Fasciola hepatica Tegumental Coat Impairs Mast Cells’ Ability To Drive Th1 Immune Responses

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J Immunol published online 15 February 2013
http://www.jimmunol.org/content/early/2013/02/15/jimmunol.1203011
Fasciola hepatica Tegumental Coat Impairs Mast Cells’ Ability To Drive Th1 Immune Responses

Krisztina V. Vukman,* Paul N. Adams,* Martin Metz,† Marcus Maurer,† and Sandra M. O’Neill*

The parasitic worm Fasciola hepatica induces strong Th2 and T-regulatory immune responses while simultaneously suppressing Th1-driven immune responses to bystander microbial infections. It also prevents the initiation of Th1-mediated autoimmune disorders in mice through the suppression of Th17 and Th1 immune responses, and this can be mimicked by parasite-derived molecules. We have isolated F. hepatica tegument coat Ag (FhTeg) and demonstrated its suppressive effect in vivo by directly targeting dendritic cells, impairing their ability to drive Th1 responses. Mast cells are critical in promoting Th1 protective immunity during bacterial infection and in driving Th1-mediated pathological conditions in autoimmune diseases. In this article, we show that FhTeg inhibits the ability of mast cells to drive the Th1 immune response by suppressing cytokine secretion (TNF-α, IL-6, IFN-γ, and IL-10) and ICAM1 expression in mast cells stimulated with LPS or heat-inactivated Bordetella pertussis Ag. These heat-inactivated B. pertussis Ag/LPS–stimulated mast cells fail to promote Th1 immune responses in CD4+ T cells when pretreated with FhTeg, and a role for ICAM1 in this process was demonstrated. FhTeg suppresses the activation of transcription factors in the TLR signaling pathway, which explains the decrease in cytokine production and cell surface marker expression. We demonstrated that FhTeg suppresses MAPK and NF-κB activation and enhances SOCS3 expression, which could explain its negative effect on the TLR pathways. We conclude that FhTeg targets innate immune cells, inhibiting their ability to drive Th1 immune responses. The Journal of Immunology, 2013, 190: 000–000.

Mast cells are associated with helminth infection but are mainly studied in the context of IgE-mediated responses and Th2 immunity. Mast cells express pathogen recognition receptors such as TLRs and, when activated with TLR ligands, are a potent source of Th1 proinflammatory mediators, such as TNF-α and IFN-γ. Mast cells promote Th1-driven host defense against bacterial and protozoan infections (1–4), and in the absence of mast cells these infections are significantly exacerbated (5, 6), demonstrating a critical role for mast cells. Mast cells are also associated with many Th1-mediated autoimmune disorders, such as multiple sclerosis and Crohn’s disease, in which TNF-α–secreting mast cells are important contributors to immune disorders (7–9).

Helminths and the products they release exert effects that impair Th1 immunity to bystander microbial infections, such as malaria and tuberculosis (10, 11), and this impairment of Th1 immunity significantly impacts populations in the developing world (12). However, they can prevent the initiation and perpetuation of many Th1 immune disorders, such as multiple sclerosis and Crohn’s disease (13, 14), in these populations. Helminth-driven regulatory networks, through the induction of regulatory cytokines TGF-β and IL-10, can suppress these proinflammatory Th1 immune responses (14, 15). Furthermore, helminths release molecules that directly impair the ability of innate immune cells to drive Th1/Th17 immune responses (10, 16). Understanding these mechanisms could lead to the development of novel anti-inflammatory therapeutic targets and expand our understanding of helminth–microbial interactions in human populations.

Fasciola hepatica, a parasitic flatworm also known as the common liver fluke, can suppress protective Th1-driven immunity against bystander infections such as tuberculosis and Bordetella pertussis infection (17–19). It can also attenuate experimental autoimmune encephalomyelitis (EAE) through the suppression of Th17 and Th1 immune responses (20).

Fasciola tegumental coat is shed from the parasite every 2–3 h and is in constant contact with host immune cells. Previously, we have isolated F. hepatica tegumental coat Ag (FhTeg) and examined its interactions with innate immune cells. Most of the isolated proteins from FhTeg (21–23) and the contents of the glyocalyx (24) are identified. More than 50 different proteins are present in the preparation, and the biological active component or components of FhTeg have yet to be identified. Currently, the focus is on elucidating FhTeg’s function and role in immunomodulation. We have demonstrated the Th1-suppressive effect of FhTeg in vivo by inhibiting the release of proinflammatory cytokines in the mouse model of septic shock (25). We also demonstrated that FhTeg alters the ability of dendritic cells to prime Th1 immune responses by rendering them hyporesponsive to TLR activation, suppressing the production of a select panel of inflammatory cytokines and costimulatory molecules. The sup-

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This work was supported by the Programme for Research in Third Level Institutions (PRTLI) Cycle 4—the PRTLI is cofunded through the European Regional Development Fund, part of the European Union Structural Funds Programme 2007–2013; and by European Cooperation in Science and Technology Action BM1007, “Mast Cells and Basophils—Targets for Innovative Therapies.”

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Abbreviations used in this article: BMMC, bone marrow–derived mast cell; BP, heat-inactivated Bordetella pertussis Ag; EAE, experimental autoimmune encephalomyelitis; FhTeg, Fasciola hepatica tegumental coat Ag; PCMC, peritoneum-derived cultured mast cell; qPCR, quantitative PCR; SOCS3, suppressor of cytokine signaling 3.

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pressive effect is not mediated through the common MAPKs found in the TLR pathway and is independent of MyD88 and TLR4.

We have shown that FhTeg suppresses the transcription factor NF-κB p65, which is involved in the transcription of many TH1-promoting factors (25). This study examines FhTeg’s interaction with mast cells to determine if its modulatory effect can impair mast cells’ ability to promote TH1 immunity, and the possible mechanism involved in this process will be discussed.

Materials and Methods

**Animals and Ages**

C57BL/6 mice, 6–8 wk old, were purchased from Charles River (Carrentrilla, Ireland). Mice were kept under specific pathogen-free conditions at the Bioresource Unit, Faculty of Health and Science, Dublin City University, Dublin, Ireland. All mice were maintained according to the guidelines of the Irish Department of Children and Health. Ethical approval for mouse experiments was obtained from the Dublin City University ethics committee and the Irish Department of Children and Health.

FhTeg was prepared as previously published (25). Endotoxin levels were determined using the PyroGene Endotoxin Detection System (Cambrex/Lonza, Walkersville, MD). FhTeg yielded levels similar to background levels and they were less than the lower limit of detection in this assay (<0.01 EU/ml). Heat-inactivated Bordetella pertussis (BP) was a kind gift from Prof. Bernie Mahon, National University of Ireland Maynooth, Maynooth, Ireland (26). All protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce/Fischer, Dublin, Ireland).

**Animal experiments**

C57BL/6 mice were i.p. injected with PBS or FhTeg (10 μg) 1 h prior to the injection with BP (5 μg) every third day for 10 d (total of four injections). Peritoneal cells were obtained from the mice 24 h after the last injection by injecting 10 ml PBS into the peritoneal cavity. Cell numbers and viability were monitored using trypan blue staining. Percentages of mast cells were determined by measuring cell surface expression of c-kit and FcεRI and by Kimura staining (27). Cells were stained for intracellular TNF-α expression immediately after peritoneal lavage.

**Isolation, maturation, and characterization of bone marrow– and peritoneum-derived cultured mast cells**

Bone marrow–derived mast cells (BMMCs) were generated from the femoral and tibial bone marrow cells of C57BL/6 mice and maintained in complete IMDM in the presence of 10% heat-inactivated FCS, 100 U/ml penicillin/streptomycin, and 50% WEHI-3-conditioned IMDM medium (ATCC TIB-68) as a source of the murine growth factor IL-3 for 4 wk (28). Peritoneal cells were obtained from C57BL/6 mice after i.p. injection of 10 ml sterile PBS and then cultured in RPMI 1640 medium supplemented with 10% FCS and 100 U/ml penicillin/streptomycin, 2 mM l-glutamine, 10 ng/ml recombinant mouse IL-3 (Calbiochem/Merck, Darmstadt, Germany), and 30 ng/ml recombinant mouse stem cell factor at 37˚C, as previously described (29). At 48 h later, nonadherent cells were removed and replaced by fresh culture medium for an additional 7 d. Of the total cells, >95% were identified as mast cells on the basis of c-kit and FcεRI cell surface expression or Kimura staining. Cell number and viability were monitored using trypan blue staining. To test functionality, β2-hexosaminidase release from mast cells was measured as previously described (29).

BMMCs and PCMCs were cultured with LPS (100 ng/ml; Alexis, San Diego, CA); BP (100 bacteria per cell); PMA (25 ng/ml), or A23187 (1 μg/ml) 2.5 h prior to stimulation with LPS or BP. Cells were harvested and washed after 5, 15, 30, and 60 min before resuspending in TRISure (Bionik, London, U.K.), and RNA was extracted according to the manufacturer’s instructions. To eliminate genomic DNA contamination, RNA samples were resuspended in RNase-free water (Ambion, Paisley, U.K.); cDNA was synthesized using a reverse transcriptase kit (Promega, Madison, WI) according to the manufacturer’s protocol.

The quantitative PCR (qPCR) transcription analysis was carried out on a real-time thermal cycler ABI7000F0H (Applied Biosystems, Paisley, U.K.), using Maxima SYBR Green Master Mix according to the manufacturer’s protocol (Fermentas/Fischer, Dublin, Ireland) in triplicate on 96-well MicroAmp Optical Plates (Applied Biosystems). Table 1 was obtained using gene primers sequences were used: β-actin (NM_007393.3), GAPDH (NM_008084.2), and Gusb (β-glucuronidase, NM_010368.1). Samples were maintained for 10 min at 95˚C as the initial step, then 15 s at 95˚C and 60 s at 60˚C, through 40 cycles. After amplification, melting curves were recorded (from 90 to 55˚C) to check product size and homogeneity. Pfaffl’s method was used to calculate relative changes in gene transcription (30). The PCR efficiency was determined using eight-step dilution series (0.3–20 ng cDNA). Primers are listed in Table 1.

**Protein extraction and Western blot analysis**

BMMCs and PCMCs were stimulated with or without FhTeg (10 μg/ml) 2.5 h prior to stimulation with LPS (100 ng/ml) or BP (100 bacteria per cell) for 5, 15, 30, or 60 min. Total protein was extracted from cell lysisates, using radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitor cocktails. Protein concentrations (10–40 μg) and protein size markers (See Blue Plus 2; Invitrogen) were separated by SDS-PAGE and visualized with a chemiluminescent HRP substrate (Millipore), exposed to X-ray film, and developed as per the manufacturer’s protocol (Fermentas/Fischer, Dublin, Ireland) in triplicate on 96-well MicroAmp Optical Plates (Applied Biosystems). Table 1 was obtained using gene primers. Protein bands were visualized and analyzed using ImageJ analysis software (imagej.nih.gov). Levels of phospho-ERK, phospho-p38, phospho-JNK, and phospho–NF-κB were measured by Western blot analysis. Western blots were performed with antibodies to phospho–ERK (phospho-ERK; Cell Signaling Technology, Beverly, MA), anti-total-ERK, anti-phospho-p38, anti-total-p38, anti-phospho-JNK, anti-total-JNK, anti-NF-κB p65, anti-GR1, and anti-β-actin primary Abs and peroxide-conjugated anti-rabbit IgG (Sigma-Aldrich, Dublin, Ireland) secondary Ab. Proteins were visualized with a chemiluminescent HRP substrate (Millipore), exposed to X-ray film, and processed using an FPM 100A processor (Fuji Film). Protein bands were quantified using ImageJ analysis software (imagej.nih.gov). Levels of phospho-ERK, phospho-p38, phospho-JNK, and phospho–NF-κB p65 were normalized to total-ERK, total-p38, total-JNK, and β-actin and expressed in arbitrary units as percentage increases over the medium control levels.

**Coculture studies and cytokine measurements**

CD4+ T cells were isolated using the MACS CD4+ T Cell Isolation Kit (Miltenyi Biotec, Surrey, U.K.) and were used if the purity was ≥95%, as determined by flow cytometry (FITC-conjugated CD4, clone: L3T4; BD Biosciences). BMMCs and peritoneum-derived cultured mast cells (PCMCs) were cultured in the presence of FhTeg (10 μg/ml) 2.5 h before stimulation with LPS (100 ng/ml) or BP (100 bacteria per cell) for 24 h. Washed BMMCs and PCMCs or supernatant was added to CD4+ cells (1:1 cell/cell or supernatant/ex vivo media ratio) in plates coated with anti-CD3 (1 μg/ml; BD Biosciences) for 72 h, and supernatants were tested for IFN-γ, IL-4, IL-5, and IL-10 by commercial ELISA (BD Biosciences). In some experiments, anti-ICAM1 blocking Ab (clone: 7C11/1.4; Abcam, Cambridge, U.K.) was added to mast cells for 30 min, and then mast cells were washed prior to coculturing with CD4+ cells.

**RNA extraction and RT-PCR**

BMMCs were stimulated with or without FhTeg (10 μg/ml) 2.5 h prior to stimulation with BP, LPS, or PBS. Cells were harvested and washed after 5, 15, 30, and 60 min before resuspending in TRISure (Bionik, London, U.K.), and RNA was extracted according to the manufacturer’s instructions. To eliminate genomic DNA contamination, RNA samples were resuspended with RNase-free water (Ambion, Paisley, U.K.). cDNA was synthesized using a reverse transcriptase kit (Promega, Madison, WI) according to the manufacturer’s protocol.

The quantitative PCR (qPCR) transcription analysis was carried out on a real-time thermal cycler ABI7000F0H (Applied Biosystems, Paisley, U.K.), using Maxima SYBR Green Master Mix according to the manufacturer’s protocol (Fermentas/Fischer, Dublin, Ireland) in triplicate on 96-well MicroAmp Optical Plates (Applied Biosystems). Table 1 was obtained using gene primers sequences were used: β-actin (NM_007393.3), GAPDH (NM_008084.2), and Gusb (β-glucuronidase, NM_010368.1). Samples were maintained for 10 min at 95˚C as the initial step, then 15 s at 95˚C and 60 s at 60˚C, through 40 cycles. After amplification, melting curves were recorded (from 90 to 55˚C) to check product size and homogeneity. Pfaffl’s method was used to calculate relative changes in gene transcription (30). The PCR efficiency was determined using eight-step dilution series (0.3–20 ng cDNA). Primers are listed in Table 1.

**Table 1. List of primers for qPCR transcription analysis**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ → 3’)</th>
<th>Product Size (bp)</th>
<th>Tm (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>ACTGCTCCCCAATCCCACTCTCCAA</td>
<td>266</td>
<td>61.83</td>
</tr>
<tr>
<td>GAPDH</td>
<td>CTCTCTACCTACCTCTCTCTCAC</td>
<td>59.25</td>
<td></td>
</tr>
<tr>
<td>Gusb</td>
<td>AGAAATGAAAGTGTGAAACCGAG</td>
<td>112</td>
<td>56.92</td>
</tr>
<tr>
<td>SOC3S</td>
<td>GATGCTCTCTTCTGTCTTCTATT</td>
<td>60.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GTGGTGGTGCTTCCTTGTTG</td>
<td>60.79</td>
<td></td>
</tr>
</tbody>
</table>

Tm, Temperature.
Statistics
All data were analyzed for normality prior to statistical testing by Origin 6.1 (OriginLab) software. When multiple group comparisons were made, data were analyzed using one-way ANOVA. For comparisons between two groups, the Student t test was used. In all tests, p < 0.05 was deemed significant (*p ≤ 0.05, **p ≤ 0.01).

Results
FhTeg inhibits TNF-α expression in mast cells in vivo
F. hepatica suppresses protective Th1-driven immunity against B. pertussis (18), and because mast cells have an important role in protection against bacterial infections, we wanted to determine if FhTeg can inhibit the production of proinflammatory cytokines by mast cells in vivo. In this study, we first demonstrated that BP, when injected into the peritoneal cavity of C57BL/6 mice, induced high numbers of TNF-α-expressing mast cells (Fig. 1). When these mice were coinjected with FhTeg, mast cells expressed significantly less TNF-α than those in BP-injected mice, confirming that FhTeg can inhibit the production of proinflammatory cytokines by mast cells in vivo (Fig. 1).

FhTeg suppresses cytokine secretion from LPS- or BP-stimulated mast cells
To confirm our in vivo findings, we stimulated BMMCs and PCMCs in vitro with BP or LPS as a positive control in the presence or absence of FhTeg, and cytokine secretion was measured in supernatant after 24 h. Both BMMCs (IL-6 and TNF-α) and PCMCs (IL-6, TNF-α, IL-10, and IFN-γ) secreted significant levels of cytokines; however, in the presence of FhTeg, cytokine secretion was significantly suppressed (Fig. 2; p ≤ 0.05 to p ≤ 0.001). We repeated these experiments with BMMCs from STAT6−/− mice, and we demonstrated that FhTeg had the same suppressive effect in STAT6−/− mast cells (data not shown). However, STAT6−/− mast cells secreted more Th1 cytokines after BP stimulation.

CD4+ T cells cocultured with FhTeg-treated mast cells secrete less IL-10, IFN-γ, and IL-5
Mast cells, when cocultured with naive CD4+ cells in the presence of anti-CD3, can promote Th1 immune responses, and this can be enhanced when mast cells are pretreated with bacterial Ags or TLR ligands (31–33). BMMCs and PCMCs were stimulated with BP and LPS in the presence or absence of FhTeg for 24 h to determine if mast cell ability to promote cytokine production from naive T cells can be inhibited. After 24 h, supernatants were removed and cells were washed. Supernatant was added to CD4+ T cells, or CD4+ T cells were cocultured with washed mast cells. When cocultured with BP- and LPS-stimulated BMMCs or PCMCs, or when cultured with supernatant from these cells, CD4+ T cells secreted significant levels of IFN-γ, IL-10, and IL-5 (Fig. 3), but no IL-4 (data not shown). Significantly lower levels of IFN-γ, IL-10, and IL-5 cytokines were obtained when T cells were cocultured with BP- and LPS-stimulated BMMCs or PCMCs in the presence of FhTeg, or when the T cells were cultured with supernatant from these cells (Fig. 3). BMMCs, PCMCs, and T cells alone were used as negative controls; and levels of IFN-γ, IL-10, IL-4, or IL-5 were not significantly higher than background levels and were significantly less than levels in cocultured samples when measured after 72 h.

ICAM1 expression on mast cells is important in mast cell–T cell communication, and its expression is also inhibited by FhTeg
OX40L, CD40L, CD28, and ICAM1 are thought to have a critical role in mast cell–T cell communication (33–35). BMMCs and PCMCs express low levels of OX40L, CD40L, and CD28 (data not shown), and these were not significantly enhanced in the presence of LPS and BP. In contrast, ICAM1 was highly expressed on these cells, and this was enhanced following stimulation with LPS and BP (Fig. 4). FhTeg did not enhance OX40L, CD40L, CD28, and ICAM1 on mast cells (data not shown). Because ICAM1 was highly expressed, we stimulated mast cells with BP and LPS in the presence of FhTeg and demonstrated that FhTeg significantly inhibited ICAM1 expression on BMMCs (data not shown) and PCMCs (Fig. 4; p ≤ 0.05). We confirmed that ICAM1 was important in mast cell–CD4+ T cell communication by adding anti-ICAM1 blocking Ab to activated mast cells a half hour prior to washing and coculturing with CD4+ T cells. Like FhTeg, it caused a significant decrease in IFN-γ, IL-10, and IL-5 secretion (Fig. 5A–C).

The FhTeg suppressive effect is not specific to NF-κB p65 expression, as it inhibits MAPK activation in mast cells
We previously showed that FhTeg suppresses the activation of NF-κB in LPS-stimulated dendritic cells (25), and this was examined in mast cells. It was first confirmed that BP can induce NF-κB p65 (at 15 and 30 min) expression in BMMCs (Fig. 6A–C). FhTeg suppressed the activation of NF-κB in LPS- and BP-stimulated mast cells (Fig. 6A–C), and this was confirmed in PCMCs at 15 and 30 min (Fig. 6D–F).

To test whether this effect was specific to NF-κB, the phosphorylation of ERK, JNK, and p38 was measured. First it was confirmed that BP can induce phospho-ERK, -JNK, and -p38 expression in mast cells, and in this study it was demonstrated that BP can significantly enhance phospho-ERK, but not phospho-JNK and -p38. We also showed that FhTeg suppressed the activation of phospho-ERK in LPS- and BP-stimulated mast cells (at 5, 30, and 60 min), and these results were confirmed in PCMCs at 15 and 30 min (Fig. 6D–F).

SOCS3 transcription and expression is up-regulated by FhTeg in mast cells
As both the NF-κB and MAPK pathways are suppressed by FhTeg, we hypothesize that this Ag targets signaling molecules further up the TLR4 signaling pathway or FhTeg may induce negative regulators of the TLR pathway. First, the expression of suppressor of cytokine signaling 3 (SOCS3), a known inhibitor of the TLR4 signaling pathway, was measured by qPCR (36) (Table I). The SOCS3 RNA level was significantly higher at 5 and 15
min after LPS stimulation and at 5 min after BP stimulation in FhTeg-treated BMMCs than in control cells (Fig. 7A, 7B). As SOCS3 was upregulated by FhTeg in early time points and it is known to be one of the most important inhibitors of the TLR4 signaling pathway, these results were confirmed by Western blot analysis. The FhTeg/LPS treatment experiment was repeated, and proteins at 5, 15, 30, and 60 min were extracted. As with the qPCR results, in the early time points SOCS3 was upregulated in FhTeg-treated samples, followed by LPS- and BP-induced SOCS3 expression in latter time points (Fig. 7C).

Discussion

This study sheds light on the anti-inflammatory effects of helminth Ags on the Th1 proinflammatory properties of mast cells. FhTeg impairs mast cells’ ability to drive Th1 immune responses by inhibiting the release of key mediators, such as TNF-α, IL-6, IFN-γ, and IL-10. It also suppresses the expression of ICAM1, a cell surface molecule that has an important role in mast cell–T cell crosstalk (35). Although studies have shown the importance of ICAM1 expression on T cells in mast cell–T cell communication, here we demonstrate that ICAM1 expression on mast cells is also

![FIGURE 2. FhTeg suppresses cytokine expression in LPS- or BP-stimulated BMMCs and PCMCs. Mast cells were stimulated with and without FhTeg (10 μg/ml) for 2.5 h prior to stimulation with vehicle (PBS), LPS (100 ng/ml), BP (100 bacteria per cell), or PMA (20 ng/ml) for 24 h. With a commercial ELISA, TNF-α (A) and IL-6 (B) levels were measured in the supernatants of BMMCs, whereas TNF-α (C), IL-6 (D), IFN-γ (E), and IL-10 (F) cytokine expression was measured in supernatants of PCMCs. Data are presented as the mean ± SEM of three independent experiments. *p ≤ 0.05, **p ≤ 0.01 compared with vehicle (PBS) group, *p ≤ 0.05, **p ≤ 0.01 compared with BP and LPS group.](http://www.jimmunol.org/)

![FIGURE 3. FhTeg suppresses the activation of T cells by LPS- or BP-stimulated mast cells. BMMCs and PCMCs were incubated with and without FhTeg (10 μg/ml) for 2.5 h following stimulation with vehicle (PBS), LPS (100 ng/ml), or BP (100 bacteria per cell) for 24 h. Anti-CD3-stimulated naive CD4+ cells were cocultured with the washed cells (A–F) (1:1) or with the supernatants (G–L) for 72 h. Supernatants were collected, and IFN-γ (A, B, G, H), IL-10 (C, D, I, J), and IL-5 (E, F, K, L) expression was measured using a commercial ELISA. Data are presented as the mean ± SEM of three independent experiments. *p ≤ 0.05, **p ≤ 0.01 compared with BP and LPS group.](http://www.jimmunol.org/)
Supernatants were tested for IFN-γ with BP and LPS group and cocultured with anti-CD3–stimulated naive CD4+ T cells for 72 h. Ab (1 ng/ml) was added to mast cells 30 min before cells were washed and without FhTeg (100 ng/ml) for 2.5 h prior to stimulation with vehicle (PBS), LPS (100 ng/ml), or BP (100 bacteria per cell) for 24 h. ICAM1 expression was measured by flow cytometry. Data are presented as the mean ± SEM of three independent experiments. *p < 0.05 compared with BP and LPS group.

![Figure 4](image-url)  
**FIGURE 4.** FhTeg suppresses the upregulation of ICAM1 expression on LPS- or BP- stimulated mast cells. PCMCs were stimulated with or without FhTeg (10 µg/ml) for 2.5 h prior to stimulation with vehicle (PBS), LPS (100 ng/ml), or BP (100 bacteria per cell) for 24 h. ICAM1 expression was measured by flow cytometry. Data are presented as the mean ± SEM of three independent experiments. *p < 0.05 compared with BP and LPS group.

Critical in this process. Our results suggest that in T cell–mast cell communication both the mast cell–derived cytokines and cell-to-cell contact are crucial to induce sufficient immune responses, as we showed that the inhibition of cytokine secretion and cell surface molecule upregulation by FhTeg both impaired the mast cells’ ability to prime T cells. Our study supports previous findings demonstrating that FhTeg can suppress Th1 immunity in vivo by directly rendering innate immune cells, such as dendritic cells, hyporesponsive to TLR activation (25), a characteristic of helminth-activated dendritic cells (37). The suppression of Th1 immune response is critical for *F. hepatica* to survive within its host, as immunity to *Fasciola* infection requires strong Th1 inflammatory responses (38). We have shown previously that IL-4−/− mice, which are predisposed toward a Th1 immune response, are less susceptible to *F. hepatica* infection, with reduced liver damage, compared with IFN-γR−/− mice, which elicited strong Th2 responses and are highly susceptible to infection (39). Vaccine efficacy using *F. hepatica* excretory–secretory molecules or using recombinant *F. hepatica* secretory enzymes is associated with enhanced lymphocyte proliferation and IFN-γ expression (10, 40).

We have previously shown that mice coinfected with *Fasciola* exhibit delayed clearance of *B. pertussis* from their lungs, which is associated with a decrease in Ag-specific Th1 immune responses (17). This finding was also observed in mice coinfected with *B. pertussis* and *Fasciola* Ags (41). In this article, we demonstrated that FhTeg injected into mice suppresses *B. pertussis*–induced TNF-α expression in mast cells, suggesting that mast cells may be targeted by helminth Ags during bystander infections. This idea could explain the failure of *F. hepatica* coinfected mice to clear *B. pertussis* infection because mast cells secrete proinflammatory mediators like TNF-α and IFN-γ that mediate host defense against *B. pertussis* (3, 4). Similarly, mast cells have a critical role during EAE in mice, as mast cell knockout mice are protected from developing this disease (42); this could also explain how *Fasciola* could attenuate EAE in mice (20). Further studies are required to determine the role of mast cells in this process, which would shed further light on the impact of helminths on communicable and noncommunicable diseases.

FhTeg suppressed LPS-induced NF-κB and MAPK pathway (ERK) activation in mast cells. NF-κB and MAPKs are important signaling molecules that lead to the expression of ICAM1 (43) and the secretion of proinflammatory cytokines. Our findings correlate with other studies on dendritic cells, as *Schistosoma mansoni* egg Ags reduce LPS-stimulated phosphorylation of p38, JNK, and ERK in murine dendritic cells (44). *S. haematobium* and *Ascaris lumbricoides* infections were shown to suppress LPS-induced p38 phosphorylation, but not ERK activation, in dendritic cells (45, 46). Studies demonstrate that NF-κB−/− mice infected with *Trichuris muris* show increased susceptibility to infection, with impaired Th2/Treg immune responses and the phosphorylation of NF-κB and ERK supposed to be crucial in helminth-induced Th2 immune responses (47, 48). The fact that FhTeg does not induce, but rather suppresses, the activation of these proteins supports our unpublished finding that FhTeg does not drive Th2 immunity; rather, it has a role in the suppression of Th1-type responses.

FhTeg induces negative regulators of the TLR pathway because FhTeg enhanced expression of SOCS3 in mast cells. SOCS3 is a member of a family of molecules that are one of the main inhibitory groups playing an important role in autoregulation of pathogen-induced inflammatory responses (49). Previous studies showed increased SOCS3 RNA levels in blood cells from children with helminth (*Ascaris spp.*, *Trichuris spp.*, or *S. haematobium*) infection. They suggested that the higher level of SOCS3 might be responsible for the increased severity of bystander *Plasmodium* infection, which requires Th1 immune responses (50). It is likely that *F. hepatica* has the same mechanism to suppress Th1 immune responses during bystander infection, but further studies are required.

Mast cells were shown to contribute to the immune response against *Strongyloides ratti*, *Trichinella spiralis*, and *Nippostrongylus*...
*brasiliensis* in rat and mouse models (51–53). The fecundity of *Heligmosomoides polygyrus* was higher in mast cell–deficient mice than in wild-type controls (54). However, these studies indicate that the mast cell phenotype is important in driving Th2 immunity that is essential for clearing gut parasites (55). We previously showed that mast cell number increases markedly in the peritoneal cavity and liver of *F. hepatica*–infected and FhTeg-injected mice and that the interaction of FhTeg with mast cells does not promote Th2 immune response or suppress IgE-mediated mast cell activation (56). Given that Th1 immune responses are important in immune protection against *Fasciola* infection, it is possible that in the early stages of infection mast cells are recruited to contribute to the overall Th1 milieu and this is inhibited by the release of FhTeg by *Fasciola* into the microenvironment. This idea is supported by FhTeg’s interaction with mast cells and dendritic cells, as it directly inhibits their ability to drive Th1 immune responses.

In summary, FhTeg impairs mast cells’ ability to drive Th1 immune responses, which sheds further light on how helminths leave populations susceptible to bystander infections while simultaneously protecting them against Th1-mediated autoimmune disorders. Further studies will help us to determine how FhTeg enhances SOCS3 expression, which may lead to potential identification of novel means by which helminths modulate innate immune cells. Because these cells are involved in Th1 immune disorders like autoimmune diseases, inflammatory bowel disease, or Crohn’s disease, in which TNF-α secretion from these cells is critical to pathological characteristics (8, 9), in the future this finding might lead to the development of a therapeutic inhibitor for pathogenic cell phenotypes.

**Acknowledgments**

We thank Dr. Tamás Visnovitz (National University of Ireland Maynooth, Maynooth, Ireland) and Keith Rochfort (Dublin City University, Dublin, Ireland) for helpful advice; Dr. Phil Cummins (Dublin City University) for providing facilities during qPCR experiments; and the staff of Bioresource Unit, at Dublin City University, for excellent technical assistance.

**Disclosures**

The authors have no financial conflicts of interest.

**FIGURE 6.** FhTeg suppresses phospho-ERK and NF-κB p65 expression in BP- and LPS-stimulated mast cells. BMMCs and PCMCs were stimulated with and without FhTeg (10 μg/ml) for 2.5 h prior to stimulation with PBS, LPS (100 ng/ml), or BP (100 bacteria per cell) for 5, 15, 30, and 60 min. Protein samples were analyzed by Western blot for phosphorylated-ERK (p-ERK), total-ERK (t-ERK), NF-κB p65, and β-actin expression (A, D). Values of p-ERK and NF-κB p65 were normalized to t-ERK and β-actin and are expressed in arbitrary units as percentage increases over PBS control value (B, C, E, F). Densitometry data are presented as the mean ± SEM of three independent experiments. *p ≤ 0.05, **p ≤ 0.01 compared with control group.

**FIGURE 7.** SOCS3 transcription and expression are upregulated by FhTeg in mast cells. BMMCs were stimulated with and without FhTeg (10 μg/ml) for 2.5 h prior to stimulation with PBS (P), LPS (L; 100 ng/ml), or BP (B; 100 bacteria per cell) for 5, 15, 30, and 60 min. Total RNA was extracted, and after reverse transcription cDNA was analyzed with qPCR for SOCS3 (A, B). RNA expression was normalized to GAPDH, β-actin, and Gusb control genes and was shown relative to PBS control, using Pfaffl’s method. Protein samples were analyzed by Western blot for SOCS3 and β-actin expression (C). Values of proteins were normalized to β-actin and are expressed in arbitrary units as percentage increases over PBS control value. Data are presented as the mean ± SEM of three independent experiments. *p ≤ 0.05, **p ≤ 0.01 compared with control group.
References


