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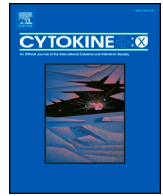
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Cytokines and splenic remodelling during *Leishmania donovani* infection

Marcela Montes de Oca^a, Christian R. Engwerda^b, Paul M. Kaye^{a,*}

^a York Biomedical Research Institute, Hull York Medical School, University of York, Heslington, York, United Kingdom

^b QIMR Berghofer Medical Research Institute, Infectious Diseases Division, Brisbane, Queensland, Australia

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ABSTRACT

Visceral leishmaniasis (VL) causes extensive splenic pathology that contributes to dysfunctional immune responses, in part through displacement and destruction of cell populations involved in maintaining splenic structural integrity. The expression of pro and anti-inflammatory cytokines and chemokines is crucial in orchestrating the delicate balance that exists between host resistance and tissue pathology. In an effort to restore homeostatic balance to the local microenvironment, remodelling of the splenic architecture occurs in a compartmentalised manner to retain some level of functionality, despite persistent inflammatory pressures. Animal models of VL as well as human studies have significantly contributed to our understanding of the architectural changes that occur in the spleen during VL. Here, we review the role of cytokines in mediating micro-architectural changes associated with the development of splenomegaly during VL.

1. Introduction

1.1. Visceral leishmaniasis and splenomegaly

Leishmaniasis is a parasitic disease caused by protozoan parasites of the genus *Leishmania* and is found in 90 countries throughout the tropics, subtropics and southern Europe [1]. Transmission occurs when a human host is bitten by an infected female hematophagous *Phlebotomine* sand fly during the taking of its blood meal [1]. Three main clinical forms of leishmaniasis include cutaneous, mucocutaneous and visceral leishmaniasis (VL; kala-azar). The latter is usually fatal if left untreated [2]. The World Health Organisation (WHO) estimates 700,000–1,000,000 cases of leishmaniasis occur annually [2]. VL predominantly affects systemic organs, notably the spleen, liver and bone marrow, though other sites may be involved particularly in the case of HIV-VL coinfection [3]. VL is predominantly caused by infection with *Leishmania donovani* and *L. infantum (chagasi)* [4]. Clinical features of VL include persistent low-grade fever, pancytopenia, hypergammaglobulinemia and hepatosplenomegaly resulting in distension of the abdomen and cachexia [5]. There is currently no licensed vaccine available and increasing drug resistance, costs and toxicity exacerbate the global burden of this disease [6]. Animal models of VL have significantly contributed to our understanding of disease progression and have aided the development of therapeutic strategies to combat the

devastating effects of this disease [reviewed in [7,8]]. Traditionally, experimental VL in mice is achieved by infection with amastigotes or metacyclic promastigotes of *L. donovani* or *L. infantum (chagasi)* intravenously via the lateral tail vein [9]. Natural transmission models of VL emphasise the importance of vector-transmitted VL to study responses to vaccination and better understand early events that occur at the bite site [10–14]. Importantly, a key feature of experimental VL in mice is the development of splenomegaly, a hallmark feature of human VL and numerous studies have highlighted changes in splenic architecture during chronic infection-induced inflammation [reviewed in [9,15]]. The study of splenomegaly in animals with VL has provided a useful model for studying the processes of normal splenic architectural remodelling that may be exaggerated during chronic disease [7,9,15].

Inflammation is a key driver of splenic remodelling in infection and other chronic inflammatory diseases. During VL, the balance between inflammatory and regulatory networks that serve to dampen inflammation in order to protect against host-tissue damage often determines disease outcome [9,15]. Numerous studies to date have shown how this cytokine-mediated war contributes to parasite persistence and consequently immunopathology [reviewed in [16,17]]. Splenic pathology in VL can be characterised by the loss of specific cell populations and the onset of vascular remodelling in an apparent effort to restore homeostasis [18–23]. This remodelling process combined with parasite presence disrupts the normal functioning of the spleen and as

* Corresponding author.

E-mail address: paul.kaye@york.ac.uk (P.M. Kaye).

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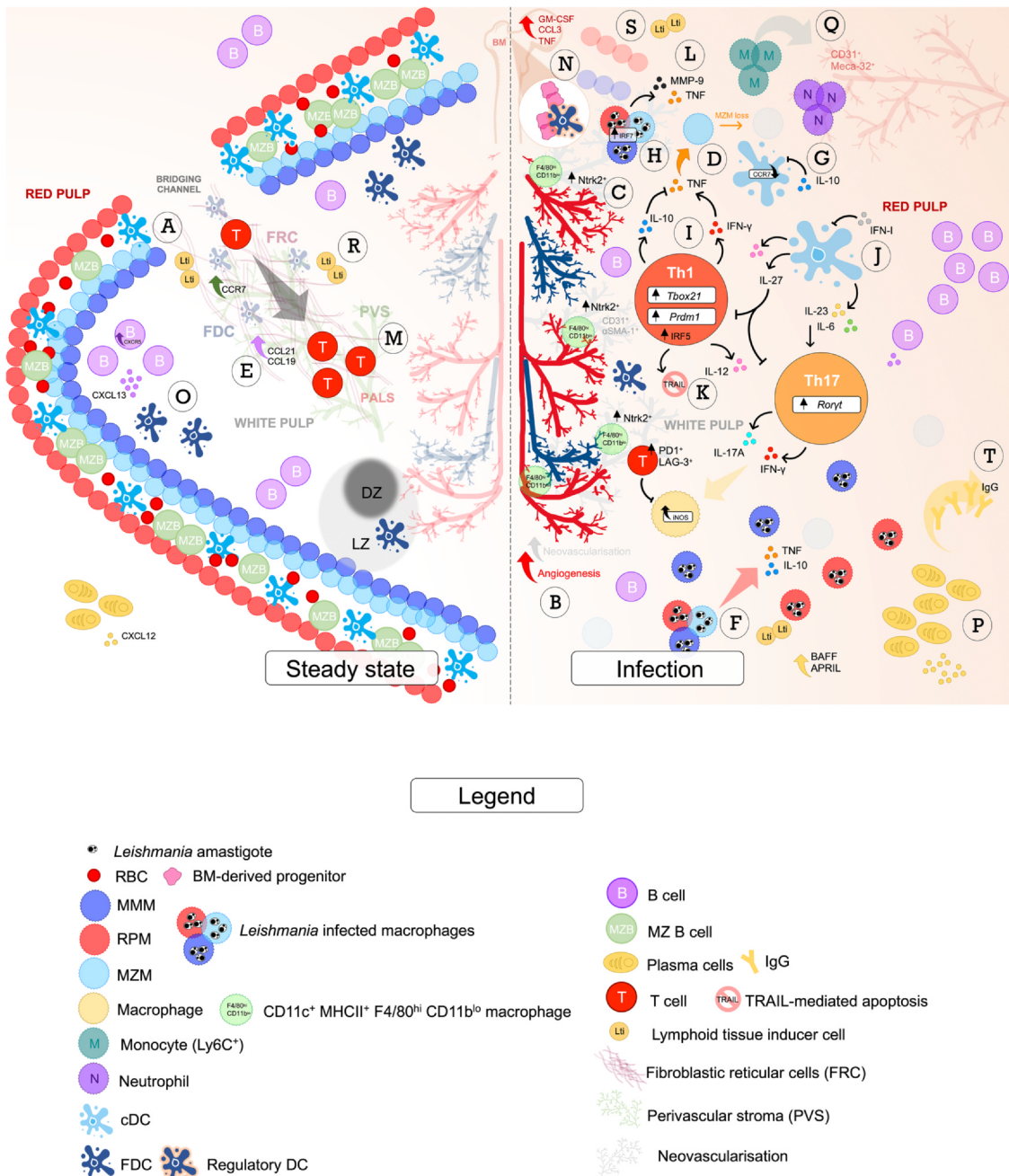


Fig. 1. Cytokine regulated alterations to the splenic microenvironment during experimental visceral leishmaniasis. Splenic architecture is highly compartmentalised in the steady state (left hand panel) and alters significantly post infection (right hand panel). Open circled letters A- T indicate key changes or underlying mechanism involved in this remodelling process (see text for further details). (A) In the steady state red and white pulp are segregated by a marginal zone punctuated by marginal zone bridging channels (B) Increased angiogenesis and neovascularisation during infection. (C) Neovascularisation of the white pulp was found to be closely associated with F4/80^{hi} CD11b^{lo} macrophages during infection. (D) TNF mediates killing of MZM (marginal zone macrophages) during infection. (E) MZM localisation maintained by CCL21 and CCL19 expression under steady state conditions. (F) Excessive TNF production in the red and white pulp induces IL-10 production. (G) IL-10 suppresses CCR7-dependent DC migration causing spatial segregation between DCs and T cells during infection. (H) IRF7 is upregulated in infected macrophages and is a source of TNF, IL-10 and MMP-9 during infection. (I) Blimp-1 (encoded by *Prdm1*) induces IL-10 production which serves to protect against TNF-mediated pathology during infection. (J) IFN-I suppress protective CD4⁺ T cell responses but is required for IL-10 production during infection. (K) TLR7-IRF5 pathway sensitises IFN γ ⁺ CD4⁺ T cells to TRAIL-mediated cell death during infection. (L) Disorganisation of the white pulp has been associated with high levels of MMP-9 during infection. (M) The perivascular stromal system (PVS) act as a pathway to guide T cells and DCs into appropriate areas of the spleen under steady state conditions. (N) Increased hematopoietic activity and stromal cells supports the development of regulatory DCs during infection. (O) CXCL13 production by FDCs and the stromal network is required for the maintenance of lymphoid follicles under steady state conditions. (P) Increased CXCL12, BAFF and APRIL prolongs plasma cell survival during infection. (Q) inflammatory monocytes specifically impaired the remodelling of Meca32⁺ and CD31⁺ vasculature in the red pulp during infection. (R) lymphoid tissue inducer (Lti) cells interact with immune and stromal populations to maintain the splenic architecture under steady state conditions. (S) Lti cells are displaced from the PALS to the red pulp during infection. (T) Increased production of IgG in the red pulp during infection. Abbreviations: FRC (fibroblast reticular cell) network, FDC (follicular dendritic cell) network, PALS (periarteriolar lymphoid sheath), DZ (dark zone), LZ (light zone). B: B cell, T: T cell, MZB: marginal zone B cell, M: monocyte, N: neutrophil. Size and shapes of cells are drawn for illustrative purposes only.

such results in dysfunctional immune responses. Here, we review recent developments in understanding how cytokines and their receptors play a role in coordinating microarchitectural changes in this organ during infection-associated splenomegaly. Most data are drawn from studies of the mouse, though reference to other models of VL are made where appropriate. Fig. 1 provides a pictorial framework for discussing these changes.

1.2. The spleen as a lymphoid organ

The development of the spleen can be broadly categorised into four stages; emergence of the vasculature, formation of the red pulp, development of the white pulp and periarteriolar lymphoid sheaths (PALS) and the assembly of follicles, the follicular dendritic cell (FDC) and fibroblastic reticular cell (FRC) networks [24]. The overall splenic architecture enables close contact with the blood, whereas the microvasculature serves to provide migratory routes specialised for lymphocyte trafficking to specific compartments. Under steady state conditions (Fig. 1A), the spleen is the largest, most highly organised lymphoid organ and is comprised of stromal and parenchymal structures [reviewed in [25]]. Comparative studies of VL across species are complicated by differences in the normal structure of rodent, canine and human spleen. In the murine spleen, the parenchyma can be divided into the red pulp, containing many red pulp macrophages (RPM) and white pulp which are separated by the marginal zone (MZ). The PALS and the lymphatic follicles are situated in the white pulp. The inner region of the PALS is populated mainly by T cells and the outer region mainly comprises B cells transiting to the follicles. The lymphatic follicles are made up of primary follicles containing small immature lymphocytes whereas secondary follicles are predominantly made up of germinal centres (GC) which include B cells and FDCs. The MZ demarcates the boundary between the white and red pulp and forms a sinus into which blood from branches of the central arteriole enters, carrying lymphocytes and other immune cells. The MZ is punctuated by marginal zone bridging channels that form entry points for migration of lymphocytes into the white pulp. Recent intravital microscopy showed that T cells attach to the perivascular stroma at these points via G protein coupled receptor (GPCR)-dependent mechanisms and subsequently engage in c-c chemokine receptor type 7 (CCR7)-dependent uni-directional migration via lymphocyte function-associated antigen (LFA)-1 and very late antigen (VLA)-4. This process provides one pathway by which infection/inflammation can alter T cell entry along perivascular homing paths [26].

1.3. Infection associated changes in splenic vasculature

In dogs, *Leishmania* infection resulted in a marked scarcity of the sinusoidal sheet in the white pulp, enhanced development of pulp venules, veins and reticular fibre development [27]. The first direct evidence that vascular remodelling accompanied progressive splenomegaly during murine VL was reported in 2010 [23]. Administration of the receptor tyrosine kinase inhibitor (RTKI) sunitinib maleate (Sm) restored splenic architecture by inhibiting vascular remodelling, and when combined with a first-line anti-leishmanial drug enhanced protective CD4⁺ T cell responses as well as interferon (IFN) γ and tumour necrosis factor (TNF) production [23]. Murine red and white pulp vasculature can be identified by the endothelial cell marker, Meca32 and was found to increase over the course of infection in the spleens of *L. donovani* infected mice [23]. Specifically, the red pulp vessels (Meca32⁺) show angiogenic potential; neovascularisation in the white pulp lead to the appearance of numerous CD31⁺ α SMA-1⁺ vessels that developed concurrent with compromise of the FRC and FDC networks (Fig. 1B) [23]. Collectively, these studies have begun to map the pathways of vascular remodelling in the spleen and how this may impact cytokine responses. For example, sunitinib treatment restored the FRC network and reduced *Il10* transcripts, suggesting enhanced CD4⁺ T

cell-APC interactions as a result of preserved structural integrity [23].

Mononuclear phagocytes (MPs) play a key role in inflammation-induced angiogenesis [28,29]. Interestingly, the neurotrophic tyrosine kinase receptor (Ntrk2) was found to be abnormally expressed on endothelial cells that form new, maturing blood vessels in the spleen during murine VL [30]. Neovascularisation of the white pulp was found to be closely associated with F4/80^{hi} CD11b^{lo} CD11c⁺ macrophages (Fig. 1C) [30]. This interaction of Ntrk2 with ligands produced by MPs was found to mediate pathological angiogenesis [30]. The microvasculature of the spleen and its close association with the stromal cell networks is thus becoming increasingly recognised as exerting an influence on local immunity in VL and as a model for other diseases involving chronic inflammation and splenomegaly.

1.4. White pulp remodelling during experimental VL

The tumour necrosis factor (TNF) family of cytokines play key roles in the development of secondary lymphoid organs, therefore it is perhaps not surprising that they are also important in splenic remodelling during infection [9]. In experimental murine VL, chronic infection becomes established in the bone marrow and the spleen [3,20]. Remodelling of the MZ, along with impaired lymphocyte trafficking, was shown to be mediated by TNF during murine VL (Fig. 1D) [20]. Previous studies reported the importance of marginal zone macrophages (MZM) localisation which was maintained by localised c-c chemokine ligand (CCL)21 and CCL19 expression (Fig. 1E) [31]. Importantly MZMs were shown to colocalise with CD31⁺ and CCL21⁺ endothelial cells [31]. Excessive TNF production in the red and white pulp regions of the spleen consequently induced IL-10 production (Fig. 1F) [3,32]. TNF was shown to mediate loss of MZM, since no direct role for cytotoxic T-lymphocyte (CTL) activity or CD95-mediated apoptosis was evident [20]. TNF also contributes to the loss of gp38⁺ stromal cells and consequently reduces CCL21 and CCL19 expression which leads to impaired cellular migration to the PALS, incomplete DC activation and reduced IL-12 production [21]. Ato *et al.*, described defective localisation of DCs due to TNF-dependent, IL-10 mediated inhibition of CCR7 (Fig. 1G) [32]. This spatial segregation of DCs and T cells, was reported to be the cause of impaired immune responses leading to ineffectual host resistance and consequently perpetuating splenic pathology [32]. More recently, interferon regulatory factor (IRF)7 was shown to be required for promoting IL-12 production by DCs and suppressing IL-10 production [33]. MZM and metallophilic macrophages (MMM) are well-positioned to encounter blood borne antigens and pathogens [34,35]. While MZM and MMM are highly phagocytic, MMM produce IFN α and are required for transport of antigen into B cell follicles [34,36,37]. During *L. donovani* infection, MZM and MMM were found to upregulate *Irf7*, associated with their intrinsic capacity to limit parasite multiplication (Fig. 1H) [34]. The suppressive role of IL-10 in VL has been extensively documented in promoting parasite persistence [17,38–44]. Recent studies showed that CD11c^{hi} DCs promote type-I regulatory (Tr1) cell expansion and contribute to splenomegaly independently of Tr1 cells [45]. Tr1 cells were shown to protect against IFN γ -dependent TNF-mediated killing of MZM, though DCs were not assessed in this latter study [46]. Tr1 cell induction occurred via IL-12-dependent upregulation of B-lymphocyte induced maturation protein (Blimp)-1 and was essential for IL-10 production (Fig. 1I) [46]. Importantly, these studies highlighted the protective role of IL-10 at the expense of parasite persistence. Furthermore, CD4⁺ T cells and DCs were critical sources of IL-10 during VL where IL-10 deficiency in either cell population improved parasite control at the expense of host tissue pathology and early MZM loss [38]. Regulatory T cells (Tregs) delayed the onset of splenic pathology and restricted leukocyte expansion [38]. Collectively, these studies identified the establishment of cell-specific immunoregulatory networks mediated by IL-10 that serve to protect against immunopathology during VL. Type I interferons (IFN-I) have been shown to be required for IL-10 induction and generation of Tr1

cells [47]. In VL, IFN-I signalling suppresses IFN γ production by CD4⁺ T cells, but is required to promote IL-10 production and Tr1 cells during infection and as such was found to regulate maintenance of MZM during infection and consequently splenic pathology (Fig. 1J) [48]. IL-27 and IL-21 have also been reported to maintain IL-10 levels during VL [49]. Consistent with these findings, IL-21 mRNA was upregulated by 4-fold even at day 60 post infection in the spleen [50]. These findings highlight the therapeutic potential for targeting IL-10 and IFN-I signalling pathways in the context of drug treatment for the purposes of improving disease outcome in VL.

During VL, T cell responses to leishmanial antigens are reduced [51], suggesting that these cells potentially become exhausted [52–54], undergo increased rates of apoptosis or develop into memory T cells [9]. It was initially thought that the memory T cell compartment was of a finite size [9]. Since then, it has been proposed that during chronic VL, splenomegaly promoted heterologous immunity and by-stander T cell expansion by resetting the size of the memory T cell pool [55]. Up until recently, the study of antigen-specific CD4⁺ T cell expansion, effector function, contraction and maintenance at the clonal level during VL remained challenging. Mou *et al.*, identified a dominant naturally processed peptide (PEPCK₃₃₅₋₃₅₁), that was found to be conserved across all *Leishmania* species and elicited strong, protective CD4⁺ T cell responses in humans and mice [56]. Future studies will provide insights into the molecular machinery that governs antigen-specific T cell responses and their contribution to splenomegaly during VL. An increased rate of apoptosis of CD4⁺ and CD8⁺ T cells was recently reported in the spleens of infected dogs and was rescued upon CD95 and CD95L blockade [57]. Fabie *et al.*, found that the TLR7-IRF5 pathway sensitised IFN γ ⁺ CD4⁺ T cells to TNF-related apoptosis inducing ligand (TRAIL)-mediated cell death via death receptor (DR)5 and caspase 8 to protect host tissue from excessive inflammatory damage (Fig. 1K) [58]. Another study showed CD4⁺ T cells exhibited increased programmed cell death protein (PD)-1 expression, whereas macrophages had elevated levels of PD-L2, suggesting that the PD-1 pathway promotes a macrophage phenotype during VL that is more permissive to the establishment of infection [53]. Given the highly diverse populations of macrophages within the spleen, this suggests that the promotion of infection-permissive macrophages may contribute to parasite persistence and such a microenvironment may foster the development of exhausted T cells. In accordance with these studies, cytotoxic T-lymphocyte-associated protein (CTLA)-4, T cell immunoglobulin and mucin domain-containing protein (TIM)-3 and lymphocyte activating (LAG)-3 were all found to be associated with splenic white pulp disorganisation and parasite load in dogs naturally infected with *L. infantum* [59]. Early reports identified a requirement for IFN γ -priming of macrophages and for their ability to produce TNF to control parasite growth [60]. In line with these reports, c-x-c motif chemokine ligand (CXCL)10 treatment was associated with a negative correlation between IL-10 and Tr1 cells [61]. Consequently, this resulted in reduced parasite burdens and splenomegaly but did not affect the ratio of white pulp area to total spleen area [61]. Since CXCL10 induces IFN γ secretion, it was suggested that improved macrophage activation contributed to parasite control as well as maintenance of the splenic architecture [61]. A transcriptional profiling study of the spleen during VL in the hamster model revealed that the IFN γ response did not restrain parasite growth or disease, supporting the accumulating evidence that macrophages are ineffectively activated to kill the parasite [62]. During *L. donovani* infection, γ/δ and CD4⁺ T cells are the main sources of IL-17 in the liver and spleen [63]. Additionally, IL-17A has been shown to synergistically act with IFN γ to induce nitric oxide (NO) production and leishmanicidal activity in infected macrophages in *L. infantum* infection [64]. Together, these data highlight the importance of cytokines acting in synergy to control infection. Moreover, the positive association between splenic architecture breakdown and disease severity has also been shown to contribute to parasite persistence in canine VL [65–67]. Associations between extracellular matrix

alterations and high parasite burdens have been previously reported [68,69]. Given the role of matrix metalloproteinase (MMP)-9 in cell migration and extracellular matrix degradation, it was postulated that aberrant MMP-9 levels seen in the serum of dogs with VL [69], would contribute to the development of splenomegaly by impairing cell migration. Moderate to intense white pulp disorganisation was associated with high levels of MMP-9 and lower CD4⁺ T cell numbers supporting a role for MMP-9 in assisting the migration of CD4⁺ T cells to appropriate areas in the spleen during infection (Fig. 1L) [68]. Collectively, these studies demonstrate the impact of architectural remodelling in the white pulp resulting in altered macrophage and T cell function, all of which aid parasite persistence in VL.

1.5. The impact of VL on FDC and FRC networks, germinal centers and follicles

The production of chemokines, adhesion molecules and growth factors are coordinated by specialised fibroblastic stromal cells in the spleen to maintain its highly organised structure [70]. All reticular cell subsets were shown to descend from multipotent periarterial progenitors depending on the microenvironment within the white pulp [71]. Stromal cells act as a pathway to guide T cells and DCs into appropriate areas of the spleen (Fig. 1M) [19]. Stromal cells are an important source of CCL21 and CCL19 [31], binding CCR7 which is expressed on mature DC, naïve T cells and a subset of memory T cells [32]. During VL, TNF mediates loss of gp38⁺ stromal cells [32]. DCs increase in number and in the presence of sufficient CCL21 and CCL19, they can migrate to PALS, but CCR7 expression is critical for their migratory capacity [32]. Previous reports identified a correlation between increased hematopoietic activity and stromal cells in supporting the development of regulatory DCs via CXCL12 and CCL8 [9,19,70]. CCL8 production by stromal cells demonstrated an enhanced capacity to promote hematopoietic progenitor differentiation into regulatory DCs (Fig. 1N) [70]. Collectively, these data show that increased haematopoietic activity gave rise to increased progenitor cells and when combined with regulatory stromal cell activity, favoured regulatory DC development, thus impeding development of effective anti-parasitic CD4⁺ T cells and parasite clearance.

Secondary lymphoid follicles or B cell zones within the white pulp play important roles in the formation of germinal centres. Splenic GCs fail to develop in *Lta*^{-/-}, *Ltb*^{-/-}, *Tnfr*^{-/-} or *Tnfr1*^{-/-} mice, suggesting an important role for the TNF-family of cytokines in GC formation [72–75]. The maintenance of lymphoid follicles is dependent on CXCL13 production by FDCs and the stromal network (Fig. 1O). B cells require CXCL13 mediated entry into follicles near DCs. Concurrent with these studies, low CXCL13 mRNA was found in dogs with disorganised lymphoid tissue during VL [76]. The number of DCs was decreased in follicles and B cells were reduced in follicles as well as the marginal zone [76]. Previous studies have shown that aberrant CXCL13 or CXCR5 expression impairs B cell migration into the follicle and GC formation [77,78]. In support of this, infected dogs had a lower frequency of lymphoid follicles and a higher density of plasma cells (Fig. 1P) [79]. Polyclonal B cell activation and hypergammaglobulinemia are other key features of VL [80]. *L. donovani* amastigotes were shown to activate B cells by triggering endosomal TLRs which induced IL-10 and IFN-I and promoted hypergammaglobulinemia during VL [80].

Within germinal centres, FDCs regulate B cell proliferation, isotype switching and somatic hypermutation [reviewed in [15,81]]. Earlier studies showed that lymphotoxin signalling was required for functional development of FDCs in the spleen [82,83]. During VL, FDC destruction leads to GC loss and is associated infiltration of heavily parasitised macrophages into the GC [18]. FDC destruction was associated with high parasite load, where drug treatment prolonged FDC survival [18]. Parasite-derived molecules excreted from infected macrophages or released when heavily infected macrophages burst may be directly

responsible for FDC destruction [18].

Taken together, these studies emphasise the importance of the stromal network in the spatial organisation of DCs, T cells and macrophages during VL. Importantly, the pathogenic role of TNF in mediating the destruction of these networks highlights the need to further understand the molecular mechanisms governing TNF signalling during disease.

1.6. Red pulp remodelling during experimental VL

Expansion of the red pulp vascular system is a key feature that accompanies splenomegaly during VL [22]. The mechanisms regulating compartment-specific remodelling of the vasculature in the spleen have recently been partially uncovered. Depletion of inflammatory monocytes specifically impaired the remodelling of Meca32⁺ and CD31⁺ vasculature in the red pulp, but did not affect FDC, FRC networks, nor white pulp vascularisation (Fig. 1Q) [22]. This study identified that during the development of splenomegaly, specific cell populations can only modify their local microenvironment. MMP-9 has been implicated as a proangiogenic factor in neutrophils [84] and was highly abundant in neutrophils in VL [22]. However, since depletion of neutrophils had no effect on vascular remodelling of the red pulp during VL, it is unlikely that MMP-9 plays a major role in this process [22]. Lymphoid tissue inducer (Lti) cells are known to interact with immune and stromal populations, indicating their importance in maintaining the splenic architecture (Fig. 1R) [85]. A recent study reported on the displacement of Lti cells from the PALS to the red pulp, supporting their role in maintaining the structural integrity of the spleen (Fig. 1S) [85]. The correlation between increased plasma cell numbers in the red pulp during VL-induced splenomegaly was initially reported in 2008 [65]. CXCL12, BAFF and APRIL are important in prolonging plasma cell survival [reviewed in [86]]. Elevated serum IgG and BAFF have been shown to contribute to splenomegaly in VL, since *Baff*^{-/-} mice have smaller spleens [87,88]. Consistent with previous reports, increased levels of BAFF, APRIL and CXCL12 was associated with increased plasma cell retention, production of IgG and extended survival within the red pulp during VL (Fig. 1T) [89]. Collectively, the promotion of plasma cell retention in the red pulp, rather than migration to the bone marrow, suggests extrafollicular differentiation of these cells where inflammatory conditions in the spleen create a survival niche for plasma cells and thereby contributes to hypergammaglobulinemia during VL [89]. Recently, hyperactive plasmablasts were shown to impede GC responses via nutrient deprivation in malaria, suggesting a similar mechanism may also favour plasma cell survival in VL [90]. Polyclonal B cell activation and hypergammaglobulinemia are characteristic features of VL [80]. Silva-Barrios *et al.*, demonstrate that *L. donovani* amastigotes activate B cells via endosomal TLRs which induces IL-10 and IFN-I to signal in a positive feedback loop to sustain cytokine production by B cells to further exacerbate disease and promote hypergammaglobulinemia [80]. Importantly, these studies illustrate that *Leishmania* induces compartment specific remodelling in the spleen and in doing so, contributes to exacerbated pathological outcomes observed in VL. Furthermore, these studies delineate the differing role of cytokines and their influences in the red pulp compared to the white pulp, further supporting the evidence of compartment-specific mechanisms of remodelling.

1.7. Outlook

Understanding immune cell biology during experimental VL has been a challenge, as the function of these cells heavily relies on their local microenvironment and is significantly altered once removed from their original environment. Recent studies have focused on establishing correlations between degree of structural tissue breakdown and cytokine levels or other signalling molecules that may provide insights into the underlying factors that contribute to the architectural breakdown of

the spleen during VL. Intravital imaging studies have been key in studying cellular interactions in real-time within their local microenvironment. Future spatially resolved highly multiplexed analysis of protein expression and mRNA abundance transcriptomic studies will provide greater insights into the key processes that occur within each defined compartment of the spleen and thus our understanding of this important aspect of VL pathogenesis.

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CRediT authorship contribution statement

Marcela Montes de Oca: Writing - original draft, Writing - review and editing, Visualisation - drawing of schematic and Formal analysis of literature. **Christian R. Engwerda:** Writing - review & editing. **Paul M. Kaye:** Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Centers-for-Disease-Control-and-Prevention, Parasites - Leishmaniasis; Epidemiology and Risk Factors, 2020. < <https://www.cdc.gov/parasites/leishmaniasis/epi.html> > .
- [2] World-Health-Organization, Leishmaniasis, 2020. < <https://www.who.int/news-room/fact-sheets/detail/leishmaniasis> > .
- [3] C.R. Engwerda, M.L. Murphy, S.E.J. Cotterell, S.C. Smelt, P.M. Kaye, Neutralization of IL-12 demonstrates the existence of discrete organ-specific phases in the control of *Leishmania donovani*, Eur. J. Immunol. 28 (1998) 669–680, [https://doi.org/10.1002/\(SICI\)1521-4141\(199802\)28:02<669::AID-IMMU669>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1521-4141(199802)28:02<669::AID-IMMU669>3.0.CO;2-N).
- [4] R. Baker, C. P., P.M. Kaye. Leishmaniasis, The Granulomatous Diseases, Cambridge University Press, 1999, pp. 212–234.
- [5] E.E. Zijlstra, A.M. El-Hassan, 3. Visceral leishmaniasis, Transactions of the Royal Society of Tropical Medicine and Hygiene 95 (2001) S27–S58. [https://doi.org/10.1016/S0035-9203\(01\)90218-4](https://doi.org/10.1016/S0035-9203(01)90218-4).
- [6] A. Kumar, S.C. Pandey, M. Samant, A spotlight on the diagnostic methods of a fatal disease Visceral Leishmaniasis, Parasite Immunol. (2020), <https://doi.org/10.1111/pim.12727>.
- [7] E.N. Loría-Cervera, F.J. Andrade-Narváez, Animal models for the study of leishmaniasis immunology, Rev Inst Med Trop Sao Paulo 56 (2014) 1–11, <https://doi.org/10.1590/S0036-46652014000100001>.
- [8] R.J. Faleiro, R. Kumar, L.M. Hafner, C.R. Engwerda, Immune Regulation during Chronic Visceral Leishmaniasis, PLoS Negl. Trop. Dis. 8 (2014) e2914, <https://doi.org/10.1371/journal.pntd.0002914>.
- [9] P.M. Kaye, et al., The immunopathology of experimental visceral leishmaniasis, Immunol. Rev. 201 (2004) 239–253, <https://doi.org/10.1111/j.0105-2896.2004.00188.x>.
- [10] H. Aslan, et al., A new model of progressive visceral leishmaniasis in hamsters by natural transmission via bites of vector sand flies, J Infect Dis 207 (2013) 1328–1338, <https://doi.org/10.1093/infdis/jis932>.
- [11] N.C. Peters, et al., Vector transmission of leishmania abrogates vaccine-induced protective immunity, PLoS Pathog 5 (2009) e1000484, <https://doi.org/10.1371/journal.ppat.1000484>.
- [12] N.C. Peters, et al., In vivo imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies, Science 321 (2008) 970–974, <https://doi.org/10.1126/science.1159194>.
- [13] I. Martín-Martín, M. Jiménez, E. González, C. Eguiluz, R. Molina, Natural transmission of *Leishmania infantum* through experimentally infected *Phlebotomus perniciosus* highlights the virulence of *Leishmania* parasites circulating in the human visceral leishmaniasis outbreak in Madrid, Spain, Veterinary Res. 46 (2015) 138, <https://doi.org/10.1186/s13567-015-0281-1>.
- [14] S. Kamhawi, H. Aslan, J.G. Valenzuela, Vector saliva in vaccines for visceral leishmaniasis: a brief encounter of high consequence? 99–99, Front Public Health 2 (2014), <https://doi.org/10.3389/fpubh.2014.00099>.
- [15] A.C. Stanley, C.R. Engwerda, Balancing immunity and pathology in visceral leishmaniasis, Immunol. Cell Biol. 85 (2007) 138–147, <https://doi.org/10.1038/sj.icb7100011>.
- [16] A. Dayakar, S. Chandrasekaran, S.V. Kuchipudi, S.K. Kalangi, Cytokines: Key

- 2010.03.017.
- [70] A.T. Nguyen Hoang, et al., Stromal Cell-Derived CXCL12 and CCL8 Cooperate To Support Increased Development of Regulatory Dendritic Cells Following Leishmania Infection, *J. Immunol.* (2010) 0903673, <https://doi.org/10.4049/jimmunol.0903673>.
- [71] H.-W. Cheng, et al., Origin and differentiation trajectories of fibroblastic reticular cells in the splenic white pulp, *Nat. Commun.* 10 (2019) 1739, <https://doi.org/10.1038/s41467-019-09728-3>.
- [72] P.A. Koni, et al., Distinct roles in lymphoid organogenesis for lymphotoxins alpha and beta revealed in lymphotoxin beta-deficient mice, *Immunity* 6 (1997) 491–500, [https://doi.org/10.1016/s1074-7613\(00\)80292-7](https://doi.org/10.1016/s1074-7613(00)80292-7).
- [73] H. Korner, et al., Distinct roles for lymphotoxin-alpha and tumor necrosis factor in organogenesis and spatial organization of lymphoid tissue, *Eur J Immunol* 27 (1997) 2600–2609, <https://doi.org/10.1002/eji.1830271020>.
- [74] M. Matsumoto, et al., Role of lymphotoxin and the type I TNF receptor in the formation of germinal centers, *Science* 271 (1996) 1289–1291, <https://doi.org/10.1126/science.271.5253.1289>.
- [75] M. Pasparakis, L. Alexopoulou, V. Episkopou, G. Kollias, Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response, *J Exp Med* 184 (1996) 1397–1411, <https://doi.org/10.1084/jem.184.4.1397>.
- [76] J.S. Silva, et al., Low CXCL13 Expression, Splenic Lymphoid Tissue Atrophy and Germinal Center Disruption in Severe Canine Visceral Leishmaniasis, *PLoS ONE* 7 (2012) e29103, , <https://doi.org/10.1371/journal.pone.0029103>.
- [77] R. Förster, et al., A Putative Chemokine Receptor, BLR1, Directs B Cell Migration to Defined Lymphoid Organs and Specific Anatomic Compartments of the Spleen, *Cell* 87 (1996) 1037–1047, [https://doi.org/10.1016/S0092-8674\(00\)81798-5](https://doi.org/10.1016/S0092-8674(00)81798-5).
- [78] K.M. Ansel, et al., A chemokine-driven positive feedback loop organizes lymphoid follicles, *Nature* 406 (2000) 309–314, <https://doi.org/10.1038/35018581>.
- [79] S.O. Santos, et al., A minimally invasive approach to spleen histopathology in dogs: A new method for follow-up studies of spleen changes in the course of Leishmania infantum infection, *Comp. Immunol. Microbiol. Infect. Dis.* 48 (2016) 87–92, <https://doi.org/10.1016/j.cimid.2016.08.007>.
- [80] S. Silva-Barrios, et al., Innate Immune B Cell Activation by Leishmania donovani Exacerbates Disease and Mediates Hypergammaglobulinemia, *Cell Reports* 15 (2016) 2427–2437, <https://doi.org/10.1016/j.celrep.2016.05.028>.
- [81] V. Rodrigues, A. Cordeiro-da-Silva, M. Laforge, R. Silvestre, J. Estaquier, Regulation of immunity during visceral Leishmania infection, *Parasites Vectors* 9 (2016) 118, <https://doi.org/10.1186/s13071-016-1412-x>.
- [82] J. Den Haan, R. Mebius, G. Kraal, Stromal cells of the mouse spleen, *Front. Immunol.* 3 (2012) 201.
- [83] A. Fütterer, K. Mink, A. Luz, M.H. Kosco-Vilbois, K. Pfeffer, The Lymphotoxin beta Receptor Controls Organogenesis and Affinity Maturation in Peripheral Lymphoid Tissues, *Immunity* 9 (1998) 59–70, [https://doi.org/10.1016/S1074-7613\(00\)80588-9](https://doi.org/10.1016/S1074-7613(00)80588-9).
- [84] V.C. Ardi, T.A. Kupriyanova, E.I. Deryugina, J.P. Quigley, Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis, *Proc Natl Acad Sci U S A* 104 (2007) 20262–20267, <https://doi.org/10.1073/pnas.0706438104>.
- [85] C.V.B.D. Melo, et al., Phenotypical Characterization of Spleen Remodeling in Murine Experimental Visceral Leishmaniasis, *Front. Immunol.* 11 (2020) 653.
- [86] L. Khodadadi, Q. Cheng, A. Radbruch, F. Hiepe, The Maintenance of Memory Plasma Cells, 721–721, *Frontiers in immunology* 10 (2019), <https://doi.org/10.3389/fimmu.2019.00721>.
- [87] S. Omachi, et al., B-cell activating factor deficiency suppresses splenomegaly during Leishmania donovani infection, *Biochem. Biophys. Res. Commun.* 489 (2017) 528–533, <https://doi.org/10.1016/j.bbrc.2017.06.005>.
- [88] Y. Goto, S. Omachi, C. Sanjoba, Y. Matsumoto, Elevation of Serum B-Cell Activating Factor Levels During Visceral Leishmaniasis, *The American Journal of Tropical Medicine and Hygiene* 91 (2014) 912–914, <https://doi.org/10.4269/ajtmh.14-0260>.
- [89] J. Silva-O'Hare, et al., Disruption of Splenic Lymphoid Tissue and Plasmacytosis in Canine Visceral Leishmaniasis: Changes in Homing and Survival of Plasma Cells, *PLoS ONE* 11 (2016) e0156733, , <https://doi.org/10.1371/journal.pone.0156733>.
- [90] R. Vijay, et al., Infection-induced plasmablasts are a nutrient sink that impairs humoral immunity to malaria, *Nat Immunol* (2020), <https://doi.org/10.1038/s41590-020-0678-5>.