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STAT1 Is Essential for Antimicrobial Effector Function but Dispensable for Gamma Interferon Production during *Toxoplasma gondii* Infection

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The opportunistic protozoan *Toxoplasma gondii* is a prototypic Th1-inducing pathogen inducing strong gamma interferon (IFN-γ) cytokine responses that are required to survive infection. Intracellular signaling intermediate STAT1 mediates many effects of IFN-γ and is implicated in activation of T-bet, a master regulator of Th1 differentiation. Here, we show that *T. gondii*-infected STAT1-null mice fail to upregulate the IFN-γ-dependent effector molecules inducible nitric oxide synthase (iNOS), IGTP, and LRG-47, which are required for mice to survive infection. Both T-bet and interleukin-12 receptor β2 (IL-12Rβ2) failed to undergo normal upregulation in response to *T. gondii*. Development of IFN-γ-producing $CD4^+$ and $CD8^+$ T lymphocytes was severely curtailed in the absence of STAT1, but a substantial level of STAT1-independent non-T-cell-derived IFN-γ was induced. Absence of STAT1 also resulted in increased IL-4, Arg1, Ym1, and Fizz1, markers of Th2 differentiation and alternative macrophage activation. Together, the results show that *T. gondii* induces STAT1-dependent T-lymphocyte and STAT1-independent non-T-cell IFN-γ production, but that effector functions of this type 1 cytokine cannot operate in the absence of STAT1, resulting in extreme susceptibility to acute infection.

Infection with the opportunistic protozoan parasite *Toxoplasma gondii* is widespread in humans and animals, and toxoplasmosis emerges as a life-threatening risk in situations of immunodeficiency or congenital disease (33, 39). In immunocompetent hosts, the parasite induces strong T-cell-mediated type 1 immunity (8), with production of proinflammatory cytokines, such as interleukin-12 (IL-12) and gamma interferon (IFN-γ). *T. gondii* infection is lethal in the absence of these cytokines (13, 44). However, the strong Th1 response generated during *T. gondii* infection must be tightly regulated by anti-inflammatory factors such as IL-10, without which the immune response triggered by the parasite leads to immunopathology (14, 34). Thus, while IFN-γ is required for resistance to *Toxoplasma*, excessive levels of the cytokine are lethal (11, 29, 47).

The signaling intermediate signal transducer and activator of transcription 1 (STAT1) is central to mediating the effects of IFN-γ. Activation of STAT1 is initiated when IFN-γ binds and induces receptor oligomerization. This is followed by a series of phosphorylations leading to activation of IFN-γ receptor (IFN-γR)-associated Janus kinases Jak1 and Jak2, IFN-γR1 phosphorylation, and ultimately recruitment and activation of STAT1. Upon IFN-γ stimulation, STAT1 dimerizes and translocates to the nucleus, where it binds gamma-activated sequence elements in promoter regions of IFN-γ-inducible genes (37). Mice deficient in STAT1 signaling are extremely susceptible to viral and bacterial infections (9, 27).

Three IFN-γ-inducible genes are of particular importance for resistance to *T. gondii*: the gene coding for inducible nitric oxide synthase (iNOS) and genes coding for the related proteins LRG-47 and IGTP. The latter molecule has been shown to localize to the endoplasmic reticulum, but its function is so far obscure. Mice deficient in either IGPT or LRG-47 die early during acute infection, with kinetics similar to those of IFN-$\gamma^{-/-}$ mice (5, 6, 44, 51). Mice lacking the iNOS enzyme are also susceptible to *T. gondii*, but in this case, death does not occur until chronic-stage infection (22, 43). IGTP, LRG-47, and iNOS genes have been shown to be induced in macrophages after IFN-γ stimulation, in a STAT1-dependent manner (15).

Nevertheless, several STAT1-independent genes are now known to be inducible by IFN-γ (15, 37, 38). For example, arginase 1 (Arg1) and IL-1β may be upregulated in response to IFN-γ stimulation in the absence of STAT1. Arg1, a hepatic enzyme, is also highly inducible in macrophages and dendritic cells under Th2 conditions, such as in the presence of IL-4 or glucocorticoids (31). Alternatively activated macrophages process L-arginine to L-ornithine and urea through the activity of Arg1 (18, 28). This results in suppressed NO production, since L-arginine is the common substrate for both iNOS and Arg1 enzymes.

Recent advances in understanding Th1 development give STAT1 a central role. The transcription factor T-bet was con-

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sidered a prerequisite for IFN-γ expression and has been shown to be induced by IL-12 stimulation through STAT4 signaling (48). However, a series of more recent studies provide evidence that STAT4 is not essential for T-bet expression (30) and that T-bet expression is induced in a STAT1-dependent fashion through IFN-γ or the IL-27/WSX-1 signaling pathway (24, 50). Transcriptional activity of T-bet also appears necessary for IL-12Rβ2 induction (1).

The present study was conducted to assess the role of STAT1 signaling during a well-documented type 1 immune response to in vivo microbial infection. We infected STAT1-deficient mice with a low-virulence *T. gondii* strain and monitored the ability of the animals to mount a protective response to the parasite. As recently reported, lack of STAT1 confers extreme susceptibility to *Toxoplasma* (5). We show here that susceptible STAT1$^{-/-}$ mice display a failure to develop IFN-γ-secreting CD4$^+$ and CD8$^+$ T lymphocytes, despite the emergence of STAT1-independent non-T-cell sources of IFN-γ. The incomplete development of a strong Th1 response was associated with defective induction of critical type 1 regulators of differentiation: namely, T-bet and IL-12Rβ2. We found a corresponding increase in induction of the anti-inflammatory cytokine IL-4, as well as Arg1, Ym1, and Fizz1, which are markers of alternative macrophage activation. The extreme susceptibility of the STAT1 knockout (KO) animals to *T. gondii* infection could be attributed to a complete lack of IGTP, LRG-47, and NO, effector molecules required for resistance to the parasite.

MATERIALS AND METHODS

Mice. Wild-type (WT) mice of the 129Sv/Ev strain were purchased from Taconic (Germantown, N.Y.). STAT1 gene KO mice, 129S6/SvEv-*Stattm1Rds* (STAT1$^{-/-}$) on the same background, were also purchased from Taconic, and a breeding colony was established in the Transgenic Mouse Facility of the College of Veterinary Medicine at Cornell University. STAT1$^{-/-}$ mice were originally engineered by homologous recombination designed to replace the first three exons of the STAT1 gene with a neomycin resistance cassette (27). We verified the genotype of the homozygous STAT1$^{-/-}$ mice by PCR, following a protocol supplied by Taconic Farms (data not shown). Mice were used at 6 to 8 weeks old. The Transgenic Mouse Facility is a specific-pathogen-free facility accredited by the American Association for Accreditation of Laboratory Animal Care.

Parasites, antigen, and infection. Tachyzoites of the RH strain were maintained in human foreskin fibroblast monolayers by biweekly passage, as previously described (11). Macrophage and peritoneal exudate cells were infected at a 1:1 parasite/cell ratio for 24 h to measure cytokine production. ME49 strain cysts were maintained in Swiss Webster mice. Three mice per group were inoculated intraperitoneally (i.p.) with 20 cysts obtained from brain homogenates of 5- to 6-week-infected Swiss Webster mice. Animals were monitored for survival or sacrificed at 0, 4, and 7 days postinfection in order to collect samples (peritoneal exudate cells, blood, and spleens). Samples from each mouse were individually processed and analyzed except where noted.

Bone marrow-derived and thioglycolate-elicited macrophages. Bone marrow cells were recovered from the tibia of 129Sv/Ev and STAT1$^{-/-}$ mice and grown for 4 days in RPMI (Life Technologies, Gaithersburg, Md.) medium supplemented with 10% fetal calf serum (FCS; HiClone, Logan, Utah), 5% horse normal serum (Invitrogen, Carlsbad, Calif.), 30% L29 cell culture supernatant (containing granulocyte-macrophage colony-stimulating factor), 1% penicillin–streptomycin, 1% nonessential amino acids, and 1% sodium pyruvate (the last three reagents from Invitrogen). Fresh medium was added at 3 days of culture. At 4 days, the medium was removed, cells were washed twice with sterile phosphate-buffered saline (PBS), covered with cold PBS, and incubated at 4°C for 20 min. Bone marrow-derived macrophages (BMMØ) were then recovered by gentle scraping, washed, and plated at 4×10^5 cells per well in 96-well flat-bottom tissue culture plates, in fibroblast medium composed of Dulbecco's modified Eagle's medium (DMEM) (Life technologies) supplemented with 1% FCS (HiClone), 100-U/ml penicillin, and 0.1-mg/ml streptomycin (Invitrogen) in the presence or absence of 500-U/ml mouse recombinant IFN-γ (rIFN-γ) (R&D Systems, Minneapolis, Minn.). After18 h of incubation, cells were infected with RH tachyzoites at a 1:1 parasite/cell ratio. Cell supernatants were collected 24 h later and stored at −20°C.

Thioglycolate-elicited peritoneal macrophages (PMØ) were obtained by injecting 1 ml of sterile, 4% thioglycolate i.p. and recovering peritoneal exudate cells 5 days later by lavage. At this time, peritoneal exudate cells were composed of 99% macrophages. Cells were washed, resuspended in fibroblast medium in 96-well flat-bottom plates at 4×10^5 cells per well, and treated as day 4 BMMØ.

Spleen cell cultures. Spleens were homogenized, erythrocytes were lysed (RBC lysis buffer; Sigma, St. Louis, Mo.), and splenocytes were resuspended in DMEM supplemented with 10% FCS (HiClone), 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 30 mM HEPES, 100-U/ml penicillin, 0.1-mg/ml streptomycin (Life Technologies), and 50 μM 2-mercaptoethanol (Sigma). Cells were then plated at 2×10^6 cells per well in 96-well flat-bottom tissue culture plates at 37°C in 5% CO_2, in the presence or absence of 25-μg/ml soluble tachyzoite antigen (STAg), prepared according to a previously published protocol (3). Cell supernatants were collected 72 h later and stored at −20°C.

Cytokine and NO quantitation. The cytokines IL-12 p40 and IFN-γ were quantitated by using two-site enzyme-linked immunosorbent assay (ELISA), as previously described for IL-12 p40 (7), and using a commercially available kit according to the manufacturer's protocol for IFN-γ (BD Biosciences, San Diego, Calif.). For quantifying cytokines in the serum, the same amount of serum was used from each mouse. NO was measured in the spleen cell culture supernatant by the Greiss reaction (16).

Parasite quantitation. PMØ from ME49-infected mice, recovered by i.p. lavage with PBS, were washed, counted, and cytospun onto glass microscope slides. After Diff Quick staining (American Scientific Products, McGraw Park, Calif.), the percentage of infected cells was determined by randomly choosing at least three fields and counting at least 300 cells per slide.

Western blotting. Splenocyte samples from WT and KO mice were mechanically homogenized in lysis buffer, composed of 1% Triton X-100, 150 mM NaCl, 50 mM Tris HCl, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitors leupeptin and aprotinin (reagents from Sigma). Samples were centrifuged 10 min at $18{,}000 \times g$, and the supernatant was collected. We measured the protein content by using a Lowry assay kit (Bio-Rad, Hercules, Calif.) and ran 8 μg of protein per lane on 10% acrylamide gels. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were further processed for immunoblotting as previously described (12). Briefly, proteins were transferred to a nitrocellulose membrane, which was then blocked in Tris-buffered saline containing 5% nonfat dry milk and 0.05% Tween 20. Primary antibody was added for overnight incubation on a rocker at 4°C. Bound antibodies were detected with horseradish peroxidase-linked antirabbit secondary antibody and an enhanced chemiluminescence system (Cell Signaling, Beverly, Mass.).

Detection of gene expression by real-time PCR. Spleen cell samples from WT and KO animals were stored in RNA-Later for subsequent isolation of RNA, with an RNA-Later Easy kit, following the manufacturer's protocol (Qiagen, Hilden, Germany). Briefly, spleens were mechanically homogenized in lysis buffer and then passed over an RNA affinity column. After several washes, RNA was eluted and quantified. Reverse transcription was carried out on 1 μg of RNA, as previously described (2). The resulting cDNA was diluted and stored at −20°C until used for real-time PCR.

Equal amounts of cDNA were submitted to PCR, in the presence of SYBR green dye, using a master mix and following the manufacturer's protocol (Applied Biosystems, Foster City, Calif.). Samples from each mouse were run in triplicate. Real-time PCR was conducted on an Applied Biosystems 7700 sequence detector. The following sets of primer sequences, kindly provided by M. Hesse (Cornell University) were used: IL-4 sense 3′-ACGAGGTCACAGGAG AAGGGA-5′ and antisense 3′-ACGCCTACAGACGAGCTCACTC-5′, IL-10 sense 3′-ATGCTGCCTGCTCTTACTGACTG-5′ and antisense 3′-CCCAAGT AACCCTTAAATCCTGC-5′, iNOS sense 3′-TGCCCCTTCAATGGTTGGT A-5′ and antisense 3′-ACTGGAGGGACCAGCCAAAT-5′, and Arg1 sense 3′-ACAGTCTGGCAGTTGGAAGCATC-5′ and antisense 3′-GGGAGTCCCC AGGAGAATCCT-5′. Sequences for the T-bet, IL-12Rβ2, HPRT, Fizz1, and Ym1 primers have been published previously (4, 19, 30). The HPRT endogenous housekeeping gene was used as a control for sample size loading and was routinely amplified on the same plate as the experimental gene of interest. Each sample was normalized by using the difference in critical thresholds (CT) between the gene of interest and HPRT, $\Delta CT_{sample} = CT_{gene} - CT_{HPRT}$. Each sample was then compared to a standard, using the expression $2^{-\Delta\Delta CTsample}$, where $\Delta\Delta CTsample = \Delta CT_{sample} - \Delta CT_{standard}$. The standard was defined as the noninfected WT sample with the lowest ΔCT. We then averaged the results

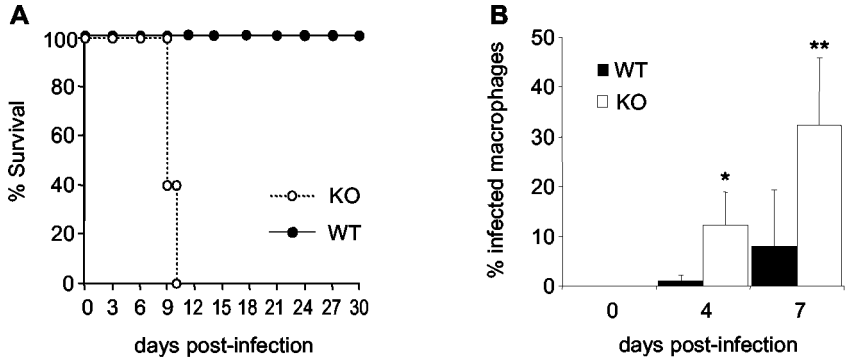

FIG. 1. STAT1$^{-/-}$ mice are highly susceptible to *T. gondii* infection. (A) STAT1$^{-/-}$ mice die by 10 days postinfection with 20 ME49 cysts, while WT 129Sv/Ev mice survive infection until termination of the experiment at 30 days. Data are representative of two independent experiments with 10 mice per group. (B) Increased parasitemia in macrophages of infected STAT1$^{-/-}$ mice. Peritoneal exudate cells were recovered at 0, 4, and 7 days postinfection, and the percentage of infected macrophages was determined at 4 and 7 days postinfection. Student's *t* test revealed statistically significant differences between WT (solid bars) and KO (open bars) samples (*, $P = 0.008$; and **, $P = 0.014$). Data were pooled from two independent experiments with three mice per group; bars represent standard error.

of each group (three mice per group per experiment) and expressed the final results as fold increase compared to the WT noninfected sample.

Flow cytometric analysis. Splenocytes were prepared from noninfected mice or animals infected 7 days previously by i.p. injection of 20 ME49 cysts. Cells were immediately stimulated with phorbol myristate acetate (PMA; 50 ng/ml) and ionomycin (500 ng/ml). After 90 min, Golgi-Plug (BD Pharmingen; La Jolla, Calif.) was added, and cultures were incubated for an additional 90 min according to the manufacturer's instructions. Cells were washed in PBS–1% bovine serum albumin (BSA) and stained with anti-CD4 or anti-CD8 fluorescein isothiocyanate (FITC)-conjugated antibody (BD Pharmingen). After surface staining, cells were washed with PBS-BSA, fixed in Perm-Wash solution, and stained with anti-IFN-γ antibody conjugated to phycoerythrin by using BD Pharmingen's Cyto-Fix Cyto-Perm reagents.

Statistical analysis. Student's *t* test was used for comparison of percentages of infected macrophages between samples from infected 129SvEv and STAT1$^{-/-}$ mice. Values of $P < 0.05$ were considered significant.

RESULTS

STAT1$^{-/-}$ mice are highly susceptible to low-virulence *T. gondii* infection. We infected STAT1$^{-/-}$ mice (KO mice) and 129Sv/Ev WT control mice with low-virulence *Toxoplasma* strain ME49. While WT mice resisted infection, KO mice uniformly succumbed within 10 days postinfection (Fig. 1A). We therefore determined parasite levels in macrophages derived from WT and STAT1$^{-/-}$ peritoneal exudate cells at 4 and 7 days postinfection. The percentage of infected macrophages in STAT1$^{-/-}$ mice was significantly higher than the corresponding population in WT mice at both time points ($P = 0.008$ and 0.014 at 4 and7 days postinfection, respectively) (Fig. 1B). Furthermore, differential counts revealed increased levels of polymorphonuclear cells infiltrating at 7 days postinfection in KO lavages (data not shown). These collective results are similar to those obtained with IFN-γ KO mice (44).

Impaired innate immune response to *T. gondii* in the absence of STAT1. Macrophages are one of the first lines of defense during *T. gondii* infection and can produce the type 1-inducing cytokine IL-12 and the antimicrobial factor NO, which are important in protection during infection (40). IFN-γ primes macrophages for activation, potentiating their antiparasitic activity. Given the high level of in vivo macrophage infection in the absence of STAT1, we sought to determine if these cells displayed an impaired response to the parasite. We infected BMMØ or PMØ after prestimulation with IFN-γ or medium only and quantitated the amounts of IL-12 and NO secreted into the culture supernatant (Fig. 2). For both PMØ and BMMØ, IL-12 was only secreted when cells were infected with tachyzoites (Fig. 2A and B). IL-12 production was higher in WT exudate cells pretreated with IFN-γ than in PMØ-infected cells in the absence of IFN-γ. The cytokine had no effect on KO PMØ, which produced barely detectable levels of IL-12. Interestingly, IL-12 production in response to *T. gondii* was high for both WT and KO BMMØ and occurred independently of IFN-γ prestimulation. Thus, IL-12 production by BMMØ is largely STAT1 independent, but IFN-γ signaling through STAT1 is required for maximal parasite induction of IL-12 by the PMØ.

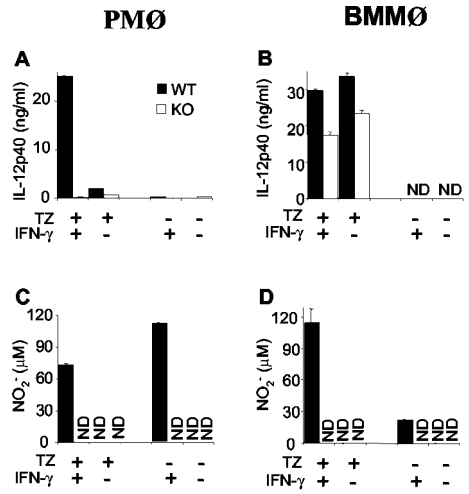

FIG. 2. Defective macrophage responses to *T. gondii* infection. PMØ (A and C) and BMMØ (B and D) were infected with RH tachyzoites (TZ; 1:1 parasite/cell ratio) after 18 h of stimulation with 500-U/ml rIFN-γ or medium alone. IL-12 p40 (A and B) and NO (C and D) were measured in the culture supernatants after 24 h of infection. Bars represent the standard deviation of duplicate samples. Solid bars, WT; open bars, KO. Data are representative of four independent experiments.

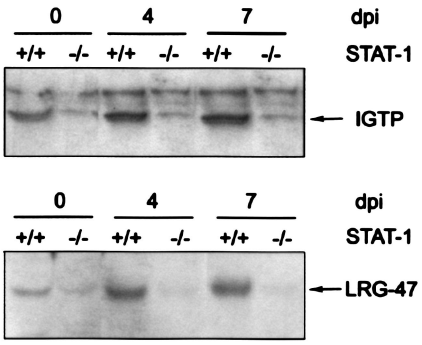

FIG. 3. Failure to induce IGTP and LRG-47 in STAT1$^{-/-}$ animals. At the indicated number of days postinfection (dpi), splenocytes were recovered and lysed. Samples were resolved by SDS-PAGE and transferred to a nitrocellulose membrane. Blots were then submitted to IGTP (upper panel) and LRG-47 (lower panel) Western blot analysis. Data are representative of two independent experiments, with independently processed samples from three mice per group at each time point.

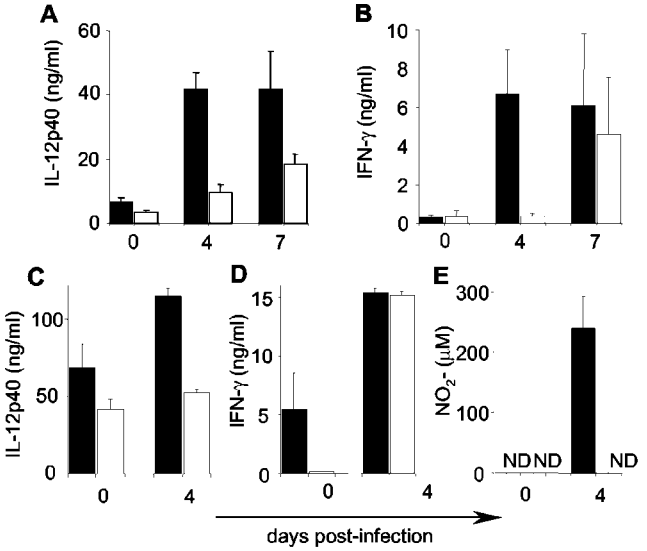

FIG. 4. Production of proinflammatory cytokines in infected STAT1$^{-/-}$ versus STAT1$^{+/+}$ animals. Sera were collected from mice at 0 and 4 and 7 days postinfection and subjected to ELISA for IL-12 p40 (A) and IFN-γ (B) cytokine detection. Splenocytes were cultured for 72 h in the presence of STAg, supernatants were assayed for IL-12 p40 (C) and IFN-γ (D) production by ELISA, and NO was measured by the Greiss assay (E). Cytokines were not detected in medium-only cultures. Samples were run in duplicate. Bars represent the standard error of three mice per group. Solid bars, WT; open bars, KO. ND, not detected. Data are representative of two independent experiments.

NO was not detected in STAT1$^{-/-}$ culture supernatants under any condition (Fig. 2C and D). In WT cell cultures of both PMØ and BMMØ, NO was produced, but only if macrophages were prestimulated with IFN-γ. In addition, IFN-γ alone was sufficient to enable NO production, particularly in PMØ, Thus, unlike IL-12, NO induction displays an absolute requirement for IFN-γ-mediated STAT1 activation.

Lack of IGTP and LRG-47 expression in the absence of STAT1 signaling. IFN regulatory factor 1 (IRF-1), induced by IFN-γ, is required for iNOS expression (22). However, mice deficient in either IRF-1 or iNOS are resistant to the acute phase of *T. gondii* infection and survive as long as 21 to 30 days after parasite inoculation (22, 43). The STAT1 KO mice, on the other hand, succumb early (Fig. 1A). We therefore sought to determine if the expression of other IFN-γ-inducible factors might be deficient. An endoplasmic reticulum-located GTP-binding protein, IGTP, was recently shown to be induced in a STAT1-dependent manner upon IFN-γ stimulation (5, 15). Both IGTP and the related protein LRG-47 are required for mice to survive acute *T. gondii* infection (5, 6, 51). We confirm here (Fig. 3) that STAT1 function is necessary for IGTP induction during *T. gondii* infection and further demonstrate that parasite-induced LRG-47 expression is also STAT1 dependent, because neither of these proteins could be detected in STAT1$^{-/-}$ samples.

Defects in early proinflammatory cytokine production during in vivo infection of STAT1$^{-/-}$ mice. In vitro infection of macrophages with RH tachyzoites revealed an impaired innate immune response. We therefore examined cytokine responses during in vivo infection. Blood samples were obtained from ME49-infected mice, and sera were collected. As shown in Fig. 4A and B, while WT mice displayed large amounts of circulating IL-12 and IFN-γ in the blood at 4 days postinfection, these cytokines were much lower in STAT1-deficient mice. Nevertheless, by day 7 postinfection, the STAT1 KO mice at least partially recovered their ability to produce IL-12 and IFN-γ.

Spleen cells were incubated for 72 h in the presence or absence of STAg, and supernatants were tested for cytokines and NO. WT cell cultures displayed a similar pattern of increased cytokine production at 4 days postinfection for both IL-12 and IFN-γ (Fig. 4C and D), and high levels of NO production were detected at 4 days postinfection (Fig. 4E). However, while IL-12 levels in STAT1$^{-/-}$ culture supernatants mimicked serum results at day 4 (Fig. 4C), surprisingly high levels of IFN-γ were produced in cultures of 4-day-infected splenocytes in response to STAg (Fig. 4D). We could detect no NO production in cultures from KO mice—results similar to those from the macrophage cultures (Fig. 4E and 2C and D). We traced the absence of NO production by STAT1$^{-/-}$ cells to a lack of iNOS gene expression, which failed to be upregulated after infection, in contrast to the situation in WT mice (see Fig. 6). Taken together, the results indicate that the type 1 cytokine response is defective but not absent in STAT1$^{-/-}$ mice. Thus, STAT1-deficient cells retain an intrinsic ability to secrete IL-12 and IFN-γ, as shown by BMMØ IL-12 production in response to tachyzoites (Fig. 2B), IFN-γ secretion after STAg stimulation of splenocytes (Fig. 4D), and serum IL-12 and IFN-γ concentrations at late time points (Fig. 4A and B).

STAT1 is required for T-lymphocyte, but not non-T-lymphocyte, IFN-γ production. To determine whether IFN-γ-secreting T cells developed in infected STAT1$^{-/-}$ animals, splenocytes were isolated and cultured in vitro with PMA and ionomycin followed by intracellular cytokine staining. In the absence of STAT1, there was a major decrease in the percent of both CD4$^+$ and CD8$^+$ T lymphocytes expressing IFN-γ (Fig. 5). Thus, in WT animals, 30% of CD4$^+$ T cells were IFN-γ positive compared to 13% in KO mice. Similarly, for CD8$^+$ T cells, 30% of WT cells were IFN-γ positive, whereas

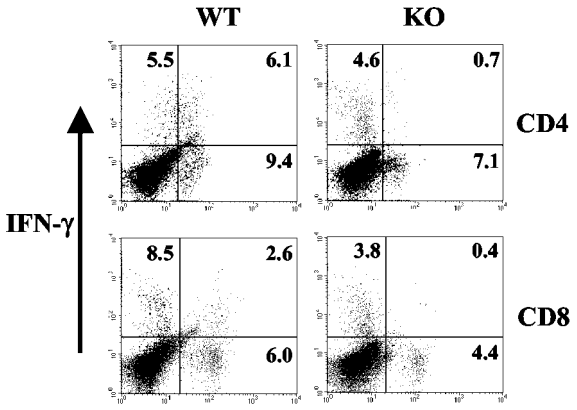

FIG. 5. Defective T-lymphocyte production of IFN-γ in STAT1$^{-/-}$ mice. Splenocytes were obtained from mice 7 days after infection, immediately stimulated with PMA and ionomycin, and stained for T-cell markers and IFN-γ as described in Materials and Methods. The numbers in each quadrant indicate the percentage of cells in that quadrant. This experiment was repeated three times with essentially identical results.

only 8% were positive in the absence of STAT1. Interestingly, in the STAT1 KO situation, there was a substantial population of non-CD4$^+$, non-CD8$^+$ cells that expressed IFN-γ. This non-T-cell population accounted for approximately 80% of the total IFN-γ-positive cells in the STAT1-deficient splenocyte population. A similar population was also detected in WT animals. It is likely that this non-T-cell population accounts for most of the IFN-γ detected during in vitro culture of STAT1-null cells (Fig. 4D).

Defective induction of inflammatory genes in STAT1$^{-/-}$ mice. Type 1 differentiation is tightly linked to expression of genes such as T-bet, a transcription factor important for IFN-γ expression, and IL-12Rβ2, whose expression allows IL-12 responsiveness (40). Furthermore, a positive feedback loop has recently been described, in which T-bet and IL-12Rβ2 expression are dependent upon STAT1 activation after IFN-γ stimulation (1, 24). We therefore sought to determine if the defect in type 1 differentiation in STAT1$^{-/-}$-infected mice was linked to deficient induction of T-bet and IL-12Rβ2 gene expression. Indeed, real-time reverse transcription-PCR analysis of these genes revealed severely impaired upregulation of T-bet and IL-12Rβ2 in spleens from infected KO mice, but strong induction in those from WT mice (Fig. 6). Thus, lack of STAT1 responsiveness has a major impact on genes involved in type 1 differentiation during *T. gondii* infection.

Induction of macrophage alternative activation genes and anti-inflammatory cytokines in the absence of STAT1. Although STAT1 is the only known signal transducer and activator of transcription responding to IFN-γ, a STAT1-independent pathway for gene activation in response to IFN-γ stimulation has recently been reported, involving a range of protein kinases and adaptor proteins (38). One such gene is the

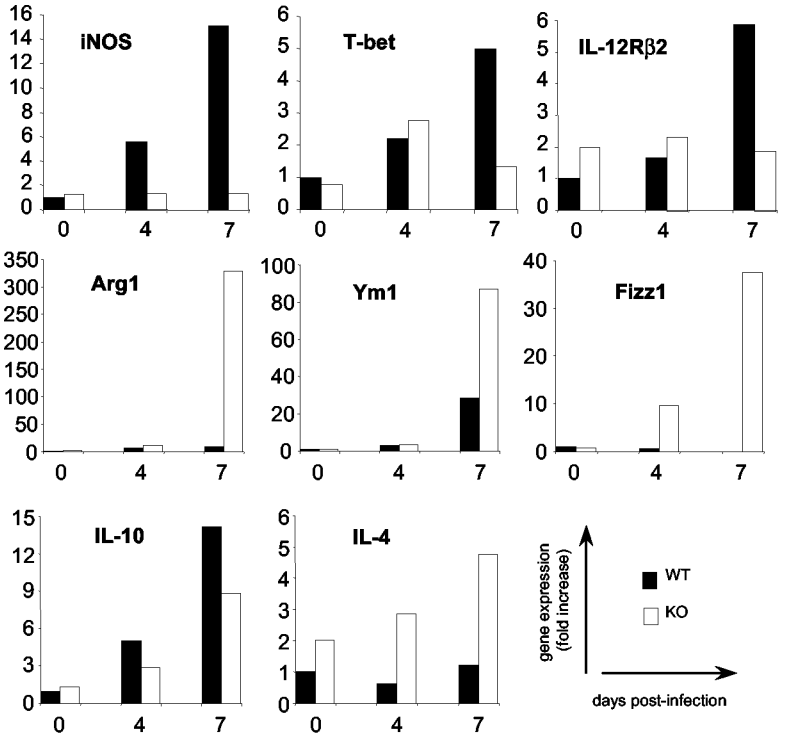

FIG. 6. Lack of induction of Th1 differentiation markers, but activation of anti-inflammatory markers, in infected STAT1 KO mice. RNA was isolated from splenocytes at the indicated times after infection and subjected to real-time PCR analysis. RNA samples were individually processed (three mice per group). Real-time PCR was performed, and the results were analyzed as described in Materials and Methods and compared to the level of gene expression found in noninfected WT animals, arbitrarily assigned the value 1. Bars represent the fold increase in gene expression over the expression level in the noninfected WT samples. Solid bars, WT; open bars, KO. Data are representative of two independent experiments.

gene coding for arginase 1 (Arg1), a liver enzyme also known as a marker of alternative macrophage activation (15). Alternatively activated macrophages metabolize arginine to ornithine and urea by using Arg1, instead of producing NO and citrulline by using iNOS. As with the iNOS gene, Arg1 is inducible in non-liver cells. Since we could not detect NO production in the in vitro-infected macrophage cultures, we hypothesized that induction of alternative activation markers might occur during *T. gondii* infection of STAT1$^{-/-}$ mice. We used real-time PCR analysis of spleen RNA expression for three alternative activation markers, Arg 1, Fizz1, and Ym1 (28, 36) (Fig. 6). Interestingly, we detected major increases in expression for all of these genes in samples from infected KO mice, but not in samples from WT mice.

Because Th1 differentiation in response to *T. gondii* infection appeared defective in STAT1$^{-/-}$ mice, we questioned a possible induction of anti-inflammatory gene expression in infected KO mice. IL-10 is normally expressed during *T. gondii* infection as a means to down-regulate the type 1 response that can otherwise be lethal (14, 47). Real-time PCR analysis of IL-10 expression showed induction of the gene at 4 and 7 days postinfection in both WT and KO mice, although the levels appeared slightly lower in the latter animals (Fig. 6). We also sought to determine if IL-4, a key cytokine in Th2 differentiation, might be up-regulated in the infected STAT1$^{-/-}$ animals. Indeed, while no IL-4 gene up-regulation was detected in WT samples, we detected a significant up-regulation of this gene in the KO samples (Fig. 6).

DISCUSSION

We used infection of STAT1-deficient mice with the Th1 cytokine-inducing parasite *T. gondii* as a means to elucidate the importance of this transducing molecule during in vivo infection. IFN-γ is well known as the major mediator of resistance to *T. gondii* (46), and we found that susceptibility of STAT1 KO mice is linked to increased parasitemia and lack of expression of IFN-γ-induced molecules IGTP, LRG-47, and NO. The initial phase of the in vivo immune response was also defective, with low levels of circulating IL-12 and IFN-γ in infected STAT1$^{-/-}$ mice compared to infected WT mice (Fig. 4). Nevertheless, it is interesting to note that cells from STAT1-deficient mice remained able to produce both IL-12 and IFN-γ (Fig. 2B and 4D), and at later times (7 days postinfection), secretion of IFN-γ was comparable to that in their WT counterparts (Fig. 4). Thus, while STAT1 appears necessary for induction of IFN-γ-induced effector molecules, it is ultimately not required for production of type 1 cytokines during *T. gondii* infection.

T-bet expression and IL-12Rβ2 gene transcription in response to T-bet have previously been shown to depend upon IFN-γ/IFN-γR or IL-27/WSX-1 signaling through STAT1 during in vitro stimulation (24, 30, 50). Our study establishes the in vivo relevance of interactions between these important players of Th1 differentiation (Fig. 6). Although absence of functional STAT1 did not prevent eventual production of substantial levels of IFN-γ, both CD4$^+$ and CD8$^+$ T lymphocytes were defective in production of this cytokine in the absence of STAT1. It is possible that STAT1-independent IFN-γ derives from NK cells in this situation (23). Indeed, earlier studies have demonstrated the importance of NK cell-derived IFN-γ in early resistance to *T. gondii* (20, 45). In addition, both MØ and dendritic cells are emerging as biologically important sources of IFN-γ (10, 26) The source of STAT1-independent IFN-γ is currently under investigation.

Induction of STAT-1-independent IFN-γ in non-T cells could be due to the low level of T-bet activation detected at 4 days postinfection (Fig. 6). In support of this, optimal IFN-γ production by dendritic cells requires T-bet (26). Alternatively, STAT-4 activation, mediated by an IL-12/IL-12Rβ2 pathway, might promote type 1 cytokine production in non-T cells (10, 49). Although IL-12Rβ2 failed to upregulate in the absence of STAT1, constitutive expression of his receptor could be sufficient to promote non-T-cell IFN-γ by a STAT4-dependent pathway. Further experiments are required to address these important issues.

Several genes were identified whose expression was unaffected or increased by STAT1 deficiency. Induction of the anti-inflammatory cytokine IL-10, a downregulatory factor during the acute phase of toxoplasmosis, was not affected by the absence of STAT1 during *T. gondii* infection. Furthermore, we detected IL-4 gene activation in the infected STAT1 KO mice by real-time PCR. IL-4 expression during *T. gondii* infection has been reported after challenge of IFN-γ$^{-/-}$ mice with irradiated RH tachyzoites in mice previously vaccinated with a killed parasite (44). Lack of MyD88, the common adaptor molecule of the Toll-like receptor signaling pathway required for IL-12 production, also results in switching to a Th2 phenotype during *T. gondii* infection (21, 42). In the present study, it is interesting that emergence of IL-4 occurred in the absence of STAT1, despite substantial amounts of IL-12 and IFN-γ. The results are in line with data suggesting a major function of STAT1-dependent T-bet induction in blocking GATA-3, which itself acts to suppress STAT4-driven Th1 induction (52).

An important finding that emerged from these studies was that PMØ produced IL-12 in response to the parasite strictly in dependence upon exogenous IFN-γ and an intact STAT1 signaling molecule. This was in dramatic contrast to BMMØ that released IL-12 in response to *Toxoplasma* alone in a manner that did not require the IFN-γ/STAT1 signaling pathway. We do not presently understand the basis for this result. However, the finding that *T. gondii* can elicit IL-12 without STAT1 is consistent with cytokine KO studies showing in vivo IL-12 production during *T. gondii* infection in IFN-γ$^{-/-}$ mice (44).

Infected STAT1$^{-/-}$ mice express extremely high levels of gene transcripts for Arg1, Ym1, and Fizz1, alternative macrophage activation markers that arise under Th2 conditions (17, 28, 36). In vivo, the alternative activation macrophage phenotype has been associated with helminth infection (18, 25, 41). However, the presence of alternatively activated macrophages has also been reported during chronic infection with an attenuated strain of the extracellular protozoan parasite *Trypanosoma brucei brucei* and during *Trypanosoma congolense* infection in resistant mouse strains (32, 35, 36). At present, little is known regarding control of these genes and how their activity is prevented by STAT1.

We show here that absence of IFN-γ signaling through STAT1 results in complete absence of effector molecules known to be critical for control of *T. gondii* and other microbial pathogens, namely, NO, IGTP, and LRG-47. STAT1 defi-

ciency also results in defective infection-driven upregulation of T-bet and IL-12Rβ2, molecules associated with Th1 generation. Nevertheless, STAT1 KO animals were able to generate normal levels of IFN-γ, although with delayed kinetics relative to WT mice and the cytokine derived mainly from non-T-cell sources. Similarly, *Toxoplasma*-induced IL-12 occured in the absence of STAT1 signaling, although levels were generally lower. The results implicate STAT1 as an important signaling intermediate in the amplification of the Th1 response during *T. gondii* infection, but show that this signaling molecule is not essential to drive IFN-γ production by other cell types. Despite IFN-γ production, antimicrobial type 1 effector mechanisms cannot function, resulting in inability to control parasite replication and leading to early host death.

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ADDENDUM IN PROOF

The effects of STAT1 deficiency during *Toxoplasma gondii* infection have recently been reported by another group (L. A. Lieberman, M. Barica, S. L. Reiner, and C. A. Hunter, J. Immunol. **172:**457–463, 2004).

REFERENCES

1. **Afkarian, M., J. R. Sedy, J. Yang, N. G. Jacobson, N. Cereb, S. Y. Yang, T. L. Murphy, and K. M. Murphy.** 2002. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. Nat. Immunol. **6:**549–557.
2. **Bliss, S. K., A. J. Marshall, Y. Zhang, and E. Y. Denkers.** 1999. Human polymorphonuclear leukocytes produce IL-12, TNF-α, and the chemokines macrophage-inflammatory protein-1α and -1β in response to *Toxoplasma gondii* antigens. J. Immunol. **162:**7369–7375.
3. **Bliss, S. K., Y. Zhang, and E. Y. Denkers.** 1999. Murine neutrophil stimulation by *Toxoplasma gondii* antigen drives high level production of IFN-γ-independent IL-12. J. Immunol. **163:**2081–2088.
4. **Chang, N. C., S. I. Hung, K. Y. Hwa, I. Kato, J. E. Chen, C. H. Liu, and A. C. Chang.** 2001. A macrophage protein, Ym1, transiently expressed during inflammation is a novel mammalian lectin. J. Biol. Chem. **276:**17497–17506.
5. **Collazo, C. M., G. S. Yap, S. Hieny, P. Caspar, C. G. Feng, G. A. Taylor, and A. Sher.** 2002. The function of gamma interferon-inducible GTP-binding protein IGTP in host resistance to *Toxoplasma gondii* is Stat1 dependent and requires expression in both hematopoietic and nonhematopoietic cellular compartments. Infect. Immun. **70:**6933–6939.
6. **Collazo, C. M., G. S. Yap, G. D. Sempowski, K. C. Lusby, L. Tessarollo, G. F. Vande Woude, A. Sher, and G. A. Taylor.** 2001. Inactivation of LRG-47 and IRG-47 reveals a family of interferon-γ-inducible genes with essential, pathogen-specific roles in resistance to infection. J. Exp. Med. **194:**181–187.
7. **Del Rio, L., S. Bennouna, J. Salinas, and E. Y. Denkers.** 2001. CXCR2 deficiency confers impaired neutrophil recruitment and increased susceptibility during *Toxoplasma gondii* infection. J. Immunol. **167:**6503–6509.
8. **Denkers, E. Y., and R. T. Gazzinelli.** 1998. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. Clin. Microbiol. Rev. **11:**569–588.
9. **Durbin, J. E., R. Hackenmiller, M. C. Simon, and D. E. Levy.** 1996. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral infection. Cell **84:**443–450.
10. **Fukao, T., D. M. Frucht, G. Yap, M. Gadina, J. J. O'Shea, and S. Koyasu.** 2001. Inducible expression of Stat4 in dendritic cells and macrophages and its critical role in innate and adaptive immune responses. J. Immunol. **166:** 4446–4455.
11. **Gavrilescu, L. C., and E. Y. Denkers.** 2001. IFN-γ overproduction and high level apoptosis are associated with high but not low virulence *Toxoplasma gondii* infection. J. Immunol. **167:**902–909.
12. **Gavrilescu, L. C., and E. Y. Denkers.** 2003. IL-12 p40 and Fas ligand-dependent apoptotic pathways involving STAT-1 phosphorylation are triggered during infection with a virulent strain of *Toxoplasma gondii.* Infect. Immun. **71:**2577–2583.
13. **Gazzinelli, R. T., M. Wysocka, S. Hayashi, E. Y. Denkers, S. Hieny, P. Caspar, G. Trinchieri, and A. Sher.** 1994. Parasite-induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection with *Toxoplasma gondii.* J. Immunol. **153:**2533–2543.
14. **Gazzinelli, R. T., M. Wysocka, S. Hieny, T. Scharton-Kersten, A. Cheever, R. Kuhn, W. Muller, G. Trinchieri, and A. Sher.** 1996. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent upon CD4+ T cells and accompanied by overproduction of IL-12, IFN-γ, and TNF-α. J. Immunol. **157:**798–805.
15. **Gil, M. P., E. Bohn, A. K. O'Guin, C. V. Ramana, B. Levine, G. R. Stark, H. W. Virgin, and R. D. Schreiber.** 2001. Biologic consequences of Stat1-independent IFN-γ signaling. Proc. Natl. Acad. Sci. USA **98:**6680–6685.
16. **Granger, D. L., J. B. J. Hibbs, J. R. Perfect, and D. T. Durack.** 1990. Metabolic fate of L-arginine in relation to microbiostatic capability of murine macrophages. J. Clin. Investig. **81:**264–273.
17. **Gratchev, A., P. Guillot, N. Hakiy, O. Politz, C. E. Orfanos, K. Schledzewski, and S. Goerdt.** 2001. Alternatively activated macrophages differentially express fibronectin and its splice variants and the extracellular matrix. Scand. J. Immunol. **53:**386–392.
18. **Hesse, M., M. Modelell, A. C. La Flamme, M. Schito, J. M. Fuentes, A. W. Cheever, E. J. Pearce, and T. A. Wynn.** 2001. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. J. Immunol. **167:**6533–6544.
19. **Holcomb, I. N., R. C. Kabakoff, B. Chan, T. W. Baker, A. Gurney, W. Henzel, C. Nelson, H. B. Lowman, B. D. Wright, N. J. Skelton, G. D. Frantz, D. B. Tumas, F. V. J. Peale, D. L. Shelton, and C. C. Herbert.** 2000. FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. EMBO J. **19:**4046–4055.
20. **Hunter, C. A., C. S. Subauste, V. H. Van Cleave, and J. S. Remington.** 1994. 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:**2818–2824.
21. **Jankovic, D., M. C. Kullberg, S. Hieny, P. Caspar, C. M. Collazo, and A. Sher.** 2002. In the absence of IL-12, CD4+ T cell responses to intracellular pathogens fail to default to a Th2 pattern and are host protective in an IL-10−/− setting. Immunity **16:**429–439.
22. **Khan, I. A., T. Matsuura, S. Fonseka, and L. H. Kasper.** 1996. Production of nitric oxide (NO) is not essential for protection against acute *Toxoplasma gondii* infection in IRF-1−/− mice. J. Immunol. **156:**636–643.
23. **Lee, C.-K., D. T. Rao, R. Gertner, R. Gimeno, A. B. Frey, and D. E. Levy.** 2000. Distinct requirements for IFNs and STAT1 in NK cell function. J. Immunol. **165:**3571–3577.
24. **Lighvani, A. A., D. M. Frucht, D. Jankovic, H. Yamane, J. Aliberti, B. D. Hissong, B. V. Nguyen, M. Gadina, A. Sher, W. E. Paul, and J. J. O'Shea.** 2001. T-bet is rapidly induced by interferon-γ in lymphoid and myeloid cells. Proc. Natl. Acad. Sci. USA **98:**15137–15142.
25. **Loke, P., M. G. Nair, J. Parkinson, D. Guiliano, M. Blaxter, and J. E. Allen.** 2002. IL-4 dependent alternatively-activated macrophages have a distinctive in vivo gene expression phenotype. BMC Immunol. **3:**7. [Online.] http://www.biomedcentral.com.
26. **Lugo-Villarino, G., R. Maldonado-Lopez, R. Possemato, C. Penaranda, and L. H. Glimcher.** 2003. T-bet is required for optimal production of IFN-γ and antigen-specific T cell activation by dendritic cells. Proc. Natl. Acad. Sci. USA **100:**7749–7754.
27. **Meraz, M. A., J. M. White, K. C. Sheehan, E. A. Bach, S. J. Rodig, A. S. Dighe, D. H. Kaplan, J. K. Riley, A. C. Greenlund, D. Campbell, K. Carver-Moore, R. N. DuBois, R. Clark, M. Aguet, and R. D. Schreiber.** 1996. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell **84:**431–442.
28. **Mills, C. D., K. Kincaid, J. M. Alt, M. J. Heilman, and A. M. Hill.** 2000. M-1/M-2 macrophages and the Th1/Th2 paradigm. J. Immunol. **164:**6166–6173.
29. **Mordue, D. G., F. Monroy, M. La Regina, C. A. Dinarello, and L. D. Sibley.** 2001. Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. J. Immunol. **167:**4574–4584.
30. **Mullen, A. C., F. A. High, A. S. Hutchins, H. W. Lee, A. V. Villarino, D. M. Livingston, A. L. Kung, N. Cereb, T. P. Yao, S. Y. Yang, and S. L. Reiner.** 2001. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science **292:**1907–1910.
31. **Munder, M., K. Eichmann, J. M. Moran, F. Centeno, G. Soler, and M. Modolell.** 1999. Th1/Th2-regulated expression of arginase isoforms in murine and dendritic cells. J. Immunol. **163:**3771–3777.
32. **Namangala, B., P. De Baetselier, W. Noel, L. Brys, and A. Beschin.** 2001. Alternative versus classical macrophage activation during experimental African trypanosomosis. J. Leukoc. Biol. **69:**387–396.
33. **Navia, B. A., C. K. Petito, J. W. M. Gold, E. S. Cho, B. D. Jordon, and J. W. Price.** 1986. Cerebral toxoplasmosis complicating the acquired immune deficiency syndrome: clinical and neuropathological findings in 27 patients. Ann. Neurol. **19:**224–238.
34. **Neyer, L. E., G. Grunig, M. Fort, J. S. Remington, D. Rennick, and C. A. Hunter.** 1997. Role of interleukin-10 in regulation of T-cell-dependent and T-cell-independent mechanisms of resistance to *Toxoplasma gondii.* Infect. Immun. **65:**1675–1682.

35. **Nöel, W., G. Hassanzadeh, G. Raes, B. Namangala, I. Daems, L. Brys, F. Brombacher, P. D. Baetselier, and A. Beschin.** 2002. Infection stage-dependent modulation of macrophage activation in *Trypanosoma congolense*-resistant and -susceptible mice. Infect. Immun. **70:**6180–6187.
36. **Raes, G., P. De Baetselier, W. Noel, A. Beschin, F. Brombacher, and G. G. Hassanzadeh.** 2002. Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. J. Leukoc. Biol. **71:**597–602.
37. **Ramana, C. V., M. Chatterjee-Kishore, H. Nguyen, and G. R. Stark.** 2000. Complex roles of Stat1 in regulating gene expression. Oncogene **19:**2619–2627.
38. **Ramana, C. V., M. P. Gil, R. D. Schreiber, and G. R. Stark.** 2002. Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. Trends Immunol. **23:**96–101.
39. **Remington, J. S., R. McLeod, and G. Desmonts.** 1995. Toxoplasmosis, p. 140–267. *In* J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant. W. B. Saunders Co., Philadelphia, Pa.
40. **Robinson, D. S., and A. O'Garra.** 2002. Further checkpoints in Th1 development. Immunity **16:**755–758.
41. **Rodriguez-Sosa, M., A. R. Satoskar, R. Calderon, L. Gomez-Garcia, R. Saavedra, R. Bojalil, and L. I. Terrazas.** 2002. Chronic helminth infection induces alternatively activated macrophages expressing high levels of CCR5 with low interleukin-12 production and Th2-biasing ability. Infect. Immun. **70:**3656–3664.
42. **Scanga, C. A., J. Aliberti, D. Jankovic, F. Tilloy, S. Bennouna, E. Y. Denkers, R. Medzhitov, and A. Sher.** 2002. 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.
43. **Scharton-Kersten, T., G. Yap, J. Magram, and A. Sher.** 1997. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii.* J. Exp. Med. **185:**1–13.
44. **Scharton-Kersten, T. M., T. A. Wynn, E. Y. Denkers, S. Bala, L. Showe, E. Grunvald, S. Hieny, R. T. Gazzinelli, and A. Sher.** 1996. In the absence of endogenous IFN-γ mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. J. Immunol. **157:**4045–4054.
45. **Sher, A., I. O. Oswald, S. Hieny, and R. T. Gazzinelli.** 1993. *Toxoplasma gondii* induces a T-independent IFN-γ response in NK cells which requires both adherent accessory cells and TNF-α. J. Immunol. **150:**3982–3989.
46. **Suzuki, Y., M. A. Orellana, R. D. Schreiber, and J. S. Remington.** 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii.* Science **240:**516–518.
47. **Suzuki, Y., A. Sher, G. Yap, D. Park, L. Ellis Neyer, O. Liesenfeld, M. Fort, H. Kang, and E. Gufwoli.** 2000. IL-10 is required for prevention of necrosis in the small intestine and mortality in both genetically resistant BALB/c and susceptible C57BL/6 mice following peroral infection with *Toxoplasma gondii.* J. Immunol. **164:**5375–5382.
48. **Szabo, S. J., S. T. Kim, G. L. Costa, X. Zhang, C. G. Fathman, and L. H. Glimcher.** 2000. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell **100:**655–669.
49. **Szabo, S. J., B. M. Sullivan, S. L. Peng, and L. H. Glimcher.** 2003. Molecular mechanisms regulating Th1 immune responses. Annu. Rev. Immunol. **21:**713–758.
50. **Takeda, A., S. Hamano, A. Yamanaka, T. Hanada, T. Ishibashi, T. W. Mak, A. Yoshimura, and H. Yoshida.** 2003. Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. J. Immunol. **170:**4886–4890.
51. **Taylor, G. A., C. M. Collazo, G. S. Yap, K. Nguyen, T. A. Gregorio, L. S. Taylor, B. Eagleson, L. Secret, E. A. Southon, S. W. Reid, L. Tessarollo, M. Bray, D. W. McVicar, K. L. Komschlies, H. A. Young, C. A. Biron, A. Sher, and G. F. Vande Woude.** 2000. Pathogen-specific loss of host resistance in mice lacking the IFN-γ-inducible gene IGTP. Proc. Natl. Acad. Sci. USA **97:**751–755.
52. **Usui, T., R. Nishikomori, A. Kitani, and W. Strober.** 2003. GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rβ2 chain or T-bet. Immunity **18:**415–428.

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