Am. J. Trop. Med. Hyg., 100(4), 2019, pp. 816–821
doi:10.4269/ajtmh.18-0350

Abnormal B-Cell Subset and Blimp-1–Mediated Humoral Responses Associated With Visceral Leishmaniasis Pathogenesis

Bhawana Singh, Om Prakash Singh, Neetu Singh, Siddharth Sankar Singh, and Shyam Sundar*
Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India

Abstract. B-cells have a spectrum of functions ranging from antibody production to antigen presentation and have additional vital roles in immune mechanisms. There is rudimentary knowledge about the role of B-cells in intracellular infections with contradictory findings. We explored the role of B-cell dysfunctions in visceral leishmaniasis (VL) pathogenesis in terms of the phenotypic and functional properties of B-cells during the course of disease. This study was performed on blood and splenic aspirates (SA) of VL cases pre- and post-treatment. Whole blood was used for flow cytometric studies for determining the profiles of B-cells at different time-points of treatment. Peripheral blood mononuclear cells were used for magnetic purification of B-cells, for transcriptional studies by real-time polymerase chain reaction (RT-PCR). Serum/plasma was used for direct agglutination test for determining parasite-specific antibodies and SA were used for scoring the presence of parasite by microscopic examination. Flow cytometric studies depicted decreased B-cell percentages during the entire course of disease and attainment of exhaustive phenotype with tissue-like memory cell markers, indicative of B-cell dysfunctions in VL. In addition, B-cells had compromised abilities of antigen processing and presentation and altered levels of B-lymphocyte–induced maturation protein-1 (Blimp-1). Blimp-1 expression goes hand in hand with B-cell maturation antigen and transmembrane activator and calcium modulator (TACI) and cyclophilin ligand interactor, suggestive of its role in promoting plasma cell survival and antibody production. Elevated level of VL-specific antibody titre was directly correlated with exhausted phenotype and also with disease severity during VL. This study indicated for impaired B-cell functions during chronic infection which may lead to pathological consequences in human VL.

INTRODUCTION

Visceral Leishmaniasis (VL) is one of the major neglected tropical diseases caused by the protozoan parasite *Leishmania donovani* and characterized by prolonged fever, anemia, splenomegaly, pancytopenia, hypergammaglobulinemia, and death if left untreated. B-cell dysfunctions as marked by hypergammaglobulinemia and presence of nonspecific (polyclonal) antibodies are hallmarks of human VL.[1] Furthermore, B-cells have been known to cause disease exacerbation during *Leishmania* infection,[2] with controversial role of antibodies in disease pathogenesis.[3,4]

Apart from the classical role of antibody production, B-cells also serve as professional antigen presenting cells, provide costimulatory signals, produce cytokines, and can have immunoregulatory properties. B-cell–associated dysfunctions have mostly been reported in viral infections such as human immunodeficiency virus (HIV).[5] In the recent past, a lot of studies have been performed to understand the role of B-cells in intracellular infections, without any clear evidence for the role of B-cells and its effector mechanisms in determining the disease pathogenesis. Not much is known about their cytokine producing, antigen processing, and presentation capacity. However, the less appreciated B-cells have been divided depending on the lineage and differentiation markers into naïve B-cells, immature B-cells, plasma cells, regulatory B-cells (Bregs), and memory B-cells. Memory B-cell subsets have further been classified into classical, active, and tissue-like memory (TLM) or atypical B-cells, based on the combined expression patterns of CD21 and CD27.

The outcomes of B-cell–mediated humoral responses are under the regulatory control of broad-spectrum regulatory cytokine, interleukin-10 (IL-10).[6] In addition, molecular factors such as Blimp-1 (B-lymphocyte induced maturation protein-1), BCMA (B-cell maturation antigen), and TACI (Transmembrane activator and calcium modulator and cyclophilin ligand interactor) from tumor necrosis factor (TNF) receptor superfamily serve as decision-makers for B-cell homeostasis, controlling their proliferation, differentiation, and apoptosis.[7]

Several hypotheses explain the role of B-cells in pathology or protection, but scarce data are available for the functional characteristics of B-cells in human VL. There are numerous phenotypic and functional anomalies in B-cells of VL cases. We found B-cell exhaustion in VL and decreased resting memory B-cells throughout the course of disease. The exhausted B-cell phenotype was also marked by compromised human leukocyte antigen (HLA-DR)–dependent antigen presentation abilities that were under the influence of the master regulator, Blimp-1. Nevertheless, antibodies have been major immune regulators, produced by B-cells. We found that antibodies could potentially serve as a crucial factor driving VL pathogenesis by improving the plasma cell survival and differentiation via. IL-10, Blimp-1, and TNF receptor superfamily members—BCMA and TACI—therefore, providing better understanding of the mechanism of B-cell–mediated antibody-dependent and independent immune mechanisms, and why these responses are compromised during *Leishmania* infection could be of great relevance to vaccine biology. Most of the studies on the contribution of B-cells in VL development have been performed on the animal model, mostly with B-cell–deficient mice. Nevertheless, in human VL, B-cell functions have not been clearly understood. By studying B-cells as targets for immune augmentation, we can explore the ways of targeting the B-cells and its antigen-presentation machinery for therapeutic advantage.

MATERIALS AND METHODS

Study subjects. The study was carried out at the Infectious Diseases Research Laboratory of the Department of Medicine, Banaras Hindu University, Varanasi, India, and at its field

*Address correspondence to Shyam Sundar, Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India. E-mail: drshyamsundar@hotmail.com

site Kala-Azar Medical Research Centre (KAMRC), Muzaffarpur, Bihar, India. The study was approved by the Ethics Committee of the Institute of Medical Sciences, Banaras Hindu University (BHU), and all subjects provided written informed consent.

The diagnosis was based on the presence of typical symptoms and confirmed by detection of amastigotes in splenic aspirates (SA) and/or serologically by a presence of antibodies to recombinant K39-antigen using dip-stick assay. In total, 235 subjects were used in the study, and venous blood was collected from patients and endemic controls (EC); age > 12 years, negative for human immunodeficiency virus, and non-pregnant candidates were enrolled for the study under the inclusion criteria. All EC were healthy household members of patients. Blood and SA samples were transported from KAMRC, at 15–18°C and 4–8°C, respectively, to BHU, Varanasi, where they were processed within 24 hours of collection.

Direct agglutination test (DAT). Direct agglutination test was performed following the standard procedures described elsewhere[8] and using a freeze-dried version of fixed, trypsin-treated and stained promastigotes of *L. donovani* prepared in ITM-Antwerp, Belgium.[9] Direct agglutination test reagent was supplemented with 2% Ultroser G (Pall, Bio Sepra, Port Washington, NY) to inhibit nonspecific agglutination[8]; 2.5 µL of serum/plasma sample was diluted in 2,000 µL of DAT dilution buffer (phosphate buffer saline [PBS], pH 7.2, supplemented with protein). One-hundred microliters of this prepared stock was transferred to a well of the first column of the V-shaped microtitre plate (8 × 12 wells; Greiner, Frickenhausen, Germany). The rest of the wells were then dispensed with 50 µL of DAT diluents with 2 mercapto-ethanol (preparation: 0.24 mL 2-ME per vial of 30 mL of DAT diluent). A serial dilution of 1:400 up to 1:3,276,800 was obtained by mixing and by transferring 50 µL from the first well to the subsequent wells. One positive and one negative control were included in each plate. Wells in the last row were kept for antigen control. Fifty microliters of properly mixed and re-suspended DAT antigen was dispensed to every well. Plates were then sealed, shaken gently, and incubated overnight in a horizontal position at ambient temperature.

The DAT results (i.e., agglutinating titre) were read against a white background by two independent readers. Samples with a titre ≥ 1:1,600 were considered positive. This titre, which is lower than the one used for VL diagnosis in clinical suspects (1:3,200), was chosen to increase the sensitivity to detect *L. donovani* infection.[10,11] The different grades of splenic scores were used for correlational analysis with the antibody titres obtained from DAT (Table1).

Flow cytometry. Peripheral blood cells were stained with fluorescent markers conjugated antibodies at KAMRC, fixed and transported to Banaras Hindu University for analysis. Antibodies for CD27, CD21, CD19, CD20 (from BD Biosciences), CD267 (TACI) PE, and CD269 (BCMA) (from Miltenyi Biotec, Bergisch Gladbach, Germany) were used for B-cell analysis in pretreatment (D-0), posttreatment (D-30 or DIS), 6 months after cure/follow-up (6M), and endemic controls. The samples were used for B-cell analysis in separate staining panels, incubated for 30 minutes in dark. Staining was performed according to manufacturer's instructions, followed by washing in PBS by centrifugation and resuspension in PBS with 0.1% bovine serum albumin (BSA) for flow cytometry. Gates for positive and negative samples were set using unstained samples, single stained controls and in method setup with fluorescence minus one controls. All the samples were acquired using FACS CALIBUR model 4CS, (BD Biosciences, Franklin Lakes, NJ) and analyzed by Flowjo.

Magnetic purification of B-cells and real-time PCR. Peripheral blood mononuclear cells were isolated from venous blood and subsequently used for B-cell enrichment using $CD19^+$ microbeads (Milteneyi), suspended in RNA later (Qiagen GmbH, Hilden, Germany), and used for total RNA isolation using RNeasy tissue kit (Qiagen GmbH) according to the manufacturer's instructions. Sample quality and integrity was assessed by ND-2000 spectrophotometer (Thermo Fischer Scientific, Wilmington, DE) and agarose (Sigma Aldrich Chemicals, St. Louis, MO) gel electrophoresis. Thousand nanograms of RNA was reverse transcribed using the high-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA) as per manufacturer's instructions. Real-time PCR was performed on ABI 7500 platform (Applied Biosystems) using SYBR green–based assays for the following genes—Blimp-1 (forward 5′-AACGTGTGGGTACGACCTTG-3′; reverse, 5′-ATTTTCATGGTCCCCTTGGT-3′),[12] TaqMan-based gene expression assays were performed using cDNA-specific FAM-MGB–labeled primer/probe (carboxyfluorescein) for HLA-DR, MHC class II transactivtor (CIITA), and CD74. Genes were normalized using glyceraldehyde-3-phosphatepi (GAPDH)[12] for SYBR green–based assays and VIC-MGB–labeled (2′-chloro-7′phenyl-1,4-dichloro-6-carboxyflurescein) 18S rRNA as endogenous control for Taqman assay, respectively, with appropriate no-reverse transcriptase (RT) and no-template controls included in each plate. The relative quantification of products was determined by the number of cycles over GAPDH or 18S mRNA endogenous control required to detect the gene expression of interest. The results obtained were analyzed by using 7500 software v.2.0.1 (Applied Biosystems, Foster City, CA).

Statistical analysis. Statistical analysis was performed using Graph pad prism 5 (version 5.00 for Windows; Graph Pad Software, San Diego, CA). Groups were compared using nonparametric Mann–Whitney test. When applicable, paired *t*-test or Wilcoxon matched paired tests were used. For association studies between different variables correlation analysis was performed using Pearson's test.

TABLE 1
Different splenic score grade in VL cases with reference to their corresponding range of antibody titres

S.No.	Splenic Slide Score (Chulay and Bryceson 1983)	Number of VL cases	Antibody titers
1	1 + (1–10 parasite/1,000 field)	11	1:25,600–1:819,200
2	2 + (1–10 parasite/100 field)	14	1:102,400–1:3,276,800
3	3 + (1–10 parasite/10 field)	12	1:819,200–1:3,276,800
4	4 + (1–10 parasite/field)	12	1:3,276,800

VL = visceral leishmaniasis.

RESULTS AND DISCUSSION

Visceral leishmaniasis infection leads to noticeable B-cell defects as lymphocytopenia, hyperactivation, and hypergammmaglobulinemia that are normally absent or appear at low levels in healthy individuals. Chemotherapy induces reversible alterations after successful treatment, suggesting a relationship with parasite survival and disease pathogenesis. The diverse functions of B-cells in the context of VL pathogenesis have not been assessed in human VL. There are contradictory

reports on B-cells without any clear evidences for protective or pathological functions during disease pathogenesis. In this study, we tried to explore this area to demonstrate the significance of B-cells in VL pathogenesis.

We hypothesized that VL is associated with significant B-cell dysfunctions and looked at phenotype and transcriptional properties of B-cells and their significance in VL manifestation. Considering the total of 235 subjects included in the entire study, B-cell kinetics study by flow cytometric analysis of $CD19^+$ cells in peripheral blood was performed. Figure 1 shows the representative gating strategy used for the analyzing the B-cell population. We observed reduced frequency of $CD19^+$ B-cells in active cases which tend to increase (almost 2-fold) after 30 days of drug treatment and 6 months posttreatment (Figure 2A). This might be suggestive of detrimental effects of *Leishmania* infection on the B-cell functioning and responsiveness that gradually resolves on treatment and with reduction in the parasite load.

We were next interested to phenotypically determine the frequency of different B-cells subsets. Flow cytometric studies revealed for the subsets of B-cells as naïve B-cells ($CD27^-CD21^+$), plasma cells ($CD20^-CD21^-$), activated memory B-cells ($CD21^-CD27^+$), resting memory ($CD21^+CD27^+$), and TLM B-cells ($CD21^-CD27^-$). Flow cytometric analysis provided the evidences for the elevated frequencies of plasma cells at the pretreated stage, whereas TLM B-cell population significantly came up during course of VL at posttreatment stages (Figure 2B and C). The increased plasma cell proportions can be attributed to the hypergammaglobulinemia during the disease, whereas the TLM population has been known to be functionally impaired and termed as exhausted B-cells as reported from HIV[13] and malaria.[14] These TLM B-cells were increased during chronic phase of disease but gradually declines on treatment over a period of 6 months as reported in HIV infection.[13] We propose that the aberration in B-cell distribution during VL reflects the alterations in B-cell populations.

We next examined the antigen presentation capacity as readout of functional impairment in B-cells during the course of disease. We observed decreased HLA-DR transcripts and its invariant chain CD74 during active disease as compared with post-treated cases and endemic controls. Looking for the upstream regulatory elements of antigen-presentation machinery of HLA-DR viz. MHC class II transactivtor and Blimp-1, we found dampened expression of CIITA at pre-treatment. This was accompanied with significantly elevated transcript levels of master regulator Blimp-1 in pre-treated cases which is assumed to negatively orchestrate transcriptional regulators of antigen presentation machinery in B-cells and also drives differentiation of B-cells into plasma cells (Figure 3A) in line with the previously reported research.[15] It can be assumed that Blimp-1 represses the B-cell–specific promoter of the human gene that encodes CIITA in a binding site-dependent manner as already known.[16] Decreased CIITA also correlates with increased BLIMP-1 during plasma cell differentiation in

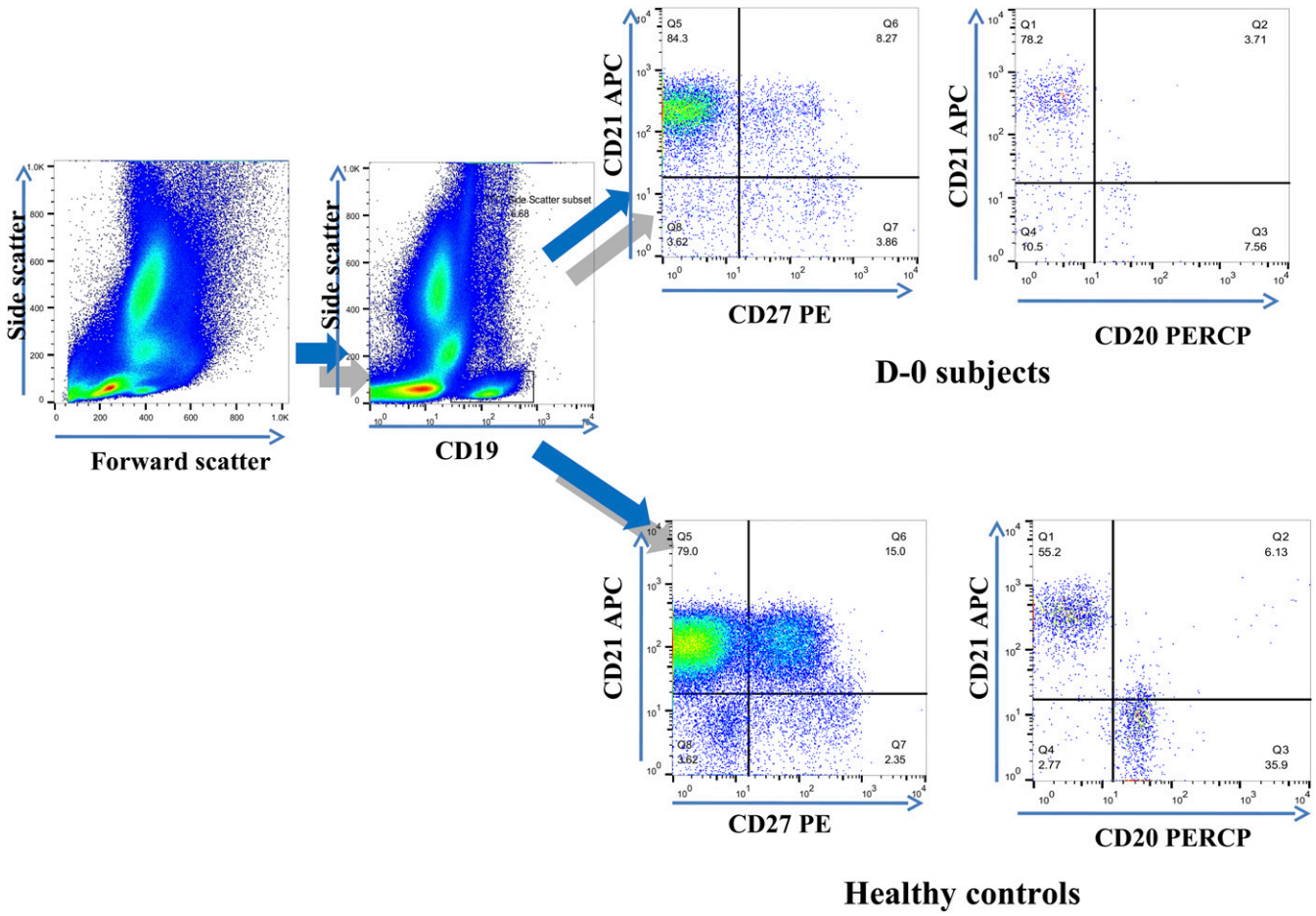

FIGURE 1. Gating strategy for analysis of B-cell populations. This figure appears in color at www.ajtmh.org.

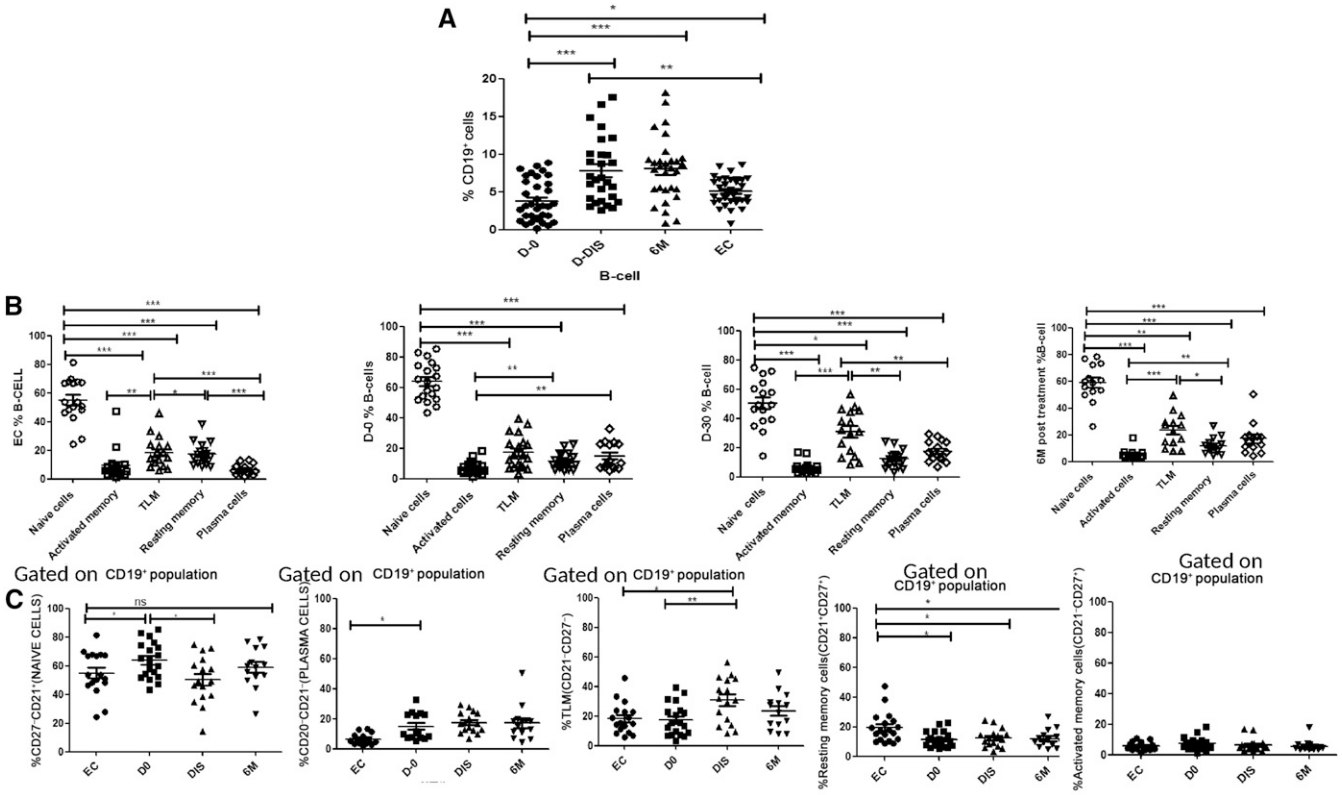

FIGURE 2. Distribution of different B-cell subsets at different stages of disease (pre- and posttreatment, and follow-up after 6 months of treatment) as compared with healthy controls. (**A**) Effect of visceral leishmaniasis infection on the frequency of B-cells at different time-points (pre-treatment, 30 days, and 6 months) of treatment compared with the healthy controls. (**B**) Differential kinetics of distribution of naïve B-cells, activated B-cells, tissue-like memory (TLM) B-cells, and resting memory B-cells. (**C**) Comparative distribution of each B-cell subsets at different time-points as compared with healthy controls (EC). ns = nonsignificant and significant differences are indicated by *P*-values (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

cultured cells[17] therefore, justifying our findings for the increased frequency of plasma cells at pre-treated stage as compared with healthy controls. Thus, ectopic expression of Blimp-1 represses endogenous mRNA for CIITA and the CIITA targets, class II MHC, invariant chain,[16] going hand in hand with the observation in VL where upregulation of Blimp-1 was accompanied with the downregulation of CIITA, HLA-DR, and CD74. The persistent exposure to the antigen, during chronic infection causes B-cell exhaustion, which was seen in the form of gradual appearance of TLM population as the treatment progresses (Figure 2B and C). Furthermore, B-cells during active disease starts attaining regulatory characteristics, by serving as the non-redundant source of IL-10, seen as increased transcripts at pre-treatment. This key regulatory cytokine is assumed to be involved in promoting disease pathogenesis, which was in direct concordance with antibody titres (Pearson's coefficient $R^2 = 0.7433$, *P*-value = 0.0056) (Figure 3B) in accordance with the previously reported findings,[18] therefore inhibiting anti-microbial immunity contributing toward the disease pathogenesis and could serve as a driving factor for visceralization. Indeed, IL-10–producing B-cells have been known to inhibit $CD4^+CD25^+$ T-cell proliferation in vitro.[19] These findings imply that manipulating IL-10 production by human B-cells could be a useful therapeutic strategy for modulating immune responses in humans; in line with the studies from murine model.[20] However, the elevated level of IL-10 has been known to cause immune suppression in VL and also serves to stimulate DNA replication and immunoglobulin secretion.[6]

In addition, insight into the key drivers of B-cell differentiation, proliferation, plasma cell survival, maturation, and antibody production phenomenon emphasized on the study of BCMA and TACI. These markers were anti-apoptotic, survival signals for plasma cells that were elevated in the active diseased state which gradually declined during the course of the treatment (Figure 3C), as already documented in myeloma model,[21] established as essential marker of disease pathophysiology.

There are literatures which suggested for protective role, whereas others advocated for the pathogenic role of antibodies during *Leishmania* infection,[2,3,22,23] as they were considered irrelevant for VL unless recent literature suggested for strong correlation of serological status with the disease progression.[24] Similarly, studies of *Leishmania major*[3] and *Leishmania amazonensis*[25] suggest for the pathological role of antibodies in the murine system. Considering hypergammaglobulinemia as one of the major immune dysfunctions in VL along with emergence of TLM B-cells during disease led us to explore the role of these hypo-responsive B-cells as determinant for the humoral responses. We observed a positive correlation between VL-specific antibody responses with the percentage of TLM B-cells (Pearson's coefficient, $R^2 = 0.4855$, $P = 0.0009$) (Figure 3D). This study raised the concern to expedite the suspicious role of anti-leishmanial antibody

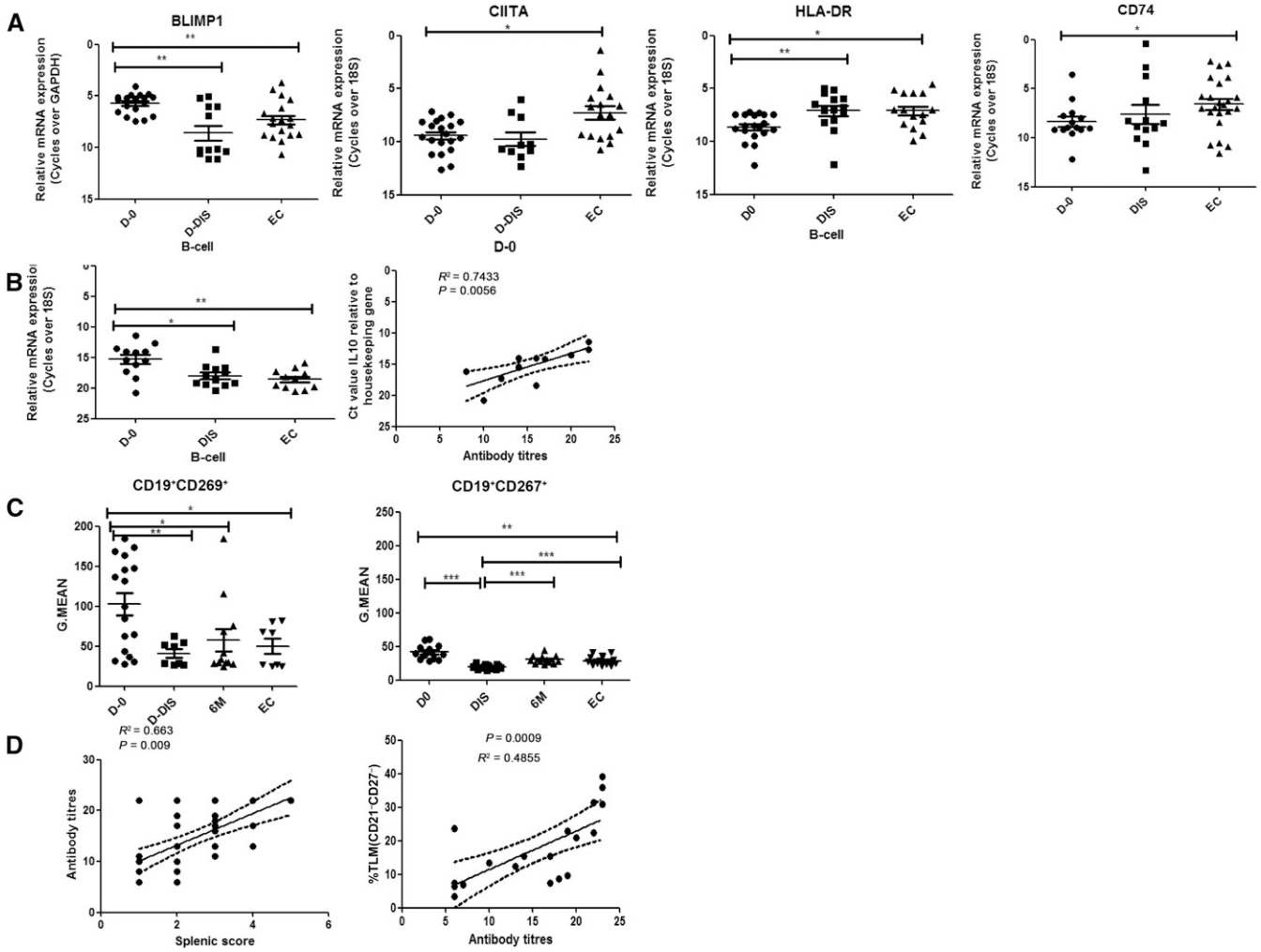

FIGURE 3. Diagrammatic representation of B-cells effector functions. (**A**) Real-time PCR–based transcript analysis of antigen-presentation capacity by master regulator B-lymphocyte–induced maturation protein-1 (Blimp-1) which regulates the transcription of MHC class II transactivator (CIITA), being the upstream regulator of human leukocyte antigen (HLA-DR), alteration in CIITA directly affects the antigen presentation seen as differential expression of HLA-DR and its invariant chain CD74. (**B**) Increased transcript level of interleukin-10 produced by B-cells during disease and linear regression plot for establishing its correlation with the antibody titres. (**C**) Expression of BCMA (CD269) and TACI (CD267) on B-cells determines their differentiation toward plasma cell and antibody production. Significant differences are indicated by *P*-values (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). (**D**) B-cell exhaustive functions in terms of linear regression plots. Correlation studies for the antibody titres with the percentage of tissue-like memory (TLM) B-cells or atypical B-cells (Pearson's coefficient, $R^2 = 0.4855$ and $P = 0.0009$). Positive correlation revealed from the analysis of antibody titres with the disease severity in terms of splenic scores from microscopic examinations (Pearson's coefficient, $R^2 = 0.663$, and $P < 0.0001$).

during VL. We observed strong correlation of VL-specific antibody titres with the parasite burden in the spleen as a direct measurement for disease severity ($R^2 = 0.3663$, $P < 0.0001$) (Figure 3D). Further dissection of the antibody titres with disease severity in terms of splenic scores indicated for the abundance of antibodies even at 10^7-fold dilution in cases with high parasite burden in the spleen (Table1). These findings provided clues regarding the devastating role of antibodies during VL, which was in line with the report by Miles et al.[3] Thus, changes in the magnitude of humoral responses and emergence of TLM cell population depend on the parasite burden.

Miles et al.[3] have shown for role of antibodies to promote disease progression by boosting IL-10 production from macrophages while switching off their IL-12 production machinery. Therefore, all these events prevented parasite elimination, in accordance with previously reported study which was also in dengue infection.[26] Furthermore, mice lacking immunoglobulin-producing B-cells were resistant to infection with different *Leishmania* strains,[27,28] whereas passive transfer of serum/purified antibodies restored their susceptibility. Similar findings were also reported from mice lacking mature B-cells toward *L. major*[2] or *L. donovani*[29] infections.

In conclusion, our data suggested for the decreased B-cell percentages from active VL cases with gradual attainment of TLM cell markers contributing to the B-cell dysfunctions in VL cases during the course of treatment. In addition, B-cells during VL had compromised abilities of antigen processing and presentation, increased production of anti-inflammatory cytokines and increased VL-specific antibody titres. This elevated titre of VL-specific antibodies directly correlated with the TLM B-cell phenotype and in turn disease severity seen in the form of parasite load in the spleen by microscopy. Anti-leishmanial treatment is assumed to gradually restore the functional features of B-cells. Together, this dataset indicated for the decreased B-cell percentages and impaired B-cell

functions in human VL, which may have important consequences for T-cell responses and affecting the overall immune status of the host in fighting against intracellular *Leishmania* parasite. We hypothesize that the emergence of TLM B-cells during the course of disease provides preliminary clues regarding B-cell dysfunctions as a consequence of chronic antigen exposure and over-activation. Thus, this study provides the preliminary evidences about the B-cell–mediated disease pathology in human VL and opens way for further insight into this cell population for better understanding the immunotherapeutic aspect of disease intervention. Perhaps, future studies are likely to examine the functional relevance of B-cell exhaustion in disease pathogenesis.

Received April 24, 2018. Accepted for publication October 14, 2018.

Published online February 18, 2019.

Acknowledgments: This work was supported in part by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID); by NIAID, NIH Tropical Medicine Research Centers (TMRC; grant 1P50AI074321); and by Council of Scientific and Industrial Research (CSIR, New Delhi).

Authors' addresses: Bhawana Singh, Om Prakash Singh, Neetu Singh, Siddharth Sankar Singh, and Shyam Sundar, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, E-mails: bhavanasonali9@gmail.com, opsbhu07@gmail.com, neetu24sep@gmail.com, singhsiddharth164@gmail.com, and drshyamsundar@hotmail.com.

REFERENCES

1. Ghose AC, Haldar JP, Pal SC, Mishra BP, Mishra KK, 1980. Serological investigations on Indian kala-azar. *Clin Exp Immunol 40:* 318–326.
2. Ronet C, Voigt H, Himmelrich H, Doucey MA, Hauyon-La Torre Y, Revaz-Breton M, Tacchini-Cottier F, Bron C, Louis J, Launois P, 2008. Leishmania major-specific B cells are necessary for Th2 cell development and susceptibility to L. major LV39 in BALB/c mice. *J Immunol 180:* 4825–4835.
3. Miles SA, Conrad SM, Alves RG, Jeronimo SM, Mosser DM, 2005. A role for IgG immune complexes during infection with the intracellular pathogen *Leishmania*. *J Exp Med 201:* 747–754.
4. Woelbing F et al., 2006. Uptake of *Leishmania* major by dendritic cells is mediated by Fcgamma receptors and facilitates acquisition of protective immunity. *J Exp Med 203:* 177–188.
5. Moir S, Fauci AS, 2008. Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease. *J Allergy Clin Immunol 122:* 12–19; quiz 20-1.
6. Rousset F, Garcia E, Defrance T, Peronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Banchereau J, 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci U S A 89:* 1890–1893.
7. Rickert RC, Jellusova J, Miletic AV, 2011. Signaling by the tumor necrosis factor receptor superfamily in B-cell biology and disease. *Immunol Rev 244:* 115–133.
8. el Harith A, Kolk AH, Leeuwenburg J, Muigai R, Huigen E, Jelsma T, Kager PA, 1988. Improvement of a direct agglutination-test for field studies of visceral leishmaniasis. *J Clin Microbiol 26:* 1321–1325.
9. Jacquet D, Boelaert M, Seaman J, Rijal S, Sundar S, Menten J, Magnus E, 2006. Comparative evaluation of freeze-dried and liquid antigens in the direct agglutination test for serodiagnosis of visceral leishmaniasis (ITMA-DAT/VL). *Trop Med Int Health 11:* 1777–1784.
10. Davies CR, Mazloumi Gavgani AS, 1999. Age, acquired immunity and the risk of visceral leishmaniasis: a prospective study in Iran. *Parasitology 119:* 247–257.
11. Saha S, Ramachandran R, Hutin YJ, Gupte MD, 2009. Visceral leishmaniasis is preventable in a highly endemic village in West Bengal, India. *Trans R Soc Trop Med Hyg 103:* 737–742.
12. Avery DT et al., 2010. B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J Exp Med 207:* 155–171.
13. Moir S et al., 2008. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med 205:* 1797–1805.
14. Portugal S et al., 2015. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. *Elife 4:* e07218.
15. Minnich M, Tagoh H, Bonelt P, Axelsson E, Fischer M, Cebolla B, Tarakhovsky A, Nutt SL, Jaritz M, Busslinger M, 2016. Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation. *Nat Immunol 17:* 331–343.
16. Piskurich JF, Lin KI, Lin Y, Wang Y, Ting JP, Calame K, 2000. BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. *Nat Immunol 1:* 526–532.
17. Silacci P, Mottet A, Steimle V, Reith W, Mach B, 1994. Developmental extinction of major histocompatibility complex class II gene expression in plasmocytes is mediated by silencing of the transactivator gene CIITA. *J Exp Med 180:* 1329–1336.
18. Ghalib HW, Piuvezam MR, Skeiky YA, Siddig M, Hashim FA, el-Hassan AM, Russo DM, Reed SG, 1993. Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. *J Clin Invest 92:* 324–329.
19. Bouaziz JD, Calbo S, Maho-Vaillant M, Saussine A, Bagot M, Bensussan A, Musette P, 2010. IL-10 produced by activated human B cells regulates CD4(+) T-cell activation in vitro. *Eur J Immunol 40:* 2686–2691.
20. Deak E, Jayakumar A, Cho KW, Goldsmith-Pestana K, Dondji B, Lambris JD, McMahon-Pratt D, 2010. Murine visceral leishmaniasis: IgM and polyclonal B-cell activation lead to disease exacerbation. *Eur J Immunol 40:* 1355–1368.
21. Carpenter RO, Evbuomwan MO, Pittaluga S, Rose JJ, Raffeld M, Yang S, Gress RE, Hakim FT, Kochenderfer JN, 2013. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin Cancer Res 19:* 2048–2060.
22. Moreno I, Molina R, Torano A, Laurin E, Garcia E, Dominguez M, 2007. Comparative real-time kinetic analysis of human complement killing of *Leishmania infantum* promastigotes derived from axenic culture or from *Phlebotomus perniciosus*. *Microbes Infect 9:* 1574–1580.
23. Dominguez M, Moreno I, Lopez-Trascasa M, Torano A, 2002. Complement interaction with trypanosomatid promastigotes in normal human serum. *J Exp Med 195:* 451–459.
24. Hasker E et al., 2014. Strong association between serological status and probability of progression to clinical visceral leishmaniasis in prospective cohort studies in India and Nepal. *PLoS Negl Trop Dis 8:* e2657.
25. Kima PE, Constant SL, Hannum L, Colmenares M, Lee KS, Haberman AM, Shlomchik MJ, McMahon-Pratt D, 2000. Internalization of *Leishmania mexicana* complex amastigotes via the Fc receptor is required to sustain infection in murine cutaneous leishmaniasis. *J Exp Med 191:* 1063–1068.
26. Halstead SB, O'Rourke EJ, 1977. Antibody-enhanced dengue virus infection in primate leukocytes. *Nature 265:* 739–741.
27. Chu N, Thomas BN, Patel SR, Buxbaum LU, 2010. IgG1 is pathogenic in *Leishmania mexicana* infection. *J Immunol 185:* 6939–6946.
28. Wanasen N, Xin L, Soong L, 2008. Pathogenic role of B cells and antibodies in murine *Leishmania amazonensis* infection. *Int J Parasitol 38:* 417–429.
29. Smelt SC, Cotterell SE, Engwerda CR, Kaye PM, 2000. B cell-deficient mice are highly resistant to *Leishmania donovani* infection, but develop neutrophil-mediated tissue pathology. *J Immunol 164:* 3681–3688.