IL-22 Mediates Host Defense against an Intestinal Intracellular Parasite in the Absence of IFN-γ at the Cost of Th17-Driven Immunopathology

Jörg Stange, Matthew R. Hepworth, Sebastian Rausch, Lara Zajic, Anja A. Kühl, Catherine Uyttenhove, Jean-Christophe Renauld, Susanne Hartmann and Richard Lucius

*J Immunol* 2012; 188:2410-2418; Prepublished online 20 January 2012;
doi: 10.4049/jimmunol.1102062
http://www.jimmunol.org/content/188/5/2410

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/01/20/jimmunol.1102062.DC1

References This article cites 41 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/188/5/2410.full#ref-list-1

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

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-22 Mediates Host Defense against an Intestinal Intracellular Parasite in the Absence of IFN-γ at the Cost of Th17-Driven Immunopathology

Jörg Stange,*1 Matthew R. Hepworth,*1 Sebastian Rausch,* Lara Zajic,* Anja A. Kühl, ‡ Catherine Uyttenhove, ‡ Jean-Christophe Renauld, ‡ Susanne Hartmann,* and Richard Lucius*‡

The roles of Th1 and Th17 responses as mediators of host protection and pathology in the intestine are the subjects of intense research. In this study, we investigated a model of intestinal inflammation driven by the intracellular apicomplexan parasite Eimeria falciformis. Although IFN-γ was the predominant cytokine during E. falciformis infection in wild-type mice, it was found to be dispensable for host defense and the development of intestinal inflammation. E. falciformis-infected IFN-γR−/− and IFN-γ−/− mice developed dramatically exacerbated body weight loss and intestinal pathology, but they surprisingly harbored fewer parasites. This was associated with a striking increase in parasite-specific IL-17A and IL-22 production in the mesenteric lymph nodes and intestine. CD4+ T cells were found to be the source of IL-17A and IL-22, which drove the recruitment of neutrophils and increased tissue expression of anti-microbial peptides (RegIIIγ, RegIIIβ) and matrix metalloproteinase 9. Concurrent neutralization of IL-17A and IL-22 in E. falciformis-infected IFN-γR−/− mice resulted in a reduction in infection-induced body weight loss and inflammation and significantly increased parasite shedding. In contrast, neutralization of IL-22 alone was sufficient to increase parasite burden, but it had no effect on body weight loss. Treatment of an E. falciformis-infected intestinal epithelial cell line with IFN-γ, IL-17A, or IL-22 significantly reduced parasite development in vitro. Taken together, to our knowledge these data demonstrate for the first time an antiparasite effect of IL-22 during an intestinal infection, and they suggest that IL-17A and IL-22 have redundant roles in driving intestinal pathology in the absence of IFN-γ signaling. The Journal of Immunology, 2012, 188: 2410–2418.

Interferon-γ is classically considered the key protective cytokine in response to infections with numerous intracellular bacteria, viruses, and protozoan parasites, including Eimeria spp., Toxoplasma gondii, and Plasmodium spp. (1–4). IFN-γ is a potent proinflammatory cytokine derived from multiple cells of both the innate and adaptive immune system, including CD4+ Th1 cells, CD8+ T cells, and NK cells. It also plays a key role in driving immunopathology in response to microbial stimuli or pathogen-induced tissue damage, resulting in the development of inflammatory disorders of the intestine (5). Recently, many studies have attributed similar roles to the cytokines IL-17A and IL-22. Cells of the Th17 lineage are the predominant source of IL-17A and IL-22, although innate immune cells such as lymphoid tissue inducer cells and NK cells have also been shown to be potent producers of these cytokines (6). In particular, both IL-17A and IL-22 have been implicated in the development of colitis in humans and multiple murine models (7, 8) and have host-protective effects during bacterial infections, including Klebsiella pneumoniae, Citrobacter rodentium, and Staphylococcus aureus (9–11). Currently little is known about the roles of IL-17A and IL-22 in driving immunopathology or mediating host defense during intestinal protozoan parasite infections.

The development of Th1- and Th17-driven inflammation is closely linked by the common requirement for IL-12p40, which constitutes one subunit of both the biologically active IL-12 and IL-23 heterodimers. Th17 differentiation from naive CD4+ T cells is characterized by the expression of the master transcription factor retinoic acid receptor-related orphan receptor γt (RORγt) (12), which is driven by IL-6 and TGF-β and stabilized by IL-23 (13, 14). Interestingly, the priming of IFN-γ–producing Th1 and IL-17A–/IL-22–producing Th17 lineages can be cross-regulated. For example, IFN-γ can directly inhibit the development of Th17 cells via the suppression of T cell IL-23R expression, or ameliorate IL-17A–driven pathology via inhibition of IL-23 (15, 16). Furthermore, the development of Th1 and Th17 lineages is cross-regulated via their master transcription factors. T-box transcription factor (T-bet), expressed during Th1 commitment, concurrently suppresses Th17 development via interaction with Runx-1 and subsequently prevents transcription of the Rorc gene that codes for RORγt (17).

Eimeria falciformis is a natural intracellular pathogen of the murine cecum and proximal colon, where it develops a self-limiting...
infection in epithelial cells of the crypts (18). Moreover, intestinal *E. falciformis* infection induces strong inflammation in the cecum and colon and has many parallels with colitic disease, thus making it an excellent model for studying mucosal inflammation in the large intestine. This coccidian parasite belongs to the phylum of Apicomplexa that also contains major human pathogens such as *T. gondii*, *Plasmodium spp.*, and *Cryptosporidium parvum*. Infections with *Eimeria* spp. are of economic relevance, particularly in the poultry industry, with the costs attributed to lost revenue and drug treatment estimated to be in excess of £2 billion per year worldwide (19). Administration of infective oocysts via the natural oral route typically leads to the rapid development of an IFN-γ–dominated response, which is associated with body weight loss and the development of transient inflammation and immunopathology in the large intestine (2). Although adoptive transfer of IFN-γ–producing CD8+ T cells was previously shown to partially control *E. falciformis* infection (2), it is currently not known whether other cytokines can compensate in the absence of IFN-γ. Moreover, there is currently very little information available concerning the roles of IL-17A and IL-22 in the control of intracellular parasite infections at mucosal barriers or their ability to induce inflammation and immunopathology in response to parasite infections.

In the present study we investigated whether mice deficient in the IFN-γ receptor (IFN-γR−/−) or the cytokine itself (IFN-γ−/−) are impaired in their ability to control *E. falciformis* infection, or in the development of infection-driven immunopathology. We identified a novel compensatory role for IL-22 in the control of parasitic infection in the large intestine at the expense of severe mucosal immunity.

**Materials and Methods**

**Mice and parasites**

Female-specific pathogen-free C57BL/6 wild-type (Wt) mice (Charles River), C57BL/6 IFN-γ−/− mice (T. Schütler, Charité University Medicine, Berlin) and C57BL/6 IFN-γR−/− mice (U. Klemm, Max Planck Institute for Infection Biology, Berlin) were used for parasite infections. All experiments were performed in accordance with the National Animal Protection Guidelines, approved by the Animal Ethics Committee. *E. falciformis* was maintained by serial passage in NMRI mice, bred at the Department of Molecular Parasitology (Humboldt University of Berlin), and oocysts were purified by flotation in sodium hypochlorite as described elsewhere (2).

**Infections and quantification of parasite burden**

For experimental infections, mice aged 10–14 wk were inoculated with 50 *E. falciformis* oocysts in 100 µl water via oral gavage, weighed daily, and sacrificed at the end of latency, or at day 10 postinfection (p.i.) where indicated. Mice were euthanized upon 20% body weight loss. For analysis of challenge infections mice received a dose of 10 oocysts followed by a challenge infection with 50 oocysts 4 wk after the primary dose. During latency, feces were collected every 24 h (≥2 h), soaked in water, homogenized, and floated in saturated sodium chloride solution. The number of oocysts was quantified using a McMaster chamber.

**Neutralization of cytokines**

Two hundred micrograms of anti–IL-17A (MM17AF3; C. Uyttenhove, Brussels) and/or anti–IL-22 (AM22.1; Jean-Christophe Renauld, Brussels) mAb in PBS was injected i.p. into mice on days −1, 2, 5, and 8 p.i. Control groups received 200 µg mouse IgG control Ab (Dianova) according to the same treatment regimen.

**Histology**

For histopathological analysis, colon and cecum samples were fixed with 3% paraformaldehyde and embedded in paraffin. Sections (2 µm) were cut, deparaffinized, and stained with H&E. To quantify intestinal inflammation, infiltration (0, none; 1, mild, minimal mucosal; 2, moderate, mucosal and submucosal; 3, marked mucosal and submucosal, sometimes transmural; 4, marked infiltrates, often transmural; 5, marked transmural inflammation) and crypt architecture (0, normal; 1, mild hyperplasia, normal architecture and goblet cell number; 2, moderate hyperplasia, goblet cell loss; 3, architecture disrupted, severe goblet cell loss; 4, severe loss of crypt architecture; 5, complete loss of crypt architecture and ulceration) were scored separately and values were combined to yield a score from 0 to 10. Periodic acid–Schiff (PAS) histochemistry was conducted in a Dako Autostainer Plus according to the manufacturer’s instructions. *E. falciformis* was quantified in PAS-stained cecal thin sections from day 5 p.i. by counting the number of mature schizonts per high power field (HPF; ×400 magnification, 20 replicates).

**In vitro assays with CMT-93 cells**

CMT-93 cells (a gift from T. Schütler, Berlin) were cultured in DMEM supplemented with 10% FCS, 20 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were harvested using 1 × trypsin/EDTA (PAA Laboratories) and washed with 2 µg/ml of mitomycin C (AppliChem) for 30 min at 37°C to inhibit proliferation. After washing, cells were seeded in media containing 50 ng/ml respective cytokine (IFN-γ, IL-17A, IL-22, and IL-10; PeproTech) and 5 × 10^5 cells were seeded in 24-well plates on coverslips (diameter 12 mm). *E. falciformis* sporozoites were purified as described before (20), and 8 h after seeding cells were infected with 1 × 10^5 *E. falciformis* sporozoites suspended in DMEM without FCS. Extracellular parasites were washed off 4 h p.i., and DMEM with 10% FCS was added for further culture. For histological examination, monolayers were fixed for 10 min using 4% paraformaldehyde and stained with PAS. Invasion was determined 4 h p.i. by counting intracellular sporozoites per HPF (×400 magnification, 10 HPFs per sample). Development was assessed at 39 h p.i. by scanning the whole colony for mature schizonts. Results are displayed relative to the mean of the control group.

**Lymphocyte isolation and culture**

Mesenteric lymph nodes (MLN) and spleens were isolated from euthanized mice and single cell suspensions were obtained by passing through a 70µm cell strainer (BD Biosciences). Cells were resuspended in RPMI 1640 containing 10% FCS, 20 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (complete RPMI 1640; PAA Laboratories) and counted using a Casy model TT cell counter (Innovatis). Lamina propria lymphocytes (LPL) were isolated from the cecum and proximal colon. Briefly, mesenteric fat and the cecal patch were removed and the intestine was opened longitudinally, washed thoroughly in HBSS containing 2% FCS, and cleaned further by vigorous shaking in the same buffer twice. Epithelial cells were removed by a 20-min incubation in HBSS 2% FCS containing 2 mM EDTA. Tissue was then minced and incubated for 1 h at 37°C in RPMI 1640 medium containing 10% FCS, 200 U/ml collagenase VIII, and 0.1 U/ml collagenase D (Sigma-Aldrich). The digested tissue was filtered, centrifuged, and the cell pellet was resuspended and layered onto a 40/70% Percoll gradient (GE Healthcare) and centrifuged for 20 min at 3000 rpm. LPL were isolated from the interface and analyzed by flow cytometry.

**Lymphocyte restimulations**

MLN cells (5 × 10^5) were cultured in a total volume of 200 µl in 96-well round-bottom plates in complete RPMI 1640. Cultures were restimulated with total sporozoite Ag equivalent to 1.2 × 10^5 sporozoites per well as described before (2) or 1 µg/ml anti-CD3 Ab (BD Biosciences) at 37°C and 5% CO2 for 48 h. Supernatants were harvested for cytokine detection, and in some experiments medium was replaced and 1 µCi μl−1 [3H]thymidine (Amersham Pharmacia Biotech) was added. Cultures were placed at 37°C and 5% CO2 for an additional 20 h to measure radiolabeled thymidine incorporation, which was detected using a 1450 MicroBeta TriLux microplate scintillation and luminescence counter (PerkinElmer).

**Flow cytometric analysis**

The following mouse mAbs were used for flow cytometry: CD8-PE, GR-1-Cy5 (DBA/2N Mice; BioLegend), CD44-FITC, IL-17A-PE (BD Biosciences), RORγt-allophycocyanin, T-bet-PE, CD49b-PE-Cy7, IL-17A-FTTC, IL-22-PE, IFN-γ-eFluor 450, CD4-PercP-eFluor 710, stem cell Ag-1 (Sca-1)-PE-Cy7, CD117-allophycocyanin (c-Kit), lineage-eFluor 450 (eBioscience), and Ly-6C-Pacific Blue (BioLegend) in pretitrated 1:100 dilutions. The following mouse mAbs were used for flow cytometry: CD8-PE, GR-1-Cy5 (DBA/2N Mice; BioLegend), CD44-FITC, IL-17A-PE (BD Biosciences), RORγt-allophycocyanin, T-bet-PE, CD49b-PE-Cy7, IL-17A-FTTC, IL-22-PE, IFN-γ-eFluor 450, CD4-PercP-eFluor 710, stem cell Ag-1 (Sca-1)-PE-Cy7, CD117-allophycocyanin (c-Kit), lineage-eFluor 450 (eBioscience), and Ly-6C-Pacific Blue (BioLegend) in pretitrated 1:100 dilutions. The following mouse mAbs were used for flow cytometry: CD8-PE, GR-1-Cy5 (DBA/2N Mice; BioLegend), CD44-FITC, IL-17A-PE (BD Biosciences), RORγt-allophycocyanin, T-bet-PE, CD49b-PE-Cy7, IL-17A-FTTC, IL-22-PE, IFN-γ-eFluor 450, CD4-PercP-eFluor 710, stem cell Ag-1 (Sca-1)-PE-Cy7, CD117-allophycocyanin (c-Kit), lineage-eFluor 450 (eBioscience), and Ly-6C-Pacific Blue (BioLegend) in pretitrated 1:100 dilutions.
nation with T-bet or RORγt. Stained cells were acquired using a BD Fortessa cytometer and data were analyzed using FlowJo software (Tree Star).

ELISA

Cytokines were quantified in cell culture supernatants in duplicates using an IFN-γ ELISA (eBioscience) and IL-22 and IL-17A DuoSet ELISAs (R&D Systems) according to the manufacturer’s instructions. ELISA plates were read with a Synergy HT reader together with Gen5 data analysis software (BioTek).

Quantitative real-time PCR

 Cecum samples were snap frozen in liquid nitrogen and stored at −80°C. The tissue was homogenized using a tissue homogenizer (MP Biomedicals), samples were centrifuged (10 min, 20,000 × g, 4°C), and total RNA was isolated using an innuPREP RNA kit (Analytik Jena) according to the manufacturer’s instructions. Following reverse transcription of 2 μg RNA using the High Capacity RNA-to-cDNA kit (Applied Biosystems), quantitative real-time PCR was performed in duplicates with 10 ng cDNA using the FastStart Universal SYBR Green Master Mix (Roche) in an ABI 7300 real-time PCR system (Applied Biosystems). The following primers (MWG Biotech) were used for detection: Actb forward, 5′-TCTTTGCTATCTGGTTCGCA-3′, reverse, 5′-TCTCTACCTGACCTGTCAGTTC-3′, 5′-CCAGCCACTCAGGCGTATCA-3′, reverse, 5′-GGCTCGACAGGATTTTGCAGAGAC-3′, reverse, 5′-GGCTGCAGAGCAGTCCGTCCT-3′, reverse, 5′-TGCATGGGCCAGTAATGTTTCT-3′, reverse, 5′-CCAAATCCCGAGCTTTGTATCTT-3′, reverse, 5′-ATGGAATCCTGTGGCA-3′, forward, 5′-GCTTCAGAAGCAGCTCTCC-3′, 5′-AGCAA-3′, reverse, 5′-TGGTCCTTAGCCACTCCTTC-3′, Reg3β forward, 5′-CTCCTCGGCTGTAGTCTT-3′, reverse, 5′-GTAGGAGCATAAAGCTGGG-3′, Reg3γ forward, 5′-TCAGTGCAGAAGTGATTG-3′, reverse, 5′-GGCCACTGTTACCACTGCTT-3′, Mmp9 forward, 5′-GGCTGTCAGAGCCTCGTGGCTTTGG-3′, reverse, 5′-GGCTGGTCAGAGCCTCGTGGCTTTGG-3′; Nos2 forward, 5′-GGCCACTGTTACCACTGCTT-3′, reverse, 5′-AGTGTTTCCTCTACCCAGCAC-3′, forward, 5′-GTGGTGTGGTGGCTCCCATC-3′, reverse, 5′-ATGAGACACTCTGACCTGTC-3′, reverse, 5′-CAGCGGAGCTGAAACGAT-3′, forward, 5′-AGTGTTTCCTCTACCCAGCAC-3′, reverse, 5′-GTGGTGTGGTGGCTCCCATC-3′, reverse, 5′-ATGAGACACTCTGACCTGTC-3′, reverse, 5′-CAGCGGAGCTGAAACGAT-3′. Following normalization to β-actin (housekeeping gene), the results were plotted as relative expression compared with naive controls using the 2−ΔΔCT method.

Statistical analyses

All experiments were performed with four to eight mice per group, and representative data are shown as means ± SEM for three independent experiments unless stated otherwise. The nonparametric Mann–Whitney U test was used for statistical analysis. Values of p ≤ 0.05 were considered to be statistically significant.

Results

IFN-γ− and IFN-γR−/− deficient mice show increased weight loss and intestinal pathology following E. falciformis infection

To determine whether IFN-γ is required for host protection during E. falciformis infections, we assessed infection-induced immunopathology by monitoring body weight loss in mice deficient in IFN-γ (IFN-γ−/−) and its receptor (IFN-γR−/−) in comparison with C57BL/6 wild-type (WT) mice. In contrast to WT mice, which only transiently lost weight during primary infection (days 8–10 p.i.; Fig. 1A), IFN-γR−/− mice showed a dramatically exacerbated body weight loss and an increased histopathology score at day 10 p.i. (IFN-γR−/−, 18.7 ± 1.4% versus WT, 2.2 ± 1.7%, p < 0.01; Fig. 1A–C). Mortality was also clearly aggravated in IFN-γR−/− mice, which was apparent from bloody feces, crouched posture, and ruffled fur (data not shown). Infection of mice lacking the cytokine (IFN-γ−/−) resulted in a comparable body weight loss and signs of morbidity at day 10 p.i. (IFN-γ−/−, 19.7 ± 1.0% versus WT, 2.4 ± 1.0%, p ≤ 0.01; Supplemental Fig. 1A). The increased infection-induced pathology was even more pronounced during secondary infections of IFN-γR−/− mice; a primary dose of 50 oocysts followed by a challenge infection with the same dose led to severe intestinal pathology and mortality in 100% of IFN-γR−/− mice (Fig. 1A–C). In contrast, WT mice showed no significant weight loss during challenge infection and developed only mild pathology (Fig. 1A–C).

MLN and spleen cells from IFN-γR−/− mice isolated at day 10 after primary infection showed a 14.9-fold (MLN) and 6.9-fold (spleen) increase in proliferation to parasite Ag in comparison with cells isolated from WT mice (p < 0.01; Fig. 1D), implying a dysregulation of the immune response to E. falciformis in the absence of IFN-γ signaling.

FIGURE 1. Pathology and parasite development in C57BL/6 WT and IFN-γR−/− mice infected with E. falciformis. (A) Body weight loss of WT and IFN-γR−/− mice after primary (1st) and challenge (2nd) infections with 50 (E. falciformis) oocysts. Mice were euthanized upon loss of 20% body weight (dotted line). (B) H&E-stainedecal thin sections of WT and IFN-γR−/− mice and (C) pathological scoring. (D) Parasite Ag-specific proliferation of MLN and spleen cells of WT (filled bars) and IFN-γR−/− (open bars) mice isolated 10 d.p.i. with E. falciformis. (E) Quantification of shed oocysts from WT and IFN-γR−/− mice following primary or challenge E. falciformis infection. (F) Quantification of the asexual development (schizogony, day 5 p.i.) of E. falciformis in cecal crypts of WT and IFN-γR−/− mice during primary infection. Scale bars, 20 μm. Data shown are means ± SEM of six mice per group and representative of four independent experiments. *p ≤ 0.05, **p ≤ 0.01, ****p ≤ 0.0001.
Signaling via the IFN-γ receptor is not required for the control of parasite replication or for the development of immunity

Because IFN-γ is a key cytokine for the control of numerous intracellular pathogens (1–5), we analyzed the development of *E. falciformis* in IFN-γ−/− and IFN-γR−/− mice. Surprisingly, significantly fewer parasites developed in IFN-γR−/− mice compared with Wt mice, as indicated by a reduced number of shed oocysts (−78%, p < 0.01; Fig. 1E). As described above (Fig. 1A–C), IFN-γR−/− mice developed severe pathology, which was associated with hemorrhagic diarrhea. To confirm the reliability of the quantification of shed oocysts as an adequate parameter, we assessed parasite development directly at the site of infection at an early time point. In line with the oocyst data, analysis of cecal thin sections on day 5 p.i. revealed 86% (p < 0.01) fewer parasites in IFN-γR−/− mice compared with Wt mice (Fig. 1F). Because a primary infection with a dose of 50 *E. falciformis* oocysts followed by a secondary challenge infection with the same dose led to 100% mortality in IFN-γR−/− mice (Fig. 1A), we instead infected mice with a primary dose of 10 oocysts and challenged them with 50 oocysts to assess whether signaling via the IFN-γR is required for the development of immunity. Quantification of shed parasites after challenge infection revealed a comparable reduction of oocyst shedding in both IFN-γR−/− and Wt mice in comparison with primary infected control mice (55 versus 58%, p < 0.01; Fig. 1E). Thus, IFN-γR signaling is dispensable for the control of *E. falciformis* replication.

Deficiency in IFN-γ signaling leads to an expansion of IL-17A− and IL-22-producing Th17 cells

Signaling via the IFN-γR has the ability to limit effector T cell responses and to reciprocally regulate other arms of the adaptive immune system, thereby preventing immunopathology caused by an overactive immune response (21). Because enhanced proliferation of draining lymph node cells was seen following *E. falciformis* infection in IFN-γR−/− mice (Fig. 1D), we sought to assess the cytokine profile and phenotype of the exaggerated immune response 10 d after *E. falciformis* infection.

Strikingly, both *E. falciformis*-infected IFN-γ−/− and IFN-γR−/− mice had higher frequencies and numbers of CD4+ T cells producing IL-17A and IL-22 in the MLN and LPL and produced higher amounts of IL-17A (IFN-γR−/−, 8.9 ± 1.0 ng/ml versus Wt, ND) and IL-22 (IFN-γR−/−, 0.43 ± 0.08 versus Wt, 0.05 ± 0.02 ng/ml) in comparison with *E. falciformis*-infected Wt controls, as measured in Ag-specific restimulated MLN cultures via ELISA (Fig. 2A, 2B, Supplemental Figs. 1B–D, 2A, 2B). No increase in Th2-associated cytokine production was detected in IFN-γR−/− mice (data not shown).

Surprisingly, IFN-γ levels were also strongly increased in IFN-γR−/− mice (IFN-γR−/−, 59.4 ± 7.8 ng/ml versus Wt, 7.2 ± 3.9 ng/ml, p < 0.01; Fig. 2A, 2B, Supplemental Fig. 2A, 2B). In line with previous studies in our group (2), the major cellular source of IFN-γ during *E. falciformis* infection of Wt mice was found to be CD8+ T cells, with smaller numbers of IFN-γ+ CD49b+ NK cells and relatively few CD4+ T cells (Supplemental Fig. 2B). In contrast, the enhanced IFN-γ production observed in culture supernatants of cells from IFN-γR−/− mice was found to be almost entirely derived from an increase in CD4+ IFN-γ–producing T cells (MLN: IFN-γR−/−, 0.36 ± 0.05 × 10^6 versus Wt, 0.08 ± 0.01 × 10^6, p < 0.01; Supplemental Fig. 2B). CD4+ IFN-γ−/− T cells also expressed T-bet (Fig. 2C), confirming an increase in committed Th1 cells following loss of IFN-γR signaling.

Similarly, *E. falciformis*-elicited IL-17A+ T cells expressed the master transcription factor RORγt, indicating committed Th17 cells (Fig. 2C). Interestingly, we noted a subset of T cells that coexpressed

---

**FIGURE 2.** Expansion of Th17 cells in IFN-γR−/− mice infected with *E. falciformis*. (A) Frequencies of IL-17A+, IL-22−, and IFN-γ–producing CD4+ T cells in the MLN and (B) total cell numbers of IL-17A+, IL-22−, and IFN-γ–producing CD4+ cells in the MLN and isolated LPL of Wt and IFN-γR−/− mice 10 d after primary infection with *E. falciformis*. (C) Coexpression of IL-17A/RORγt and IFN-γ/T-bet in the MLN cells of *E. falciformis*-infected IFN-γR−/− mice. (D) Frequencies and total cell numbers of LPL Ly-6C+ (Gr-1+) monocytes, Gr-1+ neutrophils, and CD4+ T cells. Data shown are means ± SEM of four to six mice per group and representative of three independent experiments. *p < 0.05, **p < 0.01.
both IL-17A and IFN-γ in the MLN and LPL (Fig. 2A). These cells were found to coexpress both Th1 (T-bet)- and Th17 (RORγt)-associated transcription factors (Supplemental Fig. 2C), indicating the presence of a mixed Th1/Th17 cell phenotype and suggesting a degree of T cell plasticity during *E. falciformis* infection.

Although CD4+ T cells were the major source of IL-17A and IL-22 in both the MLN and LPL compartments (Supplemental Figs. 1D, 2B), we also noted production of both cytokines by non-CD4+ T cells. In the MLN, ~14% of IL-17A- and IL-22-producing cells lacked CD4 expression, but were found to be lineage-positive (CD4+Lin+) (Supplemental Fig. 3A). In contrast, a significant proportion of IL-17A- and IL-22-producing cells in the intestinal LPL compartment were found to be CD4- lineage marker negative (Lin-) (Supplemental Fig. 3B). The number of Lin- IL-17A-/IL-22-producing cells was significantly increased in the LPL compartment following *E. falciformis* infection (Supplemental Fig. 3E). The Lin- populations expressing IL-17A and IL-22 were apparently heterogeneous, as only a small proportion of cells coproduced both cytokines (Supplemental Fig. 3C). Despite this, lamina propria Lin- IL-17A+ and Lin+ IL-22+ cells were phenotypically similar and were found to express Sca-1, but not c-Kit (Supplemental Fig. 3D).

Next we assessed recruitment of Th1/Th17-associated innate inflammatory cells to the intestinal tissue. In comparison with *E. falciformis*-infected Wt mice, the number of Gr1+ cells was found to increase by 3.8-fold in the LPL compartment in IFN-γ- and decreased parasite development observed during infections of IFN-γR−/− mice indicated a possible anti-parasite role of IL-17A and/or IL-22 against *E. falciformis*. To test this hypothesis, the mouse intestinal epithelial cell line CMT-93 was treated with IL-17A, IL-22, and IFN-γ (or IL-10 as a control) and the development of the parasite was assessed in vitro. The cell line was found to be immunocompetent, as demonstrated by the expression of the relevant cytokine receptors (Fig. 4A). *E. falciformis* sporozoites invaded CMT-93 cells efficiently and developed into the first asexual generation of their life cycle (schizonts, Fig. 4B). Invasion of CMT-93 cells by *E. falciformis* was unaffected by treatment with any of the cytokines (data not shown), whereas IL-17A, IL-22, and IFN-γ all reduced the development of *E. falciformis* to the first asexual parasite generation (Fig. 4C). IL-22 and IL-17A significantly reduced the number of mature schizonts by 52% (p ≤ 0.001) and 47% (p ≤ 0.01), respectively, whereas the Th1 cytokine IFN-γ inhibited the development to a lesser extent (35%, p < 0.01). In contrast, treatment with IL-10 had no significant effect on development of *E. falciformis* in this intestinal epithelial cell line.

**FIGURE 3.** Expression profile of Th1/Th17-associated genes in the cecum of *E. falciformis*-infected Wt and IFN-γR−/− mice. (A) Th1-regulated genes, (B) Th17 commitment-associated genes, and (C) Th17 cytokine-regulated genes were assessed by quantitative real-time PCR in the cecum of *E. falciformis*-infected Wt (filled bars) and IFN-γR−/− mice (open bars) 10 d p.i. Data shown are means ± SEM of five to six mice per group and representative of two independent experiments. *p ≤ 0.05, **p ≤ 0.01.

The expression profile in the infected cecum of IFN-γR−/− mice reflects the dominant Th17 response

Analysis of the expression profile of downstream effector genes in the cecum tissue of Wt mice infected with *E. falciformis* revealed a strong increase in Th1-associated genes. The mRNA encoding for CXCL9 and CXCL10, two chemokines that are important for migration and function of Th1 cells, and inducible NO synthetase, a major downstream effector of IFN-γ, were strongly induced in Wt mice compared with naive mice (Cxc9, 90-fold; Cxc10, 17-fold; Nos2, 107-fold). This effect was almost completely abrogated in IFN-γR−/− mice with only low expression of Cxc9, Cxc10, and Nos2 in comparison with naive mice (Cxc9, 0.9-fold; Cxc10, 0.6-fold; Nos2, 18-fold; Fig. 3A).

Although the levels of several transcripts important for commitment of cells to the Th17 lineage, including Il6, Tgfb1, and Il23a, did not differ in the cecal tissue of Wt and IFN-γR−/− mice, the mRNA coding for the IL-23R was significantly upregulated (6-fold, p ≤ 0.05; Fig. 3B). Furthermore, the dominant Th17 response observed in IFN-γR−/− mice was reflected by a dramatic upregulation of the transcripts encoding two IL-22-dependent antimicrobial peptides, Reg3β and Reg3γ (Reg3b, 2976-fold; Reg3g, 663-fold; p ≤ 0.01; Fig. 3C). The expression of matrix metalloproteinase (MMP) 9 was also found to be significantly increased in IFN-γR−/− mice (13-fold, p ≤ 0.05; Fig. 3C).

**IL-17A and IL-22 inhibit parasite development in vitro**

The correlation between increased levels of IL-17A and IL-22 and decreased parasite development observed during infections of IFN-γR−/− mice indicated a possible anti-parasite role of IL-17A and/or IL-22 against *E. falciformis*. To further dissect the roles of IL-17A and IL-22 in our model in vivo, we concurrently depleted IL-17A and IL-22 during infections of Wt and IFN-γR−/− mice with *E. falciformis*. Simultaneous neutralization of IL-17A and IL-22 significantly reduced *E. falciformis*-induced body weight loss in IFN-γR−/− mice, whereas Ab neutralization had no significant effects in Wt mice in comparison with control/IgG-treated mice (Fig. 5A). Furthermore, combined neutralization of IL-17A and IL-22 led to a significant reduction in *E. falciformis*-induced histopathology and an almost complete restoration of normal intestinal architecture (Fig. 5B, 5C). IL-17A/IL-22 neutralization correlated with a significant decrease in inflammation as indicated by a reduction in the total number of infiltrating CD4+ T cells and Gr-1+ neu-
trophils in the lamina propria of *E. falciformis*-infected IFN-γR−/− mice following Ab treatment (p ≤ 0.05, Fig. 5D). Moreover, concurrent neutralization of IL-17A and IL-22 led to significantly higher numbers of shed oocysts in comparison with IgG-treated mice (anti–IL-17/IL-22, 0.23 ± 0.02 × 10⁶ versus IgG, 0.13 ± 0.01 × 10⁶, p ≤ 0.01; Fig. 5E). The increased oocyst shedding was further found to correlate with significantly reduced expression of the IL-22–dependent antimicrobial peptides RegIIIβ and RegIIIγ in the intestinal tissue of infected mice following cytokine neutralization (p ≤ 0.01, Fig. 5F).

In contrast to double neutralization of IL-17A and IL-22, single neutralization of either IL-17A or IL-22 failed to reverse *E. falciformis*-induced body weight loss in IFN-γR−/− mice (Fig. 5G), although a slight but significant reduction in histopathology score was seen in IFN-γR−/− mice following neutralization of IL-22 (Fig. 5H, 5I). This correlated with a marked trend toward fewer infiltrating CD4+ T cells in IL-22–depleted IFN-γR−/− mice, whereas the number of Gr1+ neutrophils was significantly reduced in IFN-γR−/− mice following either IL-17A or IL-22 neutralization (Fig. 5J). Importantly, total numbers of both cell populations were still significantly higher than in infected WT mice, and the decrease was less pronounced than in combined IL-17A/IL-22–neutralized mice (Fig. 5D, 5J). Whereas neutralization of both IL-17A and IL-22 resulted in enhanced *E. falciformis* oocyst output in IFN-γR−/− mice, neutralization of IL-17A alone had no effect (Fig 5K). In contrast, single neutralization of IL-22 led to a significant increase in oocyst shedding in IFN-γR−/− mice in comparison with IgG-treated controls (Fig. 5K). The increase in oocyst shedding following IL-22 single neutralization was comparable to the effect seen in IL-17A/IL-22 double-neutralized mice, suggesting that IL-22 was the dominant anti-parasite cytokine in *E. falciformis*-infected IFN-γR−/− mice.

**Discussion**

In this study we used a potent model of intestinal inflammation driven by the large intestine-dwelling intracellular parasite *E. falciformis* to dissect the relative contributions and interplay of Th1 and Th17 immune responses in the generation of mucosal inflammation and host immunity. We demonstrate that in the absence of IFN-γ signaling, *E. falciformis*-infected mice developed an increase in immunopathology and morbidity driven by the Th17 cytokines IL-17A and IL-22. Moreover, we identify a novel anti-parasite role for IL-22, which reduced parasite development in the absence of IFN-γ during infection in vivo and in an epithelial cell line (summarized in Supplemental Fig. 4). Importantly, these findings represent the first report, to our knowledge, that demonstrates a role for IL-22 in host defense against intracellular intestinal parasites.

IL-17A production is highly correlated with disease severity in patients suffering intestinal inflammatory disorders such as ulcerative colitis and Crohn’s disease, and RORγt-expressing Th17 cells are known to be essential for the development of colitis in murine models (7). In contrast, IL-22 has seemingly pleiotropic functions during colitis. It is abundantly expressed in the mucosa of patients suffering from inflammatory bowel disease and drives production of proinflammatory cytokines and MMPs (22). Conversely, IL-22 also has protective roles in murine ulcerative colitis models by promoting mucosal wound healing responses (23). Following *E. falciformis* infection of IFN-γR−/− or IFN-γ−/− mice, CD4+ RORγt+ Th17 cells were found to be the predominant cellular source of IL-17A and IL-22, although we also noted a significant increase in innate cells producing IL-17A and IL-22 in the lamina propria of *E. falciformis*-infected IFN-γR−/− mice. These cells were found to be Lin−c-Kit−Sca-1+, a phenotype resembling a population of IL-17A−/IL-22−producing innate intestinal lymphoid cells recently identified in a T cell–independent model of colitis (24).

Our data suggest that IL-17A and IL-22 have redundant proinflammatory roles during *E. falciformis* infection, as dramatic reductions in cellular infiltration and, in particular, attenuation of body weight loss were only seen following concurrent neutralization of both cytokines. However, we cannot rule out the possibility that other Th17 cytokines such as IL-17F and GM-CSF contribute to *E. falciformis*–induced inflammation (7, 25). The severe Th17-driven inflammation and pathology seen during the self-limiting *E. falciformis* infection in the absence of IFN-γ have many parallels to murine models of colitis, and thus may prove to be a powerful model for dissecting the immunological mechanisms behind Th1/Th17 disease. For example, MMPs are potent mediators of intestinal pathology, and mice deficient for MMP9 develop less severe inflammation and disease during a mouse model of colitis (26). In line with these findings an increase in MMP9 was observed in IFN-γR−/− mice following *E. falciformis* infection. Furthermore, IL-22 but not IL-17A was shown to be necessary to drive small intestinal inflammation and tissue damage following oral infection of mice with *T. gondii* via induction of proinflammatory cytokines and MMP2 (27).

Th17 responses are implicated in the etiology of many diseases of intestinal inflammation. For example, polymorphisms in IL-23R are highly associated with altered susceptibility to colitis (28, 29). During *E. falciformis* infection of IFN-γR−/− mice we observed an increased expression of IL-23R in the large intestine. Because IFN-γ can directly prevent Th17 expansion via suppression of IL-23R (15), it is possible that IFN-γ regulates parasite-induced Th17 responses in this manner during *E. falciformis* infection. We were
unable to detect any significant changes in the expression of essential Th17-inducing cytokines, including TGF-β, IL-6, and IL-23, in the intestines of IFN-γR−/− mice, and thus it is possible that IFN-γ may limit Th17 differentiation and expansion via direct effects on Th17 cells, such as induction of apoptosis (30). Furthermore, IFN-γ was shown to suppress Th17 expansion via induction of IDO expression in the lung during tuberculosis infection (31). Interestingly, IDO expression was completely abrogated in the intestine during *E. falciformis* infection of IFN-γR2/2 and IFN-γR2/2 mice (data not shown). The regulation of Th17 cytokines by IFN-γ may be of relevance in a wide range of diseases, and deficiencies in IFN-γ or its receptor result in aggravated disease associated with enhanced IL-17A and/or IL-22 and neutrophilia in murine models of arthritis, cancer, and tuberculosis (31–33). Interestingly, in human populations, polymorphisms in IFN-γ signaling-associated genes correlate with decreased Th17 immune responses. In particular, it was recently shown that patients with gain of function mutations in STAT-1, an essential IFN-γ signaling factor, have impaired IL-17A and IL-22 production (34). Intriguingly, IFN-γ and IL-22 are found on a highly conserved locus in a wide range of species, suggesting a close evolutionary relationship between these two important cytokines (35). Thus, understanding the interplay between IFN-γ signaling and development of Th17 lineages may be of clinical relevance, and IFN-γ signals may directly regulate Th17 cytokine production during *E. falciformis* infection.

Surprisingly, the loss of IFN-γ or IFN-γR and the subsequent increase in Th17 responses were not associated with attenuated

![Figure 5](http://www.jimmunol.org/Download.png)
host defense, but rather with a decreased parasite shedding. Previous reports utilizing the large intestine-dwelling species *Eimeria praegensis* also noted that host defense was not impaired in the absence of IFN-γ, but clinical signs were exacerbated (36). Although CD8+ cell-derived IFN-γ may partially protect against *E. falciformis* (2), we found that the switch to Th17-dominated responses in the absence of IFN-γ resulted in enhanced control of asexual replication, decreased oocyst shedding, and comparable protection from reinfection. IL-22 mediated parasite control, both in vitro and in vivo, whereas IL-17A reduced parasite development in our in vitro system only. The mechanisms by which these cytokines inhibit *E. falciformis* development in vitro and in vivo are currently unclear; however, both cytokines have critical roles in host protection to other pathogens. IL-17A and IL-22 are important for host defense during oral candidiasis (37, 38). Similarly, IL-22 is essential for maintaining mucosal barrier function and for the enhanced epithelial cell proliferation and cytokine production required to control *K. pneumoniae* infection (9). High levels of IL-17A and IL-22 also strongly correlate with protective immunity in human populations infected with *Leishmania donovani* (39), and IL-17A−/− mice fail to control *Trypanosoma cruzi* infection, which leads to multiple organ failure and mortality (40). However, to date no protective role for IL-22 has been shown in parasite infections.

Neutralization of IL-22 in vivo or treatment of infected cells in vitro highlighted the anti-parasite role of IL-22 during *E. falciformis* infection. This is of particular relevance as IL-22R1−/− mice lack the interferon-γ, but clinical signs were exacerbated (36). Although IFN-γ is a central mediator of antiparasitic responses against the pre-erythrocytic and blood stage of malaria, *J. Leukoc. Biol.* 81: 1131–1143.


matrix metalloproteinase-2 and IL-22 but independent of IL-17. J. Exp. Med. 206: 3047–3059.
SUPPLEMENTAL FIGURE 1. Expansion of IL-17A- and IL-22-producing CD4⁺ T cells in *E. falciformis*-infected IFN-γ⁻/⁻ mice. (A) Body weight loss of Wt and IFN-γ⁻/⁻ mice after infection with 50 *E. falciformis* oocysts. (B) Cytokine concentrations (ELISA) in the supernatants of MLN cell cultures of *E. falciformis*-infected Wt (shaded bars) and IFN-γ⁻/⁻ (open bars) mice from d 10 p.i., stimulated with total sporozoite antigen for 48 h. (C) Frequencies of IL-22- and IL-17A-producing CD4⁺ T cells in MLN cells of Wt and IFN-γ⁻/⁻ mice. (D) Total cell numbers of IL-17A⁻, IL-22- and IFN-γ⁻/⁻-producing CD4⁺, CD8⁺ and CD49b⁺ MLN cells of Wt (shaded bars) and IFN-γ⁻/⁻ mice (open bars). Data shown are means ± SEM of six to eight mice per group representative of two independent experiments. **p ≤ 0.01, ***p ≤ 0.001.

SUPPLEMENTAL FIGURE 2. Production and cellular sources of IL-17A, IL-22 and IFN-γ in Wt and IFN-γR⁻/⁻ mice. (A) Cytokine concentrations in the supernatants of *E. falciformis*-antigen-restimulated MLN cells from Wt (shaded bars) and IFN-γR⁻/⁻ mice (open bars). (B) Total cell numbers of IL-17A⁻, IL-22- and IFN-γ⁻/⁻-producing CD4⁺, CD8⁺ and CD49b⁺ MLN cells of Wt (shaded bars) and IFN-γR⁻/⁻ mice (open bars). (C) Expression of T-bet and/or RORγt in IFN-γ⁻/IL-17A⁻ producing subsets from MLN cells of IFN-γR⁻/⁻ mice. All cells were isolated 10 days after primary infection with 50 *E. falciformis* oocysts. Data are shown as means ± SEM representative of three (A, B) and two (C) independent experiments with six to eight mice per group, respectively. n.d., not detected; **p ≤ 0.01.
SUPPLEMENTAL FIGURE 3. Innate sources of IL-17A and IL-22 in IFN-γR−/− mice during *E. falciformis* infection. (A) MLN and (B) LPL frequencies of CD4− and Lin− cells, gated on total IL-17A+ or IL-22+ cells, in *E. falciformis*-infected IFN-γR−/− mice. (C) IL-17A and IL-22 coexpression by Lin− cells derived from LPL of *E. falciformis*-infected IFN-γR−/− mice. (D) Expression of c-kit and Sca-1 on Lin− cells (grey fill) expressing IL-17A or IL-22 and non-cytokine producing cells (dotted line) derived from LPL of *E. falciformis*-infected IFN-γR−/− mice. (E) Total cell numbers of Lin− IL-17A- or IL-22-producing cells in LPL of naïve and *E. falciformis*-infected Wt and IFN-γR−/− mice. Data shown are means ± SEM of four to six mice per group and representative of two independent experiments. *p ≤ 0.05, **p ≤ 0.01.

SUPPLEMENTAL FIGURE 4. Th17 cytokines compensate for the lack of IFN-γR signaling and drive host defense at the expense of increased inflammation during *E. falciformis* infection. Left panel: Infection of C57BL/6 wild type mice with the intestinal intracellular parasite *E. falciformis* leads to a dominant CD8+ T cell-derived, IFN-γ-driven response that is sufficient to mediate host protection. IFN-γ drives infiltration of Ly6C+ monocytes in the lamina propria. Right panel: In the absence of the IFN-γR the number of IL-17A- and IL-22-producing Th17 cells (and IFN-γ-producing Th1 cells) is dramatically increased in the draining lymph nodes and the lamina propria. Th17 responses, in particular IL-22, mediate host defense by preventing parasite maturation in the epithelial cell. *E. falciformis* antigen-specific IL-17A and IL-22 drives infiltration of Gr-1+ neutrophils into the intestinal tissue, which contributes to increased pathology.
**A**

Graph showing cytokine levels (log ng/ml) for IL-17A, IL-22, and IFN-γ. The bars are labeled with "WT" and "IFN-γ−R−/−." **B**

Bar charts showing the number of IL-17A+ T cells, IL-22+ T cells, and IFN-γ+ T cells for CD4+, CD8+, and CD48+ subtypes. **C**

Flow cytometry plots showing the expression of RORγt and T-bet in different subsets of T cells induced by IFN-γ, IL-17A, and IL-17A+.