RESEARCH ARTICLE

Blimp-1-Dependent IL-10 Production by Tr1 Cells Regulates TNF-Mediated Tissue Pathology

Marcela Montes de Oca1,2, Rajiv Kumar1,3, Fabian de Labastida Rivera1, Fiona H Amante1, Meru Sheel1, Rebecca J. Faleiro1,4, Patrick T. Bunn1,5, Shannon E. Best1, Lynette Beattie1, Susanna S. Ng1,6, Chelsea L. Edwards1,2, Werner Muller7, Erika Cretney8,9, Stephen L. Nutt8,9, Mark J. Smyth1, Ashrafal Haque1, Geoffrey R. Hill1, Shyam Sundar10, Axel Kallies8,9, Christian R. Engwerda1*

1 QIMR Berghofer Medical Research Institute, Brisbane, Australia, 2 University of Queensland, School of Medicine, Brisbane, Australia, 3 Netaji Subhas Institute of Technology, New Delhi, India, 4 Queensland University of Technology, Institute of Health and Biomedical Innovation, Brisbane, Australia, 5 Griffith University, Institute of Glycomics, Gold Coast, Australia, 6 Griffith University, School of Natural Sciences, Nathan, Australia, 7 University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom, 8 Walter and Eliza Hall Medical Research Institute, Division of Molecular Immunology, Melbourne, Australia, 9 The University of Melbourne, Department of Medical Biology, Melbourne, Australia, 10 Banaras Hindu University, Institute of Medical Sciences, Varanasi, Uttar Pradesh, India

Abstract

Tumor necrosis factor (TNF) is critical for controlling many intracellular infections, but can also contribute to inflammation. It can promote the destruction of important cell populations and trigger dramatic tissue remodeling following establishment of chronic disease. Therefore, a better understanding of TNF regulation is needed to allow pathogen control without causing or exacerbating disease. IL-10 is an important regulatory cytokine with broad activities, including the suppression of inflammation. IL-10 is produced by different immune cells; however, its regulation and function appears to be cell-specific and context-dependent. Recently, IL-10 produced by Th1 (Tr1) cells was shown to protect host tissues from inflammation induced following infection. Here, we identify a novel pathway of TNF regulation by IL-10 from Tr1 cells during parasitic infection. We report elevated Blimp-1 mRNA levels in CD4+ T cells from visceral leishmaniasis (VL) patients, and demonstrate IL-12 was essential for Blimp-1 expression and Tr1 cell development in experimental VL. Critically, we show Blimp-1-dependent IL-10 production by Tr1 cells prevents tissue damage caused by IFN-α-dependent TNF production. Therefore, we identify Blimp-1-dependent IL-10 produced by Tr1 cells as a key regulator of TNF-mediated pathology and identify Tr1 cells as potential therapeutic tools to control inflammation.

* chrisE@qimr.edu.au
Author Summary

Many parasitic diseases are associated with the generation of potent inflammatory responses. These are often needed to control infection, but can also cause tissue damage if not appropriately regulated. IL-10 has emerged as an important immune regulator that protects tissues by dampening inflammation. Recently, some T cells that initially produce inflammatory cytokines have been found to start producing IL-10 as a mechanism of auto-regulation. We identified an important transcriptional regulator called B lymphocyte-induced maturation protein 1 (Blimp-1), which promotes IL-10 production by IFNγ-producing CD4+ T (Tr1) cells during malaria and visceral leishmaniasis, two important diseases caused by protozoan parasites. We found that Tr1 cell-derived IL-10 suppressed anti-parasitic immunity, but played a critical role in preventing tissue damage caused by the potent pro-inflammatory cytokine TNF. Specifically, IL-10 protected macrophages from TNF-mediated destruction, and this enabled lymphocytes to continue to migrate to regions in the spleen where T and B cell responses are generated. These findings allow us to better understand how parasites persist in a host, but also identify new opportunities to control inflammation to prevent disease.

Introduction

TNF is a key pro-inflammatory cytokine required to control intracellular pathogens and kill tumours [1]. However, excessive TNF production can cause diseases such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, ankylosing spondylitis, graft-versus-host disease and sepsis [2,3]. As such, TNF is a major target for the prevention of inflammatory diseases, and inhibitors of TNF activity are widely used in the clinic [3,4]. An important drawback to this approach is that it can increase susceptibility to infection, especially intracellular pathogens [5,6]. Therefore, a better understanding of how TNF is regulated during inflammation is needed to identify more selective ways to control disease while minimizing risk of infection.

CD4+ T cells play critical roles in coordinating immune responses by helping B cells produce high affinity antibodies, CD8+ T cells to kill infected and transformed cells and innate immune cells to recognize and control pathogens and tumour cells [7,8]. Many diseases caused by protozoan parasites require the generation of IFNγ- and TNF-producing CD4+ T (Th1) cells for the activation of macrophages and dendritic cells to kill captured or resident pathogens [9,10]. However, these potent pro-inflammatory cytokines, along with other T cell-derived cytokines such as IL-17, can also damage tissues, and as such, CD4+ T cell responses need to be tightly regulated so they themselves do not cause disease [11].

IL-10 is a major regulatory cytokine, and its secretion by conventional CD4+ T cells can suppress inflammation by directly inhibiting T cell functions, as well as upstream activities initiated by antigen presenting cells (APC’s) [12]. Initially, IL-10 production was identified in Th2 cells [13], but has since been described in Th1 [14–16], FoxP3-expressing regulatory T (Treg) [17,18] and IL-17-producing CD4+ T (Th17) [19] cell populations. Thus, CD4+ T cell-derived IL-10 production is emerging as an important mechanism to prevent immune pathology. In mice infected with protozoan parasites, Th1 cells are an important source of IL-10 that can promote parasite survival, but also limit pathology [20–28]. These IL-10-producing Th1 (Tr1) cells have also been identified in humans with visceral leishmaniasis (VL) caused by Leishmania donovani [29] and African children with Plasmodium falciparum malaria [30–32]. Tr1 cells are increasingly recognized as a critical regulatory CD4+ T cell subset that prevent immune pathology during disease and protect tissue from damage caused by
excessive inflammation [12,33–35]. Despite these protective functions, Tr1 cells may also promote the establishment of infection [34] and suppress Th1-mediated, tumour-specific immunity [36]. However, it is not clear how much of this activity can be attributed to Tr1 cells or other IL-10-producing cell types. Therefore, a better understanding of IL-10 regulation by different cell types is required for the development of new therapeutic approaches targeting this cytokine.

Lymphoid tissue remodelling occurs in many chronic inflammatory settings associated with infectious, autoimmune and metabolic diseases [37–39]. This includes parasitic diseases such as malaria and VL that are associated with pronounced splenomegaly and disruption of lymphoid follicles [40,41]. In experimental models, this is accompanied by extensive vascular remodelling, white pulp atrophy and increased numbers of tissue macrophages [42–46], features also reported in human [47,48] and canine [49] VL. This remodelling results in dramatic changes to leukocyte movements in the spleen, and despite identifying excessive TNF production as a major contributor to these alterations [50,51], the immunoregulatory networks that fail are unknown.

Here, we identify a novel pathway of IL-10-dependent control of tissue pathology during parasitic infection. We show that IL-10 produced by Tr1 cells protects against IFNγ-dependent, TNF-mediated tissue damage, but limited the control of parasites that cause malaria and VL. This pathway is critically dependent on the transcriptional regulator B lymphocyte-induced maturation protein 1 (Blimp-1), which promotes IL-10 production by Tr1 cells. These findings provide new insights into the regulation and function of Tr1-derived IL-10, and thus reveal new opportunities to harness the therapeutic potential of these cells to protect against TNF-mediated diseases.

Results

Blimp-1 is required for CD4+ T cell IL-10 production in experimental malaria

The transcriptional regulator Blimp-1 (encoded by the Prdm1 gene) has recently been implicated in the generation of IL-10-producing Tr1 cells [52,53]. To explore the relationship between Blimp-1 and IL-10 production in T cells during protozoan infections, we made use of Prdm1fl/fl x Lck-Cre C57BL/6 (Prdm1ΔT) mice [54]. Strikingly, mice lacking Blimp-1 expression in T cells controlled non-lethal, P. chabaudi AS growth more efficiently than Cre-negative (Prdm1fl/fl) litter mate controls (Fig 1A). This corresponded with an increased frequency and number of activated CD4+ T cells and Th1 cells, but severely impaired development of Tr1 cells in the spleen (Fig 1B–1E). A similar, but much smaller effect (50 fold less) was also seen in CD8+ T cells (S1 Fig). The pattern of immune response observed in control Prdm1fl/fl mice was similar to what we (S2 Fig) and others [23] observe in wild type C57BL/6 mice, suggesting the presence of the flox transgene is having minimal influence over immune responses. In addition, the changes described above in mice lacking Blimp-1 expression in T cells were observed as early as 4 days p.i., and clearly evident at day 7 p.i. (S3 Fig). When mice lacking Blimp-1 expression in T cells were infected with lethal P. berghei ANKA, they had reduced parasite burdens (S4A Fig), but despite delayed onset of severe disease and a small survival advantage, all mice ultimately succumbed with severe neurological symptoms (S4B Fig). Again, the reduced parasite burden in mice lacking Blimp-1 expression in T cells was associated with an increased frequency and number of Th1 cells, but impaired development of Tr1 cells (S4C and S4D Fig). Thus, Blimp-1 expression in T cells enhanced parasite growth and was critical for the generation of IL-10-producing Tr1 cells during experimental malaria.
Elevated Blimp-1 expression and IL-10 production by CD4+ T cells is promoted by IL-12

We next examined Blimp-1 expression in CD4+ T cells using transgenic Blimp-1/GFP reporter mice [55] infected with *P. chabaudi* AS. Blimp-1 expression in CD4+ T cells was highest in IL-10-producing cells, lowest in TNF-producing cells, while those producing IFNγ expressed intermediate levels of Blimp-1 (Fig 2A and 2B). A similar pattern of association between CD4+ T cell cytokine production and Blimp-1 expression was found in mice with experimental VL caused by infection with the human protozoan parasite *L. donovani* (Fig 2C). Interestingly, there was no difference in Blimp-1 expression between CD4+ T cells expressing IL-10 alone and Tr1 cells in both infections (Fig 2B and 2C). Consistent with the finding that IL-12 is an important driver of Blimp-1-dependent Tr1 cell differentiation in autoimmunity [52], we found that Blimp-1 and IL-10 expression by IFNγ-producing CD4+ T cells from *L. donovani*-infected mice required IL-12 (Fig 2D and 2E). This reliance on IL-12 was most apparent for CD4+ T cells because although IL-12 blockade caused a small, but significant, reduction in IL-
and IFNγ double-producing CD8+ T cell frequency, this was not accompanied by a significant reduction in Blimp-1 levels. In addition, we found no significant reduction in IFNγ-producing CD8+ T cells, although we did measure a small, but significant reduction in Blimp-1 levels (Fig 2E). Importantly, we found increased accumulation of PRDM1 mRNA in CD4+ T cells isolated from the blood of VL patients, compared with the equivalent cell population in the same individuals after completion of drug treatment (Fig 3A). This was associated with elevated IL-10 mRNA levels in the same cells (Fig 3B), as well as increased plasma IL-10 (Fig 3C), as previously reported [29,56].

Fig 2. Blimp-1 controls CD4+ T cell derived IL-10 production. (A) Prdm1GFP/+ (black-filled histograms) and WT (grey-filled histograms) mice were infected with PcAS. Blimp-1/GFP expression measured on CD4+ T cells producing IL-10, IFNγ and TNF at day 15 p.i.. (B) Kinetics of Blimp-1/GFP expression measured in various cytokine producing CD4+ T cells, as indicated, beginning at day 5 p.i.. (C) Prdm1GFP/+ and WT mice were infected with L. donovani and the kinetics of Blimp-1 expression measured in the spleen and liver. (D) FACS plots showing gated CD4+ T cell IL-10 and IFNγ production from Prdm1GFP/+ mice treated with 500 μg of polyclonal rat IgG (black bars in (E)) or anti-IL-12 (C17.8; white bars in (E)), as indicated, prior to infection and every 3 days following infection until day 14 p.i.. (E) The frequencies of IL-10 and IFNγ-producing CD4+ and CD8+ T cells, as well as Blimp-1 expressing CD4+ and CD8+ T cells, were determined by flow cytometry at day 14 p.i.. Representative of 3 similar experiments, mean ±SEM, n = 5 in each group in each experiment, **p<0.01, Mann-Whitney U test.

doi:10.1371/journal.ppat.1005398.g002
Blimp-1-dependent IL-10 production by CD4+ T cells limits parasite killing in VL

VL caused by *L. donovani* in mice is characterized by a chronic infection of macrophages in the spleen, but acute infection of macrophages in the liver [57]. Strikingly, and in contrast to littermate controls, mice lacking Blimp-1 expression in T cells controlled infection in the spleen effectively (Fig 4A). This improved control of parasite growth was again associated with an increased frequency of activated CD4+ T cells and Th1 cells, but restricted development of Tr1 cells (Fig 4B). However, despite improved control of parasite growth in mice with Blimp-1 deficient T cells, these mice presented with significantly larger spleens, associated with an increased frequency of TNF-producing CD4+ T cells (Fig 4C). Similar effects were also observed in the liver (Fig 4D–4F), and these were also associated with elevated serum TNF and IFNγ levels (Fig 4G). Enhanced CD4+ T cell responses were antigen-specific, as shown by increased IFNγ and TNF production in response to stimulation with parasite antigen (Fig 4H and 4I). Although IL-10 was not detected in serum, IL-10 production was measured in response to antigen re-stimulation, and consistent with the above Tr1 data, was significantly reduced in cells from mice lacking Blimp-1 expression in T cells, compared with littermate controls (Fig 4I). Blimp-1 has previously been shown to restrain CD4+ T cell IL-17 production [58], but we found no significant changes in serum IL-17 levels (S5A Fig) or parasite-specific IL-17 production (S5B Fig) in mice with Blimp1-deficient T cells. In addition, *L. donovani*-infected mice lacking Blimp-1 expression in FoxP3+ T (Treg) cells (*Prdm1ΔTreg* mice) showed none of the above changes (Fig 5). Thus, lack of Blimp-1 expression by conventional CD4+ T cells dramatically improved anti-parasitic immunity, but also promoted tissue pathology. We also observed relatively minor changes in the myeloid cell compartments of mice with Blimp-1-deficient T cells, relative to littermate controls (S6 Fig), most notably, an increased frequency of DC’s in the spleen at day 7 p.i., but decreased frequency in the liver.

---

Fig 3. Elevated Blimp-1 levels in CD4+ T cells from VL patients were associated with increased IL-10 production. (A) *PRDM1* and (B) IL-10 mRNA accumulation in purified CD4+ cells isolated from the PBMCs, as well as (C) plasma IL-10 levels from VL patients before (closed circles; Pre) and 28 days after (open circles; Post) drug treatment. Data are from the same 10 paired samples, **p<0.01, *p<0.05, Wilcoxon signed-rank test. doi:10.1371/journal.ppat.1005398.g003
Blimp-1-dependent IL-10 signaling to macrophages protects from tissue pathology

To test whether Blimp-1-dependent IL-10 production by T cells was responsible for inefficient control of parasite growth, we infected Il-10fl/fl x Lck-Cre (Il-10ΔT) mice [59] that lacked IL-10 production by T cells with L. donovani. Similar to mice lacking Blimp-1 expression in T cells, control of parasite growth was dramatically improved, relative to Cre-negative (Il-10fl/fl) littermate controls (Fig 6A). As indicated above, the improved parasite clearance in the absence of Blimp-1 in T cells was associated with more severe splenomegaly. IL-10 has previously been found to protect against tissue damage caused by parasite-mediated inflammation [23,24,60], and we show that in L. donovani-infected mice, increased spleen size was a consequence of a lack of IL-10 production by T cells (Fig 6B). Splenomegaly in mice lacking either Blimp-1 or IL-10 expression in T cells was also associated with a dramatic loss of splenic marginal zone macrophages (MZM) by day 14 p.i. (Fig 6C). Direct IL-10 signaling to myeloid cells
contributed to the above phenotypes because mice lacking IL-10 receptor (IL-10R) expression specifically on these cells (IL-10R<sup>−/−</sup> x LysM-Cre (IL-10r<sup>ΔM</sup>)) [61] and infected with <i>L. donovani</i> displayed dramatically improved control of parasite growth, which was again accompanied by splenomegaly, increased TNF and IFNγ production, and accelerated loss of MZM, relative to Cre-negative (IL-10r<sup>fl/fl</sup>) litter mate controls (Fig 6D–6G). Therefore, Blimp-1-dependent IL-10 produced by CD4<sup>+</sup> T cells acts on myeloid cells, including MZM, to impair parasite killing, but also acts to limit splenomegaly.

**IL-10 signaling to macrophages protects them from TNF-mediated destruction**

We previously showed that following the establishment of chronic <i>L. donovani</i> infection, TNF mediates the loss of MZM in the spleen, associated with severe disruption of lymphocyte trafficking [51]. Indeed, the accelerated development of splenomegaly and loss of MZM in mice lacking Blimp-1 expression in T cells was associated with disrupted cell trafficking into the spleen, which could be rescued by TNF blockade (Fig 7B–7E). Specifically, MZM were retained following TNF blockade, and this was associated with improved retention of injected, fluorocently-labelled lymphocytes in the T and B cell zones of the white pulp regions of the spleen.
Interestingly, despite the loss of MZM in mice lacking Blimp-1 expression in T cells, T and B cell zones were largely preserved at day 14 p.i. (Fig 7E and 7F). Importantly, improved parasite control in the absence of Blimp-1 in T cells was also dependent on TNF (Fig 7A), indicating that both beneficial and pathogenic effects of TNF were controlled by Blimp-1-regulated IL-10 production by T cells. While these data demonstrate that TNF blockade can prevent tissue damage during infection, they also highlight the importance of TNF for controlling parasite growth. However, in accordance with previous results [62], established infection could be controlled and anti-parasitic immunity maintained when TNF was blocked when mice were also treated with anti-parasitic drug (S7 Fig), thereby identifying a strategy for controlling TNF-mediated pathology while also controlling parasite growth.

IFNγ signaling promotes TNF production and associated tissue damage

As outlined above, improved parasite control and exacerbated tissue pathology in mice lacking Blimp-1 or IL-10 expression in their T cells was strongly associated with increased numbers of Th1 cells and serum IFNγ levels (Fig 4). We therefore examined how IFNγ production influenced TNF-mediated consequences of L. donovani infection described above. Strikingly, despite
uncontrolled hepatic parasite growth, as reported in IFNγ-deficient mice [63], mice lacking IFNγ receptor (IFNγR) showed no splenomegaly and complete preservation of MZM after 28 days of infection when MZM were lost in C57BL/6 controls (Fig 8A–8D). Critically, these mice produced minimal amounts of TNF, relative to wild type controls (Fig 8E). Thus, our results show that following L. donovani infection, IFNγ promoted TNF production, and this pathway was regulated by Blimp-1-mediated IL-10 production by T cells. Importantly, this regulatory pathway determined the balance between control of parasite growth and TNF-mediated pathology.

Discussion

Here we show that Blimp-1 mRNA was elevated in CD4+ T cells from VL patients, along with IL-10 mRNA and elevated levels of plasma IL-10. Furthermore, in an experimental model of
VL, Blimp-1-dependent IL-10 produced by Tr1 cells acted on myeloid cells to limit parasite killing, but was critical to prevent TNF-mediated tissue disruption. In the absence of IL-10 production by T cells, MZM were lost and this was associated with disrupted lymphocyte trafficking and splenomegaly. Thus, we have identified Tr1 cells as potent suppressors of anti-parasitic immunity, but critical regulators of IFN-γ-dependent, TNF-mediated pathology.

The transcriptional regulator Blimp-1 is important for IL-10 production by both Treg [64] and Tr1 [52,53] cells. However, Blimp-1 deficiency in Treg cells has minimal impact on immune responses and disease outcome in mice infected with *L. donovani*. Data from malaria [30,31] and VL [29] patients indicates that Tr1 cells are a major regulatory T cell population during protozoan diseases, and our results show a critical role for Blimp-1 in Tr1 cell function by promoting IL-10 production. Furthermore, results from mice that lack IL-10 in the T cell compartment indicate that IL-10 is a critical immune mediator being controlled by Blimp-1 following *L. donovani* infection. Another striking feature in mice lacking Blimp-1 expression in T cells was the increase in number and frequency of activated CD4+ T cells. Earlier work
showed that Blimp-1-deficient T cells do not have an intrinsically better ability to proliferate [65,66], but they are more resistant to activation-induced cell death [65]. In addition, Blimp1 is an important repressor of T follicular helper (Tfh) cell development by suppressing Bcl6 [67]. Thus, one potential explanation for increased CD4+ T cell activation in our disease models is less cell death, as well as cell differentiation favoring Th1 cell development.

The control of many intracellular infections requires Th1 cells which are generated in response to macrophage and dendritic cell derived IL-12 [9]. These Th1 cells produce IFNγ and TNF to activate phagocytes and kill intracellular pathogens [10]. However, if pathogens persist, there is a danger that these pro-inflammatory cytokines will damage tissue, and consequently, Tr1 cells develop to control this inflammation [12,33–35]. Our results suggest that Tr1 cells generated in our experimental setting derive from Th1 cells, a notion supported by recent results showing IL-12, along with IL-27, promoted Blimp-1-dependent IL-10 production by Tr1 cells [52]. Thus, our data suggest that IL-12 is not only required to generate Th1 cells following L. donovani infection, but also provides additional signals for the transition from Th1 to Tr1 cells in this model. Previous work by others has already identified an important role for IL-27 in Tr1 cell development in experimental malaria [23,68] and leishmaniasis [56,69].

TNF is involved in the pathogenesis of a range of diseases, including infectious and autoimmune diseases, and more recently, complications arising in immune-related adverse events as a consequence of immune check point inhibition [3,70]. Our results identify TNF as a major mediator of tissue pathology in the absence of T cell-derived IL-10. Hence, understanding how TNF is regulated in different disease settings is of major medical importance. Despite differences in the structure of rodent and human spleens [38], post-mortem studies on VL patients revealed extensive disruption to white pulp areas, associated with substantial changes in macrophage populations [47,48], also reported in experimental VL [50,51]. The MZ of the spleen is a specialized collection of cells separating the predominantly non-lymphoid red pulp regions and lymphoid dominated white pulp regions. It is also vascular and plays an important role in removing particulate antigen, as well as dead and dying cells, from the circulation [38,71]. Remarkably, the accelerated loss of MZM in mice lacking Blimp-1 or IL-10 expression in T cells resulted in significant disruption of lymphocyte trafficking into T and B cell zones of the white pulp that could be rescued by TNF blockade. These results support earlier studies showing important roles for MZM in directing lymphocyte traffic into the splenic white pulp [72,73]. Another striking feature of L. donovani-infected mice lacking Blimp-1 or IL-10 expression by T cells was dramatic splenomegaly. Angiogenesis is a dominant feature of splenomegaly during experimental VL [44], driven by inflammation-induced expression of neurotropic receptor on vascular endothelium and interactions with ligands produced by mononuclear phagocytes [43]. TNF produced by macrophages can promote angiogenesis [74,75], and chronic inflammation and vascular remodelling are intimately linked in autoimmune disease settings [76]. Critically, inhibition of vascularization with receptor tyrosine kinase inhibitors in mice with established L. donovani infection resulted in reduced mononuclear phagocyte number and reversed splenomegaly [44]. Therefore, our data supports a model whereby TNF-driven angiogenesis is regulated by Blimp-1-dependent IL-10 production by Tr1 cells.

By identifying IL-10 produced by Tr1 cells as critical regulators of TNF, we can consider IL-10-related strategies for modulating TNF production. However, given the important role of TNF in controlling intracellular infections [62,77], these strategies should be designed to limit pathogenic functions of TNF while maintaining anti-microbial effects. TNF is produced by many different cell populations, and acts on a range of target cells [78]. We previously reported that TNF from CD4+ T cells was required for anti-parasitic functions in L. donovani-infected mice [77], but the cellular source of pathogenic TNF is not known. In ulcerative colitis patients, TNF from T cells appeared to be an important driver of disease [79], while in graft versus host...
disease, both T cell [80] and macrophage/monocyte-derived TNF cause gastrointestinal damage [81]. Therefore, different cellular sources of TNF are likely to be important for pathogen control and promoting disease in different immune environments. Further work is needed to better understand the cellular and molecular aspects of TNF regulation that will allow selective targeting of these two distinct functional outcomes of TNF biology.

Treg cells are currently being generated and tested for a range of inflammatory conditions [82,83]. However, in situations where this approach fails, the use of Tr1 cells may be beneficial [84]. Our data indicate that Tr1 cells play a critical role in regulating inflammation in organs such as the spleen, in contrast to inflammation in the lung or gut, where Treg cells play critical protective roles [18,85,86]. Hence, under different clinical situations, either Treg or Tr1 cells may help to treat disease. Our findings identify Blimp-1-dependent IL-10 produced by Tr1 cells as a critical regulator of IFNγ-dependent, TNF-mediated tissue damage in the spleen in parasitic infections. Thus, Tr1 focused therapy may be an attractive modality in settings where TNF-mediated immunity is needed, but TNF-induced immunopathology instead dominates.

**Methods**

**Ethics statement**

All patients presented with symptoms of VL at the Kala-azar Medical Research Center (Muzaffarpur, Bihar, India). VL diagnosis was confirmed either by the microscopic detection of amastigotes in splenic aspirate smears or by rk39 dipstick test. Patients were treated either with Amphotericin B or Ambisome. In total, 10 patients were enrolled in the study. The use of human subjects followed recommendations outlined in the Helsinki declaration. Written informed consent was obtained from all participants and/or their legal guardian when under 18 years of age. Ethical approval (Dean/2011-12/289) was obtained from the ethical review board of Banaras Hindu University (BHU), Varanasi, India.

All animal procedures were approved by the QIMR Berghofer Medical Research Institute Animal Ethics Committee. This work was conducted under QIMR Berghofer animal ethics approval number A02-634M, in accordance with the “Australian Code of Practice for the Care and Use of Animals for Scientific Purposes” (Australian National Health and Medical Research Council).

**Mice**

Female C57BL/6j mice, 8–12 weeks old were purchased from the Australian Resource Centre (Canning Vale, WA, Australia) and the Walter and Eliza Hall Institute (Melbourne, VIC, Australia). Prdm1β/β, Prdm1AT, Prdm1GFP/+ IL-10β/β, IL-10-AT, IL-10β/β and IL-10r-AM mice were bred in-house under specific-pathogen free conditions. Prdm1ATreg mice were bred at the Walter and Eliza Hall Institute. All Prdm1β/β [54] and Prdm1GFP/+ [55] mice were on a pure C57BL/6 background, while IL-10β/β [59] and IL-10rβ/β [61] mice were backcrossed to C57BL/6 for at least 10 generations.

**Parasites and infections**

*Leishmania donovani* (LV9) parasites were maintained by passage in B6.Rag1-/- mice and amastigotes were isolated from the spleens of chronically infected mice. Mice were infected with 2 x 10⁷ LV9 amastigotes intravenously (i.v.) via the lateral tail vein. Spleen and liver impression smears were used to determine parasite burdens and were expressed as Leishman Donovan Units (LDU; number of amastigotes per 1000 host nuclei multiplied by the organ weight (in grams)).
**Plasmodium chabaudi chabaudi** AS (PcAS) and **Plasmodium berghei** ANKA (PbA) strains were used in all experiments after one in vivo passage in a C57BL/6 mouse. All mice received a dose of $10^5$ pRBCs i.v. via the lateral tail vein. Thin blood smears from tail bleeds were stained with Culti Pure- stains (HD Scientific Supplies, Willawong, Australia). Parasitemia was used to monitor the course of infection and was determined by flow cytometry (see below).

**Monitoring parasitemia by flow cytometry during experimental malaria**

Briefly, 1–2 drops of blood from a tail bleed was diluted and mixed in 250 μl RPMI/PS containing 5 U/ml heparin sulphate. Diluted blood was stained simultaneously with Syto84 (5 μM; Life Technologies, Mulgrave, Australia) to detect RNA/DNA and Hoechst33342 (10 μg/ml; Sigma-Aldrich, Castle Hill, Australia) to detect DNA for 30 minutes at room temperature, protected from light. 2 ml RPMI/PS was added to stop the reaction, and samples were immediately placed on ice until acquisition on a BD FACSCanto II Analyzer (BD Biosciences, Franklin Lakes, NJ). Data was analysed using FlowJo software (Treestar), where pRBC were readily detected as being Hoechst33342$^+$ Syto84$^+$, with lymphocytes excluded on the basis of size, granularity, and higher levels of Hoechst33342/Syto84 staining compared with pRBCs.

**Human VL patient samples**

Heparinized blood was collected from patients before and 28 days after commencement of drug treatment, and PBMC were isolated by Ficoll-Hypaque (GE Healthcare, NJ) gradient centrifugation and used for the positive selection of CD4$^+$ T cells using magnetic beads and columns (Miltenyi Biotech, Bergisch Gladbach, Germany). Cells were collected directly into RNealter (Sigma), and stored at -70°C until mRNA isolation and analysis. Total RNA was isolated using RNeasy mini kits and QiaShredder homogenizers (Qiagen, Valencia, CA), according to the manufacturer’s protocol. The quality of RNA was assessed by denaturing agarose gel electrophoresis. cDNA synthesis was performed in 20 μL reactions on 0.5–1.0 μg RNA using High-Capacity cDNA Archive kit (Applied Biosystems, CA, USA). Real-time PCR was performed on an ABI Prism 7500 sequence detection system (Applied Biosystems) using cDNA-specific FAM–MGB labelled primer/probe for PRDM1. The relative quantification of products was determined by the number of cycles over 18S mRNA endogenous control required to detect PRDM1 gene expression.

**L. donovani** antigen re-stimulation assay

Spleens were processed through a 100μm cell strainer in order to obtain a single-cell suspension. Splenocyte cell suspensions were then counted and adjusted to a concentration of $2 \times 10^6$ cells/ml. LV9 amastigotes (fixed in 4% PFA) were thawed and washed in RPMI media containing Penicillin-Streptomycin and then counted and adjusted to a final concentration of $4 \times 10^7$/ml. Cells and parasites were plated into a 96-U well plate at a 1:20 ratio, where each well contained $1 \times 10^5$ cells and $2 \times 10^6$ parasites. Cells were cultured in the presence of antigen for a period of 24 and 72 hours. Culture supernatants were harvested at 24 and 72 hours and intracellular cytokine staining was performed at both time points.

**Monitoring PbA infection and clinical scoring of ECM symptoms**

A transgenic PbA line (231c11) expressing luciferase (PbA-luc) and GFP under the control of the efl-α promoter [87] was used for all PbA experiments. PbA-infected mice were monitored and scored, as previously described [88].
In vivo bioluminescence imaging

The *in vivo* imaging system 100 (Xenogen, Alameda, CA) was used to detect the level of bioluminescence as a measure of whole body parasite burden in each mouse. At selected time-points, *PbA*-luc-infected mice were anaesthetised with isofluorane and injected with 150 mg/kg i.p. of D-luciferin (Xenogen) 5 minutes prior to imaging. Bioluminescence was measured in p/s/cm²/sr using Living Image (Xenogen), as previously described [88].

Antibody treatment

For IL-12 neutralisation experiments, mice were administered 500 μg of rat IgG (Sigma) or anti-IL-12 (clone: C17.8; BioXcell; West Lebanon, NH) i.p., on the day of infection and every 3 days post infection until day 14 p.i. For TNF blockade experiments, mice administered 200 μg of Human Normal Immunoglobulin (INTRAGAM P; CSL, Melbourne, Australia) or anti-TNF (Enbrel; Amgen, Thousand Oaks, CA) i.p., on the day of infection and every 2 days post infection until day 14 p.i.

Fluorescence microscopy

Mice were injected with 100 μg i.v. of FITC dextran (Life Technologies, Melbourne, Australia) one day prior to collection of organs. Spleen tissue was collected into 4% PFA, incubated at room temperature for 1-2hrs and then transferred to a 30% sucrose solution (in MilliQ water) (Sigma, Sydney, Australia) overnight at 4°C. Fixed spleen tissue was then preserved in Tissue-Tek O.C.T. compound (Sakura, Torrance, CA). Splenic architecture and distribution of marginal zone macrophages (MZMs) were analysed in 20 μm sections counter-stained with DAPI and visualized on the Aperio FL slide scanner. Image analysis was performed using Image Scope to determine area of the sections and Metamorph 7.8 (Integrated Morphometry analysis tool; Molecular Devices, Sunnyvale, CA) to count the MZMs. In some experiments, mice were injected intravenously with 2 x 10⁷ naïve splenocytes labelled with Cell Trace Far Red DDAO-SE (Life Technologies, Mulgrave, Australia), 2 hours prior to sacrifice. The same 20 μm sections were used to assess cell trafficking, where sections were stained with CD3 biotin (5 μg/ml) + SA AF594 (5 μg/ml), B220 PE (5 μg/ml) (Biolegend, San Diego, CA), counter-stained with DAPI (1:25000, Sigma-Aldrich, Castle Hill, Australia) and mounted with Pro-Long Gold anti-fade (Life Technologies). Slides were visualized on a Carl Zeiss 780 NLO laser scanning confocal microscope under 10x magnification. Image analysis was performed using Metamorph 7.8 (Counting App and Region Measurement tool). Briefly, since each image was identical in size (1416.30μm x 1416.30μm), the total number of cells was counted in each image. The drawing tool was used to delineate the T and B cell zones in the white pulp (WP) of the spleen and the area of these zones in each image was measured (in mm²). Number of cells in WP per mm² was calculated as the cumulative number of cells in WP in each image divided by cumulative areas of WP in each image.

Flow cytometry

Allophycocyanin-conjugated anti–IFNγ (XMG1.2), anti–IL-10 (JESS-16E3), PE-conjugated TNFα (MP6-XT22) allophycocyanin-Cy7–conjugated anti–CD4 (GK1.5), FITC-conjugated anti–CD11a (M17/4), Brilliant Violet 421–conjugated anti–IFNγ (clone XMG1.2), allophycocyanin-Cy7–conjugated anti–NK1.1 (clone PK136), Alexa Fluor 700–conjugated anti–CD8α (clone 53–6.7), biotinylated–CD49d (R1–2), PeCy7-conjugated Strepavidin and PerCP-Cy5.5–conjugated anti–TCRb-chain (H57–597), Allophycocyanin-conjugated anti–anti-CD11c (N418), Pacific Blue-conjugated anti-MHCII (I–A/I–E) (M5/114–15.3), PerCP-Cy5.5–
conjugated anti-CD11b (M1/70), FITC-conjugated anti-Ly6C (HK1.4), PeCy7-conjugated anti-F4/80 (BM8) Brilliant Violet 605-conjugated anti-TCRβ (H57-597), allophycocyanin-Cy7-conjugated anti-B220 (RA3-6B2) and Alexa Fluor 700-conjugated Streptavidin were purchased from BioLegend (San Diego, CA) or BD Biosciences. Dead cells were excluded from the analysis using LIVE/DEAD Fixable Aqua Stain or LIVE/DEAD Fixable Near Infra-Red Stain (Invitrogen-Molecular Probes, Carlsbad, CA), according to the manufacturer’s instructions. The staining of cell surface antigens and intracellular cytokine staining were carried out as described previously [89,90]. FACS was performed on a FACSCanto II or LSRFortessa (BD Biosciences), and data was analyzed using FlowJo software (TreeStar). Gating strategies used for analysis are shown in Figs 1 and 2.

Measurement of serum and culture supernatant cytokine levels

Cytokine levels in the serum and culture supernatants were measured using a BD Cytometric Bead Array (CBA) Flex sets and the HTS system plate reader on the Fortessa 5 Flow cytometer (BD Biosciences) according to the manufacturer’s instructions.

Statistical analysis

Comparisons between two groups were performed using non-parametric Mann-Whitney tests in mouse studies and Wilcoxon matched-pairs signed rank test in human studies. Comparisons between multiple groups were made using a Kruskal-Wallis test and corrected using Dunn’s multiple comparisons test. GraphPad Prism version 6 for Windows (GraphPad, San Diego, CA) was used for analysis; p<0.05 was considered statistically significant. All data are presented as the mean ± SEM.

Supporting Information

S1 Fig. Prdm1fl/fl and Prdm1ΔT C57BL/6 mice were infected with PcAS and the frequency and number of splenic CD8+ T cells expressing IFNγ and Tbet (A) and IL-10 and IFNγ (B) were measured by flow cytometry at day 15 p.i. Representative of 3 similar experiments, mean ±SEM, n = 5 in each group in each experiment. **p<0.01, *p<0.05, Mann-Whitney U test. (TIF)

S2 Fig. Female C57BL/6 mice were infected with PcAS and the frequency of activated CD4+ T cells (CD11a+ CD49d+) (A and B), Th1 cells (Tbet+ IFNγ+) (C and D) and Tr1 cells (IFNγ+ IL-10+) (E and F) were measured at the time points indicated. All Th1 and Tr1 cells were contained within the activated CD4+ T cell compartment (G). (TIF)

S3 Fig. Prdm1fl/fl and Prdm1ΔT C57BL/6 mice were infected with PcAS and the frequency of activated CD4+ T cells (CD11a+ CD49d+) Th1 cells (Tbet+ IFNγ+) and Tr1 cells (IFNγ+ IL-10+) in the spleen were measured at day 4 (A) and 7 (B) p.i. Representative of 2 similar experiments, mean ±SEM, n = 5 in each group in each experiment, **p<0.01, *p<0.05, Mann-Whitney U test. (TIF)

S4 Fig. Prdm1fl/fl and Prdm1ΔT C57BL/6 mice were infected with PbA-luc and whole body parasite burdens and blood parasitemia (A) was measured at day 6 p.i., when Prdm1fl/fl mice first began to exhibit early ECM symptoms. Clinical scores and percent survival (B) was determined. Grey area indicates time frame when neurological symptoms were apparent in Cre (-) mice. Dotted line at clinical score 4 indicates moribund threshold. Frequency and numbers of
Th1 (C) and Tr1 (D) cells were assessed by flow cytometry at day 4 p.i. Representative of 3 similar experiments, mean ±SEM, n = 5–6 in each group in each experiment, ***p<0.001, **p<0.01, *p<0.05, Mann-Whitney U test, log-rank (Mantel-Cox) test (percent survival).

(S5 Fig) Prdm1fl/fl and Prdm1ΔT C57BL/6 mice were infected with L. donovani and serum IL-17A levels (A), as well as antigen-specific IL-17A production by splenocytes were measured after 72 hours of culture in the presence of parasite antigen (B) at times indicated. In both panels, closed shapes represent Prdm1fl/fl mice, while open shapes indicate Prdm1ΔT littermates. Representative of 2 similar experiments, mean ±SEM, n = 5–6 in each group in each experiment. (TIF)

(S6 Fig) Prdm1fl/fl and Prdm1ΔT C57BL/6 mice were infected with L. donovani and monocytes, DC’s and neutrophils identified by the gating strategy shown in (A) and their frequency measured in the spleen (B) and liver (C) at time points indicated. In all panels, closed shapes represent Prdm1fl/fl mice, while open shapes indicate Prdm1ΔT littermates. Representative of 2 similar experiments, mean ±SEM, n = 5 in each group in each experiment, **p<0.01, *p<0.05, Mann-Whitney U test.

(S7 Fig) C57BL/6 mice were infected with L. donovani and received either TNF blockade (Enbrel) or control human IgG (INTRAGAM), with or without sodium stibogluconate (SSG), as indicated, from days 14–28 p.i.. Liver (A) and spleen (B) parasite burdens and spleen weights (C) were measured at day 28 p.i., as was the number of MZMs per mm² of spleen tissue ((D); as described in Fig 4C). Th1 cell frequency in splenocytes cultured in media or with parasite antigen (E), as indicated, as well as IFNγ production from antigen-stimulated cells (F) were measured after 24 hours of culture. Representative of 2 independent experiments, mean ±SEM, n = 5, **p<0.01, *p<0.05, Mann-Whitney U test.

(TIF)

Acknowledgments
We thank Paula Hall and Grace Chojnowski for assistance with flow cytometry, Nigel Waterhouse for help with microscopy and staff in the QIMR-B animal facility for animal husbandry. We thank faculty and students at the 2014 and 2015 Biology of Parasitism course (Marine Biological Laboratory, Woods Hole, MA) for contributing pilot data and ideas.

Author Contributions
Conceived and designed the experiments: MMDo AK CRE. Performed the experiments: MMDo RK FdLR FHA MS RJF PTB SEB LB SSN CLE. Analyzed the data: LB MJS AH GRH SS AK CRE. Contributed reagents/materials/analysis tools: WM EC SLN. Wrote the paper: MMDo CRE.

References
1. Kruglov AA, Kuchmiy A, Grivennikov SI, Tumanov AV, Kuprash DV, et al. (2008) Physiological functions of tumor necrosis factor and the consequences of its pathologic overexpression or blockade: mouse models. Cytokine Growth Factor Rev 19: 231–244. doi:10.1016/j.cytogfr.2008.04.010 PMID: 18502680
2. Beutler B, Millsark IW, Cerami AC (1985) Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 229: 869–871. PMID: 3895437
3. Monaco C, Nanchahal J, Taylor P, Feldmann M (2015) Anti-TNF therapy: past, present and future. Int Immunol 27: 55–62. doi: 10.1093/intimm/dxu102 PMID: 25411043
4. Feldmann M, Maini RN (2001) Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? Annu Rev Immunol 19: 163–196. PMID: 11244034
5. Crawford M, Curtis JR (2008) Tumor necrosis factor inhibitors and infection complications. Curr Rheumatol Rep 10: 383–389. PMID: 18817643
6. Kim SY, Solomon DH (2010) Tumor necrosis factor blockade and the risk of viral infection. Nat Rev Rheumatol 6: 165–174. doi: 10.1038/nrrheum.2009.279 PMID: 20142812
7. Sher A, Coffman RL (1992) Regulation of immunity to parasites by T cells and T cell-derived cytokines. Annu Rev Immunol 10: 385–409. PMID: 1590992
8. Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4+ T cell populations. Annu Rev Immunol 28: 445–489. doi: 10.1146/annurev-immunol-030409-101212 PMID: 20192806
9. O'Garra A, Murphy KM (2009) From IL-10 to IL-12: how pathogens and their products stimulate APCs to induce Th1 development. Nat Immunol 10: 929–932. doi: 10.1038/ni0909-929 PMID: 19692989
10. Tubo NJ, Jenkins MK (2014) CD4+ T Cells: guardians of the phagosome. Clin Microbiol Rev 27: 200–213. doi: 10.1128/CMR.00097-13 PMID: 24696433
11. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133: 775–787. doi: 10.1016/j.cell.2008.05.009 PMID: 18510923
12. Saraiva M, O'Garra A (2010) The regulation of IL-10 production by immune cells. Nat Rev Immunol 10: 170–181. doi: 10.1038/nri2711 PMID: 20154735
13. Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 170: 2081–2095. PMID: 25311194
14. Yssel H, De Waal Malefyt R, Roncarolo MG, Abrams JS, Lahesmaa R, et al. (1992) IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. J Immunol 149: 2378–2384. PMID: 1356125
15. Meyera L, Hovenkamp E, Otto SA, Miedema F (1996) IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. J Immunol 156: 2776–2782. PMID: 8609396
16. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, et al. (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389: 737–742. PMID: 9338786
17. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 190: 995–1004. PMID: 10510089
18. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, et al. (2008) Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 28: 546–558. doi: 10.1016/j.immuni.2008.02.017 PMID: 18387831
19. Stumhofer JS, Silver JS, Laurence A, Porrett PM, Harris TH, et al. (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol 8: 1363–1371. PMID: 17994025
20. Stager S, Maroof A, Zubairi S, Sanos SL, Kopf M, et al. (2006) Distinct roles for IL-6 and IL-12p40 in mediating protection against Leishmania donovani and the expansion of IL-10+ CD4+ T cells. Eur J Immunol 36: 1764–1771. PMID: 16791879
21. Anderson CF, Uukma M, Kuchroo VJ, Sacks D (2007) CD4+CD25(-)Foxp3(-) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J Exp Med 204: 285–297. PMID: 17283207
22. Couper KN, Blount DG, Wilson MS, Hafalla JC, Belkaid Y, et al. (2008) IL-10 from CD4CD25Foxp3CD127 adaptive regulatory T cells modulates parasite clearance and pathology during malaria infection. PLoS Pathog 4: e1000004. doi: 10.1371/journal.ppat.1000004 PMID: 18401464
23. Freitas do Rosario AP, Lamb T, Spence P, Stephens R, Lang A, et al. (2012) IL-27 promotes IL-10 production by effector Th1 CD4+ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. J Immunol 188: 1178–1190. doi: 10.4049/jimmunol.1102755 PMID: 22205023
24. Jankovic D, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, et al. (2007) Conventional T-bet(+) Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J Exp Med 204: 273–283. PMID: 17283209
25. Wilson EH, Wille-Reece U, Dzierszinski F, Hunter CA (2005) A critical role for IL-10 in limiting inflammation during toxoplasmic encephalitis. J Neuroimmunol 165: 63–74. PMID: 16005735
26. Reed SG, Brownell CE, Russo DM, Silva JS, Grabstein KH, et al. (1994) IL-10 mediates susceptibility to Trypanosoma cruzi infection. J Immunol 153: 3135–3140. PMID: 8089491

27. Roffe E, Rothfuchs AG, Santiago HC, Marino AP, Ribeiro-Gomes FL, et al. (2012) IL-10 limits parasite burden and protects against fatal myocarditis in a mouse model of Trypanosoma cruzi infection. J Immunol 188: 649–660. doi: 10.4049/jimmunol.1003845 PMID: 22156594

28. Namangala B, Noel W, De Baetselier P, Brys L, Beschin A (2001) Relative contribution of interferon-gamma and interleukin-10 to resistance to murine African trypanosomosis. J Infect Dis 183: 1794–1800. PMID: 11372033

29. Nylen S, Maurya R, Eidsmo L, Manandhar KD, Sundar S, et al. (2007) Splenic accumulation of IL-10 mRNA in T cells distinct from CD4+CD25+ (Foxp3) regulatory T cells in human visceral leishmaniasis. J Exp Med 204: 805–817. PMID: 17389235

30. Jagannathan P, Eccles-James I, Bowen K, Nankya F, Auma A, et al. (2014) IFN-gamma/IL-10 co-producing cells dominate the CD4 response to malaria in highly exposed children. PLoS Pathog 10: e1003864. doi: 10.1371/journal.ppat.1003864 PMID: 24415936

31. Portugal S, Moebius J, Skinner J, Dourinho S, Dourinbte D, et al. (2014) Exposure-dependent control of malaria-induced inflammation in children. PLoS Pathog 10: e1004079. doi: 10.1371/journal.ppat.1004079 PMID: 24743880

32. Walther M, Jeffries D, Finney OC, Njie M, Ebonyi A, et al. (2009) Distinct roles for FOXP3 and FOXP3 CD4 T cells in regulating cellular immunity to uncomplicated and severe Plasmodium falciparum malaria. PLoS Pathog 5: e1000364. doi: 10.1371/journal.ppat.1000364 PMID: 19343213

33. Hunter CA, Kastelein R (2012) Interleukin-27: balancing protective and pathological immunity. Immunity 37: 960–969. doi: 10.1016/j.immuni.2012.11.003 PMID: 23244718

34. Engwerda CR, Ng SS, Bunn PT (2014) The Regulation of CD4(+) T Cell Responses during Protozoan Infections. Front Immunol 5: 498. doi: 10.3389/fimmu.2014.00498 PMID: 25352846

35. Roncarolo MG, Gregori S, Bacchetta R, Battaglia M (2014) Tr1 cells and the counter-regulation of immunity: natural mechanisms and therapeutic applications. Curr Top Microbiol Immunol 380: 39–68. doi: 10.1007/978-3-662-43492-5_3 PMID: 25004813

36. Whiteside TL, Schuler P, Schilling B (2012) Induced and natural regulatory T cells in human cancer. Expert Opin Biol Ther 12: 1383–1397. doi: 10.1517/14712598.2012.707184 PMID: 22849383

37. Junt T, Scandella E, Ludewig B (2008) Form follows function: lymphoid tissue microarchitecture in anti-microbial immune defence. Nat Rev Immunol 8: 764–775. doi: 10.1038/nri2414 PMID: 18825130

38. Mebius RE, Kraal G (2005) Structure and function of the spleen. Nat Rev Immunol 5: 606–616. PMID: 16056254

39. Forster R, Davalos-Misslitz AC, Rot A (2008) CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol 8: 362–371. doi: 10.1038/nri2297 PMID: 18379575

40. Boelaert M, Sundar S (2014) Leishmaniasis. In: Farrer J, Hotez PJ, Junghanss T, Kang G, Lalloo D et al., editors. Manson's Tropical Diseases. Amsterdam: Elsevier. pp. 631–651. PMID: 10678964

41. White N (2014) Malaria. In: Farrer J, Hotez PJ, Junghanss T, Kang G, Lalloo D et al., editors. Manson's Tropical Diseases. Amsterdam: Elsevier. pp. 532–601.

42. Achtman AH, Khan M, MacLennan IC, Langhorne J (2003) Plasmodium chabaudi chabaudi infection in mice induces strong B cell responses and striking but temporary changes in splenic cell distribution. J Immunol 171: 317–324. PMID: 12817013

43. Dalton JE, Glover AC, Hoodless L, Lim EK, Beattie L, et al. (2015) The neurotrophic receptor Ntrk2 directs lymphoid tissue neovascularization during Leishmania donovani infection. J Exp Med 204: 805–817. PMID: 17389235

44. Dalton JE, Maroof A, Owens BM, Narang P, Johnson K, et al. (2010) Inhibition of receptor tyrosine kinases restores immunocompetence and improves immune-dependent chemotherapy against experimental leishmaniasis in mice. J Clin Invest 120: 1204–1216. doi: 10.1122/jci1281 PMID: 20234089

45. Helmy H, Jonsson G, Troye-Blomberg M (2000) Cellular changes and apoptosis in the spleens and peripheral blood of mice infected with blood-stage Plasmodium chabaudi chabaudi AS. Infect Immun 68: 1485–1490. PMID: 10678964

46. Smelt SC, Engwerda CR, McCrossen M, Kaye PM (1997) Destruction of follicular dendritic cells during chronic visceral leishmaniasis. J Immunol 158: 3813–3821. PMID: 9103448

47. Veress B, Omer A, Satir AA, El Hassan AM (1977) Morphology of the spleen and lymph nodes in fatal visceral leishmaniasis. Immunology 33: 605–610. PMID: 5909922

48. Zijlstra EE, el-Hassan AM (2001) Leishmaniasis in Sudan. Visceral leishmaniasis. Trans R Soc Trop Med Hyg 95 Suppl 1: S27–S58. PMID: 11370250
49. Keenan CM, Hendricks LD, Lightner L, Johnson AJ (1984) Visceral leishmaniasis in the German shepherd dog. IL. Pathology. Vet Pathol 21: 80–86. PMID: 6710817
50. Ato M, Stager S, Engwerda CR, Kaye PM (2002) Defective CCR7 expression on dendritic cells contributes to the development of visceral leishmaniasis. Nat Immunol 3: 1185–1191. PMID: 12436111
51. Engwerda C, Ato M, Cotterell S, Mynott T, Tschannerl A, et al. (2002) A Role for tumor necrosis factor-α in remodeling the splenic marginal zone during Leishmania donovani infection. The American Journal of Pathology 161: 429–437. PMID: 12163368
52. Heinemann C, Heinck S, Petermann F, Vasanthakumar A, Rothhammer V, et al. (2014) IL-27 and IL-12 oppose pro-inflammatory IL-23 in CD4+ T cells by inducing Blimp1. Nat Commun 5: 3770. doi: 10.1038/ncomms4770 PMID: 24796719
53. Neumann C, Heinrich F, Neumann K, Junghans V, Mashreghi MF, et al. (2014) Role of Blimp-1 in programming Th effector cells into IL-10 producers. J Exp Med 211: 1807–1819. doi: 10.1084/jem.20131548 PMID: 25073792
54. Kallies A, Hasbold J, Tarlinton DM, Dietrich W, Corcoran LM, et al. (2004) Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. J Exp Med 200: 967–977. PMID: 15492122
55. Ansari NA, Kumar R, Gautam S, Nylen S, Singh OP, et al. (2011) IL-27 and IL-21 are associated with T cell IL-10 responses in human visceral leishmaniasis. J Immunol 186: 3977–3985. doi: 10.4049/jimmunol.1003588 PMID: 21357266
56. O'Garra A, Vieira PL, Vieira P, Goldfeld AE (2004) IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. J Clin Invest 114: 1372–1378. PMID: 15545964
57. Pils MC, Pisano F, Fasnacht N, Heinrich JM, Groebe L, et al. (2010) Monocytes/macrophages and/or neutrophils are the target of IL-10 in the LPS endotoxemia model. Eur J Immunol 40: 443–448. doi: 10.1002/eji.200939592 PMID: 19941312
58. Taylor AP, Murray HW (1997) Intracellular antimicrobial activity in the absence of interferon-gamma: effect of interleukin-12 in experimental visceral leishmaniasis in interferon-gamma gene-disrupted mice. J Exp Med 185: 1231–1239. PMID: 9104810
59. Creteney E, Xin A, Shi W, Minnich M, Masson F, et al. (2011) The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. Nat Immunol 12: 304–311. doi: 10.1038/ni.2006 PMID: 21378976
60. Kallies A, Hawkins ED, Belz GT, Metcalf D, Hommel M, et al. (2006) Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. Nat Immunol 7: 457–465. PMID: 16565720
61. Martins GA, Cimmino L, Shapiro-Shelef M, Szabolcs M, Herron A, et al. (2006) Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat Immunol 7: 457–465. PMID: 16565721
62. Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, et al. (2009) Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science 325: 1006–1010. doi: 10.1126/science.1175870 PMID: 19698860
63. Findlay EG, Greig R, Stumhofer JS, Hafalla JC, de Souza JB, et al. (2010) Essential role for IL-27 receptor signaling in prevention of Th1-mediated immunopathology during malaria infection. J Immunol 185: 2482–2492. doi: 10.4049/jimmunol.0904019 PMID: 20831310
64. Anderson CF, Stumhofer JS, Hunter CA, Sacks D (2009) IL-10 regulates IL-10 and IL-17 from CD4+ cells in nonhealing Leishmania major infection. J Immunol 183: 4619–4627. doi: 10.4049/jimmunol.0904024 PMID: 19748991
65. Melero I, Grimaldi AM, Perez-Gracia JL, Ascierto PA (2013) Clinical development of immunostimulatory monoclonal antibodies and opportunities for combination. Clin Cancer Res 19: 997–1008. doi: 10.1158/1078-0432.CCR-12-2214 PMID: 23460531
Author/s: de Oca, MM; Kumar, R; Rivera, FDL; Amante, FH; Sheel, M; Faleiro, RJ; Bunn, PT; Best, SE; Beattie, L; Ng, SS; Edwards, CL; Muller, W; Cretney, E; Nutt, SL; Smyth, MJ; Haque, A; Hill, GR; Sundar, S; Kallies, A; Engwerda, CR

Title: Blimp-1-Dependent IL-10 Production by Tr1 Cells Regulates TNF-Mediated Tissue Pathology

Date: 2016-01-01

Citation: de Oca, M. M., Kumar, R., Rivera, F. D. L., Amante, F. H., Sheel, M., Faleiro, R. J., Bunn, P. T., Best, S. E., Beattie, L., Ng, S. S., Edwards, C. L., Muller, W., Cretney, E., Nutt, S. L., Smyth, M. J., Haque, A., Hill, G. R., Sundar, S., Kallies, A. & Engwerda, C. R. (2016). Blimp-1-Dependent IL-10 Production by Tr1 Cells Regulates TNF-Mediated Tissue Pathology. PLOS PATHOGENS, 12 (1), https://doi.org/10.1371/journal.ppat.1005398.

Persistent Link: http://hdl.handle.net/11343/262089

File Description: Published version

License: CC BY