F11) antibodies was
133 performed using the OPAL Automation Multiplex IHC Detection Kit (Akoya Biosciences, Catalog 160
134 #NEL830001KT) implemented onto a BOND Research Detection System (DS9455). All widefield images
135 were obtained on a Leica DM6000 microscope using the Leica Imaging Suite software. Confocal images
136 were acquired on a Leica STED 3X Super-resolution microscope. Image analysis was performed using
137 FIJI and Imaris software packages.

138 **Generation of Lymphokine Activated Killer cells**

139 Lymphokine Activated Killer cells (LAKs) were generated from Rag1^{-/-} bone marrow as described
140 previously [73],[74]. Briefly, whole bone marrow was plated at 1M cells/mL in cRPMI + 400U/mL
141 Proleukin human IL-2 (Peprotech). Fresh IL-2 was added every 3rd day, and cells were used for
142 experiments between days 7 to 10.

143 **Antibody and cytokine reagents**

144 For in vitro assays, recombinant IL-33 was purchased from Peprotech (Cat #210-33 Rocky Hill, NJ). For
145 in vivo treatment experiments, recombinant IL-33 (MedImmune), which was modified to be resistant
146 to oxidation was used, as described previously[48]. IL-33 DuoSet ELISA was purchased from R&D
147 Biosystems (Cat # DY3626, Minneapolis, MN). For flow cytometry the following combinations of
148 antibodies were used: for analysis of NK cells: CD335 Nkp46 (29A1.4, eBioscience), NK-1.1 (PK136,
149 Biolegend), IFN- γ (XMG1.2, eBioscience), CD200R1 (OX110, eBioscience), IL-33R (DJ8, MD Biosciences).
150 For analysis of myeloid cells: CD11b (M1/70, eBioscience), CD11c (N418, Biolegend), Ly6c (HK1.4,
151 Biolegend), Ly6g (1A8, Biolegend), CCR2 CD192 (SA203G11, Biolegend), CD64 Fc γ RI (X54-5/7.1,
152 Biolegend), MHC II I-A/I-E (m5/114.15.2, eBioscience), iNOS (CXNFT, eBioscience), IL-33R (DJ8, MD
153 Biosciences). Flow cytometry was performed on BD Fortessa and X-50 cytometers and data analysis
154 was performed using Flowjo 9 and Flowjo 10 (Treestar), and Prism 7 and 8 (Graphpad). Uniform
155 Manifold Approximation and Projection for Dimension Reduction (uMAP) analysis was performed using
156 the uMAP plug-in (version: 1802.03426, 2018, ©2017, Leland McInness) for Flowjo (Version 10.53).
157 The Euclidean distance function was utilized with a nearest neighbor score of 15, and a minimum
158 distance rating of 0.5.

159 **Quantification and statistical analysis** All data are expressed as means $\pm$ SEM. For comparisons
160 between two groups, the Student's t-test was applied. For data with more than two data sets, one-way
161 ANOVA coupled with Tukey's multiple comparisons test was applied. Statistical details are indicated in
162 figure legends.

Results

Toxoplasma gondii infection induces IL-33 upregulation and release

To determine the impact of *Toxoplasma* infection on IL-33 expression and secretion, C57BL/6 WT and Rag^{-/-} mice were infected intraperitoneally (i.p.) with the Me49 strain or the replication-deficient CPS strain of *T. gondii*, and the levels of IL-33 at local sites of infection and affected tissues assessed by ELISA. In the peritoneum of naïve WT and Rag^{-/-} mice the level of IL-33 was below the limit of detection (<10 pg/mL) (Fig 1A). Infection i.p. with 2x10⁵ tachyzoites of the non-replicating CPS strain did not cause parasite-induced host cell lysis and failed to elicit detectable IL-33 at 1 or 5 days post-infection (dpi) (data not shown). Infection of WT mice with 20 cysts of Me49 resulted in <1% infected cells in the peritoneum at 5 dpi, and IL-33 was not detected (Fig 1A). When Rag^{-/-} mice received the same challenge, there were 2-5% infected cells at 5 dpi, and low levels of IL-33 were detected (Fig 1A). To test if IL-33 levels were a function of parasite burden, WT and Rag^{-/-} mice were treated with anti-IFN- γ , which resulted in a 20-fold increase in parasite load (data not shown) and a 3-4-fold increase in the levels of IL-33 (Fig 1A). When these data sets were collated and quantity of parasite DNA plotted versus IL-33 concentration, there was a strong correlation between parasite burden and IL-33 levels (R = 0.7902) (Fig 1B). To determine if IL-33 was released in other tissues affected by *T. gondii*, tissue biopsies from the liver of WT mice at 10 dpi were prepared and placed in culture for 24 hours and IL-33 release measured. While basal levels of IL-33 were detected in tissues from naïve WT mice and mice injected with replication deficient CPS parasites, the biopsies from infected mice showed significantly elevated levels of IL-33 (Fig 1C). These results suggest that parasite replication and lysis of infected cells lead to IL-33 release.

To identify the cellular source of IL-33 during infection, the IL-33-IRES-GFP mouse [75], a faithful reporter for IL-33 protein production (Supp Fig 1A), was utilized. The IL-33 reporter mice were infected i.p. with a fluorescent strain of *T. gondii* (Pru-tdTom), and the expression of IL-33-GFP in the omentum

was examined by flow cytometry and IHC at 3 dpi. The omentum is an adipose tissue that contains Fat Associated Lymphoid Clusters (FALCs) which are one of the major sites for drainage from the peritoneum [76]–[78]. In naïve mice, the omentum contained a small population of CD45⁺ immune cells, most of which were IL-33-GFP⁻, whereas fibroblastic stromal cells (CD45⁻FSC^{hi} SSC^{hi} CD31^{+/-} PDPN^{+/-}) were the main source of IL-33-GFP⁺ cells (Fig 1D and Supp Fig 1B). Upon infection, there was a marked expansion of the CD45⁺ population. A small population of CD45⁺ F4/80⁺ MHCII⁺ cells expressed IL-33 (data not shown) but the majority of IL-33-GFP⁺ cells remained fibroblastic stromal cells. The ability to detect infected cells based on parasite expression of tdTomato revealed that infected cells were not associated with IL-33 expression (Supp. Fig 1C). Similarly, at 7 days post infection the use of flow cytometry and immunofluorescence revealed that CD45⁻ cells were the dominant source of IL-33 in the spleen, lung, and liver (Supp Fig 1D).

To understand the spatial organization of the IL-33-GFP⁺ cells, the omentum was used for whole tissue mount immunofluorescence. In uninfected mice, consistent with the analysis above, IL-33 was constitutively expressed by non-hematopoietic CD45⁻ cells with fibroblastic morphology distributed throughout the FALCS. At 3 dpi there was a marked increase in the size of the FALC, and an approximate 3-fold increase in number of IL-33⁺ cells (Fig 1E). These images are max projection views that illustrate the size of FALCs, but quantification of the intensity of fluorescence highlighted the 10-fold increase in the expression of IL-33-GFP (Fig 1E) associated with ERTR7⁺ fibroblastic reticular cells (Fig 1F). Imaging revealed that areas of parasite replication were inversely correlated with the presence of IL-33-GFP expression (Fig 1G). Together, these data establish that in vivo infection with *T. gondii* leads to the release of IL-33 by stromal cells that correlates with levels of parasite replication.

ILC responses to IL-33

To identify the cell populations that could respond to the local release of IL-33 during this infection, a UMAP analysis was used to provide an unbiased comparison of the changes in IL-33R

212 expression in the peritoneum of naïve and infected Rag^{-/-} mice (Fig 2A). In naïve mice, IL-33R was
 213 expressed by peritoneal macrophages (CD64⁺CD11b⁺MHCII⁺) and a small population of ILC2 (Lin⁻
 214 Nkp46⁻) when compared with IL-33R^{-/-} controls (Fig 2A). By 5 dpi there was a marked change in the
 215 cellular composition of the peritoneum with a loss of the MHCII⁻ macrophage and ILC2 populations
 216 but a prominent monocyte and neutrophil infiltration and the expansion of Nkp46⁺ NK cells and
 217 ILC1s. While there were low levels of IL-33R expressed by MHCII^{hi} CD64⁺ cells, the highest levels of IL-
 218 33R were observed on Nkp46⁺ cells. Further validation revealed that in naïve WT, IL-33R^{-/-}, and IL-33^{-/-}
 219 mice, IL-33R expression was not detected on peritoneal or splenic NK cells, but IL-33R was observed
 220 on a subset (~20%) of NK cells by 5 dpi (Fig 2B). Furthermore, NK cells from infected IL-33^{-/-} mice still
 221 upregulated IL-33R, indicating that IL-33 signaling is not required for this process. Thus, infection with
 222 *T. gondii* leads to the emergence of populations of NK cells and ILC1s that express the IL-33R.

223 Next, a population of IL-2 induced LAKs (generated from the bone marrow of Rag^{-/-} mice) were
 224 utilized to compare the impact of IL-33 (and its relative IL-18) alone or in combination with IL-12 on
 225 ILCs. Phenotyping of these LAK cultures revealed that they contained ILC1s (Nkp46⁺ CD200R1⁺), ILC2s
 226 (Nkp46⁻ CD200R1⁺) and NK cells (Nkp46⁺ CD200R1⁻) (Fig 2C). Upon withdrawal of IL-2, the addition of
 227 IL-33 preferentially stimulated the proliferation of CD200R1⁺ ILC2s, while IL-18 stimulated NK cell
 228 proliferation (Fig 2C). IL-12 alone did not induce the expansion of a specific cell type, but when
 229 combined with IL-33 maintained the heterogeneity of the LAK population and IL-12 plus IL-18
 230 resulted in a modest increase in the proportion of NK cells compared to IL-18 alone. Testing of the
 231 ability of IL-33 to stimulate LAKs to produce IFN- γ showed that IL-33 alone did not stimulate LAKs to
 232 produce IFN- γ but did act in synergy with IL-12 to enhance the production of IFN- γ in the Nkp46⁺
 233 populations (Fig 2D). Similar results were observed when splenocytes from Rag^{-/-} mice were used (Fig
 234 2D), indicating that these effects of IL-33 on NK cells were not dependent on pre-activation with IL-2.

These observations are consistent with previous reports on the ability of IL-33 to promote ILC2 activity [26],[79], but demonstrate that in the presence of IL-12, IL-33 is a potent inducer of IFN- γ .

Endogenous IL-33 is required for innate resistance to *T. gondii*

To directly test the role of endogenous IL-33 in innate resistance to *T. gondii*, Rag^{-/-} mice that lacked the IL-33R (Rag^{-/-}IL-33R^{-/-} mice) were generated and infected i.p. with *T. gondii*. Compared to Rag^{-/-} mice, at 7 dpi the Rag^{-/-}IL-33R^{-/-} mice showed an increased parasite burden based on the frequency of infected cells in the peritoneum (Fig 3A) and quantitation of parasite DNA in the peritoneum and liver (Fig 3B). Serum analysis of infected mice revealed comparable levels of IL-12p40 in Rag^{-/-} and Rag^{-/-}IL-33R^{-/-} mice, but IFN- γ was severely compromised in the absence of IL-33R (Fig 3C). At this time point, Rag^{-/-} mice had a marked expansion in ILC1s and NK cells in the liver that was reduced in the absence of the IL-33R (Fig 3D). Consistent with decreased production of IFN- γ , fewer Ly6c^{hi} monocytes were recruited to the liver in the Rag^{-/-}IL-33R^{-/-} mice, and these Ly6C^{hi} monocytes expressed lower levels of iNOS (Fig 3E). Analysis of the Ly6c^{hi} population in the peritoneum at 7 dpi after infection with Pru-tdTom showed that a proportion of infected and uninfected cells express iNOS in the Rag^{-/-} mice, but iNOS levels were markedly reduced in the Rag^{-/-}IL-33R^{-/-} mice (Fig 3F). These data sets establish that endogenous IL-33 is required for optimal production of innate IFN- γ and the recruitment of monocyte populations that express anti-microbial effector mechanisms required for resistance to *T. gondii*.

IL-33 treatment boosts IL-12 and IFN- γ dependent immunity

Based on the ability of IL-33 to stimulate IL-12-dependent IFN- γ production in ILC1s and NK cells, a recombinant version of IL-33, resistant to oxidation which has a 30 fold increase in efficacy [36],[48], was utilized to determine if exogenous IL-33 could be used to enhance innate resistance to *T. gondii*.

259 Beginning at 1 dpi, IL-33 was administered i.p. every two days until 7 dpi, which resulted in a dose-
 260 dependent reduction in the frequency of infected cells at the site of infection and a decrease in parasite
 261 DNA in multiple tissues (Fig 4A). Analysis of cytopins of PECs revealed that treatment of infected Rag^{-/-}
 262 mice with IL-33 resulted in the emergence of a highly activated monocyte population (Fig 4B). These
 263 inflammatory monocytes were larger (higher FSC) and more granular (higher SSC) (Fig 4C).
 264 Furthermore, these cells were characterized by their expression of CD11b, CD11c, Ly6c, CCR2, and
 265 MHCII (Fig 4C). IL-33 treatment also resulted in increased recruitment of Ly6c^{hi} CCR2⁺ inflammatory
 266 monocytes to the liver and lungs by 7 dpi, and these monocytes had enhanced iNOS and IL-33R
 267 expression (Fig 4D). Histological analysis of the liver confirmed that IL-33 treatment resulted in
 268 increased cellular infiltration and expression of iNOS (Fig 4E, black arrows). Importantly, these changes
 269 induced by IL-33 treatment were associated with decreased necrotic foci that were frequent in infected
 270 Rag^{-/-} mice (blue arrow). These results correlate the protective effects of IL-33 treatment with an
 271 increase in macrophage and monocytes responses required for the control of *T. gondii*.

272 To determine whether the protective effects of exogenous IL-33 depended on the ability of IL-
 273 12 to promote ILC production of IFN- γ , infected Rag^{-/-} mice were treated with IL-33 in combination with
 274 either anti-IL-12p40 or anti-IFN- γ neutralizing antibodies. Additionally, Rag^{-/-} γ C^{-/-} mice, which lack ILC,
 275 were treated with PBS or IL-33. Blockade of either IL-12 or IFN- γ entirely abrogated the protective
 276 effects of IL-33 treatment as measured by the frequency of infected cells in the peritoneum at 7 dpi
 277 (Fig 5A). Rag^{-/-} γ C^{-/-} mice were more susceptible than Rag^{-/-} mice, as expected, and IL-33 treatment did
 278 not affect parasite burden in the peritoneum. IFN- γ levels at the site of infection were increased by IL-
 279 33 treatment in ILC-sufficient Rag^{-/-} animals, but were unaffected in Rag^{-/-} γ C^{-/-} animals (Fig 5B). The
 280 expansion of Ly6c^{hi} CCR2⁺ monocytes associated with protection was also dependent on these factors,
 281 as cytokine blockade or absence of innate lymphoid cells effectively eliminated these cells (Fig 5C and

282 Supp Fig 2). These results emphasize that the protective effects of IL-33 are dependent on IL-12 and
283 ILC cytokine production and consequent recruitment of inflammatory monocytes.

284
285

286 Discussion

287 Previous studies have identified a central role for IL-12 in innate and adaptive production of
288 IFN- γ required for control of *T. gondii* [4],[5],[80]–[83] but other cytokines and costimulatory pathways
289 potentiate the effects of IL-12 on NK cells. In particular, IL-1 and IL-18 can amplify NK cell production
290 of IFN- γ but evidence that endogenous IL-1 and IL-18 are critical for control of *T. gondii* in this model is
291 limited. Thus, while IL-1 or IL-18 contribute to the development of infection-induced, microbiome-
292 dependent immune mediated pathology in the gut, there is limited evidence that loss of IL-1 or IL-18
293 leads to increased parasite replication [17],[22],[37],[39],[84]–[87]. Indeed, early studies showed that
294 neutralization of endogenous IL-18 did not affect levels of parasite replication, and that in SCID mice
295 treated with IL-12 the effects of IL-1R blockade were modest and these treated mice were still more
296 resistant than untreated SCID mice [34]. It is relevant to note that recent work has highlighted a cell
297 intrinsic role for MyD88 in NK cells to help control *T. gondii* [23], but neither IL-1 blockade or the use
298 of IL-1R^{-/-} and IL-18^{-/-} mice, replicates the susceptibility of MyD88^{-/-} mice [22],[17]. In the studies
299 presented here, the reduced NK and ILC responses observed in Rag^{-/-}IL-33R^{-/-} mice suggest that the
300 ability of IL-33 (rather than IL-1 or IL-18) to amplify the IL-12-mediated innate response to acute
301 toxoplasmosis helps explain the role for MyD88 in innate resistance to *T. gondii*. It is increasingly
302 appreciated that in addition to NK cells, tissue resident ILC1s are an early source of IFN- γ in the innate
303 response to *T. gondii* [32],[33], but the differential programming of these cells, including their
304 responsiveness to the IL-1 family member cytokines, is still being described. While IL-12 is central to
305 NK and ILC1 production of IFN- γ , there are other stimuli that can potentiate this pathway [88]. Certainly

306 in vitro, in the presence of IL-12, IL-33 can promote NK cells and ILC1 IFN- γ production. While IL-18 is a
307 more potent stimulator of IFN- γ in LAK cultures, the ability of IL-33 to promote NK and ILC1 responses,
308 combined with the defects seen in these populations in IL-33R^{-/-} mice, suggest that of all the IL-1 family
309 members IL-33 is uniquely important for innate lymphoid cell responses to *Toxoplasma*.

310 Because the signaling pathways for the IL-1 family members converge on MyD88-dependent
311 activation of NF- κ B, differences in expression patterns and tissue localization are likely to dictate the
312 relative importance of each cytokine. The release of IL-1 and IL-18 is typically considered to be
313 downstream of inflammasome mediated caspase activation and processing of pro-forms of these
314 cytokines [89]. While there is evidence that pro-IL-1 and pro-IL-18 are produced during toxoplasmosis
315 [31],[90]) several studies have concluded that *T. gondii* does not readily activate inflammasomes and
316 there is evidence that *Toxoplasma* suppresses inflammasome activity [40], [91],[92]. However, there
317 is a report that the inflammasome sensors NLRP1 and NLRP3 are required for protective immunity to
318 *T. gondii* [41]. A possible explanation for this discrepancy is that some inflammasome components are
319 not just microbial sensors but have additional functions that include a role for Caspase 8 in the
320 activation of the c-Rel transcription factor required for expression of IL-12 and resistance to *T. gondii*
321 [93]. To date, no murine sensor of *Toxoplasma* or parasite ligand has been identified that directly
322 activates inflammasomes, although there is evidence for sensor-independent routes for inflammasome
323 activation [94]. In contrast to the complex events that lead to the production and processing of IL-1
324 and IL-18, IL-33 is expressed constitutively by epithelial and endothelial cells at barrier sites and stored
325 in the nucleus and therefore may be resistant to parasite mechanisms of immune evasion and
326 suppression that target host cell transcription. The release of IL-33 can occur as a consequence of tissue
327 damage associated with allergic inflammation or viral infection [63],[50] and during toxoplasmosis IL-
328 33 levels correlated with levels of parasite replication. Thus, even though there may be non-canonical

329 pathways for IL-33 release [46],[47],[56],[95],[96] it seems likely that these levels are a consequence of
330 parasite-mediated lysis of infected cells.

331 Treatment of infected mice with exogenous IL-33 confirmed the protective effects of IL-33 and
332 highlighted the impact on the recruitment of inflammatory monocytes to sites of infection and the
333 subsequent upregulation of iNOS, a process required for control of *T. gondii* [7]–[10]. IL-33 drives ILC-
334 cell dependent recruitment of CCR2⁺ inflammatory monocytes which resemble the TipDCs (TNF and
335 iNOS-producing dendritic cells) previously recognized to be important for control of infection [57],[58].
336 This agrees with findings in an allergy model that described a role for IL-33 in the CCR2-dependent
337 recruitment of inflammatory monocytes [97]. IL-33 may also act directly on these monocytes, as
338 expression of the IL-33R was observed on monocytes in these studies, and it has been reported that IL-
339 33 can directly enhance monocyte production of iNOS [98]. It is important to note that endogenous IL-
340 33 is susceptible to rapid inactivation via oxidation in the extracellular space, which restricts its effects
341 spatially and temporally. However, the recombinant IL-33 used in these studies was engineered to
342 resist oxidation and it is possible that this treatment approach may have wider activities on
343 hematopoiesis than IL-33 produced at sites of inflammation.

344 While IL-33 is most prominently linked to the regulation of Th2 type responses, there are
345 reports that highlight the context dependent role that IL-33 plays in TH1 responses. In models of
346 Leishmaniasis and cerebral malaria, IL-33 contributes to T-cell dependent immune pathology in the skin
347 and brain, respectively [61],[99]. However, with the viral pathogens MCMV and LCMV, IL-33
348 contributes to NK and T cell expansion, and in its absence there is a delay in viral clearance, but in
349 neither case is IL-33 essential for protective immunity [37],[62]. Indeed, during intracerebral LCMV
350 infection IL-33 contributes to the development of lethal immune pathology [63], whereas for mice
351 chronically infected with *T. gondii* the loss of IL-33 results in increased parasite burden [66]. More
352 recent studies have highlighted that IL-33 promotes astrocyte responses that promote T cell responses

353 required for control of *T. gondii* in the CNS [65]. Nevertheless, the data presented here establish that
354 the ability of IL-33 to amplify ILC responses and their production of IFN- γ plays a protective role in the
355 acute innate response to *Toxoplasma*. These results are consistent with a model in which IL-33 has a
356 protective rather than pathological role in the immune response to *T. gondii*.

357

358

359 **Figure Legends**

360 **Figure 1: *Toxoplasma gondii* infection induces IL-33 expression and release**

361 Mice were infected with *T. gondii* **(A)** after 7 days, free IL-33 in the peritoneal cavity was measured by
 362 ELISA. **(B)** Measurements of IL-33 from (A) were plotted against corresponding parasite burden and fit
 363 to a linear model. **(C)** 5mm punch biopsies of liver were placed in culture for 24 hours and IL-33
 364 measured in supernatants by ELISA. 3 biopsies per mouse, 5 mice per group. **(D)** Cells from omenta of
 365 IL-33 GFP reporter mice were analyzed by flow cytometry at 3 days post infection. Cells shown are live
 366 singlets. Data are representative of 3 mice per group. **(E)** Whole mount omentum showing IL-33-GFP
 367 signal in milky spot. **(F)** 3D projection of milky spot showing stromal marker ERTR7 and IL-33 GFP signal.
 368 **(G)** Whole mount immunofluorescence of milky spot. NS, not significant ($p>0.05$); * $p<0.05$ and
 369 *** $p<0.001$ (one-way ANOVA with Tukey's multiple comparisons test). Data are representative of or
 370 are pooled from three (A and B), or two (C, D, E, F and G) independent experiments (mean + s.e.m)

371

372 Supplemental Figure 1:

373 (A-C) Intracellular staining of omenta from infected mice. Data are representative of 3 mice per group.
 374 (D) Immunofluorescence of spleen sections from naïve mouse.

375 **Figure 2: Infection sensitizes NK and ILC to IL-33**

376 **(A)** UMAP analysis of peritoneal exudate cells from naïve or 7 dpi mice, with heatmap for IL-33R
 377 expression. Data compiled from 4 mice per group. **(B)** Flow cytometry from peritoneal cells showing
 378 IL-33R staining on NKp46+ cells. Data are representative of 3-4 mice per group. **(C)** Flow cytometry of
 379 LAKs showing composition of population based on cytokine stimulation condition. Population shown
 380 is pre-gated on live singlets. **(D)** Intracellular cytokine staining of LAKs after 24-hour cytokine
 381 stimulation and 4 hour incubation with Brefeldin A. Data are representative of 3 independent
 382 experiments. NS, not significant ($p > 0.05$) (student's t-test); Data are representative of or are pooled
 383 from three independent experiments (A-D).

384

385 **Figure 3: Endogenous IL-33 promotes the anti-parasitic immune response**

386 **(A)** Cytospins of peritoneal exudate cells at 7 dpi. **(B)** qPCR for parasite DNA from indicated tissues.

387 **(C)** Serum cytokines measured by ELISA at 7 dpi. Representative of 4-5 mice per group **(D)** Flow

388 cytometric analysis and quantification of liver innate lymphoid cells. Populations shown are pre-gated

389 on live singlets that are MHCII⁺. **(E)** Quantification of inflammatory monocytes (CD11b⁺ CD64⁺ Ly6g⁻)

390 in livers of infected mice at 7 dpi. **(F)** Intracellular iNOS staining from monocytes in (E). NS, not

391 significant ($p > 0.05$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ (student's t-test). Data are

392 representative of 3 independent experiments.

393

394 **Figure 4: IL-33 treatment boosts IL-12 and IFN- γ dependent immunity**

395 **(A)** Quantification of infected cell frequencies in cytopins at 7 dpi and qPCR for parasite DNA in
 396 indicated tissues. **(B)** Representative cytopins from peritoneal lavage at 7 dpi. Data are
 397 representative of 4-6 mice per group and 5 independent experiments. **(C)** Flow cytometric analysis of
 398 inflammatory monocytes in the peritoneal exudate at 7 dpi. Populations shown are pre-gated on live
 399 Ly6g⁻ singlets. Ly6c^{hi} CCR2⁺ cells are highlighted in black. **(D)** Representative analysis of Ly6c⁺ CCR2⁺
 400 cells at 7 dpi in the liver and quantification of monocyte numbers and iNOS staining. **(E)** Histology of
 401 liver at 7 dpi, H&E showing infiltration of immune cells (left) and DAB iNOS staining (right). *p<0.05,
 402 **p<0.01, and ***p<0.001 (student's t-test). Data are representative of or are pooled from three
 403 independent experiments.

404

405 **Figure 5: Protective effect of IL-33 is dependent on IL-12, IFN- γ , and ILC**

406 **(A)** Quantification of cytopins from peritoneal exudate cells at 7 dpi. **(B)** Quantification of IFN- γ in
407 peritoneal lavage at 7 dpi. **(C)** Flow cytometric analysis of inflammatory monocytes in peritoneum at 7
408 dpi. Data are representative of 5 mice per group and 2 independent experiments.

409

410 Supplemental Figure 2:

411 (A) Flow analysis of inflammatory monocyte recruitment in IFN- γ and IL-12p40 blockaded mice

412

413

References

1. Montoya, J. G. & Liesenfeld, O. Toxoplasmosis. in *Lancet* **363**, 1965–1976 (Elsevier, 2004).
2. Weiss, L. M. & Dubey, J. P. Toxoplasmosis: A history of clinical observations. *Int. J. Parasitol.* **39**, 895–901 (2009).
3. Israelski, D. M. & Remington, J. S. Toxoplasmic encephalitis in patients with AIDS. *Infect. Dis. Clin. North Am.* **2**, 429–45 (1988).
4. Khan, I. A., Matsuura, T. & Kasper, L. H. Interleukin-12 enhances murine survival against acute toxoplasmosis. *Infect. Immun.* **62**, 1639–42 (1994).
5. Hunter, C. A., Subauste, C. S., Van Cleave, V. H. & Remington, J. S. Production of gamma interferon by natural killer cells from *Toxoplasma gondii*-infected SCID mice: regulation by interleukin-10, interleukin-12, and tumor necrosis factor alpha. *Infect. Immun.* **62**, 2818–24. (1994).
6. Yap, G. S. & Sher, A. Cell-mediated immunity to *Toxoplasma gondii*: Initiation, regulation and effector function. in *Immunobiology* **201**, 240–247 (Elsevier GmbH, 1999).
7. Yap, G. S. & Sher, A. Effector cells of both nonhemopoietic and hemopoietic origin are required for interferon (IFN)- γ - and tumor necrosis factor (TNF)- α -dependent host resistance to the intracellular pathogen, *Toxoplasma gondii*. *J. Exp. Med.* **189**, 1083–1091 (1999).
8. Dunay, I. R., Fuchs, A. & Sibley, L. D. Inflammatory monocytes but not neutrophils are necessary to control infection with *Toxoplasma gondii* in mice. *Infect. Immun.* **78**, 1564–70 (2010).
9. Serbina, N. V., Salazar-Mather, T. P., Biron, C. A., Kuziel, W. A. & Pamer, E. G. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* (2003).
10. Scharton-Kersten, T. M., Yap, G., Magram, J. & Sher, A. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. *J. Exp. Med.* **185**, 1261–1273 (1997).
11. Wang, S. *et al.* Infection-induced intestinal dysbiosis is mediated by macrophage activation and nitrate production. *MBio* **10**, (2019).
12. Chen, L. *et al.* The *Toxoplasma gondii* virulence factor ROP16 acts in cis and trans, and suppresses T cell responses. *J. Exp. Med.* **217**, (2020).
13. Scanga, C. A. *et al.* Cutting edge: MyD88 is required for resistance to *Toxoplasma gondii* infection and regulates parasite-induced IL-12 production by dendritic cells. *J. Immunol.* **168**, 5997–6001 (2002).
14. Del Rio, L. *et al.* *Toxoplasma gondii* Triggers Myeloid Differentiation Factor 88-Dependent IL-12 and Chemokine Ligand 2 (Monocyte Chemoattractant Protein 1) Responses Using Distinct Parasite Molecules and Host Receptors. *J. Immunol.* **172**, 6954–6960 (2004).
15. Yarovsky, F. *et al.* TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* **308**, 1626–9 (2005).
16. Plattner, F. *et al.* *Toxoplasma* Profilin Is Essential for Host Cell Invasion and TLR11-Dependent Induction of an Interleukin-12 Response. *Cell Host Microbe* **3**, 77–87 (2008).
17. Hitziger, N., Dellacasa, I., Albiger, B. & Barragan, A. Dissemination of *Toxoplasma gondii* to immunoprivileged organs and role of Toll/interleukin-1 receptor signalling for host resistance assessed by in vivo bioluminescence imaging. *Cell. Microbiol.* **7**, 837–848 (2005).
18. Furuta, T., Kikuchi, T., Akira, S., Watanabe, N. & Yoshikawa, Y. Roles of the small intestine for induction of toll-like receptor 4-mediated innate resistance in naturally acquired murine toxoplasmosis. *Int. Immunol.* **18**, 1655–1662 (2006).
19. Kim, L. *et al.* *Toxoplasma gondii* genotype determines MyD88-dependent signaling in infected

- macrophages. *J. Immunol.* **177**, 2584–91 (2006).
20. Sukhumavasi, W. *et al.* TLR Adaptor MyD88 Is Essential for Pathogen Control during Oral *Toxoplasma gondii* Infection but Not Adaptive Immunity Induced by a Vaccine Strain of the Parasite. *J. Immunol.* **181**, 3464–3473 (2008).
21. Mercer, H. L. *et al.* *Toxoplasma gondii* dense granule protein GRA24 drives MyD88-independent p38 MAPK activation, IL-12 production and induction of protective immunity. *PLOS Pathog.* (2020). doi:10.1371/journal.ppat.1008572
22. LaRosa, D. F. *et al.* T cell expression of MyD88 is required for resistance to *Toxoplasma gondii*. *Proc. Natl. Acad. Sci.* **105**, 3855–3860 (2008).
23. Ge, Y. *et al.* Natural killer cell intrinsic toll-like receptor MyD88 signaling contributes to IL-12-dependent IFN- γ production by mice during infection with *Toxoplasma gondii*. *Int. J. Parasitol.* **44**, 475–484 (2014).
24. Dinarello, C. A. Introduction to the interleukin-1 family of cytokines and receptors: Drivers of innate inflammation and acquired immunity. *Immunological Reviews* (2018). doi:10.1111/imr.12624
25. Powell, N. *et al.* The Transcription Factor T-bet Regulates Intestinal Inflammation Mediated by Interleukin-7 Receptor+ Innate Lymphoid Cells. *Immunity* **37**, 674–684 (2012).
26. Monticelli, L. A. *et al.* Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* **12**, 1045–1054 (2011).
27. Klose, C. S. N. & Artis, D. Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nature Immunology* **17**, 765–774 (2016).
28. Abt, M. C. *et al.* Innate immune defenses mediated by two ilc subsets are critical for protection against acute clostridium difficile infection. *Cell Host Microbe* **18**, 27–37 (2015).
29. Bancroft, G. J., Schreiber, R. D. & Unanue, E. R. Natural Immunity: A T-Cell-Independent Pathway of Macrophage Activation, Defined in the scid Mouse. *Immunol. Rev.* **124**, 5–24 (1991).
30. Tripp, C. S., Wolf, S. F. & Unanue, E. R. Interleukin 12 and tumor necrosis factor α are costimulators of interferon γ production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 3725–3729 (1993).
31. Hunter, C. A., Abrams, J. S., Beaman, M. H. & Remington, J. S. Cytokine mRNA in the central nervous system of SCID mice infected with *Toxoplasma gondii*: importance of T-cell-independent regulation of resistance to *T. gondii*. *Infect. Immun.* **61**, 4038–44 (1993).
32. Park, E. *et al.* *Toxoplasma gondii* infection drives conversion of NK cells into ILC1-like cells. *Elife* **8**, (2019).
33. Weizman, O.-E. El *et al.* ILC1 Confer Early Host Protection at Initial Sites of Viral Infection. *Cell* **171**, 795–808.e12 (2017).
34. Hunter, C. A., Chizzonite, R. & Remington, J. S. IL-1 beta is required for IL-12 to induce production of IFN-gamma by NK cells. A role for IL-1 beta in the T cell-independent mechanism of resistance against intracellular pathogens. *J. Immunol.* **155**, 4347–54 (1995).
35. Cai, G., Kastelein, R. & Hunter, C. A. Interleukin-18 (IL-18) enhances innate IL-12-mediated resistance to *Toxoplasma gondii*. *Infect. Immun.* **68**, 6932–6938 (2000).
36. Kearley, J. *et al.* Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. *Immunity* **42**, 566–579 (2015).
37. Melchor, S. J. *et al.* IL-1R Regulates Disease Tolerance and Cachexia in *Toxoplasma gondii* Infection. *J. Immunol.* **204**, 3329–3338 (2020).
38. Yap, G. S., Ortmann, R., Shevach, E. & Sher, A. A Heritable Defect in IL-12 Signaling in B10.Q/J

- 510 Mice. II. Effect on Acute Resistance to *Toxoplasma gondii* and Rescue by IL-18 Treatment . *J.*
511 *Immunol.* **166**, 5720–5725 (2001).
- 512 39. Vossenkämper, A. *et al.* Both IL-12 and IL-18 contribute to small intestinal Th1-type
513 immunopathology following oral infection with *Toxoplasma gondii* , but IL-12 is dominant over
514 IL-18 in parasite control. *Eur. J. Immunol.* **34**, 3197–3207 (2004).
- 515 40. Ewald, S. E., Chavarria-Smith, J. & Boothroyd, J. C. NLRP1 Is an Inflammasome Sensor for
516 *Toxoplasma gondii*. *Infect. Immun.* **82**, 460–468 (2014).
- 517 41. Gorfu, G. *et al.* Dual Role for Inflammasome Sensors NLRP1 and NLRP3 in Murine Resistance to
518 *Toxoplasma gondii*. *MBio* **5**, e01117-13 (2014).
- 519 42. Schmitz, J. *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related
520 protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23**, 479–490 (2005).
- 521 43. Moussion, C., Ortega, N. & Girard, J. P. The IL-1-like cytokine IL-33 is constitutively expressed in
522 the nucleus of endothelial cells and epithelial cells in vivo: A novel ‘Alarmin’? *PLoS One* **3**, 1–8
523 (2008).
- 524 44. Liew, F. Y., Girard, J.-P. P. & Turnquist, H. R. Interleukin-33 in health and disease. *Nat. Rev.*
525 *Immunol.* **16**, 676–689 (2016).
- 526 45. Rostan, O. *et al.* Crucial and diverse role of the interleukin-33/ST2 axis in infectious diseases.
527 *Infect. Immun.* **83**, 1738–1748 (2015).
- 528 46. Kouzaki, H. *et al.* The Danger Signal, Extracellular ATP, Is a Sensor for an Airborne Allergen and
529 Triggers IL-33 Release and Innate Th2-Type Responses. *J. Immunol.* **186**, 4375–4387 (2011).
- 530 47. Kakkar, R., Hei, H., Dobner, S. & Lee, R. T. Interleukin 33 as a mechanically responsive cytokine
531 secreted by living cells. *J. Biol. Chem.* **287**, 6941–8 (2012).
- 532 48. Cohen, E. S. *et al.* Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nat.*
533 *Commun.* **6**, 8327 (2015).
- 534 49. Humphreys, N. E., Xu, D., Hepworth, M. R., Liew, F. Y. & Grencis, R. K. IL-33, a Potent Inducer of
535 Adaptive Immunity to Intestinal Nematodes. *J. Immunol.* **180**, 2443–2449 (2008).
- 536 50. Silver, J. S. *et al.* Inflammatory triggers associated with exacerbations of COPD orchestrate
537 plasticity of group 2 innate lymphoid cells in the lungs. *Nat. Immunol.* **17**, 626–635 (2016).
- 538 51. Osbourn, M. *et al.* HpARI Protein Secreted by a Helminth Parasite Suppresses Interleukin-33.
539 *Immunity* **47**, 739-751.e5 (2017).
- 540 52. Ricardo-Gonzalez, R. R. *et al.* Tissue signals imprint ILC2 identity with anticipatory function.
541 *Nat. Immunol.* **19**, 1093–1099 (2018).
- 542 53. Molofsky, A. B., Savage, A. K. & Locksley, R. M. Interleukin-33 in Tissue Homeostasis, Injury,
543 and Inflammation. *Immunity* **42**, 1005–1019 (2015).
- 544 54. Matta, B. M. *et al.* IL-33 Is an Unconventional Alarmin That Stimulates IL-2 Secretion by
545 Dendritic Cells To Selectively Expand IL-33R/ST2⁺ Regulatory T Cells. *J. Immunol.* **193**, 4010–
546 4020 (2014).
- 547 55. Schiering, C. *et al.* The alarmin IL-33 promotes regulatory T-cell function in the intestine.
548 *Nature* **513**, 564–568 (2014).
- 549 56. Spallanzani, R. G. *et al.* Distinct immunocyte-promoting and adipocyte-generating stromal
550 components coordinate adipose tissue immune and metabolic tenors. *Sci. Immunol.* **4**,
551 eaaw3658 (2019).
- 552 57. Ito, M. *et al.* Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery.
553 *Nature* **565**, 246–250 (2019).
- 554 58. Franca, R. O. R. F. O. *et al.* IL-33 signaling is essential to attenuate viral-induced encephalitis
555 development by downregulating iNOS expression in the central nervous system. *J.*
556 *Neuroinflammation* **13**, 159 (2016).
- 557 59. Milovanovic, M. *et al.* Deletion of IL-33R (ST2) Abrogates Resistance to EAE in BALB/C Mice by

- 558 Enhancing Polarization of APC to Inflammatory Phenotype. *PLoS One* **7**, 1–13 (2012).
- 559 60. Xiao, Y. *et al.* Interleukin-33 deficiency exacerbated experimental autoimmune
560 encephalomyelitis with an influence on immune cells and glia cells. *Mol. Immunol.* **101**, 550–
561 563 (2018).
- 562 61. Rostan, O. *et al.* The IL-33/ST2 Axis Is Associated with Human Visceral Leishmaniasis and
563 Suppresses Th1 Responses in the Livers of BALB/c Mice Infected with *Leishmania donovani*.
564 *MBio* **4**, e00383-13 (2013).
- 565 62. Nabekura, T., Girard, J.-P. & Lanier, L. L. IL-33 receptor ST2 amplifies the expansion of NK cells
566 and enhances host defense during mouse cytomegalovirus infection. *J. Immunol.* **194**, 5948–52
567 (2015).
- 568 63. Bonilla, W. V. *et al.* The Alarmin Interleukin-33 Drives Protective Antiviral CD8+ T Cell
569 Responses. *Science (80-.)*. **335**, 984–989 (2012).
- 570 64. Baumann, C. *et al.* T-bet– and STAT4–dependent IL-33 receptor expression directly promotes
571 antiviral Th1 cell responses. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 4056–4061 (2015).
- 572 65. Still, K. M. *et al.* Astrocytes promote a protective immune response to brain *Toxoplasma gondii*
573 infection via IL-33-ST2 signaling. *PLOS Pathog.* **16**, e1009027 (2020).
- 574 66. Jones, L. A. *et al.* IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of
575 encephalitis in mice infected with *Toxoplasma gondii*. *Eur. J. Immunol.* **40**, 426–436 (2010).
- 576 67. Konradt, C. *et al.* Endothelial cells are a replicative niche for entry of *Toxoplasma gondii* to the
577 central nervous system. *Nat. Microbiol.* **1**, (2016).
- 578 68. Van Grol, J., Muniz-Feliciano, L., Portillo, J. A. C., Bonilha, V. L. & Subaustea, C. S. CD40 induces
579 anti-toxoplasma gondii activity in nonhematopoietic cells dependent on autophagy proteins.
580 *Infect. Immun.* **81**, 2002–2011 (2013).
- 581 69. Betancourt, E. D. *et al.* From entry to early dissemination-*Toxoplasma gondii*’s Initial Encounter
582 with Its Host. *Frontiers in Cellular and Infection Microbiology* **9**, 46 (2019).
- 583 70. Luu, L. *et al.* An open-format enteroid culture system for interrogation of interactions between
584 *Toxoplasma gondii* and the intestinal epithelium. *Front. Cell. Infect. Microbiol.* **9**, (2019).
- 585 71. Ju, C.-H., Chockalingam, A. & Leifer, C. A. Early Response of Mucosal Epithelial Cells during
586 *Toxoplasma gondii* Infection . *J. Immunol.* **183**, 7420–7427 (2009).
- 587 72. Hauser Jr., W. E., Sharma, S. D. & Remington, J. S. Augmentation of NK cell activity by soluble
588 and particulate fractions of *Toxoplasma gondii*. *J Immunol* (1983).
- 589 73. Hunter, C. A. *et al.* The role of the CD28/B7 interaction in the regulation of NK cell responses
590 during infection with *Toxoplasma gondii*. *J. Immunol.* **158**, 2285–93 (1997).
- 591 74. Wherry, J. C., Schreiber, R. D. & Unanue, E. R. Regulation of gamma interferon production by
592 natural killer cells in scid mice: roles of tumor necrosis factor and bacterial stimuli. *Infect.*
593 *Immun.* **59**, 1709–15 (1991).
- 594 75. Johnston, L. K. *et al.* IL-33 Precedes IL-5 in Regulating Eosinophil Commitment and Is Required
595 for Eosinophil Homeostasis. *J. Immunol.* **197**, 3445–3453 (2016).
- 596 76. Christian, D. A. *et al.* cDC1 Coordinate Innate and Adaptive Responses in the Omentum
597 required for T cell Priming and Memory. *bioRxiv* 2020.07.21.214809 (2020).
598 doi:10.1101/2020.07.21.214809
- 599 77. Jackson-Jones, L. H. *et al.* Fat-associated lymphoid clusters control local IgM secretion during
600 pleural infection and lung inflammation. *Nat. Commun.* **7**, 12651 (2016).
- 601 78. Buscher, K. *et al.* Protection from septic peritonitis by rapid neutrophil recruitment through
602 omental high endothelial venules. *Nat. Commun.* **7**, 1–7 (2016).
- 603 79. Monticelli, L. A. *et al.* IL-33 promotes an innate immune pathway of intestinal tissue protection
604 dependent on amphiregulin–EGFR interactions. *Proc. Natl. Acad. Sci.* **112**, 10762–10767
605 (2015).

80. Gazzinelli, R. T. *et al.* Interleukin 12 is required for the T-lymphocyte-independent induction of interferon γ by an intracellular parasite and induces resistance in T-cell-deficient hosts (*Toxoplasma gondii*/natural killer cells). *Immunology* **90**, 6115–6119 (1993).
81. Hunter, C. A., Candolfi, E., Subauste, C., Van Cleave, V. & Remington, J. S. Studies on the role of interleukin-12 in acute murine toxoplasmosis. *Immunology* **84**, 16–20 (1995).
82. Yap, G., Pesin, M. & Sher, A. Cutting Edge: IL-12 Is Required for the Maintenance of IFN- γ Production in T Cells Mediating Chronic Resistance to the Intracellular Pathogen, *Toxoplasma gondii*. *J. Immunol.* **165**, 628–631 (2000).
83. Wilson, D. C., Matthews, S. & Yap, G. S. IL-12 Signaling Drives CD8 + T Cell IFN- γ Production and Differentiation of KLRG1 + Effector Subpopulations during *Toxoplasma gondii* Infection. *J. Immunol.* **180**, 5935–5945 (2008).
84. Mordue, D. G., Monroy, F., La Regina, M., Dinarello, C. A. & Sibley, L. D. Acute Toxoplasmosis Leads to Lethal Overproduction of Th1 Cytokines. *J. Immunol.* **167**, 4574–4584 (2001).
85. Struck, D. *et al.* Treatment with interleukin-18 binding protein ameliorates *Toxoplasma gondii* -induced small intestinal pathology that is induced by bone marrow cell-derived interleukin-18. *Eur. J. Microbiol. Immunol.* **2**, 249–257 (2012).
86. Muñoz, M. *et al.* Interleukin-22 Induces Interleukin-18 Expression from Epithelial Cells during Intestinal Infection. *Immunity* **42**, 321–331 (2015).
87. Villeret, B. *et al.* Blockade of IL-1R signaling diminishes Paneth cell depletion and *Toxoplasma gondii* induced ileitis in mice. *Am. J. Clin. Exp. Immunol.* **2**, 107–16 (2013).
88. Walker, W., Aste-Amezaga, M., Kastelein, R. A., Trinchieri, G. & Hunter, C. A. IL-18 and CD28 use distinct molecular mechanisms to enhance NK cell production of IL-12-induced IFN- γ . *J. Immunol.* **162**, 5894–901 (1999).
89. Man, S. M. & Kanneganti, T.-D. Regulation of inflammasome activation. *Immunol. Rev.* **265**, 6–21 (2015).
90. Zediak, V. P. & Hunter, C. A. IL-10 fails to inhibit the production of IL-18 in response to inflammatory stimuli. *Cytokine* **21**, 84–90 (2003).
91. Lima, T. S., Gov, L. & Lodoen, M. B. Evasion of human neutrophil-mediated host defense during *Toxoplasma gondii* infection. *MBio* **9**, (2018).
92. Liu, Y. *et al.* *Toxoplasma gondii* Cathepsin C1 inhibits NF- κ B signalling through the positive regulation of the HIF-1 α /EPO axis. *Acta Trop.* **195**, 35–43 (2019).
93. DeLaney, A. A. *et al.* Caspase-8 promotes c-Rel-dependent inflammatory cytokine expression and resistance against *Toxoplasma gondii*. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 11926–11935 (2019).
94. Sandstrom, A. *et al.* Functional degradation: A mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. *Science (80-)*. **364**, (2019).
95. Snelgrove, R. J. *et al.* Alternaria-derived serine protease activity drives IL-33-mediated asthma exacerbations. *J. Allergy Clin. Immunol.* **134**, (2014).
96. Chen, W.-Y., Hong, J., Gannon, J., Kakkar, R. & Lee, R. T. Myocardial pressure overload induces systemic inflammation through endothelial cell IL-33. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 7249–7254 (2015).
97. Tashiro, H. *et al.* Interleukin-33 from Monocytes Recruited to the Lung Contributes to House Dust Mite-Induced Airway Inflammation in a Mouse Model. *PLoS One* **11**, e0157571 (2016).
98. Li, C. *et al.* Interleukin-33 Increases Antibacterial Defense by Activation of Inducible Nitric Oxide Synthase in Skin. *PLoS Pathog.* **10**, e1003918 (2014).
99. Reverchon, F. *et al.* IL-33 receptor ST2 regulates the cognitive impairments associated with experimental cerebral malaria. *PLoS Pathog.* (2017). doi:10.1371/journal.ppat.1006322

IL-33 1° polyclonal goat 2° anti-goat Af647
 bioRxiv preprint doi: <https://doi.org/10.1101/2021.01.10.426122>; this version posted January 11, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

FMO: all-33 primary FMO: all-33 secondary

Rag^{-/-}